TURRIALBA

REVISTA INTERAMERICANA DE CIENCIAS AGRICOLAS

VOLUMEN 34 TRIMESTRE OCTUBRE-DICIEMBRE 1984 NUMERO 4

CODEN: TURRAB 34(4)405-542

IICA INSTITUTO INTERAMERICANO DE COOPERACION PARA LA AGRICULTURA

San José, Costa Rica CR ISSN 0041 - 4360

EVOLUCION ESTACIONAL DE NUTRIMENTOS EN GUINDO DULCE (*Prunus avium* L.)[1]

*I. VIDAL**
*A. VENEGAS**
*C. HIDALGO**

Summary

*The variability of foliar N, P, K, Ca, Mg, Mn, Fe, Zn, Cu and B content as a function of time was studied in an irrigated, 18 year old cherry orchard (*Prunus avium *L.) Samples were taken during two seasons at 15 days intervals from leaves of 1, 2 to 3 and 4 to 5 years old woody branches. In general, N, P, Cu and Zn concentrations decreased as the growth period advances, while Ca, Mg, Fe and Mn concentration showed an inverse response. The behavior of K and B was very similar, having their higher concentration at the middle of the growing season. The period of higher stability of the macro and micronutrients occurs during the summer. There is an important effect on the mineral concentration according to the leave position for all the nutrients, except for N and P. Considering the good fit of the regression equation, correction factors are proposed for samples taken at any time during the growing season.*

Introducción

El cultivo del guindo dulce (*Prunus avium* L.) en Chile, presenta crecientes posibilidades de mercado como fruta fresca o industrializada, tanto para el consumo nacional como internacional. Entre los problemas que aquejan al manejo de este frutal, lo concerniente a la nutrición mineral, es de primera importancia. Esta situación se agudiza en la VIII Región por el hecho de que gran parte de los huertos están en suelos de baja fertilidad, como son los derivados de material granítico o los de origen aluvial de textura gruesa (8)

Una de las técnicas de diagnóstico que presenta mayor ventaja para estudiar el aspecto nutricional de los frutales, es el análisis foliar, el cual puede proporcionar la información necesaria para la determinación de deficiencia y/o exceso de los elementos esenciales en el árbol frutal (2). Este método ha permitido obtener muy buenos resultados en el control de la nutrición de especies permanentes y, como ejemplo de ello, se pueden citar trabajos en guindo (3, 16, 20), manzano (10, 21), damasco (1, 21), duraznero (17, 18, 19, 21, 22), vid (4, 9), almendro (6, 7), olivo (14), palto (11), pino (13) y ciruelo (15).

Para iniciar el estudio de la nutrición de un cultivo por análisis foliar, es necesario conocer una serie de factores que garanticen que la muestra represente el estado nutritivo de la planta en una determinada época (5). De aquí se deduce la importancia de fijar el tipo de hoja y la época en que ésta debe ser recogida.

Por la importancia y perspectiva futura que reviste esta especie frutal para esta zona, es que se planteó la presente investigación con el fin de: a) determinar la evolución estacional del contenido de nutrimentos (N, P, K, Ca, Mg, Fe, Zn, Cu, Mn y B) en hojas de guindo dulce, cultivar Corazón de Paloma o Royal Ann, b) establecer la mejor época de muestreo con propósitos de diagnóstico nutricional y c) medir el efecto de la posición de la hoja sobre la composición mineral.

Materiales y métodos

El estudio se realizó en un huerto de guindo dulce, cultivar Corazón de Paloma, de 17 años de edad, de riego, perteneciente a la Estación Experimental de la Facultad de Ciencias Agropecuarias y Forestales de la Universidad de Concepción en Chillán (36° 36' latitud sur y 72° 06' longitud oeste), VIII Región de Chile

1 Recibido para publicación el 3 de mayo, 1984.
Parte del Proyecto 20 26 07 de la Dirección de Investigación, Universidad de Concepción

* Dpto. Agronomía, Univ. de Concepción, Casilla 537, Chillán, Chile.

La región presenta características climáticas de transición entre el clima mediterráneo y el clima templado húmedo. Las precipitaciones son del orden de los 1 033 mm anuales, con períodos secos de 3 a 5 meses y una temperatura media anual de 14 a 15°C, presentándose las máximas en diciembre-enero y las mínimas, en julio. El suelo corresponde a la serie Diguillín, que de acuerdo a la séptima aproximación, se clasifica como Typic Dystrandept, originado de toba volcánica sedimentaria y ceniza volcánica, con buen drenaje y pendiente de 0 5 a 1.5%.

Para la selección de los árboles, se consideró la uniformidad en cuanto a desarrollo y vigor y su buen estado sanitario. Se escogieron así cuatro árboles, los cuales se usaron como repeticiones en el análisis estadístico de los resultados. El manejo sanitario comprendió la utilización de productos químicos que no interfirieran en la concentración foliar.

El muestreo se realizó durante las temporadas 1980-81 y 1981-82. Se obtuvieron hojas provenientes de maderas de 2-3 años, cada 15 días aproximadamente, entre los meses de octubre y marzo, inclusive. Con el propósito de determinar el efecto de la posición de la hoja sobre la composición mineral, en los meses de enero, febrero y marzo, se tomó tejido foliar proveniente de maderas de 1 año, de 4-5 años y más de 5 años, además del material de 2-3 años que se venía previamente muestreando.

En las muestras foliares se determinó N, por el método Kjeldahl y el resto de los nutrimentos, a excepción del B, se solubilizaron en una mezcla de ácido nítrico y perclórico. El Ca, Mg, Zn, Fe, Mn y Cu, se determinaron por espectrofotometría de absorción atómica; el K por fotometría de llama; el P por colorimetría. Para el B, se usó una mineralización vía seca y se determinó posteriormente en forma colorimétrica, de acuerdo a la metodología propuesta por Lachica (12).

Los coeficientes de variación para los diferentes elementos estudiados en las dos temporadas, se obtuvieron a partir de un análisis de varianza. Los valores del análisis con respecto a los días de postfloración de las dos temporadas, se ajustaron a ecuaciones de regresión de tipo cuadrática, cúbica, logarítmica y potencial, considerando cada elemento en forma individual. El período de mayor estabilidad en la composición mineral, se obtuvo a partir de las ecuaciones de regresión, según la metodología propuesta por Leece y Gilmours (18). Posteriormente, a partir de las mismas ecuaciones, se calculó un factor de corrección para las muestras tomadas antes o después del período de estabilidad.

El suelo correspondiente al huerto, se muestreó a tres profundidades (0-30, 30-60 y 60-90 cm), para evaluar sus características físicas y químicas). En los Cuadros 1 y 2 se indican los resultados de estos análisis. Con respecto a las características químicas, el perfil del suelo presenta contenidos normales de Ca, Mg, K, Na y relación C/N, en cambio los niveles de P son medios a bajos. El pH es ligeramente ácido y su contenido de M O es normal, disminuyendo en profundidad. El N total es más bien bajo, con tendencia a disminuir aún más en profundidad. En relación a las características físicas del suelo, llama especialmente la atención la mayor densidad aparente y una menor porosidad en el primer horizonte, atribuible esto a un mayor contenido de arcilla de el estrato superficial y el paso de la maquinaria agrícola en el huerto. En relación a las otras características éstas se encuentran dentro de los rangos considerados normales para esta serie de suelo (24).

Resultados y discusión

Variabilidad de los elementos

En el Cuadro 3, se presentan los coeficientes de variación de ambas temporadas, para los diferentes elementos estudiados. Se observa que existe un estrecho grado de asociación en el coeficiente de variación de las dos temporadas para todos los elementos, a excepción del Fe que varió de una temporada a otra. La variabilidad del Fe, se puede atribuir a que este microelemento tiene un comportamiento bastante irregular, pues es muy propenso a sufrir contaminación con partículas de polvo, las cuales son ricas en este nutrimento, o durante la molienda en micromolinos acerados del tipo Wiley.

El rango de variación de la primera temporada es de 7 a 66%, en tanto que en la segunda, éste va de 3 a 33% aproximadamente, presentando los menores valores el N, P, K, Mg y Zn, en tanto que el Ca, Mn, Fe, B y Cu mostraron valores superiores al 15%. Esta variabilidad, asociada a las fechas de muestreo, indica que es necesario contar con las curvas de evolución estacional para las diferentes condiciones edafoclimáticas en que se plante cerezo, con el propósito de estandarizar la época de muestreo.

De acuerdo a lo anterior, se infiere que es de particular importancia la época de recolección de la muestra foliar en el caso del Ca, Mn, Fe, B y Cu y de mediana importancia para N, K, P, Mg y Zn.

Evolución estacional

La evolución estacional de la composición foliar del guindo dulce, durante las temporadas 1980-81 y 1981-82, se representa en las ecuaciones del Cuadro 4 y en las Figuras 1 y 2. Se infiere que las ecuaciones

Cuadro 1. Características químicas del suelo del huerto.

Profund. (cm)	Ca	Mg	K	Na	pH	M.O. %	N total %	P Olsen ppm	Relac. C/N
	meq/100 g suelo								
0–30	9.50	2.78	1.15	0.33	6.10	6.70	0.28	13.0	13.93
30–60	8.75	2.34	0.99	1.09	6.20	3.40	0.11	3.0	18.18
60–90	8.25	3.50	1.20	1.09	6.50	2.10	0.04	4.0	10.00

Cuadro 2. Características físicas del suelo del huerto.

Profundidad (cm)	Arcilla %	Da	Dr	Porosidad %	Tensión Atm.	
		g/cc			1/3	15
					%	
0–30	26	1.04	2.46	57.5	35.71	16.13
30–60	21	0.86	2.57	64.4	36.55	20.12
60–90	19	0.98	2.71	63.9	32.44	19.69

Cuadro 3. Coeficiente de variación (C.V.) para los elementos estudiados en las dos temporadas.

Elemento	Coeficiente de variación (%)	
	Temporada 80-81	Temporada 81-82
Nitrógeno	C.V. = 9.61	C.V. = 7.68
Fósforo	C.V. = 8.62	C.V. = 8.29
Potasio	C.V. = 8.64	C.V. = 3.57
Calcio	C.V. = 14.59	C.V. = 15.78
Magnesio	C.V. = 8.46	C.V. = 7.48
Manganeso	C.V. = 14.72	C.V. = 20.56
Hierro	C.V. = 66.49	C.V. = 12.28
Cinc	C.V. = 7.43	C.V. = 10.07
Cobre	C.V. = 29.08	C.V. = 27.13
Boro	C.V. = 17.85	C.V. = 33.65

representan muy bien la evolución de los diferentes nutrimentos a través de la temporada. Los coeficientes de correlación obtenidos, fueron altamente significativos ($P \leq 0.01$).

Los niveles de N, P, Cu y Zn presentan una tendencia muy clara a disminuir con respecto al avance de la temporada de crecimiento, manteniéndose valores relativamente estables a partir del mes de enero. Esto concuerda en forma muy aproximada con trabajos realizados por Sánchez (22), Leece y Gilmour (18) y McClung y Lott (19).

El K y el B presentan un comportamiento muy similar entre sí con las mayores concentraciones a mediados de la estación de crecimiento (fines de diciembre), para posteriormente disminuir.

El Ca , Mg, Fe y Mn aumentan a medida que avanza la temporada de crecimiento. Sin embargo, el Fe y Mn presentan un período de cierta estabilidad a mediados de verano, para caer posteriormente en el mes de marzo.

Las tendencias descritas anteriormente, coincide con estudios realizados en guindo dulce (16, 20), así

Cuadro 4. Ecuaciones de regresión y coeficientes de determinación (R^2) para los diez elementos estudiados.

Elemento	Ecuación	R^2
	%	
N	$8.262F^{-0.38}$	0.87**
P	$0.595 - 0.088 \ln F$	0.59**
K	$1.087 + 0.018F - 9.12 \times 10^{-5}F^2$	0.40**
Ca	$0.19F^{0.51}$	0.72**
Mg	$0.084F^{0.311}$	0.69**
	ppm	
Fe	$211.15 - 4.96F + 0.067F^2 - 2.25 \times 10^{-4}F^3$	0.45**
Cu	$15.25 - 2.10 \ln F$	0.31**
Mn	$11.55 + 039F - 1.41 \times 10^{-3}F^2$	0.40**
Zn	$44.52 - 5.43 \ln F$	0.44**
B	$16.75 + 0.74$ F-$3.2 \times 10^{-3}F^2$	0.31**

** Significativo al $P \leqslant 0.01$.

F = Días después de floración (20 sep.).

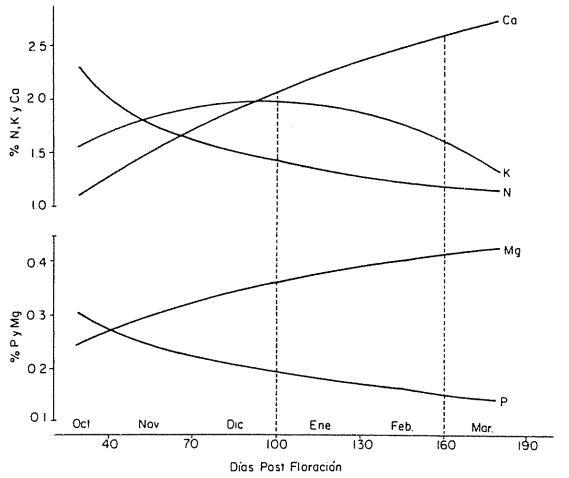

Fig. 1. Evolución estacional y período de mayor estabilidad de macronutrimentos en guindo dulce *(Prunus avium)*.

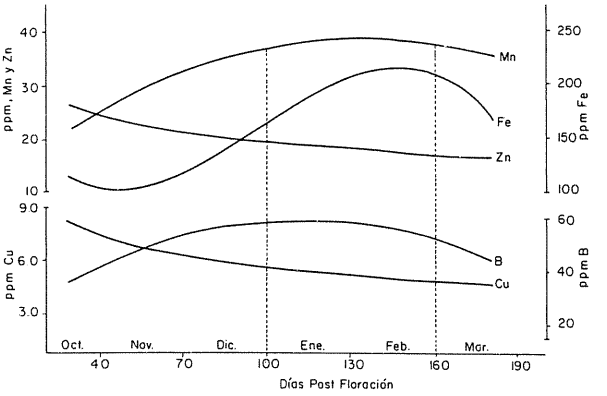

Fig. 2. Evolución estacional y período de mayor estabilidad de micronutrimentos en guinde dulce (*Prunus avium*).

como duraznero (18, 22) y damasco (1). En efecto, Smith (23) y Leece y Gilmour (18), señalan que las curvas estacionales son características para cada uno de los elementos y varían muy poco entre especies.

Las bases fisiológicas de los cambios descritos precedentemente, han sido discutidos por McClung y Lott (19), Emmert (5) y Smith (23). La tendencia descendente del N, P, Cu y Zn que ocurren en primavera, se debería a un rápido aumento en materia seca de la hoja que trae como consecuencia que estos elementos que están presentes inicialmente en altas concentraciones, manifiesten un efecto de dilución. Así, la concentración de estos elementos decrece, aunque en términos de cantidades absolutas muestran un incremento neto.

A la inversa, la tendencia ascendente del Ca, Mg, Mn, B y Fe en primavera, se puede atribuir a que estos elementos se presentan inicialmente en baja concentración y se acumulan más rápidamente que la materia seca de la hoja (5, 19, 23).

Al término de la temporada (marzo), bajan las concentraciones de N, K, P, Zn, B, Cu, Mn y Fe. Según algunos autores (5, 19, 23), este comportamiento se debe a una remobilización y traslocación de los elementos al comenzar la senescencia de la hoja. Sin embargo, llama la atención el caso de los micronutrimentos que se consideran elementos muy poco móviles a nivel foliar, por lo que su poder de traslocación sería despreciable.

Período de estabilidad de nutrimentos

La información presentada en las Figuras 1 y 2, reafirma la necesidad de caracterizar las muestras foliares con respecto a un período de muestreo definido y de cierta estabilidad. En efecto, se puede inferir que el período de mayor estabilización de los macro y mi-

cronutrimentos corresponde a los meses de enero y febrero. Al respecto, cabe señalar que esta fecha coincide con otros trabajos realizados en Chile y en el extranjero para estas especies (16, 20)

De acuerdo a lo indicado precedentemente, los muestreos foliares para propósito de diagnóstico nutricional en guindo dulce, deberían efectuarse dentro de los meses de enero y febrero. A su vez, los estándares de comparación deben provenir de muestreos dentro del mismo período. No obstante, considerando la limitación práctica de estar condicionado a un período restringido de muestreo, y de acuerdo al alto grado de ajuste ($P \leq 0.01$) de las ecuaciones de regresión (Cuadro 4), se calcularon factores de corrección para muestreos efectuados fuera de época. Estos factores de corrección, usando el 31 de enero como fecha base, se presentan en el Cuadro 5, para los meses de octubre, noviembre, diciembre y marzo. Cabe señalar, que estos factores deben considerarse sólo como una aproximación en el caso de los elementos con R^2 bajo (Cuadro 4), como es la situación del K, Cu, Mn y B.

Estos factores permitirán realizar muestreos fuera de época, aun cuando éstas no sean las de mayor estabilidad nutricional; esto significa en la práctica, una ayuda de gran importancia pues proporcionan un rango de tiempo mucho más amplio que lo recomendado por la literatura para efectuar los muestreos foliares

Influencia de la posición de la hoja

En el Cuadro 6 se presentan los resultados obtenidos en ambas temporadas, anulando la influencia de las fechas de muestreo. Para tal efecto, se obtuvo un promedio a partir de las diferentes fechas en cada uno de los distintos tipos de tejido analizado. Se puede inferir que no existe un efecto significativo ($P \leq 0.01$) de la posición de la hoja sobre los niveles de N y P mientras que para el K y el Fe, hay una variación acusada sólo en una temporada. Para el resto de los nutrimentos, hay un efecto importante de la posición de la hoja sobre su composición mineral, lo que sugiere que es decisivo mencionar para estos elementos el tipo de hoja muestreada en estudios nutricionales

Existe poca o nula información respecto de la distribución de nutrimentos en plantas de cerezo y en otras especies. En general, las experiencias que más se han realizado sobre este tema, son a nivel de invernaderos y con elementos marcados. Sin embargo, estos trabajos no aportan información que permita explicar el comportamiento de los diferentes nutrimentos, según el tipo de tejido

Llama la atención que el Ca, Mg, Mn, Zn y Fe se encuentran siempre en menores concentraciones en las hojas provenientes de madera de 1 año. Este fenómeno se atribuye a que todos estos elementos, a ex-

Cuadro 5. Factores de ajuste para la composición de muestras colectadas en octubre, noviembre, diciembre y marzo a nivel del 31 de enero.

Elementos	15 octubre	15 noviembre	15 diciembre	15 marzo
Nitrógeno	0.533	0.719	0.849	1.116
Fósforo	0.532	0.686	0.814	1.186
Potasio	1.270	1.044	0.960	1.302
Calcio	2.327	1.556	1.247	0.862
Magnesio	1.672	1.312	1.147	0.914
Manganeso	1.883	1.338	1.114	1.050
Cinc	0.667	0.793	0.885	1.096
Cobre	0.590	0.733	0.846	1.138
Hierro	1.636	1.982	1.516	1.086
Boro	1.768	1.230	1.040	1.218

Cuadro 6. Contenido de macro y micronutrimentos en relación a la posición de la hoja.

Posición	N	P	K	Ca	Mg	Mn	Zn	Cu	Fe
			%				ppm		
Temporada 1980–81									
Madera 1 año	1 19	0 16	1.90	1 79	0.32	25 3	13 9	3 81	160 9
2-3 años	1 30	0 16	1.83	2 82	0 39	34 7	17 6	5 12	230 3
4-5 años	1.25	0 12	1.95	2 37	0 37	34 2	61 9	6 41	256 9
Valor F	0.33	0.88	1 38	11 89**	4 55**	13 68**	7 45**	3 23*	16 86**
Temporada 1981–82									
Madera 1 año	1.22	0 20	1 73	1 53	0.30	26 3	14 2	6 48	148 3
2-3 años	1 21	0 18	1 62	2 39	0 39	39 3	17.5	5 62	177 7
4-5 años	1.18	0 21	1.57	2 56	0.38	36 6	15.0	8 63	148 7
Madera + 5 años	1.11	0 25	1.83	2 32	0.36	36 7	21.0	9.23	176.5
Valor F	1 96	1.56	5.24 **	36 47 **	14.23**	19.0**	15 13**	5 99**	2 9

Nota: El B se determinó sólo en las hojas provenientes de ramillas de 2-3 años.
En la temporada 1981-82 se incluyó tejido proveniente de madera de más de 5 años.

* Significativa $P \leq 0.05$

** Significativa $P \leq 0.01$.

cepción del Mg, son relativamente inmóviles a nivel foliar, por lo que su redistribución es muy reducida, tendiendo más que nada a acumularse en los tejidos más viejos. Cabe señalar que las variaciones de Ca y Mg se manifiestan de una forma paralela con respecto a la posición de la hoja

En general, se puede inferir que el tejido proveniente de madera de 2-3 años, es más representativo para ser utilizado con fines de diagnóstico nutricional, puesto que refleja condiciones minerales intermedias

Resumen

En un huerto de guindo dulce (*Prunus avium* L.), bajo riego de 17 años de edad, se estudió la variabilidad en función del tiempo del contenido foliar de N, P, K, Ca, Mg, Mn, Fe, Zn, Cu y B Se tomaron muestras en dos temporadas a intervalos de 15 días, considerando hojas provenientes de madera de 1 año, 2 a 3 años y 4 a 5 años. En general, la tendencia estacional del N, P, Cu y Zn es a disminuir, a medida que avanza la temporada de crecimiento, en tanto que el Ca, Mg, Fe y Mn presentan una situación inversa El comportamiento del K y B es muy similar entre sí, logrando sus mayores concentraciones a mediados de la temporada de crecimiento. El periodo de mayor estabilización de los macro y micronutrimentos, corresponde a los meses de enero y febrero. Para todos los nutrimentos, a excepción del N y P, hay un efecto importante de la posición de la hoja sobre su composición mineral. Considerando el alto grado de ajuste de ecua-

ciones de regresión, se proponen factores de corrección para muestreos efectuados en cualquier época dentro de la temporada de crecimiento

Literatura citada

1 BAHAMONDES, A y EMILIO, E Estudio nutricional del damasco en Chile Santiago, Chile, Universidad Católica de Chile, (Tesis Ingeniero Agrónomo) Facultad de Agronomía, 1974 66 p.

2 BOULD, C. Leaf analysis as a diagnostic method and advisory aid in crop nutrition. Experimental Agriculture 4(1):17-27. 1968

3 BOWERSOX, T W., WARD, W Seasonal variation in foliar nutrient concentration of black cherry Forest Science 23(4):429-432. 1977

4 CUMMINGS, G. A. Variation in the concentration of certain elements in muscadine grape leaves related to season, leaf portion and age. Journal of the American Society for Horticultural Science 102(3):339-342. 1977.

5 EMMERT, F. H. Chemical analysis of tissue as a means of determining nutrient requirements of deciduous fruit plants. Proceedings of the American Society for Horticultural Science 73:521-547 1959.

6. ESTEBAN, E., GOMEZ, M. y RECALDE, L. Dinámica anual de los macronutrimentos en el almendro. I. Primeros resultados Agrochimica, Italia 13(4-5):336-345 1969.

7. ESTEBAN, E., GOMEZ, M y RECALDE, L. Dinámica anual de los macronutrimentos en el almendro. II Equilibrios iónicos. Agrochmica, Italia 13(6):551-559. 1969

8. ETCHEVERS, J. y FARIAS, A. Características químicas de los principales suelos cultivados de Ñuble. Agricultura Técnica, Chile 39(2): 29:34. 1979

9 ETCHEVERS, J., MERINO, R y VIDAL, I. Prospección nutricional en viñedos de la costa de la VIII Región. Agricultura Técnica (Chile) 43(1):13-20. 1983.

10. GONZALEZ, C., DE JOANNES, A. y LACHICA, M. Estudio del contenido foliar diferencial de macro y micronutrimentos en distintas variedades de manzano, su ciclo vegetativo. Anales de Edafología y Agrobiología, España 31(7-8):543-555 1972.

11 KOO, R C. and YOUNG, T W. Effects of age position, and fruiting status on mineral composition of "Tonnage" avocado leaves. Journal of the American Society Horticultural Science 102(3):311-313. 1977.

12. LACHICA, M. Estudio sobre la determinación de B en plantas con azometina-H 4° Colloque internationale sur controle de l'alimentación de plantes cultives Vol 11:53-61. 1976.

13. LACHICA, M., KOSCHE, R. y GONZALEZ, C. El pinus radiata D. Don en Chile, determinación de los índices nutritivos óptimos de las plántulas. Anales de Edafología y Agrobiología, España 38(11-12):2141-2157 1979.

14. LACHICA, M., YAÑEZ, J. y AGUILAR, A. Determinación analítica de macro y micronutrimentos en olivo; su ciclo vegetativo In: Coloquio Europeo y Mediterráneo II. Control de la fertilización de las plantas cultivadas Sevilla, España, 1968 pp. 133-143.

15. LEECE, D. R Diagnostic leaf analysis for stone fruit. 4. Plum Australian Journal of Experimental Agriculture and Animal Husbandry 15(72):112-122. 1975.

16 LEECE, D. R. Diagnostic leaf analysis for stone fruit. 5 Sweet cherry. Australian Journal of Experimental Agriculture and Animal Husbandry 15(72):118-122. 1975.

17 LEECE, D R. and BARKUS, B. Diagnostic leaf analysis for stone fruit. 3. Nutritional status of peach orchards. Australian Journal of Experimental Agriculture and Animal Husbandry 14(71):828-834 1974

18. LEECE, D. R. and GILMOUR, A. R. Diagnostic leaf analysis for stone fruit. 2. Seasonal changes in the leaf composition of peach. Australian Journal of Experimental Agriculture and Animal Husbandry 14(71):822-827. 1974

19. McCLUNG, A. C. and LOTT, W. L. Mineral nutrient composition of peach leaves as affected by leaf age and position and the presence of a fruit crop. Proceedings of the American Society for Horticultural Science 67:113-120. 1956.

20. MANNS, J. E. Estudio nutricional del guindo en Chile. Tesis Ingeniero Agrónomo Santiago, Chile, Universidad Católica de Chile, Facultad de Agronomía, 1972. 63 p.

21. RAZATO, B. y URIU, K. Estado de la nutrición mineral de frutales de hoja caduca en Chile. Santiago, Chile, Servicio Agrícola y Ganadero, Central de Divulgación Técnica Boletín Técnico no. 47. s.f. 38 p.

22. SANCHEZ, L. Variación estacional de nutrimentos en durazno de la variedad Phillips Cling y Fortuna. (Tesis Ingeniero Agrónomo) Santiago, Chile, Universidad Católica de Chile, Facultad de Agronomía, 1970. 74 p.

23. SMITH, P. F. Mineral analysis of plant tissues. Annual Review of Plant Physiology 13:81-108. 1962.

24. VIDAL, I. y DEL CANTO, P. Propiedades físicas de los suelos de Ñuble, VIII Región de Chile. Agricultura Técnica (Chile) 43(3): 195-202. 1983.

Notas y comentarios

Hongo patógeno que ayuda a plantas

Las mariposas que van de flor en flor transmiten a veces organismos patógenos, dispersando así alguna enfermedad de la planta que visitan. Pero, por lo menos en un caso, uno de estos patógenos puede, en realidad ayudar a las plantas.

El hongo *Ustilago violacea* afecta a plantas cariofiláceas, familia que comprende a los claveles (*Dianthus*) y a las viscarias (*Lychnis*). Las esporas del hongo se producen en las anteras de las plantas víctimas, en lugar de los granos de polen; de allí el nombre de "carbón de las anteras".

Ola Jennesten de la Universidad de Upsala, en Suecia, estudió dos especies cariofiláceas silvestres, *Dianthus deltoides* y *Lychnis viscaria*, ambas adaptadas a la polinización por mariposas. Jennesten realizó su estudio en un campo soleado en el oeste de Suecia, y mientras las mariposas estaban alimentándose, recolectó algunas de ellas para análisis de las esporas fungosas y de los granos de polen que podrían estar transportando (**Oikos**, vol. 40, p. 125).

El estudio microscópico reveló esporas fungosas en por lo menos 13 diferentes especies de mariposas y una de polillas, aunque más individuos llevaban granos de polen que esporas de hongos. Así se comprobó que las mariposas eran las que diseminaban el carbón de las anteras entre esas plantas. Pero hay una variante feliz en esta triste historia.

Resulta que uno de los problemas que enfrentan todas las plantas con flores es evitar la autofertilización, que conduce a la endogamia, que tiende a debilitar a la descendencia. El problema es particularmente agudo en plantas con flores hermafroditas. Jennesten encontró que las anteras de alrededor de un tercio de la población de viscarias estaban afectadas con el hongo, y eran así incapaces de producir polen. Pero, aunque las flores eran estériles como machos, eran capaces de funcionar como hembras, y todas las plantas infectadas producían semillas. De esta manera, la infección fungosa era, en realidad, beneficiosa para las plantas: Al inhibir la producción de polen, estaba facilitando la fecundación con polen de otras plantas no infectadas. Y esto es una buena noticia para las plantas cariofiláceas. Adalberto Gorbitz.

EFFECTS OF SOIL MULCHES ON SOIL TEMPERATURE, PLANT GROWTH AND POTATO YIELDS IN AN ARIDIC ISOTHERMIC ENVIRONMENT IN PERU[1] /

*L.A. MANRIQUE**
*R. MEYER**

Resumen

Se evaluó el efecto de plásticos y paja de cebada usada como cobertura, sobre la temperatura del suelo y el crecimiento y rendimiento de variedades de papa comerciales peruanas, durante las estaciones de invierno y verano (1975-1977) en el medio árido, isotérmico de la Molina

Durante el invierno, la temperatura del suelo cubierto con plástico negro y blanco osciló entre 18 y 26°C. Bajo estas condiciones, los rendimientos de papa aumentaron en la mayoría de las variedades. En el verano, las coberturas plásticas elevaron la temperatura sobre 30°C, desfavoreciendo el crecimiento de las plantas y la formación de tubérculos.

El suelo cubierto con paja mantuvo una temperatura óptima y estable (inferior a 21°C) para la papa durante el invierno. En el verano, la paja redujo considerablemente la temperatura del suelo durante el día, pero en la noche siempre se mantuvo sobre los 20°C. El rendimiento con este tratamiento durante el verano solo alcanzó el 48% del rendimiento de invierno. Los resultados indican que si bien se logra reducir la temperatura del suelo con el uso de paja, el cambio no es suficiente para que la papa crezca bien en el verano.

Introduction

Different types of materials (straw, polyethylene plastic, gravel, and asphalt) have been used as soil mulches for different purposes. Some beneficial effects of soil mulches on soil temperature, moisture content, nutrient availability, and disease and weed control have been reported (1, 2, 3, 4, 5, 7, 8). Mulches have been used in extreme climatic conditions, either to increase night soil temperatures or to decrease day soil temperatures. However, very little information exists on the effects of soil mulches on potato performance in tropical and subtropical environments. The purposes of this paper are twofold: (1) to report the effects of soil mulches on soil temperature, and (2) to evaluate the effects of soil mulches on plant growth and tuber yields of non-heat tolerant potato varieties in an arid coastal environment of La Molina, Lima, Peru.

Potato production in the central arid coastal zone of Peru is limited to the winter season (June-September). Maximum and minimum air temperatures during this period are 22 and 12°C, respectively (Figure 1). With good water management, yield production during this season is similar to yields from high productive areas in the Andean region. However, no commercial production is conducted during the summer (December-March).

High temperatures and also high incidence of diseases and insect attack increase greatly the risk of crop failure during this season. Thus, potato production during the summer is entirely conducted in the

1 Received for publication in July 13, 1983. Journal Series No. 2760 of the Hawaii Agricultural Experiment Station. This work was supported by the International Potato Center, Lima, Peru.

* Research Associate/Agronomist, Benchmark Soils Project, University of Hawaii, Honolulu, Hawaii 96822, and former CIP Agronomist now Edaphologist, Water Management, Dryland Agriculture, S&T/AGR/RNR/AID, Washington, D.C. 20523.

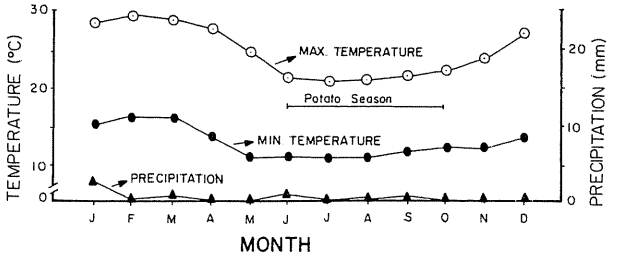

Fig. 1. Climatological data. Period 1968-1974. La Molina, Peru (Latitude 12°S, Longitude 76°57'W. Altitude 238 m.).

fairly favorable environment of the Andean region. No or very little potato is grown in the Andean region during the dry-winter season due to high risk of frost-killing temperatures and also to lack of irrigation. Thus, the potato production cycle in Peru is restricted to the winter in the Coast and to the summer in the Andean region.

Materials and methods

Experiments on soil mulches were conducted during the winter season of 1975 and summer seasons of 1976 and 1977 in a nonacid, coarse loamy, isothermic soil family of Typic Torrifluvents. The experimental site was located at the La Molina Agricultural Experiment Station, Lima, Peru.

Treatments of barley straw, and black and white polyethylene plastics were included in experiments conducted during the winter of 1975 (Experiment 1) and summer of 1976 (Experiment 2) (Table 1). A splitplot design was used with mulch treatments as main plots and varieties as subplots. Peruvian commercial varieties were planted in plots of two rows, spaced 90 cm apart and 3 m in length. The plastic mulches covered approximately 60 cm of the row width, whereas the straw mulches covered the plots completely. Plastic mulches were applied before planting. Holes 5 cm in diameter spaced 30 cm apart provided planting locations. Straw mulches were applied immediately after planting. In the unmulched + weed control treatment (control treatment), weed control was made either by repeated hand weeding (winter of 1975) or by application of the herbicide Sencor (metribuzin) after planting and 20 days after emergence (summer of 1976). The growth periods (planting to harvest) during the winter of 1975 and summer of 1976 were 110 and 93 days, respectively. During the summer of 1977, barley straw mulch rates of 5, 10, 15 and 20 metric tons/ha were applied in plots of four rows, spaced 90 cm apart and 6 m in length (Experiment 3). The control treatment (0 metric tons/ha of straw) received similar application of herbicide as the unmulched + weed control treatment in Experiment 2. Half of the mulch rate was applied after planting and the other half after 30 days. The growth period for this experiment was 105 days.

Table 1. Treatment and varieties included in mulch experiments.

Winter 1975	Summer 1976	Summer 1977
	Mulch treatments	
Black plastic	Black plastic	–
White plastic	White plastic	–
Unmulched + weed control	Unmulched + weed control	Unmulched + weed control
Straw 5 metric t/ha	Straw 5 metric t/ha	Straw 5 metric tons/ha
		Straw 10 metric t/ha
		Straw 15 metric t/ha
		Straw 20 metric t/ha
	Varieties (tbr x adg) +	
Rv, A, Y, C, R, M	Rv, M, Y, C, R	A, Y, C, MP
	Experimental Design	
Split-plot	Split-plot	Split-plot
(Rep. = 3)	(Rep. = 5)	(Rep. = 4)

+ tbr = tuberosum, adg = andigena, Rv = Revolucion, M = Mariva, A = Antarqui, Y = Yungay, R = Ranrahirca, MP = Mi Peru, C = Cusco.

Each experiment received fertilizer rates of 160, 120 and 120 kg/ha of N, P_2O_5 and K_2O, respectively. Phosphorus and K fertilizers were applied at planting, whereas half of N fertilizer was applied at planting and half at 30 days after planting. Irrigation was scheduled when the soil water tension (measured at 20 cm depth) exceeded 0.50 bars. Daily soil water tension readings were taken using tensiometers installed at 20 cm depth. Soil temperature data were recorded using thermocouples installed at 5, 10 and 15 cm depths. Only soil temperature data for days representing critical stages of the growing season are included in this study. Emergence, plant height, number of harvested plants, tuber yield and tuber size (data not included) were measured during the growing season and at harvest time.

Results and discussion

Soil temperature

Variations in soil temperature (5 cm depth) during the winter of 1975 (June 27-July 5) and summer of 1976 (December 24-February 21) are presented in Table 2. Soil temperatures during the winter of 1975 were recorded approximately 20 days after planting. Soil temperatures in straw mulched plots were below 21°C during the winter of 1975. Stable soil temperatures (small difference between daily maximum and minimum temperature) in straw mulched plots contrasted with those high variable soil temperatures in the unirrigated bare soil. Black plastic mulched plots had soil temperatures between 22 and 26°C whereas white plastic mulched plots had soil temperatures between 18 and 25°C.

Soil temperatures during the summer of 1976 were recorded approximately 20 days after planting (December 1975), during early plant development (January 1976), and during tuber initiation and early tuber development (February 1976). The daily soil temperature variation in straw mulched plots was slightly greater than that found during the winter season and the night soil temperature always was maintained above 20°C, particularly in critical stages such as tuber initiation and tuber enlargement. According to Went (9), optimum tuber formation usually occurs with night soil temperatures of 10 to 16°C and day temperatures of 16 to 20°C.

In the summer of 1976, black and white plastic mulched plots had day soil temperatures above 30°C and night soil temperatures above 20°C in most days (Table 2). The increase in soil temperature in plastic mulched plots was more a consequence of the vapor-barrier formed rather than differences in net radiation. White plastic materials have a higher reflectance than black plastic materials, therefore their net radiation should be less. However, white plastics allow a more effective entry of solar energy, producing a greenhouse effect. The stable night soil temperatures in irrigated inmulched plots during the summer of 1976, which were considerably lower than night soil temperatures in the unirrigated bare soil, show the effectiveness of irrigation in lowering soil temperatures. The accompanying reduction in soil temperature is attributed to the increase in specific heat and thermal conductivityof the soil with wetness and to greater evaporative cooling from the wet soil (6).

Soil temperatures at different straw mulch rates are shown in Figure 2. The effect of mulch rate on night soil temperature was minimal but there was a substantial decrease in diurnal soil temperature. No differences in soil temperature were found between straw mulch rates of 5 and 20 metric tons/ha, suggesting that low rates can be as effective as high rates. Although there was an overall effect of straw mulches on soil temperature, the data in Figure 2 shows again that night soil temperatures always were above 20°C. Effective decrease in day soil temperature was also found in irrigated unmulched plots.

Plant growth and tuber yields

In the winter of 1975, barley straw mulches caused initial low emergence but later increased plant height for all varieties (Table 3) and tuber yields for Revolucion and Yungay (Table 4). This delay in emergence was probably attributed to the physical barrier effect of the straw layer. Black plastic mulches initially caused a delay in plant growth, but the final tuber yields for Mariva, Yungay, Revolucion and Ranrahirca were significantly increased.

Visual observations taken during the summer of 1976 in some varieties showed important changes in plant morphology. Varieties, which in normal habitats, such as the winter season in the Peruvian coastal zone, develop great plant canopy, vigorous stems and large leaves, developed small plants with long and thin stems, small leaves, profused root development but few and small tubers during the summer of 1976. Similar changes in plant morphology were observed in potato mulched experiments conducted in an isohyperthermic Typic Taleudult at Yurimaguas (Meyer and Manrique, unpublished data). These changes are attributed to high soil temperatures, since other limiting factors, such as water and nutrient availability, were maintained at optimum levels. Such high soil temperatures probably altered important physiological processes such as water and nutrient uptake, photosynthesis and respiration rate, which are strongly reflected in the low tuber yields (Table 4).

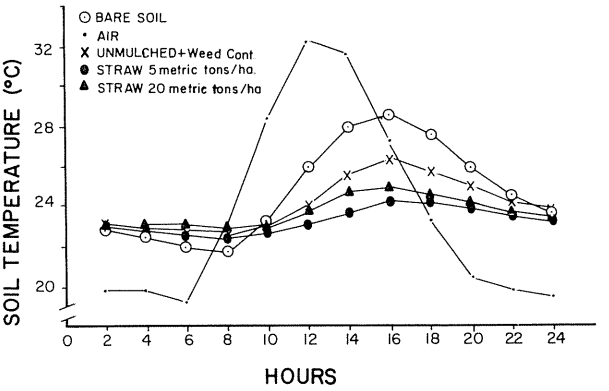

Fig. 2. Soil temperature variation (at 10 cm depth) in straw mulched plots (menas of six daily observations, February 13-18, summer of 1977).

Table 2. Effect of soil mulches on soil temperature (5 cm depth). Winter of 1975 and summer of 1976.

Season	Date		Air Temperature		Mulch Treatments									
					Bare Soil		Control*		Black Plastic		White Plastic		Straw	
			Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
			°C											
Winter 1975	June	27	10.5	18.0	15.0	25.0	–**	–	23.0	26.0	–	–	18.0	20.5
		28	10.0	18.0	15.0	20.0	–	–	23.0	25.0	–	–	18.0	19.0
		29	10.5	19.5	14.0	25.0	–	–	23.0	25.5	–	–	17.0	20.2
		30	12.0	17.0	15.0	21.0	–	–	23.0	24.0	–	–	18.0	19.5
	July	1	12.0	18.0	15.0	22.5	–	26.0	23.0	24.8	–	25.0	17.5	20.0
		2	9.0	18.0	12.0	22.0	14.0	–	22.5	–	–	–	16.5	–
		3	10.0	15.0	15.0	18.0	–	21.0	–	24.0	–	23.0	–	21.0
		4	10.0	12.0	14.0	15.0	16.0	17.0	23.0	23.5	19.0	21.0	20.5	20.5
		5	10.0	12.5	13.0	15.0	15.0	17.0	23.0	–	18.0	–	20.0	–
Summer 1976	Dec.	24	17.0	26.0	19.0	35.0	19.0	32.5	21.5	34.0	20.5	34.0	20.0	23.5
		25	16.0	25.0	20.0	32.0	20.0	30.0	21.5	32.5	–	32.5	20.0	–
		31	17.0	26.0	20.0	32.0	–	35.0	–	36.0	–	34.5	20.0	24.0
	Jan.	1	16.0	25.0	20.0	34.0	20.0	35.0	20.0	37.0	22.0	34.0	–	24.0
		2	16.0	27.0	20.0	36.0	–	–	–	–	–	–	–	–
		9	17.0	25.0	20.0	30.0	–	27.0	–	30.0	–	30.0	–	22.0
		10	17.0	27.0	19.0	39.0	20.0	27.5	21.5	31.5	23.0	31.0	21.0	23.0
		11	17.5	28.5	20.0	36.0	21.0	–	–	–	–	–	22.0	–
	Feb.	5	–	–	22.0	29.0	–	30.5	–	34.0	–	29.5	–	27.5
		6	–	–	22.0	29.0	21.0	27.5	24.8	33.0	21.0	27.0	22.5	27.0
		7	–	–	22.0	30.0	21.0	31.0	25.0	32.0	21.0	–	–	–
		19	20.0	25.5	23.0	26.0	–	23.0	–	26.5	–	–	–	23.0
		20	20.0	30.0	22.0	29.0	20.5	28.5	25.0	31.0	22.0	28.5	22.0	24.5
		21	20.0	31.0	22.0	31.0	20.8	–	24.0	–	20.0	–	21.5	25.0

* Control = Unmulched + weed control treatment.

** No available data.

Table 3. Effect of soil mulches on plant growth. Winter of 1975.

Varieties	Mulch Treatments		
	Unmulched + weed control	Black plastic	Straw
	Emergence (%)*		
Revolucion	75.0	90.0	85.0
Cusco	90.0	75.0	70.0
Antarqui	90.0	80.0	70.0
Mean	85.0	82.0	75.0
	Plant height (cm)**		
Revolucion	25.0	30.0	37.0
Cusco	33.0	27.0	38.0
Antarqui	28.0	26.0	33.0
Mean	28.7	27.7	36.0

* Measured at 20 days after planting.
** Measured at 55 days after planting

Symptoms of N deficiency in some white plastic mulched plots were also observed during the summer of 1976. Similar symptoms were reported in sorghum plants grown in gravel mulched plots (1). The author attributed such deficiency to radiation emitted from the gravel which could alter the metabolic process of N uptake and translocation. Also, symptoms of stem and leaf damage by burning were observed in black plastic mulched plots suring emergence. These effects reduced the final number of harvested plants (Table 5). Similar unfavorable effects were reported by Clarkson (5).

In the summer of 1976, yields of straw mulched plots were significantly superior to yields of other mulch treatments (Table 4). Yields in straw mulched plots, however, were only 48 percent of yields in similar plots during the winter of 1975. These results reflect the inability of Peruvian commercial varieties to perform in hot environments, even though a favorable decrease in soil temperature was obtained by the use of straw mulches.

Figure 3 shows the effects of straw mulch rates on tuber yields during the summer of 1977. Except for the variety Antarqui, which showed an almost linear response to mulch rate, significant yield response was obtained up to 10 metric tons/ha only. This response was consistent with the small variation in soil temperature at mulch rate higher than 5 metric tons/ha.

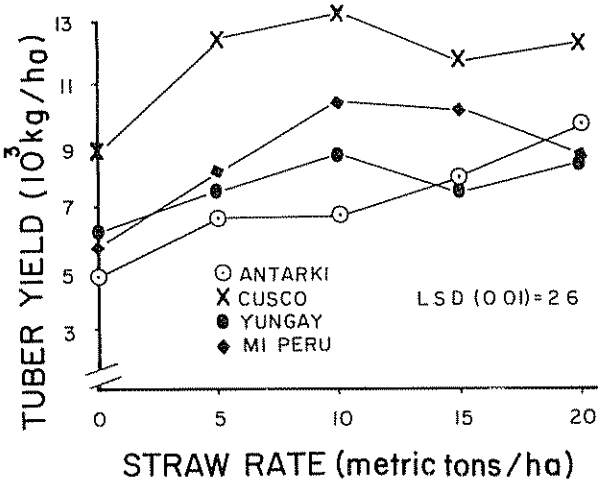

Fig. 3 Effect of straw mulch rates on tuber yield. Summer of 1977.

Conclusions

Favorable soil temperatures for potato growth was found under straw mulches during the winter season. The straw mulched environment promoted rapid plant growth for all varieties and high tuber yields for Revolucion and Yungay. During the summer, straw mulches reduced considerably day soil temperature and maintained a low daily variation, but the night soil temperature always remained above 20°C, which was significantly greater than the optimum required to assure adequate tuber initiation and tuber enlargement.

During the summer, black and white plastic mulches maintained high but also stable soil temperatures which were not conducive for tuber formation. Overall, the use of straw mulches holds better promise for increasing potato yields than plastic mulches, especially during hot and dry seasons. However, the favorable effects on soil temperature attributed to the straw mulches are not sufficient to overcome the inability of commercial varieties to produce tubers in hot environments such as coastal Peru in the summer.

Summary

Polyethylene plastic materials and barley straw mulches were used on a potato field to evaluate their effects on soil temperature, plant growth and tuber yields of Peruvian commercial varieties during the winter and summer seasons (1975-1977) in an aridic, isothermic environment at La Molina, Peru.

Table 4. Effect of soil mulches on tuber yields. Winter of 1975 and summer of 1976.

Season	Varieties	Mulch Treatments				
		Unmulched* weed control	Black plastic	White plastic	Straw	Mean for varieties
		10^3 kg/ha				
Winter 1975	Mariva	19.9	22.0	–*	19.3	20.4
	Cusco	27.0	23.3	–	23.1	24.1
	Yungay	15.9	19.7	–	17.1	17.6
	Revolucion	27.9	32.4	–	31.5	30.6
	Ranrahirca	14.0	22.3	–	14.8	17.0
	Antarqui	21.5	21.7	–	17.5	20.2
	Mean for mulches	21.0	23.4	–	20.6	–
	L.S.D. (0.01) Means for varieties				3.3	
	Means for mulches				2.7	
Summer 1976	Mariva	8.4	3.9	3.9	12.8	7.3
	Cusco	5.0	1.0	0.9	9.2	4.0
	Yungay	4.9	1.0	1.2	9.1	4.1
	Revolucion	7.7	0.7	0.6	9.7	4.7
	Ranrahirca	6.2	3.1	1.0	8.1	4.6
	Mean for mulches	6.4	1.9	1.5	9.8	–
	L.S.D. (0.01) Means for varieties					2.5
	Means for mulches					2.9

* No available data.

Table 5. Effect of soil mulches on the percentage of harvested plants. Summer of 1976.

Varieties	Mulch Treatments			
	Unmulched + weed control	Black plastic	White plastic	Straw
	%			
Mariva	67.0	57.0	55.0	72.0
Cusco	40.0	24.0	10.0	48.0
Yungay	58.0	22.0	35.0	72.0
Revolucion	67.0	39.0	34.0	65.0
Ranrahirca	58.0	46.0	27.0	70.0
Mean	58.0	37.6	32.2	65.4

During the winter, soil temperatures in black and white plastic mulched plots ranged from 18 to 26°C. The soil environment under these plastic mulches during the winter promoted relatively high tuber yields in most varieties. In the summer, plastic mulches significantly increased day soil temperatures above 30°C, resulting in a highly unfavorable environment for plant growth and tuber formation.

Straw mulches maintained optimum and stable soil temperatures (< 21°C) for economic potato production during the winter. During the summer, straw mulches reduced considerably day soil temperatures, but the night soil temperatures always remained above 20°C. Yields of straw mulched plots in the summer were 48 percent of yields of similar plots in

the winter The results indicate that straw mulches significantly lowered soil temperatures, but may not do so sufficiently to overcome excessively high soil temperatures during the summer months.

Literature cited

1. ADAMS, J. E. Effect of soil temperature on grain sorghum growth and yield . Agronomy Journal 54:257-261. 1962

2. ADAMS, J. E. Effect of mulches on soil temperature and grain sorghum development. Agronomy Journal 57:471-474. 1965

3. ADAMS, J. E. Effect of mulches and bed configurations. II. Soil temperature and growth and yield responses of grain sorghum and corn. Agronomy Journal 63:785-790. 1970.

4. BENNET, O.L., ASHLEY, D.A. and DOSS, B.D. Cotton responses to black plastic mulch and irrigation. Agronomy Journal 58:57-60. 1966

5. CLARKSON, V.A. Effect of black polyethylene mulch on soil and microclimate temperature and nitrate level. Agronomy Journal 52:307-309. 1960.

6. KHERA, K.L., KHERA, R., PRIHAR, S.S., SANDHU, B.S. and SANDHU, K.S. Mulch, nitrogen and irrigation effects on growth, yield and nutrient uptake of forage corn. Agronomy Journal 68:937-941. 1976.

7. LAL, R. Soil temperature, soil moisture and maize yield from mulched and unmulched tropical soils. Plant and Soil 40:129-143. 1974.

8. MILLER, D.E. and BUNGER, W.C. Use of plastic soil covers in sweet corn production. Agronomy Journal 55:417-419. 1963

9. WENT, F.W. Effects of parent and grandparent on tuber production by potatoes. American Journal of Botany 46:277-282. 1959.

Notas y comentarios

Premio Nobel de Química de 1984

Bruce Merrifield, de la Universidad Rockefeller, en Nueva York, quien efectuó roturas de frente cruciales en la revolución biotecnológica, ganó el premio Nobel de Química de 1984. En los primeros años de la década de los novecientos setenta, Merrifield tuvo una idea que revolucionó la química de los péptidos e inventó el procedimiento que lleva su nombre, "la síntesis péptida de estado sólido".

Para comprender muchos procesos biológicos, el científico debe trazar la secuencia de aminoácidos de una proteína. Debe saber cómo duplicar esa secuencia para manipular sus componentes en su investigación. Antes de Merrifield, los biólogos demoraban meses y aún años para sintetizar una cadena péptida. Las secuencias de aminoácidos no debía ser variada, pues ella era la que determinaba la clase de proteína resultante. La estrategia era poner las moléculas cuidadosamente en etapas. Después de cada paso, el producto deseado era aislado, trasladado a otra etapa, se efectuaba la nueva reacción, el nuevo producto se aislaba, y así sucesivamente. El tiempo empleado era enorme y los rendimientos muy pobres.

Las ideas de Merrifield irrumpieron en la escena en 1963 con una síntesis fácil de la bradikinina, una hormona péptida de nueve aminoácidos (nonapéptida). Lo que asombró a los químicos ese año fue el alto rendimiento y rapidez de la nueva síntesis: 68 por ciento en sólo ocho días. Bradikinina era la gran favorita como causa de la trasmisión del dolor, y para investigarla se necesitaban grandes cantidades de la sustancia. Para 1968, esas investigaciones demostraron que no existía tal papel importante para esta hormona.

Pero Merrifield no se había quedado quieto; tenía otras sorpresas sintéticas. Había fabricado ribonucleasa, con su secuencia de hasta 124 aminoácidos. Había automatizado el procedimiento, y con la ayuda del computador, los reactivos requeridos se podían agregar a intervalos predeterminados, y retirarlos cuando habían cumplido su misión. Y esto se realizaba sin parar, día y noche, en un solo recipiente. La ribonucleasa es importante como medicamento y para investigar estructuras de ácidos nucleicos y de cadenas proteínicas.

La idea concebida en 1961 era simple. En vez de separar la creciente cadena péptida después de cada paso de su síntesis, ¿por qué no adherirla a un soporte insoluble? De esta manera, cualquier subproducto indeseable, y las materias que no reaccionaron en cada paso, podrían ser lavados, dejando al protegido péptido pegado al soporte.

Para su soporte, escogió un plástico que era un copolímero de estireno y de divinilbenceno, en el cual algunos anillos bencénicos habían sido funcionalizados con grupos químicos reactivos. El primer aminoácido se introduce al sistema y reacciona con un grupo funcional de la resina, quedando pegado a ella. El grupo es extraído y se introduce el segundo aminoácido. La cadena péptida se va extendiendo en una serie de pasos, lavando bien después de cada uno de ellos y dejando la creciente cadena pegada en el soporte. Al final, se separa el péptido sintetizado de la bolita soporte. Este prototipo complicado se mejoró hasta llegar a modelos controlados por el computador programado para realizar todas estas operaciones. En ningún momento de la operación se separaba un producto intermedio y se purificaba, algo parecido a un acto de herejía química.

La técnica de Merrifield aceleró drásticamente la síntesis de péptidos, en especial cuando fue enteramente automatizada. Actualmente, Merrifield en la Universidad Rockefeller y Bob Sheppard en el laboratorio de Biología Molecular de Cambridge, han mejorado sustancialmente sus resinas y las condiciones para despegar los péptidos al final. Esto conduce a péptidos y proteínas grandes, de gran pureza, los que deben, por lo menos, ser tan buenos como los obtenidos por injertos de genes de la biotecnología.

Ya se está especulando sobre la aplicación, en el futuro, de esta técnica a otros grandes polímeros del mundo orgánico, los ácidos nucleicos tales como el DNA y el RNA. Esto sería otra revolución en la biotecnología y en la ingeniería genética. Merrifield probablemente sería el último en reclamar crédito por estas proyecciones de largo alcance de su idea. Modesto y de habla suave, su fortaleza de carácter es evidente en la larga batalla personal que él sostiene contra el cáncer de la piel. Adalberto Gorbitz

_ ESTUDO TAXONOMICO DO GENERO *Coffea* COM EMPREGO DE METODOS NUMERICOS[1] / _

*C. R. LOPES**
*R. A. DA CUNHA***
*L. F. BLOTTA****

Summary

Using flavonoid and phenolic compound constituents, methods of numerical taxonomy were applied to ten species and some cultivars of three species of the genus Coffea. *The purpose was to investigate the genetic relationships among them. The results pointed out* C. eugenioides *as one parent of the allotetraploid species* C. arabica, *but could not decide between* C. liberica *and* C. canephora *as the other parent. No evidence for a relationship between* C. congensis *and* C. arabica *has been found. It is suggested a taxonomic review of the genus* Coffea, *since species belonging to separated subsections according to Chevalier (3), showed more affinity than some species belonging to the same subsection.*

Introdução

Apesar do valor econômico de algumas de suas espécies, o gênero *Coffea* é ainda mal conhecido do ponto de vista sistemático e filogenético. Isto se deve à ampla distribuição geográfica das espécies do gênero no continente africano, à dificuldade de coleta do germoplasma existente, sua introdução e manutenção nos centros de investigação.

Dez espécies do gênero introduzidas no Brasil, distribuem-se na África em uma extensa área, em condições ecológicas das mais variáveis, que vão desde a floresta tropical úmida da Africa Equatorial até as regiões quase desérticas de Moçâmbique (1).

O estudo taxonômico do gênero *Coffea* tem se baseado em três critérios principais quais sejam: distribuição geográfica e características morfológicas (3), cruzamentos interespecíficos (1), análise de homologia cromossômica em híbridos interespecíficos (10) e análises serológicas (12).

A classificação proposta por Chevalier (3), baseada em investigações extensas mas incompletas e contraditórias, por considerar distribuição geográfica e quase que exclusivamente características de flores e de frutos, permaneceu por muitos anos como a mais completa classificação taxonômica do gênero.

Mais recentemente, novos métodos utilizados têm demonstrado que em certas categorias taxonômicas, como as subseções, o agrupamento de espécies proposto é notadamente artificial. Além disso, a descrição de novas espécies e a atual possibilidade de acesso a novas áreas da África têm enfatizado a necessidade de uma revisão na sistemática de *Coffea*.

1 Recebido para publicação em 23 mayo, 1984.
Trabalho parcialmente financiado pela Fundação de Amparo à Pesquisa do Estado de São Paulo e pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico.
Os autores agradecem ao Dr. Alcides Carvalho, da Seção de Genética do Instituto Agronômico de Campinas e ao Dr. Norberto da Silva, do Departamento de Agricultura e Silvicultura da Faculdade de Ciências Agronômicas, UNESP, "campus" de Botucatu, pela revisão do texto

* Departamento de Genética, Instituto Básico de Biologia Médica e Agrícola, UNESP, Botucatu, SP, Brasil.

** Instituto de Geociências e Ciências Exatas, UNESP, Rio Claro, SP, Brasil

*** Centro de Processamento de Dados da Escola de Engenharia de São Carlos, USP, SP, Brasil

Os resultados de cruzamentos interespecíficos têm questionado a classificação apresentada por Chevalier (3) e têm contribuido para esclarecer as relações filogenéticas entre as espécies. Contudo, as dificuldades inerentes a essa metodologia e o número restrito de espécies colocadas à disposição do geneticista para tais cruzamentos, não tem permitido conclusões gerais. Apesar disso, os dados obtidos por Carvalho e Monaco (1) permitiram-lhes sugerir a mudança de *C. eugenioides*, da subseção Mozambicoffea para a subseção Erythrocoffea, a de *C. stenophylla* da subseção Melanocoffea para a subseção Pachycoffea e a introdução no gênero *Coffea* da espécies *Kapakata*, considera por Chevalier como espécie monotípica do gênero *Psilanthopsis*. Similarmente, Charrier (2) tem relatado a ocorrência de alta frequência de cruzamentos e produção de grande número de híbridos, entre espécies de seções diferentes, pondo em dúvida os agrupamentos específicos feitos também nesta categoria taxonômica.

Devido às limitadas e contraditórias informações decorrentes dos estudos morfológicos, de distribuição geográfica e genéticos, espécies e cultivares foram analisados do ponto de vista da quimiotaxonomia, quanto à constituição de pigmentos flavonóides e compostos fenólicos (6, 7, 8, 9). Este método que permite uma análise mais rápida de grande número de indivíduos e oferece informações sobre a sequência evolutiva das espécies, demonstrou sua utilidade trazendo informações sobre a taxonomia do gênero *Coffea*, considerando as relações filogenéticas entre espécies. Através dele obteve-se um conhecimento mais preciso sobre as afinidades entre as espécies e variedades estudadas, sobre a origem de *C. arabica* e comprovou-se o inadequado agrupamente taxonômico dessas espécies.

No presente trabalho, os resultados referentes a composição de pigmentos flavonóides e compostos fenólicos foram analisados através de métodos de taxonomia numérica (13) com a finalidade de se obter novos informes e comparar as relações de similaridade entre espécies e variedades obtidas por este método, com aquelas resultantes das demais metodologias anteriormente utilizadas com vistas à filogenia.

Material e métodos

As espécies estudadas pertencem ao gênero *Coffea*, seção Eucoffea e de acordo com a classificação de Chevalier (3), com modificações introduzidas por Carvalho e Monaco (1) distribuem-se pelas seguintes subseções: Erytrocoffea: *C. congensis* Froehner, *C. canephora* Pierre ex Froehner, *C. arabica* L. e *C. eugenoides* Moore; Mozambicoffea: *C. racemosa* Lour, *C. salvatrix* Swyn et Phil, e *C. kapakata* Hirch; Pachycoffea: *C. liberica* Hiern e *C. dewevrey* De Wild et Durand; Melanocoffea: *C. stenophylla* G. Don.

Da espécie *C. arabica* analisaram-se as cultivares Mundo Novo, Caturra Amarelo, Caturra Vermelho, Bourbon Vermelho, Bourbon Amarelo, Laurina, Mokka, Geisha, X-321, K-7, BA-10, Cioiccie, além das cultivares Arabica, o tipo da espécie segundo Linnaeus e Abissinica, o tipo da espécie segundo Chevalier (3). Na espécie *C. canephora* analisaram-se as cultivares Kouillow e Robusta, por ser a primeira, o tipo da espécie segundo Chevalier (3) e a segunda por sua maior importância econômica e mais os cultivares Laurentii e Bukobensis. Da espécie *C. dewevrei*, foram analisadas as cultivares Dewevrei, Dybowsky e Abeokutae. Este material encontra-se em coleção na Seção de Genética do Instituo Agronômico do Estado, em Campinas, SP, e suas procedências e prováveis origens estão relacionadas em Lopes e Monaco (7 e 9).

As análises foram efetuadas na polpa (conjunto de exocarpo e mesocarpo) de frutos maduros. As amostras de polpas coletadas, foram colocadas ainda no campo em gelo seco (−80°C) a fim de evitar a ação de degradação das polifenoloxidases e nessas condições transportadas para o laboratório. O material foi então seco por liofilização e estocado em atmosfera de nitrogênio, a temperatura ambiente, até utilização. De cada espécie ou cultivar, foram analisadas, no mínimo, três plantas diferentes.

Os métodos utilizados para extração dos flavonóides e compostos fenólicos e para separação, detecção e classificação dos compostos, acham-se descritos em Lopes e Monaco (7, 9).

Os dados obtidos foram analisados através de procedimentos de taxonomia numérica, segundo Sneath e Sokal (13). Os valores de correlação cofenética (r) foram obtidos utilizando-se um conjunto de programas em métodos numéricos ("Numerical Taxonomy System") cedido por W. W. Moss da "Academy of Natural Sciences of Philadelphia" e implementado no Centro de Processamento de Dados da Escola de Engenharia de São Carlos, USP.

Resultados

No Quadro 1 se apresentam os resultados obtidos por Lopes e Monaco (9), na análise das espécies. Nela, *C. arabica* está representada pelas cultivares Arabica e Abissinica e *C. canephora* pelas cultivares Kouillow e Robusta. Nos Quadros 2 e 3 se apresentam os resultados obtidos por Lopes e Monaco (7, 8) nos estudos referentes, respectivamente, aos cultivares de *C. arabica* e *C. canephora*, estando incluidos no Quadro 3,

Quadro 1. Relação dos compostos isolados nas análises cromatograficas dos extratos de polpas de frutos maduros de espécies do gênero Coffea.

	Subseção Erythrocoffea						Subseção Mozambicoffea			Subseção Pachycoffea		Subseção Melano-coffea
Compostos	*Congensis*	*Arabica* (típica)	*Arabica* (abissinica)	*Canephora* (kouilou)	*Canephora* (robusta)	*Eugenioides*	*Racemosa*	*Kapakata*	*Salvatrix*	*Liberica*	*Dewevrei*	*Stenophylla*
1	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+		+	+	+		+	+	+	+	+
6	+	+	+	+	+	+	+	+		+	+	+
7	+	+	+	+	+	+	+	+		+	+	+
8	+	+	+	+	+	+	+	+		+	+	+
9	+	+	+	+	+	+	+	+		+	+	+
10	+	+	+	+	+	+	+	+		+	+	+
11	+	+	+	+	+	+	+	+		+	+	+
12	+	+		+	+		+	+		+	+	+
13	+	+	+	+		+	+	+	+	+	+	
14	+	+	+	+	+		+	+		+	+	+
15	+	+				+		+	+	+	+	
16		+	+	+	+		+	+		+	+	+
17	+	+	+	+	+	+	+			+		+
18	+	+		+	+		+			+	+	
19		+	+	+	+	+		+		+	+	
20		+				+	+	+			+	+
21	+			+	+		+			+	+	
22				+	+		+	+			+	+
23	+			+	+		+				+	
24		+	+					+		+	+	
25		+	+	+	+			+		+		
26				+	+					+	+	+
27		+					+			+	+	
28	+						+	+				
29		+	+	+	+	+						
30		+	+			+			+			
31					+					+	+	
32		+	+					+		+	+	
33		+	+			+	+		+			
34		+	+		+							
35								+			+	
36	+							+				
37		+						+	+			
38						+			+			

Continuación del Quadro 1.

Compostos	Subseção Erythrocoffea						Subseção Mozambicoffea			Subseção Pachycoffea		Subseção Melano-coffea
	Congensis	*Arabica* (típica)	*Arabica* (abissinica)	*Canephora* (kouillou)	*Canephora* (robusta)	*Eugenioides*	*Racemosa*	*Kapakata*	*Salvatrix*	*Liberica*	*Deverei*	*Stenophylla*
39						+			+			
40						+			+			
41						+			+			
42						+						
43		+		+	+							
44	+			+						+		
45					+		+					
46		+	+									
47	+											
48	+											
49	+											
50		+	+									
51		+	+									
52		+	+									
53										+		
54						+						
55						+						
56									+			
57									+			
58									+			
59									+			
60									+			
61									+			
62									+			
63									+			
64									+			
65									+			
66									+			
67									+			
68				+	+							
69				+	+							
70								+				
71								+				
72			+									
73			+									
74			+									
75			+									

Quadro 2. Relação dos compostos isolados nas análises cromatográficas dos extratos de polpas de frutos maduros de cultivares de *C. arabica*

Compostos	CULTIVARES													
	C. arabica	*Mundo Novo*	*B. Vermelho*	*B. Amarelo*	*Cat. Vermelho*	*Cat. Amarelo*	*Laurina*	*Mokka*	*Abissinica*	*Geisha*	*Cioccie*	X 321	K 7	BA 10
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+		+	+	+
16	+	+	+	+	+	+	+	+	+	+		+	+	+
17	+	+	+		+	+	+	+		+	+	+	+	+
18	+	+	+	+	+	+		+		+	+	+	+	+
19	+	+		+	+	+	+	+	+	+	+	+	+	
20	+	+	+	+		+	+	+		+	+	+	+	
21	+	+		+	+		+	+	+	+	+	+	+	
22	+	+	+	+		+	+	+	+	+	+		+	
23	+	+		+	+	+	+	+	+	+			+	+
24		+			+		+	+	+	+	+	+	+	+
25		+	+		+	+		+		+	+	+	+	+
26	+	+	+	+	+	+	+	+		+				
27				+	+		+		+	+	+	+		+
28	+	+	+		+	+		+						+
29	+	+		+		+	+		+					+
30	+		+		+			+	+	+	+			
31	+			+			+	+		+	+			+
32				+	+		+		+					
33	+				+				+				+	
34	+		+						+				+	
35	+		+		+									+
36		+				+		+						
37	+	+							+					
38			+			+						+		
39			+					+						
40	+													+
41		+				+								
42									+					
43											+			
44											+			
45											+			
46												+		
47										+				

Quadro 3. Relação dos compostos isolados nas análises cromatográficas dos extratos de polpas de frutos/maduros de cultivares de *Coffea dewevrei* e de *Coffea canephora*

	C. dewevrei				*C. canephora*			
Compostos	*Dewevrei*	*Dybowsky*	*Aheokutae*	Compostos	*Robusta*	*Kouillou*	*Laurentii*	*Bukobensis*
1	+	+	+	1	+	+	+	+
2	+	+	+	2	+	+	+	+
3	+	+	+	3	+	+	+	+
4	+	+	+	4	+	+	+	+
5	+	+	+	5	+	+	+	+
6	+	+	+	6	+	+	+	+
7	+	+	+	7	+	+	+	+
8	+	+	+	8	+	+	+	+
9	+	+	+	9	+	+	+	+
10	+	+	+	10	+	+	+	+
11	+	+	+	11	+	+	+	+
12	+	+	+	12	+	+	+	+
13	+	+	+	13	+	+	+	+
14	+	+	+	14	+	+	+	+
15	+	+	+	15	+	+	+	+
16	+	+	+	16	+	+	+	+
17	+	+	+	17	+	+	+	+
18	+	+	+	18	+	+	+	+
19	+	+	+	19	+	+		+
20	+	+	+	20	+	+	+	
21	+		+	21	+	+	+	
22	+		+	22	+	+	+	
23	+		+	23	+	+	+	
24	+		+	24	+	+	+	
25		+	+	25		+	+	+
26		+	+	26	+			+
27		+	+	27	+			+
28	+	+		28		+		+
29	+	+		29	+	+		
30	+			30	+	+		
31	+			31	+			
32		+						
33			+					
34			+					
35			+					
36			+					

também os dados obtidos para três cultivares de *C. dewevrei* (6).

Os dados foram usados para o cálculo do coeficiente de semelhança, SM ("Simple – Mactchin"). Por conveniência de escala mediu-se semelhança por (1-SM) entre espécies e entre cultivares. A partir dessas matrizes, espécies foram agrupadas com recurso do W.P.G.M.-A (Weighted Pair-Group Method – Arithmetic averages) obtendo-se o fenograma da

Figura 1 e, pelo mesmo método, cultivares foram agrupadas, resultando o fenograma da Figura 2.

Os valores de correlação cofenética (r), traduzindo a representatividade das matrizes de semelhança pelos fenogramas correspondes, mostraram-se como seria de esperar, mais altos quando são reunidas variedades de uma mesma espécie (r = 1.00 para as de *C. dewevrei;* r = 0.96 para as de *C. canephora* e r = 0.91 para as de *C. arabica*), do que quando as diferentes espécies são agrupadas (r = 0.80).

Discussão e conclusões

As relações taxonômicas e filogenéticas das espécies do gênero *Coffea* têm sido interpretadas por diversos autores. Estes estudos, conduziram a agrupamentos diversos, evidenciando as dificuldades encontradas para estudos dessa natureza num gênero ainda pouco conhecido quanto a vários aspectos como: a distribuição geográfica, a ecologia e a própria sistemática de suas espécies.

Pelo emprêgo de estudos quimiotaxonômicos, através da análise de flavonóides e compostos fenólicos, constatou-se ser difícil indicar a devida posição de *C. eugenioides,* uma vez que esta espécie apresentou maior afinidade por *C. arabica* da subseção Erythrocoffea onde foi incluida pelos resultados obtidos por Carvalho e Monaco (1) e por *C. salvatrix* da subseção Mozambicoffea, na qual foi colocada por Chevalier (3). Estes mesmos estudos de quimiotaxonomia comprovaram que a espécie *kapakata* deve pertencer ao gênero *Coffea,* como sugerem Carvalho e Monaco (1), pelos altos valores de afinidade apresentados por esta espécie com as espécies de *Coffea* analisadas, mas não confirmam a colocação de *C. stenophylla* na subseção Pachycoffea, como indicam esses mesmos autores, uma vez que esta espécie tem afinidades igualmente altas por espécies da subseção Mozambicoffea. Este método comprova a alta afinidade existente entre *C. liberica* e *C. dewevrei,* o mais alto obtido entre duas espécies, resultado este obtido também em todos os demais tipos de estudo realizados. A análise do fenograma ilustrativo das interrelações entre as espécies estudadas (Figura 1) corrobora as conclusões acima expostas.

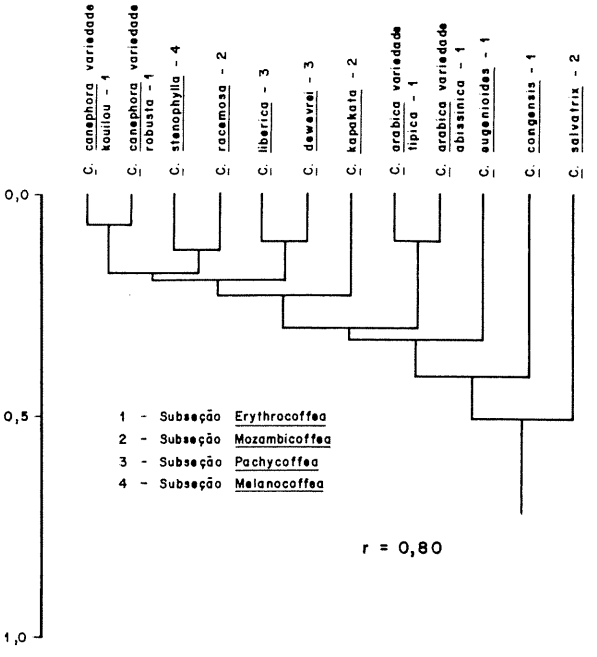

Fig. 1. Fenograma ilustrativo das interrelações de espécies do gênero *Coffea,* obtido por "W.P.G.M.–A" e correspondente correlação cofenética.

C. salvatrix revelou-se, pelos métodos numéricos empregados, a espécie mais distante das demais espécies do gênero. Seguem-se *C. congensis* e *C. eugenioides.* É interessante notar que estas espécies constituem um grupo de menor semelhança com as demais espécies analisadas e que *C. eugenioides* é entre as espécies de sua subseção, a espécie mais próxima de *C. arabica.*

O estudo quimiotaxonômico (Quadro 1) ao demonstrar a existência de alguns compostos pertencentes à classe dos diidroflavonóis (9), ocorrendo apenas em *C. salvatrix,* em *C. eugenioides* e em *C. arabica,* embora em frequências progressivamente menores, além da afinidade existente entre elas, e os resultados decorrentes deste estudo numérico, demonstrando a maior semelhança de *C. arabica* com *C. eugenioides* e desta com *C. salvatrix,* comprovam as suposições de diversos autores como Cramer (4), Carvalho e Monaco (1) e Narasimhaswamy e Vishveshwara (11), de que *C. eugenioides* seja uma das espécies que teve participação na formação de *C. arabica.* Comprovam também que *C. salvatrix* deve ter contribuido com alguns genes, por hibridações esporádicas (introgressão), para o conjunto gênico de *C. eugenioides.* As áreas geográficas limítrofes ocupadas por estas duas espécies, teriam favorecido tais hibridações ocasionais ou então pode-se supor que *C. eugenioides* seja derivada de cruzamentos naturais, nos quais *C. salvatrix* tenha sido uma das espécies envolvidas.

A análise do fenograma da Figura 1, demonstra ainda que *C. arabica* relaciona-se em mesmo nível de semelhança com a espécie *C. canephora* de sua própria subseção, com as espécies *C. liberica* e *C. dewevrei* de Pachycoffea, com *C. racemosa* de Mozambicoffea e com *C. stenophylla* de Melanocoffea. Tais espécies vistas como componentes de um grupo, relacionam-se com *C. arabica,* sendo entratanto mais seme-

lhantes a *C. kapakata*, fato este que justifica a inclusão de *C. kapakata* no gênero *Coffea*.

O mesmo nível de semelhança apresentado por *C. arabica* com as espécies *C. liberica* e *C. canephora*, torna difícil sugerir qual delas tenha sido a outra espécie a ter participado de sua formação, embora alguns autores como Cramer (4) e Krug e Carvalho (5) tenham sempre sugerido a participação de *C. canephora*, inclusive por sua área geograficamente mais próxima da de *C. arabica*.

A análise de pigmentos flavonóides e compostos fenólicos ao determinar que *C. arabica* apresenta seus maiores valores de afinidade com *C. canephora* e com C. liberica, não permite decidir entre ambas, indicando porém, afinidade ligeiramente mais alta entre esta última e *C. arabica*.

Por outro lado, Cramer (4) e Hills Ris Lambers, citado por Cramer (4), sugerem a possibilidade de *C. congensis* ter sido, além de *C. eugenioides*, a outra espécie a participar da formação de *C. arabica*, baseados no fato de ocorrerem áreas de sobreposição das três espécies no continente africano. Neste trabalho, o fenograma da Figura 1 demonstra que entre as espécies da subseção Erythrocoffea, *C. congensis* apresenta o menor grau de semelhança com *C. arabica* o que não justifica a proposição desses autores.

O mesmo fenograma da Figura 1 evidencia alto grau de semelhança entre *C. liberica* e *C. dewevrei*, de nível próximo à semelhança entre *C. racemosa* e *C. stenophylla* ou entre cultivares de *C. canephora*. Estes resultados referentes a *C. liberica* e *C. dewevrei* confirman os resultados obtidos em todos os demais métodos de estudo empregados, mas o alto nível de semelhança entre *C. racemonae* e *C. stenophylla* sugere a inclusão desta última na subseção Mozambicoffea e não em Pachycoffea como pretendem Carvalho e Monaco (1), apesar dessas espécies ocuparem áreas geográficas distantes e extremamente distintas. A análise quimiotaxonômica situa *C. stenophylla* entre as duas subseções, não permitindo nenhuma conclusão definitva (9).

No que se refere às espécies *C. eugenioides* e *C. stenophylla*, todos os resultados quando comparados indicam que elas se comportam como pontes, a primeira entre as subseções Erythrocoffea e Mozambicoffea e a segunda entre Pachycoffea e Mozambicoffea.

Na análise do fenograma para cultivares (Figura 2) verifica-se que dentro de *C. arabica*, as cultivares Mundo Novo e Caturra Amarelo, Bourbon Amarelo e Laurina, Mokka e Geisha, X-321 e K-7 são as mais semelhantes entre si. Dentre as cultivares brasileiras, aquelas consideradas originárias de cruzamentos naturais,

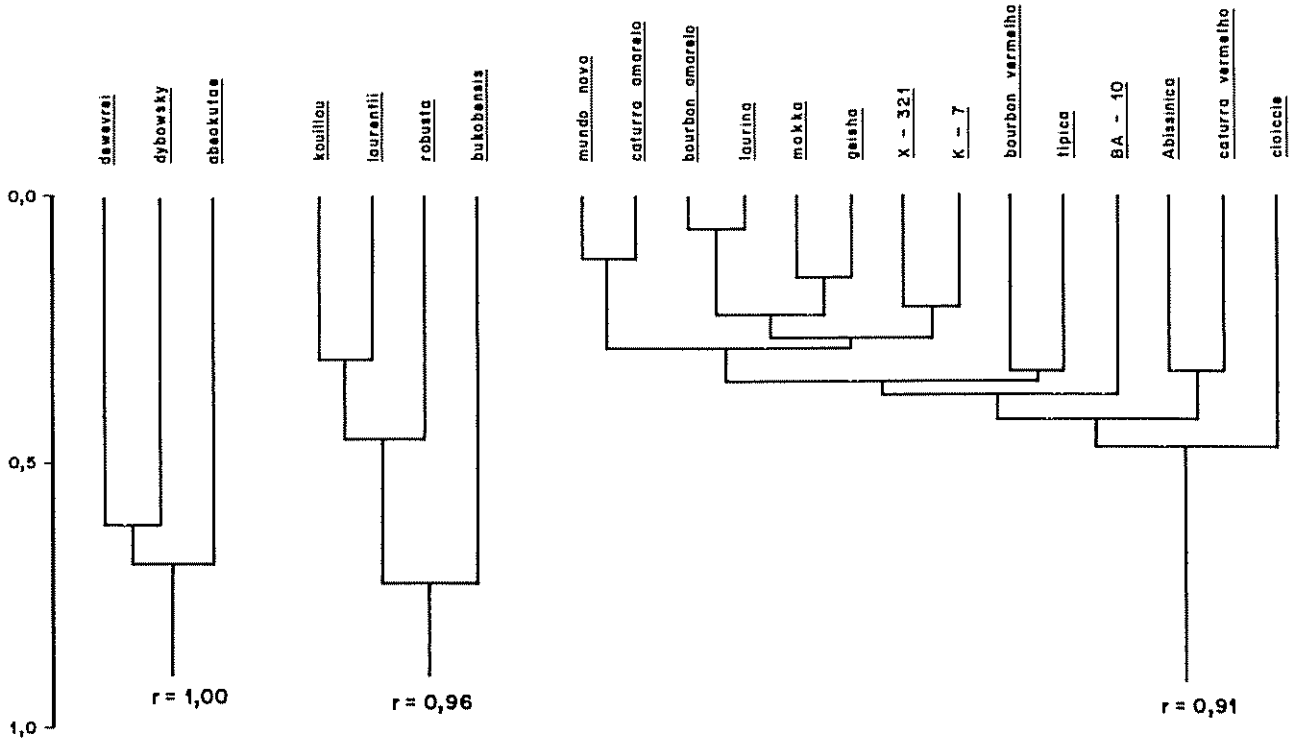

Fig. 2. Fenogramas ilustrativos das interrelações de cultivares de *C. arabica*, *C. canephora* e *C. dewevrei* obtidos por "W.P.G.M.–A" e correspondentes valores de correlação cofenética.

Mundo Novo, Bourbon Amarelo e Caturra Amarelo, são as que se apresentam mais semelhantes como era de se esperar, pois foram formadas a partir de um mesmo conjunto gênico (7).

Quanto às cultivares de *C. dewevrei*, apenas se pode constatar maior semelhança entre Dewevrei e Dybowsky e menor semelhança de ambas com Abeokutae. Entre as cultivares de *C. canephora*, os valores de semelhança mostram maior interrelação entre Kouillou e Laurentii, com Robusta menos próxima e Bukobensis bastante distante.

A análise numérica, como apresentada, confirma resultados obtidos por outros métodos quanto à artificialidade dos agrupamentos nas subseções e os dados aqui relatados evidenciam a necessidade da inclusão de outras características que permitam novas análises, para aprimorar os agrupamentos de espécies do gênero *Coffea*.

Resumo

As relações genéticas entre dez espécies de *Coffea* e algumas cultivares de três destas espécies, foram estudadas do ponto de vista quimiotaxonômico quanto à composição de pigmentos flavonóides e compostos fenólicos, sendo os dados obtidos analisados com recurso de metodologia de taxonomia numérica. Os resultados demonstraram a utilidade do método empregado para estabelecer relações de afinidade entre as espécies e entre os cultivares analisados e confirmaram a necessidade de uma revisão sistemática do gênero, uma vez que, algumas espécies de subseções diferentes apresentaram entre si maiores níveis de semelhança, do que espécies de uma mesma subseção. Comprovou-se a participação de *C. eugenioides*, juntamente com *C. liberica* ou *C. canephora* na formação da espécie tetraplóide *C. arabica*, não havendo contudo dados suficientes que justifiquem a indicação definitiva entre uma das duas últimas espécies. Foi excluída a participação de *C. congensis* como possível constituinte de *C. arabica*, baseando-se nos resultados das plantas examinadas.

Literatura citada

1. CARVALHO, A. e MONACO, L. C. Genetic relationship of selected species of *Coffea*. Ciência e Cultura 19:151-165. 1967.

2. CHARRIER, A. Contribution a l'étude genetique des Mascarocoffea. VII Colloque Scientifique International sur le café 9-14/6, Hamburg. 1975. 8 p.

3. CHEVALIER, A. Les cafeièrs du globe III. Systematique des cafeièrs et faux cafeièrs, maladies et insects nuisibles. Paul Lechevalier (ed.), Paris. 1947. 356 p.

4. CRAMER, P. J. Review of literature of coffee research in Indonesia. Interamerican Institute of Agricultural Sciences. Turrialba (Costa Rica) (Miscelaneous publ. nº 15) 1957. 262 p.

5. KRUG, C. R. and CARVALHO, A. The genetics of *Coffea*. Advanced Genetic 4:127-158. 1951.

6. LOPES LONGO, C. R. Estudo dos pigmentos flavonóides e sua contribuição à filogenia do gênero *Coffea*. Tese de Doutoramento. Escola Superior de Agricultura "Luiz de Queiroz", USP, Piracicaba, SP. 1972. 91 p.

7. LOPES, C. R. e MONACO, L. C. Estudos de quimiotaxonomia em cultivares de *Coffea arabica* L. Turrialba 27(1):55-61. 1977.

8. LOPES, C. R. e MONACO, L. C. Flavonóides em cultivares de *Coffea canephora*. Bragantia 27(5):XXXI-XXXIV. 1978.

9. LOPES, C. R. e MONACO, L. C. Chemotaxonomic studies of some species of the genus *Coffea*. Journal of Plantation Crops 7(1): 6-14. 1979.

10. MEDINA, D. M. Caracterização de híbridos interespecíficos de *Coffea*. Tese de Doutoramento. Escola Superior de Agricultura "Luiz de Queiroz". USP, Piracicaba, SP, 1972. 110 p.

11. NARASIMHASWAMY, R. L. and VISHVESHWARA, S. Report on hybrids between some diploid species of *Coffea* L. Indian Coffee 25(3):104-109. 1961.

12. OLIVEIRA, A. R., PAYNE, R. C. e FAIRBROTHERS, D. D. Disc. electrophoretic investigation of *Coffea arabica* and *Coffea canephora* general protein and malate dehydrogenase of cotyledonary leaves. Acta societatis botanicorum poloniae, XLII(2): 301-308. 1973.

13. SNEATH, P. H. A. and SOKAL, R. R. Numerical taxonomy. The Principles and Practice of Numerical Classification. W. H. Freeman and Company (ed.), San Francisco, 1973. 573 p.

Notas y comentarios

Premio Nobel de Economía de 1984

Patrones vitales, tales como el producto bruto nacional y el índice de precios al consumidor, que se consideran ahora medidas esenciales del comportamiento económico de un país, sólo se han llegado a usar extensivamente en recientes décadas. El premio Nobel de Economía de 1984 ha sido otorgado al inglés Richard Stone, el creador de estos índices durante los primeros años de la segunda guerra mundial, y que después enseñó al mundo cómo calcularlos y utilizarlos en la política económica. Según Erik Lundberg, un miembro del comité Nobel,, "Stone hizo el trabajo sucio, pesado. Lo desarrolló bajo la presión de un problema urgente y con el gran estímulo de Keynes".

Cuenta R. F. Harrod, en su biografía de Keynes (Londres 1951), que al asumir Churchill el gobierno británico en 1941, se reunió un equipo grande de economistas para coordinar el esfuerzo económico. A Robinson, quien había seguido con interés el estudio de Keynes, "How to pay for the War" (Londres 1940), presionó sobre la necesidad de estudios oficiales con la clase de datos que Keynes empleó en ese libro, e informó que Richard Stone era el hombre ideal para el propósito. Así se inició la contribución de Stone a la economía moderna. A fines de 1940, él y J. Meade (Nobel 1977) trabajaron duro en un análisis del ingreso y del gasto nacionales. En búsqueda de mayor autoridad y guía en sus trabajos, se acercaron a la oficina de Keynes, quien les brindó el mayor estímulo y apoyo. Keynes, a su vez, presionó a las autoridades sobre la enorme importancia de tales estadísticas y de su publicación al mismo tiempo de la presentación del presupuesto de 1941. El título completo de la publicación fue "An analysis of the sources of war finance and an estimate of the national income and expenditure in 1938 and 1940". Esto fue ciertamente una revolución. Por primera vez, las cuentas incluían estimados, los cuales muchos consideraban como peligrosos. Sin embargo, esta clase de contabilidad del ingreso nacional ha llegado a considerarse como la herramienta esencial de todo planeamiento, ya sea del tipo individualista o socialista, y casi todas las naciones han seguido a la Gran Bretaña en presentar tales cuentas.

Stone consideró que el trabajo de 1941 era sólo un comienzo y que era necesario mucho más trabajo posterior. En esto, Keynes cooperó también, logrando que Stone, reteniendo su cargo en la recién creada Oficina Central de Estadística, fuese nombrado como su asistente personal en el Tesoro. El trabajo sobre el ingreso prosiguió posteriormente cuando Stone pasó a colaborar en las Naciones Unidas en la difusión de los métodos de lograr estos índices. El sistema es ahora usado en todo el mundo, y las organizaciones mundiales tales como las Naciones Unidas, el Banco Mundial y el Fondo Monetario Internacional, publican anualmente sus cuadros comparativos que muestran la situación económica de cada país.

Dos consideraciones finales cabe hacer sobre los premios Nobel de este año. La primera se refiere a algo que caracteriza tanto al de Economía, como al de Medicina y al de Química. Y es que en este año, el énfasis se ha dado a la creación de sistemas y procedimientos que han hecho posible la aplicación en gran escala de contribuciones teóricas previas, abriendo el campo a la utilización práctica de estos procedimientos, por los gobiernos y por la medicina. El sistema de cuentas nacionales de Stone ha facilitado la aplicación racional de políticas económicas. La invención de los anticuerpos monoclonales por Milstein y Köhler, basados en las teorías de Jerne (premio de Medicina), ha abierto potencialidades de producción industrial de métodos de diagnóstico y de terapia, en la lucha contra las enfermedades. Y, por último, Merrifield, premio de Química, desarrolló un proceso automatizado para fabricar, paso por paso, una cadena péptida, y sintetizar en poco tiempo nuevas drogas, vacunas y reactivos, por la industria farmacéutica.

La otra consideración se refiere a la influencia keynesiana en la ciencia económica de la actualidad. Keynes murió antes de la creación de los premios de Economía, pero su influencia se siente en algunos de los premiados. Hicks (Nobel 1972), con su interpretación gráfica del modelo teórico de Keynes (las curvas IS--LM); Meade (Nobel 1977), con su invención de las contribuciones variables a los seguros sociales; son otros ejemplos de la influencia del genio de Keynes. Adalberto Gorbitz.

COMPARATIVE ANATOMY OF FLOWER BUDS WITH AND WITHOUT POTENTIAL FOR ABSCISSION IN *Phaseolus vulgaris*[1] /

*P YAÑEZ**
*E PIMIENTA***
*E MARK**
*J KOHASHI**

Resumen

Ovulos de botones florales con alto potencial de abscisión mostraron alto porcentaje (96%) de anormalidades anatómicas e irregularidades en el desarrollo del saco embrionario. En contraste pocos (31%) óvulos de flores con bajo potencial de abscisión fueron anormales. Se observaron las siguientes anormalidades anatómicas: necrosis del saco embrionario y de la nucela, ausencia o desarrollo retardado del saco embrionario. Estas y otras observaciones sugieren que la abscisión de botones florales se relaciona con las anormalidades previamente desarrolladas en los óvulos

Introduction

In beans, *Phaseolus vulgaris*, abscission of reproductive organs, buds, and young pods, accounts for the loss of up to 80% of potential seeds (2, 3, 4, 17).

Published studies on aborted ovules generally treat fruits or flowers at anthesis; the flower-bud stage has passed unnoticed. Different authors have related abortion to irregularities in the development of the embryo sac or its absence (7, 12, 16), to embryo-sac degeneration (10, 13) and to collapse of the nucellus and chalaza (10, 15). These studies examine principally woody plants. Williams (18) mentions that flowers of *Phaseolus vulgaris* usually contain normal ovules. Ormrod *et al.* (9) find that high temperatures (up to 35°C) stimulate the degeneration of the embryo sac of *Phaseolus vulgaris*.

In the present study, the ovules from flower buds of bean with high potential (more prone) for abscission, with those of buds with low potential (less prone) were anatomically compared.

Material and methods

Flower buds were collected from plants (*Phaseolus vulgaris* L. Cv. Cacahuate-72) grown in a greenhouse. This is a determinate variety with terminal, subterminal and axillary inflorescences.

It was noticed that under these greenhouse conditions, the subterminal inflorescence (two flowers in the axil of a trifoliolate leaf) of the main stem almost always produces two pods with seeds. On the other hand, flowers from the second and third nodes of the terminal inflorescence of the branch at the fourth or fifth nodes, almost never produce pods. These are the chosen sites of low and high potentials for abscission. The buds were collected at the stage when the corolla was just protruding from the enveloping bracteoles. Those with high potential for abscission (12 buds) were pale green and abscised when tapped lightly with the finger. Buds with low potential for abscission (12 buds from subterminal inflorescences of the main stem) were deep green and did not abscise when tapped lightly with the finger.

1 Received for publication in March 2, 1983

* Centro de Botánica, Colegio de Postgraduados, 56230 Chapingo, México

** Centro de Genética, Colegio de Postgraduados, 56230 Chapingo, México

The dissected ovaries were fixed in Craf III (11) and embedded in paraffin. Ten-micron sections were prepared by standard histological techniques, stained with safranin and fast green (6) and mounted in synthetic resin.

Results

The normal bean ovule is campylotropous, bitegmic and crassinucelate. The embryo sac is of the Polygonum type (Figure 1). As the embryo sac matures, the nucellar tissue desintegrates partially. The mature megagametophyte is adpressed to the inner epidermis of the inner integument.

Ovules from flower buds with high potential for abscission were characterized by higher frequencies of abnormalities. The abnormalities commonly observed were the following (Table 1): a) Necrosis of the embryo sac and the nucellus, which could occur in the same ovule (Figure 2), although in some cases necrosis of the embryo sac was not accompanied by the that of the nucellus. Necrosis could occur throughout the embryo sac or be localized (Figures 2, 3, 4), and generally was accompanied by separation between the integuments or between the inner integument and the nucellar tissue (Figures 2, 3). Necrosis in the nucellus could occur close to the micropyle, around the base of the embryo sac, or alongside the whole embryo sac. A total of 83% of ovules had necrosis in one or more parts. b) Some ovules (14.6%) lacked embryo sac c) Megagametophyte development in 23% of the ovules showed a delayed development. These abnormalities were so common that only 4% of the ovules were normal.

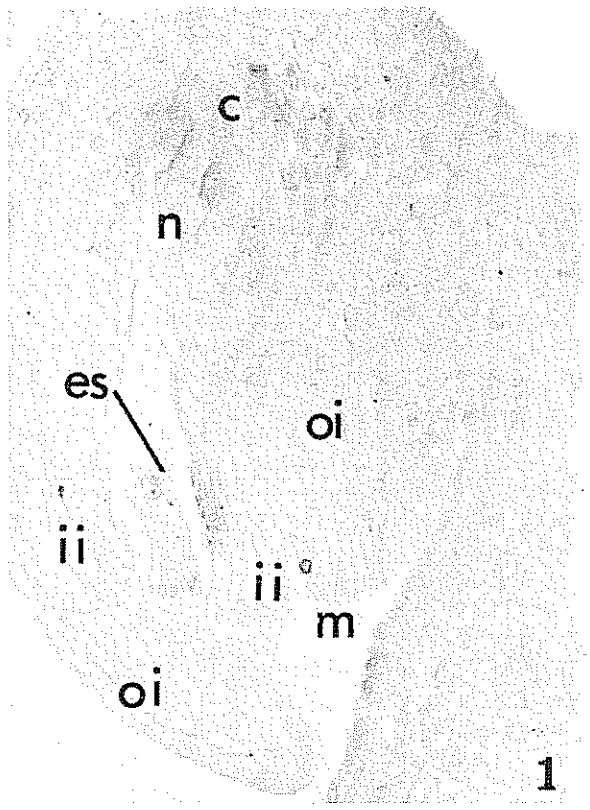

Fig. 1. Ovule from a flower bud with low abscission potential. m, micropyle; oi, outer integument; ii, inner integument; n, nucellus; c, chalaza; es, embryo sac. X285. All figures are of longitudinal sections.

Table 1. Percentages of necrosis and developmental irregularities in ovules of flower buds with high and low potentials for abscission.

	High Potential, %	Low Potential %
Necrosis in the whole embryo sac	27.1	7.8
Necrosis in antipodal cells	10.4	3.1
Necrosis in egg apparatus	8.3	9.4
Necrosis in chalaza	4.2	0
Necrosis in nucellus	43.8	6.2
Total ovules with necrosis	83.3*	25.0*
Total ovules without necrosis	16.7	75.0
Embryo sac absent	14.6	1.5
Differentiation arrested at 2-4 nuclei	14.6	6.3
Differentiation arrested at 4-8 nuclei	8.3	0
Mature embryo sacs, with or without necrosis	62.5	92.2
Total ovules with mature embryo sacs and without necrosis	4.1	68.8

* The percentage of ovules with necrosis in smaller than the sum of the percentages of necrosis in different parts, because some ovules had necrosis in more than one part.

In contrast, in ovules from flower buds with low potential of abscission, 69% of the embryo sacs developed normally to maturity (Table 1). This is because, in ovules with low abscission potential, 25% displayed necrosis, 1.5% lacked the embryo sac and 6% had delayed gametophyte development (Table 1).

Discussion

The anatomical abnormalities detected mainly in flower buds with high abscission potential are similar to those reported in studies of ovule abortion in bean (9, 18) and other species (7, 10, 12, 13, 15, 16). Different types of abnormalities frequently exist, and they occur at several stages in development.

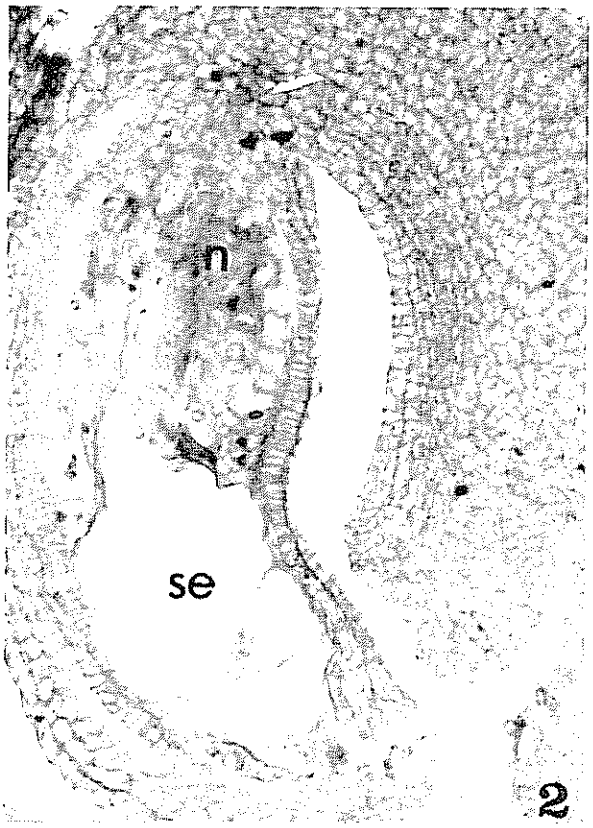

Fig 2. Ovule from a flower bud, with high abscission potential showing necrosis of the embryo sac (se) and of the nucellus (n) X373

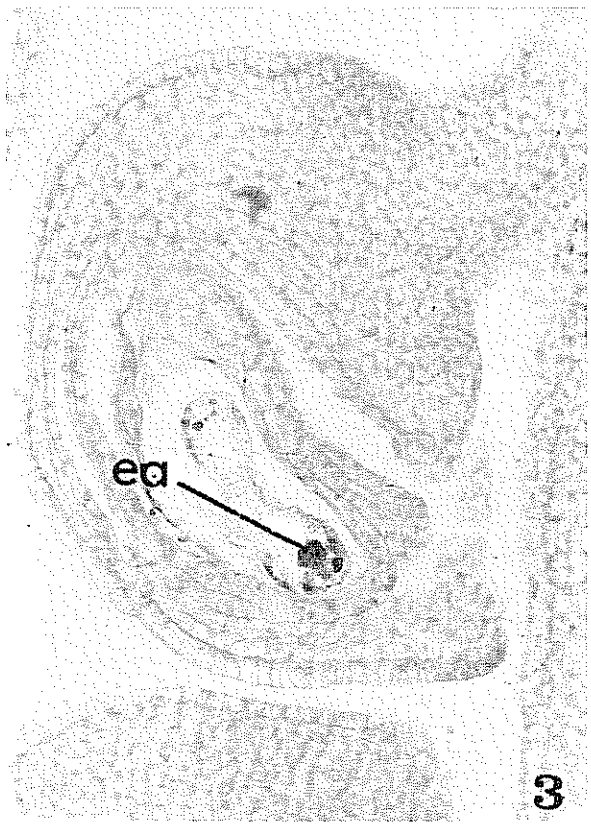

Fig 3. Ovule from a flower bud with high abscission potential, showing necrosis of the egg apparatus (ea) X405

The physiological mechanism for the control of flower bud abscission is not fully understood (14). Our observations suggest that this mechanism affects the development of the embryo sac and induces senescence of both the embryo sac and nucellar tissue.

Adato and Gazit (1) reported that young fruits of avocado produce ethylene mainly in the seed coat, and expressed the opinion that this may induce abscission in young fruits. Sedgley (12) attributes the rise in ethylene to arrested development of the fruits. In the case of *Phaseolus* buds with high potential for abscission, the possibility exists that senescent embryo-sac and nucellar tissue might be a source of synthesis of ethylene, which is well known to stimulate the processes leading to abscission by inhibiting polar auxin transport (5). Observations by one of us (PYJ) have shown that anatomical abnormalities precede the formation of the abscission zone. This suggests that anatomical abnormalities play an important role in abscission of flower buds. However, Sedgley (12) indicates that degeneration of the embryo sac does not cause abscission, but that these two phenomena possibly are due to inefficient distribution of water and nutrients, cessation of growth, and production of ethylene.

Such a mechanism may be related to correlative senescence, by which the abscission of one organ is controlled by another organ (8). If this were the case, it might be possible that flower buds with low abscission potential stimulate the abscission of those with high abscission potential, giving rise first to developmental abnormalities. The normal embryo sac might attract more photosynthates into the developing ovules. Failure or alteration of the normal development of the embryo sac thus would reduce that attraction, triggering abscission.

Summary

Ovules from flower buds with high potential for abscission showed a high percentage (96%) of anatomical abnormalities and irregularities in the development of the embryo sac. In contrast, few (31%) ovules from flowers with low potential for abscission were abnormal. The following anatomical abnormalities were identified: necrosis of embryo sac and nucellus, absence or delayed development of embryo

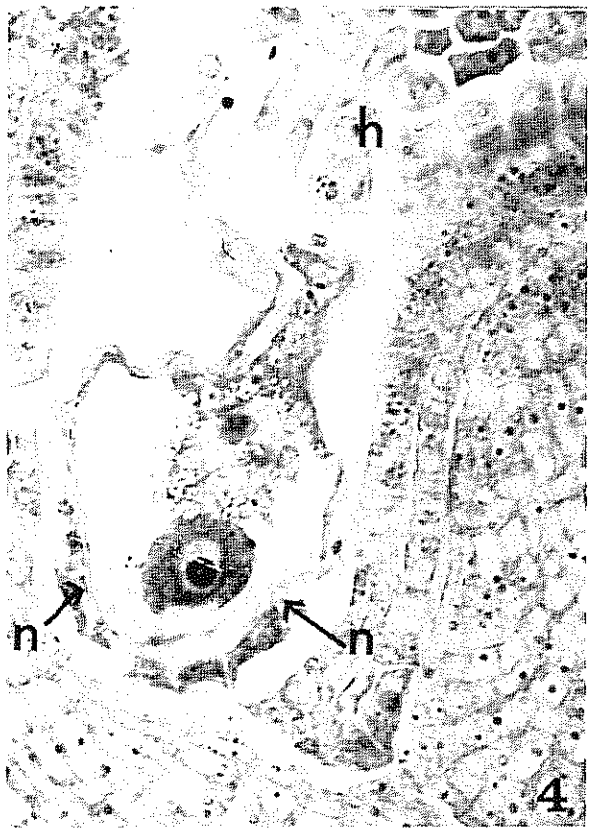

Fig. 4 Ovule from a flower bud with high abscission potential showing necrosis of the embryo sac in an eraly ontogenetic stage, collapse of the nucellus (arrows), hyperplasia (h) of the nucellus. 737X

sac. These and other observations suggest that abscission of the flower buds is related to previously developed anatomical abnormalities in the ovules.

Literature cited

1. ADATO, I., and GAZIT, S. Role of ethylene in avocado fruit development and ripening. II. Ethylene production and respiration by harvested fruits. Journal of Experimental Botany 28:644-649. 1977.

2. DIAZ M., F., and KOHASHI S., J. Distribución de materia seca en el frijol (*Phaseolus vulgaris* L.) bajo condiciones de campo. Turrialba 32:19-27. 1982.

3. ESCALANTE E., J. A. S., KOHASHI-S., J., and GOMEZ R., O. B. Efecto del sombreado artificial en tres épocas a partir de la floración sobre el rendimiento en semillas y sus componentes del frijol (*Phaseolus vulgaris* L.). Agrociencia 42:5-16. 1980.

4. FANJUL, L., KOHASHI-SHIBATA, J., and HERNANDEZ-XOLOCOTZI, E. Yield potential and stratified growth analysis of an indeterminate climbing pole bean (*Phaseolus vulgaris*) in México. Experimental Agriculture 18:167-175. 1982.

5. GOLDSMITH, M. H. M. The polar transport of auxin. Annual Review of Plant Physiology 28:439-478. 1977.

6. JOHANSEN, D. A. Plant microtechnique. McGraw-Hill, New York. 1940. 523 p.

7. MOGENSEN, H. L. Ovule abortion in *Quercus* (Fagaceae). American Journal of Botany 62:160-165. 1975.

8. NOODEN, L. D., and LEOPOLD, A. C. Phytohormones and related compounds. A comprehensive treatise. Vol II. Elsevier, North-Holland biomedical Press. 1978. pp. 329-369.

9. ORMROD, D. P., WOOLLEY, C. J., EATON, G. W., and STOBBE, E. H. Effect of temperature on embryo sac development in *Phaseolus vulgaris* L. Canadian Journal of Botany 45:948-950. 1967.

10. PIMIENTA, E., and POLITO, V. S. Ovule abortion in 'Nompareil' almond (*Prunus dulcis* (Mill) D. A. Webb). American Journal of Botany 69:913-920. 1982.

11. SASS, J. E. Botanical Microtechnique. Ioawa State College Press, Ames. 1958. 228 p.

12. SEDGLEY, M. Anatomical investigation of abscissed avocado flowers and fruitlets. Annals of Botany 46:771-777. 1980.

13. SREE RANGASAMY, S. R., and VIJENDRA DAS, L. D. Seedlessness in triploid guava (*Psidium guajava* L.). Embryological studies. Canadian Journal of Genetics and Cytology 15:331-334. 1973.

14. STEPHENSON, A. G. Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12:253-279. 1981.

15. THOMPSON, M. M., and LIU, L. J. Temperature, fruit set, and embryo sac development in 'Italian' prune. Journal of the American Society for Horticultural Sciences 98:193-197. 1973.

16. TOMER, E., and GOTTREICH, M. Defective ovules in avocado cultivars. Journal of the American Society for Horticultural Sciences 101:620-623. 1976.

17. WEBSTER, B. D., CRAIG, M. E., and TUCKER, C. L. Effects of ethephon on abscission of vegetative and reproductive structures of *Phaseolus vulgaris* L. Horticultural Science 10:154-156. 1975.

18. WILLIAMS, D. D. F. Influence of soil moisture level on flower abscission, ovule abortion and seed development in the snap bean (*Phaseolus vulgaris* L.) Ph. D. Thesis. University of Wisconsin. 1962. 74 p. (Dissertation Abstracts 22:29-33. 1962).

Notas y comentarios

Hacia la fotosíntesis artificial

La conversión artificial de la energía solar a energía química potencial, fuera del reino vegetal y en el laboratorio, está acercándose a la realidad. Hemos dado cuenta hace algún tiempo de los avances y dificultades de los equipos dirigidos por Mervin Calvin, en California, y por Sir George Porter, en Londres (**Turrialba** Vol. 29, p. 137, 1979). Los trabajos de estos dos premios Nobel (1961 y 1967, respectivamente) desatacaban la importancia de las porfirinas y quinonas en el intercambio rápido de electrones, necesario para la fotosíntesis. Ahora, una nueva molécula, que simula los procesos fundamentales que dan lugar a la fotosíntesis, ha sido elaborada y podría abrir el camino a sistemas fotosintéticos artificiales más eficientes. El trabajo ha involucrado a 12 científicos de Estados Unidos, Francia y Gran Bretaña, todos coordinados por Thomas Moore, de la Universidad del Estado de Arizona (**Nature**, Vol. 307, p. 630, 1983).

El paso clave en la fotosíntesis es lo que se ha llamado "separación de carga fotoimpelida". La luz atrapada por las "antenas" de la clorofila se cree que excita electrones, los que son sacados rápidamente a través de una membrana. Son usados para generar ciertas moléculas de alta energía que posteriormente ayudan en la conversión del dióxido de carbono a carbohidratos.

Mientras tanto, la carga positiva, o "hueco", que queda en la clorofila es rellenado con electrones procedentes de moléculas cercanas de agua. Estas son oxidadas, produciendo oxígeno en el proceso. Pero, al conducir rápidamente los electrones excitados lejos de las antenas clorofílicas (mediante moléculas atrapadoras de electrones, llamadas quinonas), la naturaleza asegura que las cargas positivas y negativas, inducidas por la luz, **permanecen** separadas. Esto reduce la pérdida de energía por recombinación de cargas (o retroreacción).

Ahora bien, si los convertidores biomiméticos se espera que funcionen por tiempos largos y eficientemente, deben atacar los dos problemas, de separación de cargas y de prevención de retroreacción. El primero de estos es relativamente fácil; los químicos han hecho moléculas con grupos especiales que pueden o aceptar electrones excitados o donar electrones para los "huecos" dejados por los primeros. La dificultad está en que las moléculas de carga separada son de una vida demasiado corta para ser de algún uso práctico. Aquí entra la esposa de Moore, uno de los arquitectos principales de la nueva molécula.

La retroacción es prevenida mediante la incorporación en la misma molécula, tanto de los grupos aceptadores de electrones como de los donadores de electrones. El corazón de este sistema capturador de luz es una porfirina, un pariente cercano de la clorofila. En un costado de la porfirina se encaja un grupo quinona. Este agarrará cualquier electrón que levante su cabecita excitada por la luz por encima del parapeto de energía potencial de la porfirina. En el otro lado

de la porfirina está un grupo betacaroteno largo y errático que rápidamente completa cualquier déficit electrónico en la porfirina. Entre ellos, los dos grupos pueden desviar cargas a través de la molécula unos 10 000 millones de veces más rápido que un servicio de John McEnroe. Funciona de la manera siguiente:

Un electrón fotoexcitado deja la porfirina y pasa a la quinona, pero antes de que pueda saltar de regreso, el betacaroteno dona un electrón a la porfirina. El resultado final es una molécula de carga separada que es de relativa longevidad (unas 3 millonésimas de segundo comparado con 3 billonésimas para una porfirina quinona simple, es decir 3^{-6} contra 3^{-12} en la notación en español). La carga negativa está situada en la quinona, mientras que la carga positiva está segura, guardada en el betacaroteno.

El parecido del comportamiento de esta molécula con las antenas de la clorofila en la fotosintesis natural es fantástico. Por una parte, el desacoplamiento de las cargas en la molécula se deriva de lo que los fotoquímicos llaman estado excitado simple ("singlet excited state"). Cuando una molécula es fotoexcitada, el electrón salta tan rápido a un estado más alto de energía que no tiene tiempo de cambiar su momento angular de rotación ("spin"). Esto significa que todos los electrones en la molécula están todavía apareados en su spin, una condición llamada el estado simple. Sin embargo, muy rápidamente, el electrón excitado puede cambiar su spin al módulo opuesto. Esto quiere decir que hay ahora **dos** electrones en la molécula con spins no apareados. Esto se llama el estado triplete y tiene menos energía que el estado simple.

En la nueva molécula, la separación de cargas es tan rápida que el electrón no tiene tiempo de cambiar su spin antes de ser arrebatado por la quinona, al igual que en la fotosintesis. No hay tiempo para que se pierda energía en formar el estado triplete.

Pero hay algo más. El betacaroteno también protege a la molécula del daño por la luz. El betacaroteno efectúa esta protección de dos maneras. Primero, enjuga cualquier oxígeno en estado simple que pueda producir la porfirina. Segundo, aceptará los electrones excitados en estado triplete que provengan de la porfirina. Ahora bien, las porfirinas en estado triplete son particularmente susceptibles al ataque del oxígeno en estado simple. El caroteno rompe la relación destructiva entre porfirinas y oxígeno.

La otra función del betacaroteno es extender el ámbito de longitudes de onda que las antenas de la clorofila pueden recoger. En la nueva molécula, el caroteno ejerce la misma función pero sólo con una eficiencia de 10 por ciento, comparado con el sistema natural. Adalberto Gorbitz

ACTIVIDAD ESTROGENICA DE CUATRO VARIEDADES DE TREBOL (*Trifolium* sp.) ASOCIADOS A GRAMINEAS Y RECONOCIMIENTO DE ISOFLAVONAS EN *T. repens* var. LADINO[1] /

*L. A. GIL**
*J. RAMIREZ**
*J. C. DIAZ**

Summary

Estrogen activity in four varieties of clover alone or in association with grasses in the Bogota Savannah in the Andean highlands at an altitude of 2 600 masl was measured by comparing radioactive estradiol by receivers. Estrogen levels increased dramatically with plant age for the two varieties of clover-Triel and Levezou. In white and red clover considered native, estrogen levels varied with age, but with no marked tendency to increase or decrease. The general average for the clovers was far below that of introduced varieties ($P < 0.01$). The average for red clover was greater ($P < 0.05$) than for white. There was no evident effect of the association with grasses ($P > 0.05$) for any variety.

*For white clover (*Trifolium repens *var. Ladino), the presence of isoflavonoides biochanine A, genistein, formononetine, daidze in and cumestrol was proven. This identification was made with the use of column and thin layer chromatography on silica gel, infrared and ultraviolet spectrophotometry, and liquid high pressure chromatography.*

Introducción

Estudios de este laboratorio (8) evidencian una mayor actividad estrogénica de trébol blanco en un altiplano andino de Colombia, que la mencionada en otros países.

Se determinó la actividad estrogénica de dos tréboles nativos: *Trifolium repens* var. Ladino (blanco) y *Trifolium subterraneum* var. indeterminada (rojo) y dos introducidos de Francia, *Trifolium subterraneum* var. Triel y var. Levezou, con el objeto de evaluar variedades que pudieran sustituirlo para vacas en reproducción sobre las cuales pudiera ser nocivo.

Los tréboles se cultivaron asociados con kikuyo (*Pennisetum clandestinum*) tetrelite (*Lolium hybridum*) y manawa (*L. perenne* x *L. multiflorum* correspondiendo las dos últimas gramíneas a raygrasses de crecimiento tan rápido como el de los tréboles introducidos.

En adición se pretendió evaluar el efecto que la demanda sobre los nutrimentos del suelo tuviera sobre la estrogenicidad de los tréboles y la forma como ésta fluctuara con la edad de las plantas en diferentes estados de su desarrollo

Ante la elevada estrogenicidad de los tréboles estudiados se hizo necesario averiguar si las sustancias causantes de este efecto fueran las mismas citadas por otros autores (1, 6, 13) o fueran otros compuestos aun no identificados. Con tal fin se analizó el trébol blanco

Materiales y métodos

Determinación de la estrogenicidad

Se evaluó la actividad biológica de los fitoestrógenos *in vitro* mediante competencia del estradiol radiactivo (17B-estradiol^{3}H New England Nuclear) contra los estrógenos extraídos del trébol por receptores uterinos aislados por ultracentrifugación, según el método de Corker y Exley (5).

1 Recibido para publicación el 21 de mayo de 1984. Se agradece al Organismo Internacional de Energía Atómica, OIEA, de Viena, por su apoyo financiero

* Instituto de Asuntos Nucleares, IAN. Apartado Aéreo 8595, Bogotá, Colombia. Facultad de Medicina Veterinaria y Zootecnia y el Departamento de Biología.

El cultivo de trébol y de tréboles asociado con pasto, se efectuó en un diseño de subparcelas divididas dispuestas como bloques al azar con tres repeticiones, de manera que la composición botánica final fuera de un 25% tréboles y 75% gramíneas.

Se efectuaron cinco recolecciones de hojas a intervalos de 15 días, iniciando la primera 30 días después de un corte de emparejamiento. Las muestras fueron secadas en aire forzado a 70°C por 48 horas, molidas y conservadas en recipientes herméticos. Los extractos acuosos fueron hechos por maceración del material seco durante 24 h lo cual activa la hidrólisis enzimática de los glucósidos. Los volúmenes fueron ajustados a 0.1 g/ml con buffer de McDougal (15).

Reconocimiento de isoflavonoides y cumestrol

Las hojas del trébol secas y molidas fueron maceradas en etanol durante 24 h, para lograr una extracción más completa. El filtrado se concentró por evaporación al vacío a 25°C y luego se redisolvió en agua hirviendo para precipitar clorofilas, las cuales fueron removidas por filtración. La solución acuosa resultante fue extraída sucesivamente con cloroformo y éter de petróleo para removerle lípidos. La fase acuosa se acidificó con HCl concentrado hasta pH 3 para hidrolizar los glucósidos isoflavonoides. Después de 12 h en acidez el extracto se neutralizó potenciométricamente con NaOH y por último los isoflavonoides se extrayeron con acetato de etilo.

Cada una de las fracciones anteriores se analizó cualitativamente mediante la reacción descrita por Bryant (4) para comprobar la presencia de isoflavonoides por enrojecimiento de la solución en ácido clorhídrico y limaduras de magnesio. Las fracciones de reactividad negativa fueron descartadas.

La solución de acetato de etilo se analizó por cromatografía a través de una columna de 76 cm de largo y 3 cm de diámetro empacada con sílica gel 60 (70-230 mesh) y eluída con cloroformo-metanol en proporciones de creciente polaridad. Se recogieron 5 200 alícuotas de 7 ml cada una con un colector de fracciones LKB que funcionó continuamente por 55 días recogiendo 2.5 ml/h. Cada alícuota se analizó por cromatografía de capa delgada sobre sílica gel 60G con cloroformo-metanol 95:5 (v:v) y los compuestos revelados bajo luz ultravioleta (UV), en presencia de vapores de amoníaco bajo luz UV, y por su reacción al fumigarlos con una mezcla de iguales volúmenes de cloruro férrico al 2% y ferricianuro de potasio al 1%. Aquellas alícuotas que contuvieran sustancias cuyo Rf y reactividades correspondieran a las de los isoflavonoides, fueron reunidas para constituir una fracción.

A cada fracción le fue establecido su espectro de absorción al infrarrojo y al UV y finalmente fue resuelta por cromatografía líquida de alta presión (HPLC) a través de una columna de Sephadex LH-20 por elución con metanol-agua 70:30 (v:v) y con un detector ultravioleta.

Resultados y discusión

Actividad estrogénica de las cuatro variedades de trébol

Se encontraron amplias diferencias en la estrogenicidad promedio de cada variedad. El trébol blanco tuvo la menor estrogenicidad, equivalente a 55.7 ng de 17B-estradiol (E_2)/g de M.S, valor aproximado a las dos terceras partes del promedio para el trébol rojo (74 ng E_2/g de M.S).

Los tréboles Levezou y Triel presentaron respectivamente actividades equivalentes a 118.2 y 114.3 ng E_2/g de M.S en promedio, no siendo significativas estas diferencias entre sí.

Estos resultados concuerdan con los de Francis *et al.* (7), quienes citan diferencias en el contenido de sustancias estrogénicas de 14 especies de trébol y aun entre variedades de la misma especie. El trébol blanco ha sido reconocido como medianamente o poco estrogénico (3) aunque Gil *et al.* (1), han demostrado que existen variaciones estacionales en su actividad estrogénica, la cual se incrementa a niveles peligrosos cuando la leguminosa es fermentada con fluído ruminal *in vitro*.

La estrogenicidad de los tréboles rojos se ha reconocido como superior a la del trébol blanco debido a que contienen altas concentraciones de formononetina (14) la cual se convierte en el rumen a ecuol que es más persistente en su acción biológica sobre animales poligástricos. Tratándose de la misma especie, es posible que las variedades Triel y Levezou compartan esta propiedad y que las condiciones del altiplano andino las estén potencializando.

Efecto de la edad de las plantas sobre su actividad estrogénica

La actividad estrogénica (Y) de las variedades introducidas tuvo una tendencia progresiva al aumento a medida que avanzó la edad (X) de las plantas (Figura 1), de acuerdo a las regresiones $Y = 96.079 - 1.348x + 0.025x^2$ ($P < 0.0001$; $R^2 = 0.76$) para Triel, y $Y = 114 - 1.98x + 0.029x^2$ ($P < 0.0001$; $R^2 = 0.709$) para Levezou. Las variedades nativas en cambio exhibieron fluctuaciones sin tendencia definida y no presentaron regresiones significativas (Figura 1).

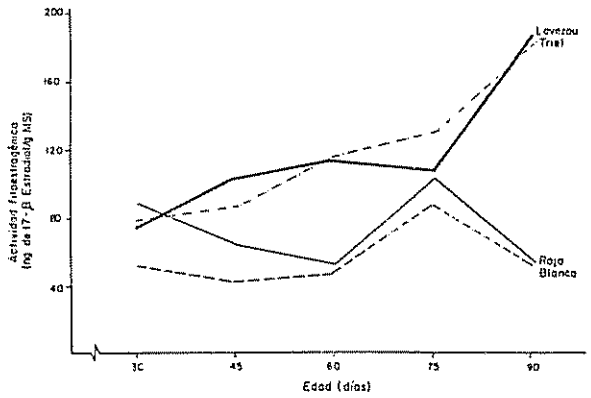

Fig. 1. Estrogenicidad de tréboles a diferente edad (Promedio para 4 parcelas por 3 replicaciones por variedad).

Al comparar los resultados obtenidos con trébol blanco y rojo con los de Rossiter y Beck (12), sobre las fluctuaciones de los fitoestrógenos de acuerdo al desarrollo ontogénico, del trébol subterráneo, variedades Yarloop y Dwalganup, se encuentra un comportamiento semejante. Así, los isoflavonoides de la variedad Dwalganup presentan una tendencia a disminuir entre los 14 y 28 días, desapareciendo toda la formononetina y la biochanina A hacia los 56 días cuando se incrementó la daidzeína para descender hacia los 70 días.

La daidzeína, producto de la desmetilación de la formononetina (10) aunque presente en menor concentración tiene mayor afinidad por receptores estrogénicos y por ende una mayor potencia fisiológica; la transformación de la formononetina en daidzeína podría explicar la actividad fluctuante de los tréboles rojo y blanco durante etapas tardías de su desarrollo.

Efecto de gramíneas asociadas con tréboles, sobre la actividad estrogénica

Estadísticamente los promedios para cada pasto no indicaron ($P > 0.05$) ningún efecto de las gramíneas sobre la actividad estrogénica de los tréboles durante el período de establecimiento y por lo tanto reflejan un efecto a corto plazo. Podría esperarse que a largo plazo las demandas nutricionales sobre el suelo impuestas por cada pasto, incidieran sobre los niveles fitoestrogénicos de los tréboles ya que Rossiter y Beck (11) han demostrado que la deficiencia en fósforo incrementa la concentración de isoflavonoides en los tréboles.

Actividad estrogénica de los tréboles según su edad y la gramínea asociada

La Figura 2 ilustra la actividad estrogénica de cada trébol a diferentes edades, asociados al pasto manawa.

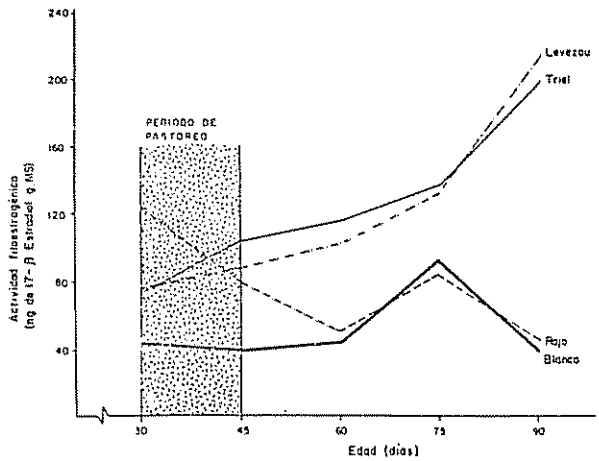

Fig. 2 Estrogenicidad de tréboles asociados al pasto Manawa (promedio de 3 replicaciones).

Este pasto se caracteriza por un rápido crecimiento que lo capacita para el corte o pastoreo entre los 30-45 días, edad a la cual el trébol blanco asociado presenta la más baja estrogenicidad (43 y 48 ng E_2/g de M S), sin embargo su escaso desarrollo a esta edad le impide competir con la gramínea.

El trébol rojo no sólo es pequeño a esta edad sino que su actividad estrogénica de 122 y 77 ng E_2/g de M S es similar o más alta que la de los tréboles Triel y Levezou. De lo anterior se deduce que la asociación más apropiada del pasto manawa sería con Levezou o Triel.

La Figura 3 ilustra las fluctuaciones entre los mismos parámetros anteriores con el pasto tetralite, el cual posee condiciones de rendimiento y precocidad similares a las del manawa, por lo tanto los criterios de selección del trébol para asociación son los ya anotados.

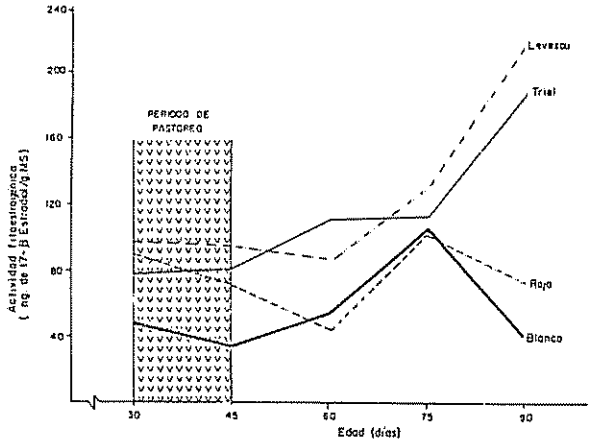

Fig. 3 Estrogenicidad de tréboles asociados al pasto tetrelite (promedio de 3 replicaciones).

La situación con el pasto kikuyo se ilustra en la Figura 4. Puesto que el período de pastoreo para este pasto está entre los 75 y 90 días, el trébol deseable para establecimiento de praderas mixtas sería el rojo y/o blanco que a esta edad tienen un desarrollo competitivo con el kikuyo y además presentan la más baja estrogenicidad.

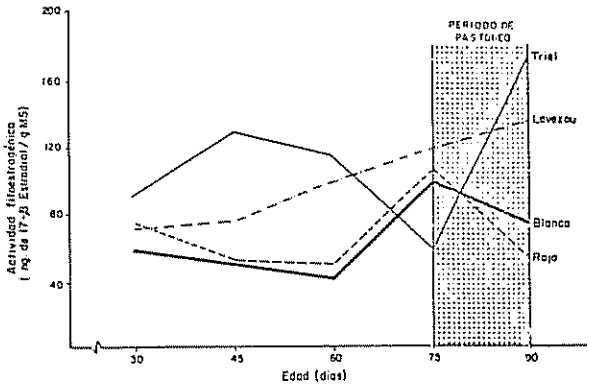

Fig. 4. Estrogenicidad de tréboles asociados al pasto kikuyo. (Promedio de 3 replicaciones

La estrogenicidad promedio para el trébol blanco fue de 36.8 ng E_2/g de M S para todo el período y coincide con el promedio general para esta variedad, sin fermentar y durante el tiempo lluvioso, o de crecimiento activo, reportado por Gil *et al* (8) Las condiciones de no fermentación y riego periódico fueron las observadas en el presente trabajo, e indican que la actividad estrogénica para las variedades estudiadas puede ser entre 8 y 15 veces superiores después de la fermentación ruminal.

Reconocimiento de isoflavonoides en el trébol blanco

De 1380 g de hojas secas y molidas se obtuvo por evaporación del extracto en acetato de etilo 1.05 g de fitoestrógenos parcialmente purificados, rendimiento comparable al logrado por Bickoff *et al.* (2).

El extracto en acetato de etilo se fraccionó por cromatografía en columna sobre sílica gel en 5 200 alícuotas. Aquellas que según su Rf en capa delgada y reacciones de conocimiento se comportaron como isoflavonoides o cumestrol se agruparon en las fracciones 4, 10, 15 y 18 (Cuadro 1). Las demás fracciones se descartaron.

Cuadro 1. Características cromatográficas sobre capa delgada de los compuestos presentes en cada serie (fracción) de alícuotas[1] eluídas de una columna con sílica gel.

Fracción	Alícuotas tubo No.	Eluente	Número de compuestos y vizualización en capa delgada[2]				
			Visible	Ultravioleta	Vap de NH_3	Revelado	Rf
1	1-275	Cloroformo	–	–	–	–	–
2	276-459	"	Verde	–	–	–	0.90
3	460-668	"	Rojo	–	–	+/–	0.95
	–		–	Azul tenue	+	–	0.73
	–		–	Azul tenue	+	–	0.60
4	669-857	"	Amarillo suave	Azul intenso	+	+	0.88
	–		–	Azul tenue	+	–	0.78
	–		–	Azul muy tenue	+	–	0.60
10	1995-2095	90:10	–	Azul violeta			
	–	Cloroformo:	–	intenso	+	+	0.69
	–	Metanol	–	Azul tenue	+	–	0.60
	–	–	–	Azul intenso	+	–	0.52
15	3037-3690	90:15	Amarillo tenue	Azul tenue	–	–	0.60
	–	–	–	Azul violeta			
				intenso	Verde-amarillo	+	0.47
	–	–	–		+	–	0.34
18	4811-4930	80:20	–	Azul intenso	+	+	0.42
	–	–	–	Violeta suave	+	–	0.35
21	5116-5200	75:25	–	Azul tenue	–	–	0.26
	–	–	Amarillo	Azul intenso	–	–	0.04

1 Alícuotas de 7 ml, eluídas de la columna y recogidas en tubos que se numeraron de 1 a 5 200

2 El número de alícuotas integrantes de cada fracción fue determinado por los cambios en el cromatograma sobre C.D. cuyas manchas se visualizaron al visible, al ultravioleta, con yapores de NH_3 o por revelado con cloruro férrico y ferricianuro de potasio.

Por comparación de los Rf (Cuadro 2) en capa delgada de isoflavonoides (biochanina A, formononetina y genisteína) puros, y cumestrol donados por el Dr. B. Tam, University of Western Australia, se dedujo que la fracción 4 contenía biochanina A, la 10 formononetina, y la 15 cumestrol y/o genisteína cuyos Rf son idénticos, y la 18 posiblemente (carencia de estándar) daidzeína.

Espectrofotometría al infrarrojo

Los espectros de absorción de las sustancias recuperadas de cromatogramas preparados sobre capa delgada, fueron prácticamente indistinguibles (Figura 5) y en consecuencia sólo útiles para revelar la naturaleza isoflavonóidea de los compuestos. Las bandas comunes más notorias fueron: a 3 600 cm^{-1} atribuible

Cuadro 2. Rf's sobre capa delgada de sustancias estándar y valores de la literatura en atmósferas a diferente saturación.

	Rf Literatura		Rf Estándares[1]		
Saturación cámara	3	2	3	3	3
Solventes desarrollo cloroformo: metanol	89:11	89:11	90:10	95:5	89:11
Biochanina – A	0.85	0.65	0.93	0.88	0.87
Formononetina	0.75	0.60	0.72	0.69	0.80
Genisteina	0.55	0.50	0.60	0.47	0.67
Cumestrol	–	–	0.60	0.47	0.67

1 Cada número es el promedio de 3 determinaciones.

2 Cámara totalmente saturada

3 Cámara parcialmente saturada.

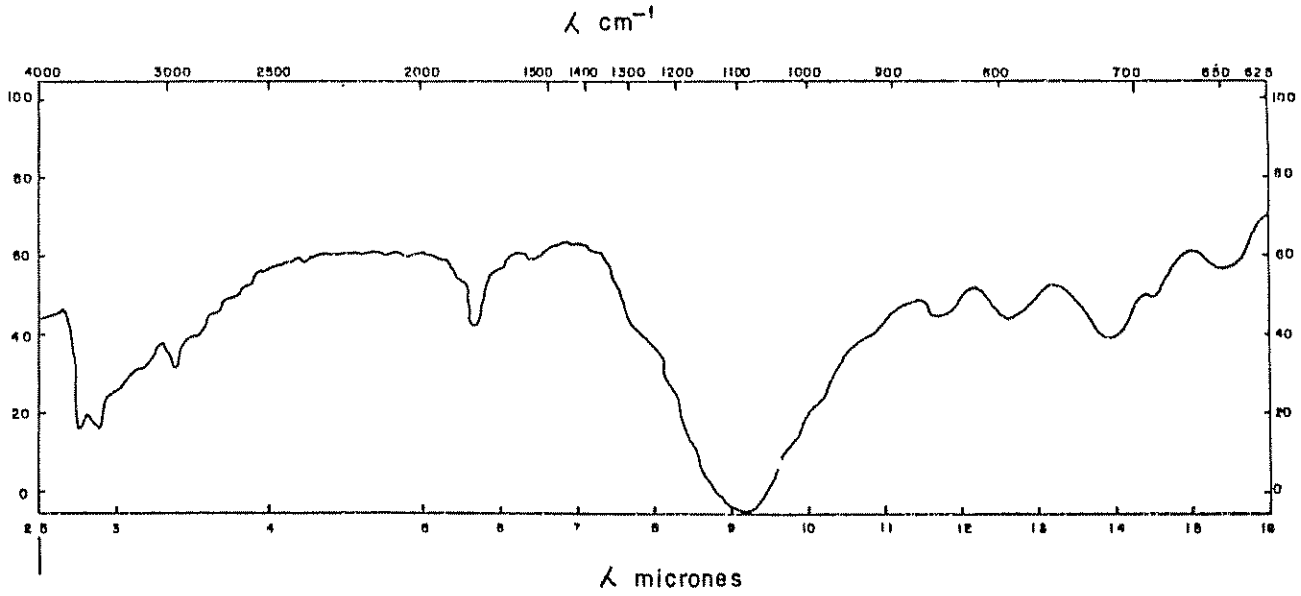

Fig 5 Espectro de absorción I.R. de la Biochanina A

a tensiones de los hidroxilos, a 3 400 cm^{-1} asociadas a tensiones C-0, a 2920 cm^{-1} atribuible a tensiones C-H y a 2850 cm^{-1} relacionada al grupo metoxilo

Espectrofotometría ultravioleta

Se obtuvo tres tipos de espectros para las sustancias analizadas, que coinciden con los citados por Markham (9): un tipo para la biochanina A y la genisteína (/max:261), otro para la formononetina y la daidzeína (/max:248-249) y otro para el cumestrol con un pico (/max 243) ancho que incluye parte de los anteriores.

A pesar de pequeñas desviaciones, tal vez causadas por impurezas, sobre los /max para las fracciones 4, 10, 15 y 18, esta información (Cuadro 3) sirvió para corroborar los postulados basados en los valores Rf obtenidos sobre capa delgada, y para decidir que la fracción 15 contiene cumestrol y genisteína mezclados.

Cromatografía líquida de alta presión

Esta técnica reveló, además de las sustancias ya identificadas por los procedimientos anteriores, alguna impureza, tal vez causante de las desviaciones en los /max al UV, tales como pequeños picos a 84 y 216 seg (Cuadro 4) para el solvente o metanol (supuestamente grado cromatográfico) y un pico adicional a 144 seg para el eluato en metanol de la sílica usada en las cromatografías y sometida al proceso cromatográfico con los solventes puros. Estos tres pequeños picos aparecieron en casi todos los cromatogramas y fueron descartados como criterio de identificación

Cuadro 3. Características espectrales al ultravioleta. Valores de λ/max. para estándares, fracciones cromatográficas y de la literatura.

Sustancia	(λ/max. nm)	
	Tomado de Markham (9)	Compuestos aislados ó estándares
Biochanina-A	261	–
Genisteina	261	–
Formononetina	248	–
Daidzeina	249	–
Estándares (mezcla)	–	245, 261
Cumestrol (estándar)	243, 343	244, 340
De la fracción 4	–	256
De la fracción 10	–	248
De la fracción 15	–	248, 260, 339
De la fracción 18	–	247

La mezcla de estándares se fraccionó a tres picos que en orden de polaridad corresponden a la biochanina A (192 seg), a la formononetina (252 seg) y a la genisteína (372 seg). Estos tiempos de retención para los estandares permitieron concluir que la fracción 4 contenía biochanina A (192 seg) y la impureza a 120 seg. La fracción 10 contenía formononetina (252 seg) y la misma impureza a 120 seg. La fracción 15 contenía genisteína (372 seg) y cumestrol (288 seg) más las impurezas mencionadas. La fracción 18 contenía un isoflavonoide (420 seg) no identificado por carencia de estándar, pero que basados en los datos espectrofotométricos y su Rf, corresponde a la daidzeína (Cuadro 1)

Conclusiones

La actividad estrogénica depende de la variedad de trébol y a temprana edad de la planta no se ve modificada apreciablemente por la gramínea con que se asocie. En cambio la actividad aumenta con la edad de algunos tréboles, lo cual hace que el periodo de pastoreo deba seleccionarse según el animal que lo utilice; con hembras en reproducción, el trébol deberá usarse joven y asociado a un pasto de crecimiento rápido.

El otro criterio de selección de plantas forrajeras asociadas, será la similitud en la velocidad de crecimiento entre las gramíneas y la leguminosa.

Las sustancias causantes de la estrogenicidad medida por competencia contra el estradiol por receptores estrogénicos *"in vitro"*, son los isoflavonoides y el cumestrol presentes en el trébol blanco

Resumen

La actividad estrogénica de cuatro variedades de trébol asociado y sin asociar a gramíneas en la Sabana de Bogotá, altiplano andino a 2 600 msnm, se determinó por una técnica de competencia del estradiol radioactivo por receptores. La estrogenicidad aumentó drásticamente con la edad de las plantas, para las variedades de tréboles introducidos Triel y Levezou. En los tréboles blanco y rojo considerados nativos, la estrogenicidad varió con la edad pero sin una tendencia

Cuadro 4. Tiempos de retención (Picos) en cromatografía líquida de alta presión para estándares y sustancias aisladas (fracciones).

Sustancia	Tiempo de retención (segundos)				
Metanol	84	216	(Impurezas)	–	–
Eluato de silica en metanol	84	144	216	(Impurezas)	
Estándares (mezcla)	84	192 (B)	216	252 (F)	372 (G)
Cumestrol (estándar)	84	216	288	–	–
Fracción 4	84	120 (I)	144	192 (B)	216
Fracción 10	84	120 (I)	144	216	252 (F)
Fracción 15	84	144	216	288 (C)	372 (G)
Fracción 18	84	144	216	420 (D)	–

I Impureza B Biochanina A F Formononetina

G Genisteína D Daidzeína C Cumestrol

definida de aumento o disminución El promedio general para los tréboles nativos fue muy inferior al de los introducidos ($P < 0.01$); el promedio para el trébol rojo fue mayor ($P < 0.05$) que para el blanco No fue evidente el efecto de la asociación con gramíneas ($P > 0.05$) para ninguna variedad

Para el trébol blanco (*Trifolium repens* var Ladino) se corroboró la presencia de los isoflavonoides biochanina A, genisteína, formononetina, daidzeína y del cumestrol El reconocimiento se hizo mediante cromatografía en columna y capa delgada sobre sílica gel, espectrofotometría infrarroja y ultravioleta y por cromatografía líquida de alta presión

Literatura citada

1 ADAMS, N. R. Cervical mucus in infertile ewes previously exposed to estrogenic subterranean clover. Research in Veterinary Science 21(1):59 1976.

2 BICKOFF, E. M., BOOTH, A. N., LYMAN, R. L., LIVINGSTON, A. L., THOMPSON, C. R. y KOHLER, G. O. Isolation of a new estrogen from Ladino clover. Journal of Agricultural and Food Chemistry 16:536. 1958.

3. BICKOFF, E. M., LIVINGSTON, A. L., BOOTH, A. N., THOMPSON, C. R., HOLLOWELL, E. A. y BEINHART, E. G. Some variation in estrogenic activity in fresh and dried white clover and Ladino variety. Journal of Animal Science 19(4):1143 1960

4. BRYANT, E. F. A note on the differentiation between flavonoid glycosides and their aglucones. Journal American of Pharmacy Assistance Science 39:480 1950

5 CORKER, C. S., EXLEY, D. y NAFTOLIN, F. Assay of 17B-oestradiol by competitive protein binding methods. Acta Endocrinologica, suppl 147, 1970 305 p.

6 FOLMAN, Y. y POPE, G. S. The interaction in the immature mouse of potent oestrogens with coumestrol, genistein and other vaginotrophic compound of low potency. The Journal of Endocrinology 34(2):215 1966.

7. FRANCIS, C. M., MILLINGTON, A. J. y BAILEY, F. T. The distribution of oestrogenic isoflavones in the Genus *Trifolium*. Australian Journal of Agricultural Research, 18(2):47. 1967.

8 GIL, P., L. A., DIAZ, M. C., RAMIREZ, J. y MAYORGA, M. Actividad fitoestrogénica

del trébol blanco *in vitro* e *in vivo*, sus fluctuaciones en relación a variables climáticas, estado metabólico y la fermentación ruminal. Turrialba 34(2):147-156. 1984.

9. MARKHAM, K. R. Techniques of flavonoid identification. Academic Press. U.S.A. 1982.

10. NILSON, A. Demethylation of the plant oestrogen formononetin to Daidzein in rumen fluid. Arkiv for Kemi. 19(39):549. 1962.

11. ROSSITER, R. C. y BECK, A. B. Physiological and ecological studies on the oestrogenic isoflavones in subterranean clover (*T. subterraneum* L.) II. Effects of phosphate supply. Australian Journal of Agricultural Research. 17(4):447. 1966.

12. ROSSITER, R. C. y BECK, A. B. Phisiological and ecological studies on the oestrogenic isoflavones in subterranean clover (*T. subterraneum* L.) V. Ontogenic changes. Australian Journal of Agricultural Research. 18(4):561. 1967.

13. SHUTT, D. A. Interaction of genistein with oestradion in the reproduction tract of the ovariectomized mouse. The Journal of Endocrinology. 37(2):231. 1967.

14. SHUTT, D. A., WESTON, R. H. y HOGAN, J. P. Quantitative aspects of phyto-oestrogen metabolism in sheep feed on subterranean clover (*Trifolium subterraneum* cultivar Clare) or red clover (*Trifolium pratense*). Australian Journal of Agricultural Research. 21(5):713. 1970.

15. TILLEY, J. M. A. y TERRY, R. A. A two-stage technique for the *in vitro* digestion of forrage crops. Journal of British Grassland Society. 18:104. 1963.

DIFFERENTIAL PLANT RESPONSES AND MORPHOMETRICS OF SOME *Meloidogyne* spp FROM COSTA RICA[1] /

*R. LOPEZ**

Resumen

Con base en los resultados de un estudio morfométrico de machos, hembras y juveniles en el segundo estadio, y en la respuesta de siete plantas diferenciales, se pudo diferenciar cinco especies de Meloidogyne *entre 16 poblaciones colectadas en diferentes localidades de Costa Rica. Las especies identificadas fueron* M. arenaria, M. incognita, M. hapla, M. exigua *y* M. salasi. *La respuesta de las plantas diferenciales dio evidencia de que la población de* M. arenaria *pertenece a la raza 2 (no infecto maní) y que entre las poblaciones de* M. incognita *existían representantes de las razas 1 y 2 de esta especie. Se encontró evidencia de variación patogénica en dos poblaciones de* M. exigua, *por cuanto una de ellas se reprodujo fácilmente en tomate, mientras que la otra no. En forma similar, dos poblaciones de* M. hapla *se reprodujeron abundantemente en chile, mientras que una tercera lo hizo pero sólo levemente*

Introduction

The broad geographical distribution, wide host range, severe pathogenic effects and synergistic interactions with many kinds of plant disease organisms, have placed root-knot nematodes (*Meloidogyne* Goeldi, 1887, Nematoda: Meloidogynidae) among the major plant pathogens affecting man's food supply (15)

Management strategies aimed at reducing the severity of the damage caused by *Meloidogyne* spp include the use of chemicals, crop rotation, resistant cultivars and other cultural practices (15). The last three tactics require extensive knowledge of the morphology, variability and ecology of the species causing the damage.

One of the problems associated with the implementation of nonchemical management tactics against root-knot nematodes is the correct identification of populations. Identification is complicated by the variation in morphology and host range commonly present in species of this genus (9). Due to this variability, approaches other than classical morphology, such as the response of differential plants, have been used to identify species (15).

The identification and/or quantification of the variability within and among species of root-knot nematodes by these different approaches could provide the basis for a better understanding of the genus from different points of view. This understanding would enable recognition of those characters which are species specific and therefore reliable for distinguishing species, as well as recognition of characters with little or no value in the identification of fields populations due to their overlap among or between species or their instability.

Having these considerations in mind research was conducted to study the variability of some populations of root-knot nematodes from Costa Rica by a morphometric characterisation of males, females and second stage juveniles, and by the reaction of certain differential plants.

Materials and methods

Sixteen populations of root-knot nematodes were collected from different localities in Costa Rica (Figure 1) and increased in a greenhouse at the Facultad de Agronomía, Universidad de Costa Rica, San

1 Received for publication on September 12, 1984. Research supported by a grant from the Consejo Nacional de Investigaciones Científicas y Tecnológicas de Costa Rica. The help provided by Mr. Federico Valverde B., Mr. A. L. Taylor and Ing. Luis A. Salazar F., is deeply appreciated.

* Laboratorio de Nematología, Escuela de Fitotecnia, Universidad de Costa Rica. San José, Costa Rica

Pedro. Some selected ecological characteristics of the collection sites of these populations, along with the hosts on which they were collected and their population designation, are presented in Table 1

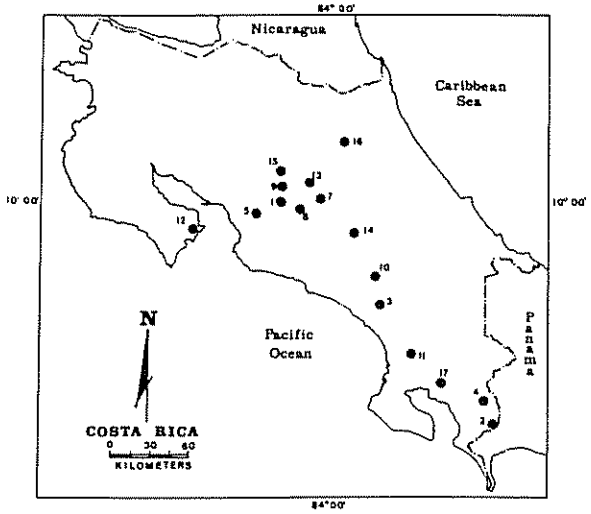

Fig 1. Map of Costa Rica showing the approximate location of the collection sites of 16 populations of *Meloidogyne* spp

The inoculum for the propagation of each population consisted of several dozen egg masses collected from roots of the host from the original locality where the population was collected. Most population were increased on tomato, cv. Rutgers. Populations CR7 and 9 were maintained on coffee, cv. Caturra, whereas population CR2 was maintained on rice, cv. C R 1113. All plants were grown in 2 000 ml clay pots that contained 1 700 ml of an Andept soil (43.2% sand, 31.4% silt, 25.4% clay, 8.7% O.M. and 5.8 pH) The soil in all cases was treated with steam at 105°C for 24 hours prior to use. Each pot was fertilized twice a week during the first five weeks of plant growth with 150 ml of a 1% 20-20-20 fertilizer formula solution Air temperatures varied between 17 and 31°C.

Twenty specimens were used for each character studied in the males, females and second-stage juveniles. All measurements were analyzed statistically with a one way classification model, and the mean values were compared using the Duncan's Multiple Range Test. The methodology used for the morphometric study, as well as that for the differential plants were the same as previously described (5), except that strawberry and sweetpotato plants were not included.

Table 1. Designations, sources and selected ecological characteristics of the collection sites of 16 populations of *Meloidogyne* spp. from Costa Rica.

Pop.	Host	Soil (%)				Soil pH	Elevation m.a.s.l.	Locality	Ecological zone
		Sand	Silt	Clay	O.M.				
CR1	*Lycopersicon esculentum* Mill.	50.2	29.7	20.1	8.8	6.1	810	La Guacima	Premontane moist forest
CR2	*Oryza sativa* L.	75.2	14.7	10.1	3.4	6.0	22	La Cuesta	Premontane wet forest, basal belt
CR3	*Nicotiana tabacum* L.	68.2	13.7	18.1	3.2	5.4	550	Repunta	Tropical moist forest
CR4	*Carica papaya* L.	55.2	26.7	18.1	2.4	6.2	42	Ciudad Neilly	Tropical wet forest
CR5	*Carica papaya* L.	30.1	31.8	38.1	5.6	6.0	220	Orotina	Tropical moist forest
CR6	*Lycopersicon esculentum* Mill.	35.1	46.8	18.1	2.7	6.1	905	Santa Ana	Premontane moist forest
CR7	*Coffea arabica* L.	44.8	32.7	23.5	8.7	6.1	1 360	San Luis	Premontane moist forest
CR9	*Coffea arabica* L.	32.1	34.8	33.1	11.6	6.2	1 020	Sarchí	Premontane wet forest
CR10	*Eupatorium subcordatum*	88.1	5.8	6.1	2.8	6.2	2 400	Division	Montane rain forest
CR11	*Musa acuminata* X *M. balbisiana*, AAB	52.1	12.7	35.2	4.3	6.0	60	Palmar Norte	Premontane wet forest
CR12	*Carica papaya* L.	26.1	31.8	42.1	5.3	6.1	10	Paquera	Premontane moist forest
CR13	*Bidens pilosa* L.	35.5	58.9	5.6	9.2	6.0	1 040	Porrosati	Lower montane rain forest
CR14	*Brassica oleracea* var. *capitata* L.	29.0	43.0	28.0	8.4	6.0	2 050	El Empalme	Lower montane wet forest
CR15	*Impatiens balsamina* L.	34.0	33.0	33.0	6.8	6.2	2 200	Palmira	Lower montane wet forest
CR16	*Musa acuminata* X *M. balbisiana*, AAB	80.6	8.0	11.4	5.6	5.8	75	Río Frío	Tropical wet forest
CR17	*Carludovica* sp	55.1	25.8	19.1	8.6	6.1	10	Piedras Blancas	Tropical wet forest

Results

Five species of *Meloidogyne* were identified among the 16 populations studied. These species were *M. incognita* (populations CR1, 3, 5, 6, 11, 12, 16 and 17), *M. exigua* (populations CR7 and 9), *M. hapla* (populations CR10, 14 and 15), *M. arenaria* (population CR4), and *M. salasi* (population CR2). Population CR13 was identified as a mixture of *M. incognita* and *M. hapla*.

Morphology

The interpretation of the predominant type of perineal pattern for each species is presented in Table 2. Only specimens of *M. salasi* (CR2) and a few from *M. exigua* (CR7 and 9) had a posterior protuberance. Populations CR16 and 17 of *M. incognita* had a few striae originating at the vulval lips and going out to the sides. *M. exigua* (CR7 and 9) had three striae in the perineum, whereas *M. hapla* (CR10, 14 and 15) and some specimens of CR13, a population consisting of a mixture of *M. incognita* and *M. hapla*, had one striae. The striae of *M. arenaria* (CR4), *M. exigua* (CR7, 9) and *M. hapla* (CR10, 14, 15) were interrupted were the lateral lines normally are, but they were not distinct enough to be considered lateral lines. The *M. incognita* populations CR1, 3, 5, 6, 11 and 17 had a few, wavy and broken striae in zones 2, 3 and 4, whereas CR12 and 16 had a moderate number of striae in these zones. In these same zones *M. exigua* (CR7, 9) had few, smooth, broken striae,

Table 2. Interpretation of the predominant type of perineal pattern of females of 16 populations of *Meloidogyne* spp. from Costa Rica.

Pop.	Posterior protuberance	Vulva lip striae	Perineum striae	Lateral incisures	Striae zone			
					1	2	3	4
M. incognita								
CR1	A	A	A	A	F	FWB	FWB	FWB
CR3	A	A	A	A	F	FWB	FWB	FWB
CR5	A	A	A	A	F	FWB	FWB	FWB
CR6	A	A	A	A	F	FWB	FWB	FWB
CR11	A	A	A	A	F	FWB	FWB	FWB
CR12	A	A	A	A	F	MWB	MWB	MWB
CR16	A	I	A	A	M	MWB	MWB	MWB
CR17	A	I	A	A	F	FWB	FWB	FWB
M. exigua								
CR7	A	A	3	I	F	FSB	FSB	FSB
CR9	A	A	3	I	F	FSB	FSB	FSB
M. hapla								
CR10	A	A	I	I	F	FSU	FSU	FSU
CR14	A	A	I	I	F	FSU	FSU	FSU
CR15	A	A	I	I	F	FSU	FSU	FSU
M. arenaria								
CR4	A	A	A	I	F	FSB	FSB	FSB
M. salasi								
CR2	P	A	A	A	F	FSU	FSU	FSU
M. incognita & *M. hapla*								
CR13	A	A	I & A	I & A	F	FSW& FWB	FSU& FWB	FSU& FWB

A: absent; P: present; F: few; M: moderate in number; W: wavy; B: broken; U: unbroken; S: smooth; I: interrupted.

whereas *M. hapla* (CR10, 14, 15) had few, smooth, unbroken striae. *M. arenaria* (CR4) had few, smooth, broken striae in zones 2, 3 and 4. In the mixture of *M. incognita* and *M. hapla* (CR13) perineal patterns with few, smooth and unbroken striae, as well as perineals with few, wavy, broken striae in zones 2, 3 and 4 were found. *M. salasi* (CR2) had few, smooth, mostly unbroken striae in zones 2, 3 and 4. Striae of *M. salasi* and *M. hapla* were relatively fine whereas they were relatively coarse in the other species.

The shape of the perineal pattern varied with the species. In *M. incognita* the perineal patterns of all populations were mostly pyriform, with a trapezoidal dorsal arch. The perineal patterns of the two *M. exigua* populations were roughly rounded, with a low rounded dorsal arch; the striae were rather coarse and far apart. In *M. hapla* populations the perineal patterns were roughly rounded, with a low and wide dorsal arch. No wings were observed in the perineal patterns, but punctations on the tail terminus area were present. The striae were closely spaced. In *M. arenaria* the perineal patterns were mostly oval, with striae forming a shoulder on the low, flat to rounded dorsal arch. In the mixture of *M. incognita* and *M. hapla* both pyriform and roughly rounded perineal patterns were found. *M. salasi* (CR2) had oval shaped perineals, with high and wide rectangular dorsal arches; the striae were far apart. A photomicrograph of one perineal pattern from each population, except of the mixture of *M. incognita* and *M. hapla*, is presented in Figures 2 and 3.

The mean values of morphometric characters of the females are presented in Table 3. Highly significant differences among populations were found in stylet, DEGO, distance between the middle of the excretory pore and the head end (excretory pore), maximum body width, body length, vulva, anus-vulva and interphasmidial distances.

Average values for the characters measured in second-stage juveniles are presented in Table 4.

Highly significant differences among populations were found in total length, tail length, maximum body width, anal width, stylet base to head end, DEGO, and the a and c ratios. Undilated recta were present in *M. exigua* (CR7, 9) and *M. hapla* (CR10, 14, 15) whereas they were dilated in the other populations. In all juveniles the hemizonid was located anterior to the excretory pore.

Average values and observations of certain male characters are presented in Table 5.

All males had areolated lateral fields, although to a variable degree. In most populations they had four lines in the lateral fields, although five were also observed in some specimens of CR14, a population of *M. hapla*. Only one gonad was observed in males of *M. salasi* (CR2), the *M. incognita* populations CR3 and 11, and the *M. hapla* population CR15; the others had a varying percentage of males with two gonads.

Highly significant differences were found among populations in the stylet, DEGO and spicules (chord of arch). The mean stylet length of *M. salasi* was the lowest, followed only by those of *M. exigua* (CR7, 9), which had stylets 2.4 and 2.8 μm longer, respectively.

Differential Plants

Similar responses were obtained in the four replicates of each differential plant-population combination, and the average values are presented in Table 6. The differential plant responses indicated that populations CR1, 5, 6 and 17 were *M. incognita* race 1, populations CR3, 11, 12 and 16 were *M. incognita* race 2, population CR4 was *M. arenaria* race 2, populations CR7 and 9 were *M. exigua*, populations CR10, 14 and 15 were *M. hapla*, population CR2 was *M. salasi*, and population CR13 was a mixture of *M. incognita* and *M. hapla*.

The responses to the differential plants gave evidence of pathogenic variation in *M. exigua*. The two populations of this species could be differentiated by their ability or inability to infect tomato, cv. Rutgers (Table 6). Population CR9 was able to reproduce well on this host but CR7 was not.

A major difference among populations of *M. hapla* was found in the reaction of pepper, cv. California Wonder. Populations CR14 and 15 reproduced abundantly on this host, but CR10 reproduced only to a limited extent.

Tomato was heavily infected and received the maximum rating value of 5 with all but *M. salasi* (CR2) and the *M. exigua* population CR7. Tobacco was not infected by *M. salasi* (CR2) and *M. exigua* (CR7, 9), only slightly by the *M. incognita* populations CR5, 6 and 17, and heavily by the remaining populations. Pepper was not a host for *M. salasi* (CR2), and was infected only slightly by CR10, a population of *M. hapla*, the other populations reproduced well on this host. Cotton was not infected, except slightly by CR6 and 16, two populations of *M. incognita*. Peanut was a good host for *M. hapla* (CR10, 14, 15), moderate for the mixture of *M. incognita* and *M. hapla* (CR13) and a poor host for CR6, a population of *M. incognita*. Watermelon was not infected by *M. salasi* (CR2), two populations of *M. ha-*

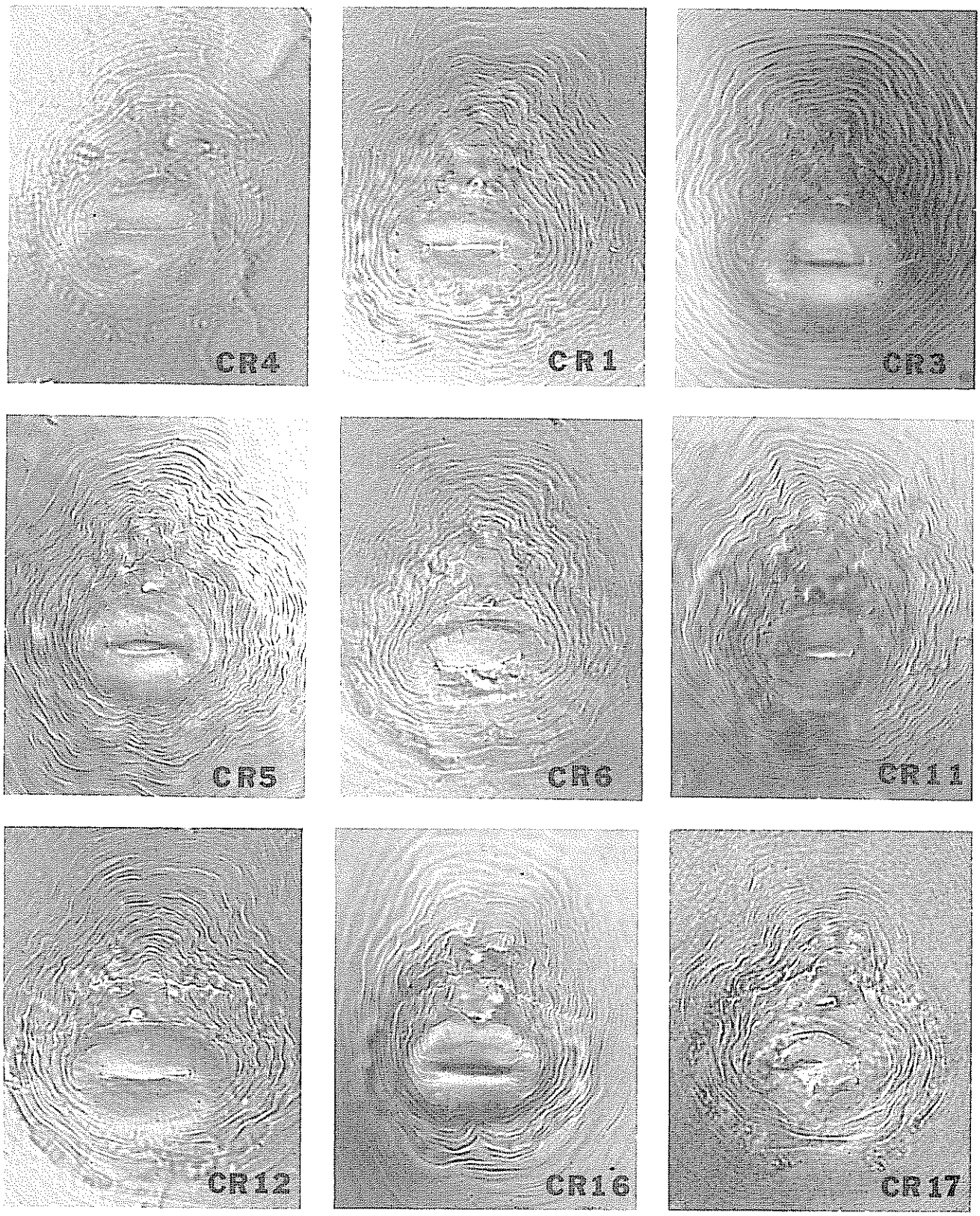

Fig. 2. Photomicrographs of female perineal patterns of nine populations of *Meloidogyne* spp. from Costa Rica. CR4: *M. arenaria* CR1, CR3, CR5, CR6, CR11, CR12, CR16 and CR17: *M. incognita*

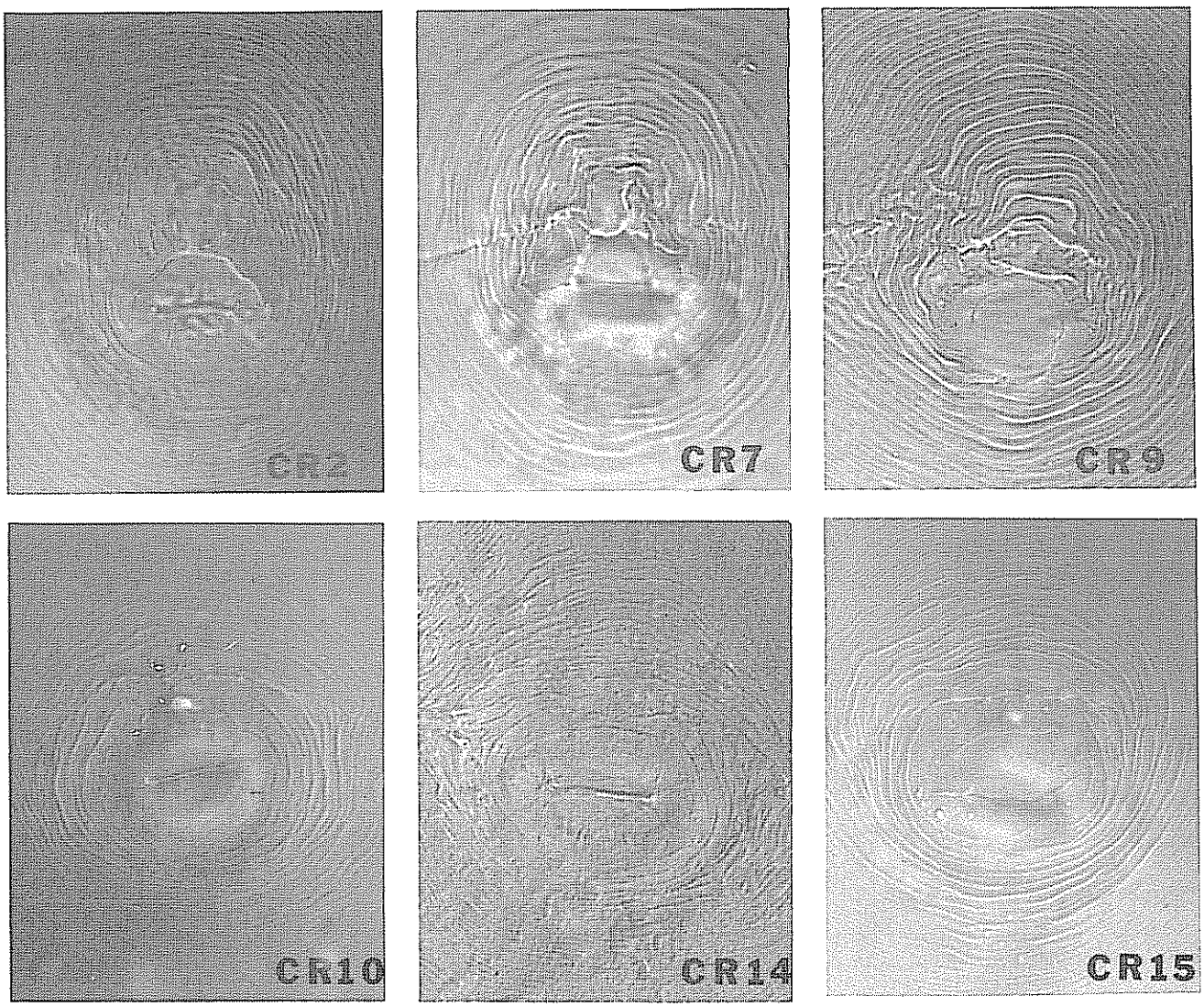

Fig. 3. Photomicrographs of female perineal patterns of six populations of *Meloidogyne* spp. from Costa Rica. CR2: *M. salasi*. CR7 and CR9: *M. exigua*. CR10, CR14, and CR15: *M. hapla*.

pla (CR14, 15), and only lightly by the third population of *M. hapla* (CR10) and by the mixture of *M. incognita* and *M. hapla*. This plant was a good host for the other populations. Finally, corn was not a host for *M. salasi* (CR2), *M. exigua* (CR7, 9), and for two populations of *M. hapla* (CR10, 14), a poor host for *M. arenaria* (CR4), the mixture of *M. incognita* and *M. hapla* (CR13), one population of *M. hapla* (CR15) and two of *M. incognita* (CR5, 17), and a good one for the other populations.

Discussion

Morphology

M. incognita

The general shape of the perineal patterns was similar among the populations studied and could be used to distinguish this species from the others. The interpretation of the characteristics exhibited by the perineal patterns agreed with the reports by previous authors in Costa Rica (3, 13). Similarly, the absence of a posterior protuberance was noted. It was noticed that when the mean values of the morphometric characters of each population were compared to those previously reported from Costa Rica, the juvenile length and the female stylet in CR1 were greater. In CR3 the juveniles were wider; CR5 had juveniles with a greater length and females with a longer stylet; CR6 had longer juveniles, with longer tails and a greater a ratio; population CR11 had longer juveniles, and the interphasmidial distance, tail length, stylet base to head end, DEGO and the a ratio in the female were slightly greater. They also had a smaller anal width. In the females, the excretory pore was longer.

Males of all populations had mean values of their characteristics similar to those reported by these authors (3, 13)

M. exigua

There are no previous reports about the morphometrics of this species in Costa Rica, so comparisons were made to the data provided by Chitwood (1) and Lordello and Zamith (7) The general shape and the characteristics of the striae of the perineal pattern agreed with previous descriptions A few females of each Costa Rican population had the neck region located on the ventral side of the body and a posterior protuberance

On the other hand, some males of the two populations from Costa Rica had twisted bodies and some untwisted bodies This observation was in agreement with the previous report by Scotto la Massese (14), and contradicts the statement by Lordello and Zamith (7), that males of *M. exigua* did not have a twisted body, thus constituting an exception among the root-knot nematodes Another contradiction with

Table 3. Morphometric characters of females of 16 populations of *Meloidogyne* spp. from Costa Rica.

Pop.	Exc. pore	Stylet	DEGO*	Body width	Body length
M. incognita					
CR1	23 7 a**	15 2 cde	4 1 bc	486 efg	634 bcd
CR3	26 4 abcd	14 3 abcd	4 0 abc	538 g	764 gh
CR5	26 2 abcd	15 9 e	3 8 ab	459 def	679 cdef
CR6	23 4 a	15 6 de	4.0 abc	519 g	721 fg
CR11	25 5 abc	13 5 ab	3 9 ab	495 efg	784 h
CR12	26 4 bcd	15 4 cde	3 4 a	439 de	656 bcde
CR16	31 7 cde	15.4 cde	4 6 cd	371 bc	642 bcde
CR17	24.7 ab	15 2 cde	4 3 bcd	471 def	659 bcde
M. exigua					
CR7	35 3 e	14 8 bcde	6 1 e	272 a	493 a
CR9	32.6 bcde	14.4 bcd	4 8 e	325 ab	491 a
M. hapla					
CR10	34 8 e	12 9 a	5.7 e	472 def	697 ef
CR14	36 5 e	13 7 ab	5 7 e	423 cd	629 bc
CR15	33 3 de	14 2 abc	5.8 e	486 efg	728 fg
M. arenaria					
CR4	34 2 e	15 5 cde	4 6 d	508 fg	698 ef
M. salasi					
CR2	35.4 e	13.6 ab	3 9 ab	468 def	602 b
M. incognita & *M. hapla*					
CR13	25 2 abc	15 6 de	4 2 bc	495 efg	690 def
CV (%)	28.4	10.1	14 6	14 6	9 9

* DEGO refers to the distance between the base of the stylet knobs and the dorsal esophageal gland orifice

** Mean of 20 observations. All measurements in μm Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test ($P = 0.01$)

Continuation Table 3.

Pop.	Vulva length	Anus-vulva	Interphasmidial distance
M. incognita			
CR1	22.1 bcde	17.8 cde	24.5 def
CR3	25.3 f	18.9 ef	28.6 h
CR5	25.0 f	18.6 def	27.2 gh
CR6	24.5 ef	17.0 abcde	27.5 gh
CR11	23.2 bcdef	19.8 f	26.2 fg
CR12	20.6 abcd	17.5 abcde	23.0 bcd
CR16	23.5 def	18.1 cdef	26.6 g
CR17	23.3 cdef	18.0 cde	26.9 gh
M. exigua			
CR7	18.9 a	17.2 abcde	24.8 ef
CR9	20.6 abcd	16.7 abcd	22.6 bc
M. hapla			
CR10	20.7 abcd	15.7 ab	21.7 b
CR14	20.8 abcd	15.6 a	24.9 ef
CR15	20.4 abc	17.6 bcde	22.8 bcd
M. arenaria			
CR4	21.5 abcd	17.8 cde	27.6 gh
M. salasi			
CR2	23.2 bcde	16.3 abc	15.1 a
M. incognita & M. hapla			
CR13	20.3 ab	17.5 abcde	23.9 cde
CV (%)	14.2	11.7	7.9

* DEGO refers to the distance between the base of the stylet knobs and the dorsal esophageal gland orifice.

** Mean of 20 observations. All measurements in μm. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (P = 0.01).

the report by Lordello and Zamith (7) was the finding of only one testis in some males of both populations. Lordello and Zamith (7) reported that all males possessed two testes.

The two Costa Rican populations had longer second-stage juveniles and females with longer stylets than previously reported. Males of CR7 had greater DEGO values than those reported by Lordello and Zamith (7). All other values found in this investigation agreed with, and in some cases were identical to, those previously reported.

M. hapla

The finding of this species outside the Central Plateau and the Central Volcanic Range, the only areas where it had been found previously (4, 6, 8, 13), widens its reported geographical distribution in Costa Rica. Both El Empalme and Division are high altitude areas with high precipitation and relatively cool temperatures all year round. This agrees with the observed tendency for the distribution of *M. hapla* in the rest of Costa Rica (6).

The shape of the perineal patterns and the characteristics of their striae were in close agreement with previous reports from Costa Rica (6, 13), except that no wings were observed in the perineal patterns. Females of the three populations had greater values for the excretory pore and the DEGO than those reported for other Costa Rican populations (6, 13). The population CR10 had greater values for the total length and tail length of the juveniles, and for the

stylet and spicules (chord of arch) of the males. The CR14 population had longer spicules than found by previous authors. The other characters had mean and range values similar to those reported earlier (6, 13).

The recta of all juveniles were undilated. When first found in Costa Rica, López and Salazar (6) observed some juveniles with dilated recta in a population collected from cabbage. Later, these authors (13) found *M. incognita* and *M. hapla* coexisting in cabbage in the same general area of their first finding. Since *M. incognita* juveniles have dilated recta (1), the possibility of a mixture of both species in the first report seems likely, and therefore makes the report of dilated recta in *M. hapla* juveniles doubtful.

M. arenaria

The finding of a population of *M. arenaria* in Ciudad Neilly is the first report of this species in Costa Rica. Comparisons were made to the values and observations given by previous authors (1, 2). The general shape and characteristics of the striae of the perineal pattern were similar to those reported by these authors, but the second-stage juveniles were shorter than the value given by Eisenback *et al* (2),

Table 4. Morphometric characters of second-stage juveniles of 16 populations of *Meloidogyne* spp. from Costa Rica.

Pop.	Total length	Tail length	Maximum body width	Anal width
M. incognita				
CR1	414 cd**	53.3 b	14.9 abc	10.8 cde
CR3	402 c	49.8 a	16.1 e	11.1 de
CR5	426 d	53.9 bc	15.2 bcd	10.8 cde
CR6	449 e	57.5 d	14.9 abc	10.9 cde
CR11	420 d	52.8 b	15.1 bcd	11.1 de
CR12	419 d	49.8 a	15.6 cde	10.3 bcde
CR16	461 e	56.4 cd	14.9 abc	9.0 a
CR17	386 b	47.4 a	15.7 de	10.3 bcde
M. exigua				
CR7	373 ab	48.5 a	14.5 ab	9.4 ab
CR9	368 a	48.8 a	14.7 ab	9.4 ab
M. hapla				
CR10	464 f	61.2 e	14.7 ab	10.6 bcde
CR14	373 ab	47.4 a	14.6 ab	10.2 bcd
CR15	373 ab	48.1 a	14.5 ab	10.0 abcd
M. arenaria				
CR4	459 e	57.3 d	14.2 a	9.8 abc
M. salasi				
CR2	466 f	69.5 f	16.1 e	11.4 e
M. incognita & *M. hapla*				
CR13	418 d	54.7 bcd	14.9 abc	10.3 bcde
CV (%)	4.0	6.3	5.5	12.0

* DEGO refers to the distance between the base of the stylet knobs and the dorsal esophageal gland orifice.

** Mean of 20 observations. All measurements in μm. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (P = 0.01). All juveniles had the hemizonid anterior to the excretory pore. In CR7, 9, 10, 14 and 15 the recta were undilated, whereas in the remaining populations they were dilated.

Continuation Table 4.

Pop.	Stylet base to head end	DEGO*	a	c
M. incognita				
CR1	15.3 de	3.2 ab	27.9 bcd	7.8 bcde
CR3	15.6 ef	3.1 ab	26.2 abc	8.1 ef
CR5	15.7 ef	3.0 a	28.3 bcd	7.9 cdef
CR6	15.5 ef	3.1 ab	30.3 def	7.8 bcde
CR11	15.1 cd	3.1 ab	28.0 bcd	8.0 def
CR12	15.4 def	3.8 d	23.5 a	8.4 g
CR16	15.8 f	3.6 cd	31.2 def	8.2 fg
CR17	15.5 ef	3.2 ab	25.0 ab	8.2 fg
M. exigua				
CR7	13.6 a	3.2 ab	25.6 ab	7.7 bcd
CR9	13.6 a	3.5 bcd	25.2 ab	7.5 b
M. hapla				
CR10	14.8 c	4.5 e	31.7 ef	7.6 bc
CR14	14.2 b	4.2 e	25.7 ab	7.9 cdef
CR15	14.0 b	3.7 d	25.8 ab	7.8 bcde
M. arenaria				
CR4	15.4 def	3.5 bcd	32.6 f	8.0 def
M. salasi				
CR2	14.2 b	3.3 abc	29.2 cde	6.7 a
M. incognita & M. hapla				
CR13	15.3 de	3.1 ab	28.2 bcd	7.6 bc
CV (%)	3.2	12.7	13.5	4.7

* DEGO refers to the distance between the base of the stylet knobs and the dorsal esophageal gland orifice

** Mean of 20 observations. All measurements in μm. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (P = 0.01). All juveniles had the hemizonid anterior to the excretory pore. In CR7, 9, 10, 14 and 15 the recta were undilated, whereas in the remaining populations they were dilated.

although similar to the values given by Chitwood (1). All other morphometric values for females, males and juveniles were similar to the reports by the previously mentioned authors.

M. salasi

The females of this root-knot nematode could be differentiated from the other species by the presence of a posterior protuberance and the neck and head regions located on the ventral side of the body. The body was usually oval, in contrast to the pyriform shape found in *M. exigua,* some of which showed a posterior protuberance and the neck on the ventral side of the body. Some, but not all specimens of *M. exigua* exhibited these characters.

The shape of the perineal pattern of *M. salasi* was also unique. Other differentiating characters were the short interphasmidial distance in the females, the longer juvenile tails, the smaller c ratio of juveniles and the shorter male stylet. The phasmids of the females were also smaller than in other root-knot nematode species.

Differential Plants

As point out by Taylor and Sasser (15), differential plants 1) provide a preliminary or corroborative indication of the root-knot nematode species being evaluated, based on the usual response of the hosts, and 2) detect pathogenic variation of a population, as determined by host responses different from the

Table 5. Morphological characters of males of 16 populations of *Meloidogyne* spp. from Costa Rica.

Pop.	Stylet	Spicules (chord of arch)	DEGO*	Areolation	Number of lateral lines	% males with one gonad
M. incognita						
CR1	23.6 f***	35.0 hi	3.5 b	yes	4	95
CR3	25.1 hi	34.5 ghi	3.0 ab	yes	4	100
CR5	24.1 fg	32.6 efg	2.8 a	yes	4	65
CR6	25.0 ghi	34.2 fghi	2.9 a	yes	4	85
CR11	22.0 e	33.5 fghi	2.8 a	yes	4	100
CR12	22.6 e	31.1 de	3.0 ab	yes	4	80
CR16	24.4 fgh	32.3 ef	3.2 ab	yes	4	70
CR17	25.7 i	35.4 i	3.5 b	yes	4	95
M. exigua						
CR7	18.4 b	24.1 a	3.2 ab	yes	4	45
CR9	18.9 b	25.0 a	4.9 de	yes	4	85
M. hapla						
CR10	21.7 de	30.0 cd	5.0 e	yes	4	60
CR14	20.4 c	29.0 bc	4.1 c	yes	4 –5	95
CR15	20.5 c	27.7 b	5.0 e	yes	4	100
M. arenaria						
CR4	24.3 fgh	33.0 efgh	3.1 ab	yes	4	95
M. salasi						
CR2	16.0 a	27.2 b	3.3 ab	yes	4	100
M. incognita & *M. hapla*						
CR13	21.0 cd	28.2 bc	4.4 cd	yes	4	85
CV (%)	6.8	9.3	16.9	–	–	–

* DEGO refers to the distance between the base of the stylet knobs and the dorsal esophageal gland orifice

** The rest of the males had two gonads.

*** Mean of 20 observations. All measurements in μm. Means in the same column followed by the same letter do not differ significantly from one another according to Duncan's Multiple Range Test (P = 0.01)

usual for the various species. However, differential plants cannot be relied upon entirely for identification, because the population being studied may be a mixture of species or a species for which no or limited host response data are available.

For example, *M. salasi* did not reproduce on any of the differential plants (Table 6). The reaction of the plants, however, was used to differentiated among the other species studied, and even for the determination of the host race among populations of *M. incognita* and *M. arenaria*.

Based on the scheme provided by Taylor and Sasser (15), the *M. incognita* populations CR1, 5, 6 and 17 were designated as race 1, whereas populations CR3, 11, 12 and 16 were designated as race 2. This is the first report of the presence of race 2 in Costa Rica. Salazar and Lopez (13) had previously reported race 1 only.

In spite of the evidence of pathogenic variation in the two populations of *M. exigua*, it seems premature at this time to call them races. This term was applied to populations of *Meloidogyne* species that were

Table 6. Response of seven differential plants to 16 populations of *Meloidogyne* spp. from Costa Rica.

Pop.	Tomato 'Rutgers'	Tobacco 'NC-95'	Pepper 'California Wonder'	Cotton 'Deltapine 16'	Peanut 'Florunner'	Watermelon 'Charleston Grey'	Corn 'Minnesota A-401'
M. incognita							
CR1	5*	3	5	0	0	5	5
CR3	5	5	5	0	0	4 5	5
CR5	5	1 5	4 5	0	0	3	3 2
CR6	5	1 5	5	1 5	0 2	5	4 7
CR11	5	5	5	0	0	5	5
CR12	5	4	3 5	0	0	5	1 5
CR16	5	5	5	0 5	0	5	5
CR17	5	2	5	0	0	5	3 7
M. exigua							
CR7	1 5	0	5	0	0	4	0
CR9	5	0	4 7	0	0	4	0
M. hapla							
CR10	5	5	1 7	0	5	5	0 2
CR14	5	4	5	0	5	0	0
CR15	5	5	5	0	5	0	1 6
M. arenaria							
CR4	5	5	5	0	0	5	3 2
M. salasi							
CR2	0**	0	0	0	0	0	0
M. incognita & *M. hapla*							
CR13	5	4 5	5	0	2 7	2	2 5

* Mean of four replicates. Responses evaluated according to the number of egg masses/root system. 0 = 0 egg masses; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; and 5 = more than 100 egg masses.

** Inoculum viability was evidenced by high reproduction on rice plants inoculated at the same time as the differential plants.

shown by numerous experiments to have unique host preferences, and that were named only after there was evidence of wide geographical distribution and/or sufficient significance for crop rotation and/or plant breeding programs (15). Most of the criteria used for the application of the term host were not fulfilled in this case. Future work could give the necessary proof that they indeed deserve to be designated as host races. From a practical point of view, this finding could be of value to farmers in the area of Sarchi, as some fields where coffee was grown were changed to tomato production.

Differences in the ability of *M. hapla* populations to reproduce on pepper were found. Reactions of the other differential plants to the three populations were similar, and agreed with the usual response given by them to this species (15). As in the case of *M. exigua*, it seems premature at this time to apply the term host races to these populations.

The population of *M. arenaria*, similar to most of the populations in the world collection of the International *Meloidogyne* Project, did not reproduce on peanut, cv. Florunner, and therefore was determined to be race 2 of this species.

The reaction given by the differential plants of CR13, the mixture of *M. incognita* and *M. hapla*, was different from the usual one given to each of the major species (15). Previous workers have found this same mixture of species in the Central Volcanic Ran-

ge of Costa Rica, on plants such as cabbage, carrot, lettuce and green peas (4, 10, 11, 12, 13). As pointed out by several of them, such mixture of species makes the management of root-knot nematodes by crop rotation and resistant cultivars even more difficult, and might require some long term studies for the development of profitable management schemes.

Summary

Based on a morphometric study of males, females and second-stage juveniles, and on the responses of seven differential plants, five species of *Meloidogyne* (*M. arenaria, M. incognita, M. hapla, M. exigua* and *M. salasi*) were distinguished among 16 populations collected at different locations in Costa Rica. The responses of the differential hosts gave evidence that the *M. arenaria* population was host race 2, and that the *M. incognita* populations included host races 1 and 2. Evidence of pathogenic variation was found between the two *M. exigua* populations. One reproduced readily on tomato, whereas the second population did not. Similarly, two populations of *M. hapla* reproduced readily on pepper, whereas the third population reproduced only to a limited extent on that host.

Literature cited

1. CHITWOOD, B. G. Root-knot nematodes – Part I. A revision of the genus *Meloidogyne* goeldi, 1887. Proceedings of the Helminthological Society of Washington 16:90-104. 1949.

2. EISENBACK, J. D., HIRSCHMANN, H., SASSER, J. N. and TRIANTAPHYLLOU, A. C. A guide to the most common species of root-knot nematodes (*Meloidogyne* species) with a pictorial key. A cooperative publication of the Departments of Plant Pathology and Genetics, North Carolina State University, and the United States Agency for International Development. Raleigh, North Carolina. North Carolina State Graphics. 1981. 48 p.

3. HIDALGO, L. and LOPEZ, R. Caracterización morfométrica de cuatro poblaciones de *Meloidogyne incognita* de Costa Rica. Turrialba 30:129-135. 1980.

4. LOPEZ, R. and AZOFEIFA, J. Reconocimiento de nematodos fitoparásitos asociados con hortalizas en Costa Rica. Agronomía Costarricense 5:29-35. 1981.

5. LOPEZ, R. and DICKSON, D. W. Morfometría y respuesta de hospedantes diferenciales a tres poblaciones de *Meloidogyne incognita* y una de *M. javanica*. Agronomía Costarricense 1:119-127. 1977.

6. LOPEZ, R. and SALAZAR, L. Morfometría y algunos hospedantes de *Meloidogyne hapla* en la Cordillera Volcánica Central de Costa Rica. Agronomía Costarricense 2:29-38. 1978.

7. LORDELLO, L. G. E. and ZAMITH, A. P. L. On the morphology of the coffee root-knot nematode, *Meloidogyne exigua* Goeldi, 1887. Proceedings of the Helminthological Society of Washington 25:133-137. 1958.

8. MATTEY, J. and LOPEZ, R. Evaluación de nematicidas y de métodos de aplicación en el combate de nematodos fitoparásitos y en la producción y calidad de la lechuga. Turrialba 28:15-18. 1978.

9. NETSCHER, C. Morphological and physiological variability of species of *Meloidogyne* in West Africa and implications for their control. Mededelingen Landbouwhogeschool, Wageningen, Netherland 78(3). 46 p.

10. PADILLA, C. and LOPEZ, R. Evaluación de nematicidas granulados para el combate de *Meloidogyne* spp. en arveja (*Pisum sativum* L.). Agronomía Costarricense 3:89-95. 1979.

11. PERLAZA, F., LOPEZ, R. and VARGAS, E. Efecto de la aplicación combinada de nematicidas y fungicidas en el combate de *Meloidogyne incognita, M. hapla* y *Alternaria* sp. en lechuga. Fitopatología (Perú) 13:90-96. 1978.

12. PERLAZA, F., LOPEZ, R. and VARGAS, E. Combate químico de *Meloidogyne* spp. y *Alternaria* sp. en zanahoria (*Daucus carota* L.). Turrialba 29:263-267. 1979.

13. SALAZAR, L. and LOPEZ, R. Caracterización morfométrica y ámbito de hospedantes diferenciales de diez poblaciones de *Meloidogyne* spp. de Costa Rica. Agronomía Costarricense 4:21-31. 1980.

14. SCOTTO LA MASSESE, C. The principal plant nematodes of crops in the French West Indies. In Peachey, J. E., ed. Nematodes of Tropical Crops. London, England, Common-

wealth Agricultural Bureaux Technical Communication No 40. 1969 pp. 164-183.

15. TAYLOR, A. L. and SASSER, J. N. Biology, Identification and Control of root-knot nematodes (*Meloidogyne* species). A cooperative publication of the Department of Plant Pathology, North Carolina State University, and the United States Agency for International Development Raleigh, North Carolina North Carolina State Graphics 1978. 111 p.

Reseña de libros

TARO A review of *Colocasia esculenta* and its potentials ed by Jaw-Kai Wang University of Hawaii Press 2840 Kolowalu Str Honolulu, Hawaii 96822 400 p. Price $ 35.00

Taro is a very complete book about the species *Colocasia esculenta,* crop of first feeding importance through all the islands of the Pacific.

The investigation shown in this book have been realized, principally in the last 15 years, by a wide group of scientifics (botanicists, agronomists, nutritionists, economists), especially of the University of Hawaii.

This team of researchers, working in all aspects of a crop, considered until now of second economic importance, due that is practised by multiple small aboriginal communities, is a real example of great vision to afford a problem. They have not only considered the plant itself, but also the thousands of peasants, distributed in islands, with very scarce technical elements and means of communications

The book cover:

A general background: botany, physiology and nutritive value.

Production technology: agronomy, pest and diseases.

Utilization: processed food, animal feed and industrial uses, and planning and development: production systems planning, socio-economic aspect of taro as food and production management considerations It calls the attention, in all chapters, to the details of the investigations carried on.

About Pest – as an example – it is presented the inventory of taro pest, cited, by separated, those that attack: leaves, petiols, corms and roots and adding the parasites and predators of the danger animals, and also cited those invertebrates and vertebrates, until now, known as associated with the crop.

The results and feasible recommendations presented, specially for the Pacific area, I am sure, will serve as well, for Asia, Africa and America, where this crop is also an important food, but technically neglected, as being practised by peasants.

Congratulations to Jaw-Kai Wang and all the equipment.

ALVARO MONTALDO
FACULTAD DE AGRONOMIA
UNIVERSIDAD CENTRAL DE
VENEZUELA, MARACAY, VENEZUELA

STUDIES ON SEED GERMINATION AND DORMANCY IN COTTON GENOTYPES (*Gossypium* spp.)[1] /

*R. V KOTI**
*K. V JANARDHAN**

Resumen

Se estudió la germinación y dormancia de semillas frescas de algunos genotipos de G. hirsutum, G. herbaceum *y* G. arboreum, *cosechados en tres etapas sucesivas, en relación a sus características y a métodos para romper la dormancia. La germinación inicial fue mayor en* G. herbaceum *seguida por* G. arboreum *y* G. hirsutum. *En* G. herbaceum *la germinación decreció con la etapa de cosecha, mientras que en* G. arboreum *se encontró una tendencia de aumento en la germinación.* G. hirsutum *mostró una baja germinación en semillas de la primer y tercera cosecha. En general, los genotipos de* G. hirsutum *mostraron períodos variables de dormancia, mientras que las otras dos especies no mostraron dormancia en ningún período de cosecha. El cv LRA-5166 (*G. hirsutum*) mostró el período de dormancia más bajo, variando entre 24 y 36 días después de la cosecha. Se observó una relación negativa y significativa entre la germinación inicial y el contenido de vella en la semilla de* G. hirsutum *(r = 0.9603), positiva con el índice de semilla en* G. herbaceum *(r = 0.5277) y negativa con el contenido de humedad inicial de la semilla de* G. arboreum *(r = 0.6128)*

Un tratamiento con calor (45°C) por siete días de las semillas con vella rompió la dormancia del cv. LRA-5166 en forma efectiva. El secado de las semillas al sol por dos días, también aumentó la germinación significativamente

Introduction

Seed dormancy indicates the inability to germinate under favourable conditions. Seeds dormancy in cotton is, however, not a serious problem, since normally, the time gap between harvest and next planting is considerably large. Hence, not much information is available on this aspect in the cultivated cotton genotypes. Nevertheless, to have basic knowledge of germination and dormancy is most important, in order to understand the nature of seed quality and viability later during the storage. In this paper, attempts have been made to obtain information on the initial germination of cotton seeds and factors controlling it, the extent of seed formancy in three cultivated cotton genotypes and methods to break the seed dormancy, if present.

Materials and methods

Experiment I: Initial germination and extent of dormancy period in cultivated cotton genotypes.

A few randomly chosen genotypes from three cultivated species of *Gossypium*, were used for the study. The crop was planted on medium black soil during the wet season of 1981, at the Agricultural Research Station, Dharwad, under rainfed conditions following a recommended package of practices for the region. The details of genotypes with dates of sowing and picking are given in Table 1.

1/ Received for publication in 3 July, 1983.
Part of M Sc (Agri.) thesis submitted to the University of Agricultural Sciences, Bangalore, by the senior author.
The work reported in this paper was supported through grants from the Indian Council of Agricultural Research, New Delhi. The authors thank Dr. Y. C. Panchal, Professor and Head, Department of Crop Physiology, Agricultural College, Dharwad and Dr. B. H. Katarki, Senior Scientist (Cotton), Agricultural Research Station, Dharwad, for providing necessary facilities.

* Department of Crop Physiology, College of Agriculture, Dharward-580 005 (Karnataka-India).

Table 1. Initial germination percent in cotton genotypes with reference to seed dormancy at different pickings.

Species variety		Pickings				For comparing		
		I	II	III	Mean	Variety (V)	Picks (P)	V x P
1		2	3	4	5	6	7	8
G hirsutum								
1.	DP-338	10	68	46	41 3			
2.	JK-236-2	72	82	82	78 7			
3.	JK-78-162	36	54	58	49 3			
4.	UAS-48-4	24	48	48	40 0			
						C D. at 5%		
5	DP-498	44	72	34	50 0			
6.	DP-342	50	66	28	48.0	9.8	5.7	17 0
7	NA-606	12	62	34	36.0			
8	LRA-5166	20	30	6	18 7			
9	DP-452	80	86	54	73 3			
Mean		38.7	63 1	43.3				
G herbaceum								
1	DB-3-12	96	88	94	92 7			
2	R-51	98	74	72	81 3	C.D at 5%		
3.	SM-6	92	70	78	80 0			
4.	72-245	88	58	80	75 3	6.2	4 8	10 8
5.	Jayadhar	90	62	32	61 3			
Mean		92 8	70 4	71.2	–			
G arboreum								
1	Lohit	52	82	82	72 0			
2	G-27	86	80	92	86 0	C D at 5%		
3	HD-11	90	80	90	86 7	10 7	NS	NS
4.	LD-135	90	88	84	87 3			
5	HD-135	78	84	82	81 3			
Mean		79.2	82 8	86 0				

Note		*G hirsutum*	*G herbaceum*	*G arboreum*
Date of sowing		3 8 1981	5 8 1981	12 8 1981
Date of picking:	I	23.12 1981	16 1.1982	23 1.1982
	II	2 1.1982	31 1.1982	3 2.1982
	III	13 1.1982	15.2 1982	13.2 1982

When cotton seed were picked, they were brought to the laboratory, ginned and immediately kept for germination. Germination tests were carried out at room temperature (28°C ± 2°C) using the standard (between) paper towel technique. Five days were allowed for germination and on sixth day only "normal seedlings" were considered for germination; The period of dormancy was calculated as the time taken for 80 per cent germination. Germination tests were continued until the desired germination was attained.

In addition, moisture content of seed (initial and final), seed index (100 seed weight) and hull to kernel ratio were also evaluated following standard procedures.

Experiment II: Methods to break dormancy in cv. LRA-5166.

The variety LRA-5166 which exhibited seed dormancy for nearly 30 days period in all the three pickings, was chosen for the study. Nine treatments were

imposed including a check (Table 3) The treatments consisted of soaking the seeds for five hours in test solutions followed by washing with distilled water and kept for germination at room temperature using the standard paper towel technique

All the seeds were kept separately in three lots of hundred each The data obtained on germination, was analysed statistically

Results

Experiment I: (a) Initial germination

The initial germination was highest in *G. herbaceum* followed by *G arboreum* and least in *G hirsutum* (Table 1). The germination percent also varied in different species with reference to pickings. Thus, the varieties of *G hirsutum* showed significantly low germination in first (38 7%) and third pickings (43 3%) than in second picking (63 1%) Germination in *G. herbaceum*, was significantly highest in first picking (92.8%) than in either the second (70 4%) or third picking (71 2%). In contrast, the varieties of *G. arboreum* exhibited a tendency for increased germination (79 2, 82 8 and 86.0% in first, second and third pickings, respectively) although the differences were non-significant.

(b) Period of seed dormancy

The data indicated the occurrence of seed dormancy for various periods in the varieties of *G hirsutum* only In the other two species, dormancy was either minimum (*G. herbaceum*) or completely absent (*G arboreum*) Table 2).

In general, in the varieties of *G. hirsutum*, the dormancy period varied with successive pickings: 12 0, 10.7 and 14 0 days in first, second and third pickings, respectively. Among the varieties, JK-236-2 and DP-452 had either negligible period of dormancy, six days in JK-236-2 in the first pick and 12 days in DP-452. The variety LRA-5166 recorded dormancy period for over a period ranging from 24 days (first pick) to 36 days (second pick). The other variety NA 606, also showed dormancy for a lesser period ranging from 12 days (second pick) to 24 days (third pick)

The other varieties had a shorter dormancy period ranging from 6 to 12 days.

(c) Seed characteristics.

The data on fuzziness, seed index, hull to kernel ratio and initial and final seed moisture are given in Table 2.

i) Fuzziness: The data indicated considerable variation in fuzziness among the species studied, which is in the decreasing order: *G. hirsutum* (8 96%), G. *arboreum* (7 10%) and *G. herbaceum* (4 10%). Even within a species, further variation was observed with different pickings Thus, in both *G. hirsutum* and *G. arboreum*, the fuzz content decreased; while in *G. herbaceum*, the fuzz increased with successive pickings

Among the varieties of *G. hirsutum*, cv. LRA-5166, had the highest fuzz content (14 10 to 14 50%), while, cv. JK-236-2 had the lowest (4 37 to 4 60%) The mean data for three successive pickings in *G. herbaceum*, indicated lowest fuzz in R-51 (3 11%) and highest in Sel. 72-245(5 27%). Unlike the other two species, the variation in fuzziness among varieties of *G arboreum* and pickings was considerably less However, HD-133(8 210%), in the first picking, LD-135 the second (7 96%) and in the third picking (8 46%) exhibited relatively higher fuzziness

ii) Seed index (Acid delinted): The mean seed index was in the increasing order of: *G arboreum* (4 74 g), *G. herbaceum* (5 88 g) and *G. hirsutum* (8 19 g). In general, all the three species showed a decreased in seed index with successive pickings. Among the genotypes of *G. hirsutum*, the seed index was relatively higher in DP-338 in the first picking (9 22 g), UAS-48-4 in the second picking (8 98 g) and DP-338 in the third picking (9 82) in comparison to the others.

Among the varieties of *G herbaceum*, Jayadhar (6 37 g), Sel. 72-245 (6 28 g), and DB-3-12 (6 23 g) recorded a relatively higher seed index over others in the successive pickings.

The seed index in HD-133 of *G. arboreum* was consistently higher in all the three pickings (5 52, 5 10 and 4 53 g, respectively).

iii) Hull to kernel ratio: In general, *G. herbaceum* (0 78) had higher full to kernel ratio, followed by *G arboreum* (0 63) and least in *G hirsutum* (0 54). In all the species, it however, increased with progressive picking.

Among the varieties of *G. hirsutum*, cv. DP-498, had a higher ratio of 0 57, 0 59 and 0 56 in the first, second and third pickings. The mean data for *G. herbaceum* indicated that hull to kernel ratio was highest in the second picking (0 81), followed by a third picking (0 78) and comparatively lower in the first picking (0 74). The variety SM-6 in the first picking, Sel. 72-245 in the second and third pickings, recorded the highest ratios of 0 75, 0 85 and 0 93 respectively. In *G. arboreum*, the ratio was almost similar in the

Table 2. Dormancy period and some seed characters in the varieties of *Gossypium* spp.

Genotype	Dormancy period (day)			Fuzz content (%)			Seed index (g)			Hull to kernel ratio ratio			Initial seed moisture			Final seeds moisture		
	Picking			Picking			Picking			Picking			Picking			Picking		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
G. hirsutum																		
DP-338	12	12	12	7.64	8.02	7.67	9.22	8.65	8.82	0.51	0.57	0.56	12.50	10.35	10.84	7.46	7.00	7.71
JK-236-2	6	0	0	4.37	4.37	4.60	8.58	7.54	7.74	0.49	0.53	0.52	12.25	11.50	11.60	7.88	–	–
JK-78-162	12	12	12	9.04	7.86	9.25	8.69	8.69	8.37	0.49	0.48	0.53	14.00	10.50	10.00	7.00	7.92	7.30
UAS-78-4	12	6	12	11.05	10.05	9.11	8.92	8.98	8.68	0.57	0.54	0.64	13.30	10.50	8.80	9.18	7.50	7.18
DP-498	12	6	12	12.20	9.31	11.22	7.86	7.09	7.24	0.57	0.59	0.66	13.50	11.00	11.00	8.87	7.92	8.00
DP-342	12	12	12	8.42	6.61	7.45	9.07	8.02	8.20	0.49	0.54	0.57	13.73	11.18	9.25	7.50	8.65	8.25
NA-606	18	12	24	10.19	7.85	6.38	8.29	8.21	8.48	0.55	0.55	0.55	14.71	10.84	10.73	7.88	2.92	7.39
LRA-5166	24	36	30	14.30	14.50	14.10	7.24	7.20	7.30	0.48	0.47	0.48	13.50	12.50	11.00	8.70	8.25	8.00
DP-452	0	0	12	7.98	4.12	8.00	7.44	8.03	6.99	0.47	0.48	0.68	13.24	9.80	9.50	–	–	7.50
Mean	12.0	10.7	14.0	9.47	8.74	8.64	8.36	8.15	8.06	0.51	0.53	0.58	13.42	10.91	10.30	8.06	7.88	7.79
G. herbaceum																		
DB-3-12	0	0	0	4.96	4.84	4.74	5.81	6.23	5.91	0.67	0.78	0.68	9.31	9.50	6.82	–	–	–
R-51	0	6	6	3.08	2.98	3.28	5.88	5.54	5.84	0.74	0.74	0.76	9.00	10.80	13.00	–	7.51	7.00
SM-6	0	6	0	2.42	2.90	4.63	6.05	5.73	6.07	0.99	0.84	0.69	8.70	9.00	9.09	7.43	7.43	–
72-245	0	6	6	2.95	5.66	7.20	5.88	6.28	5.49	0.75	0.85	0.93	7.70	9.06	7.91	–	8.42	7.50
Jayadhar	0	6	6	3.32	3.65	4.95	6.37	5.89	5.02	0.75	0.82	0.86	12.17	9.71	7.36	–	6.79	6.50
Mean	0.0	4.8	3.6	3.35	4.01	4.95	6.00	5.98	5.67	0.74	0.81	0.78	9.34	9.61	8.24	–	7.54	7.00
G. arboreum																		
Lohit	6	0	0	5.88	6.12	6.35	5.11	4.20	3.85	0.61	0.67	0.64	10.78	7.91	7.00	8.46	–	–
C-27	0	0	0	7.68	7.01	6.59	5.45	4.37	4.02	0.67	0.67	0.77	7.00	5.50	4.82	–	–	–
HD-11	0	0	0	6.78	6.57	6.80	5.26	4.83	4.35	0.63	0.61	0.64	6.50	6.00	4.89	–	–	–
LD-135	0	0	0	7.56	7.96	8.46	5.46	4.85	4.24	0.62	0.46	0.63	8.91	5.50	4.51	–	–	–
HD-133	6	0	0	8.21	7.58	6.89	5.52	5.10	4.53	0.59	0.63	0.63	9.86	6.50	4.51	6.00	–	–
Mean	2.4	0.0	0.0	7.22	7.05	7.02	5.36	4.67	4.20	0.62	0.61	0.66	8.61	6.28	5.15	7.23	–	–

Table 3. Germination per cent as influenced by various physico-chemical treatments in the freshly harvested seeds of LRA-5166.

Sl. no.	Treatments	Germination (%)	Difference over control (%)
1	Fuzzy (Control	37.3	..
2	Fuzzy – Sundried	72.0	+ 34.7
3	Fuzzy – 45°C, 3 days	66.7	+ 29.4
4	Fuzzy – 45°C, 7 days	81.3	+ 44.0
5	Acid delinted	57.3	+ 20.0
6	Delinted + water soaked	54.7	+ 17.4
7	Delinted + GA_3 20 ppm	56.0	+ 19.3
8	Delinted + etherel 50 ppm	49.3	+ 12.0
9	Delinted + water soaked-dried	59.3	+ 22.0
For comparing treatments:		S. Em±	5.0
		C.D. at 5%	10.6

first two pickings (0.61 and 0.62) which increased to 0.66 in the third picking. Among the varieties, G-27 showed consistently higher ratio of 0.67, 0.67 and 0.77 in the successive pickings.

iv) Initial seed moisture: The mean initial seed moisture was the highest in *G. hirsutum* (11.54%) followed by *G. herbaceum* (9.06%) and least in *G. arboreum* (6.68%). With reference to the time of picking, there was a reduction in initial seed moisture in all the species. In *G. hirsutum*, the initial seed moisture decreased from 13.42 percent in the first picking to 10.30 percent in the third picking. Among the genotypes, DP-342 (14.7%) followed by JK-78-162 (14.0%) in the first picking, LRA-5166 in the second picking (12.50%) and JK-236-2 in the third picking (11.60%) had relatively higher moisture contents.

The mean initial seed moisture in *G. herbaceum*, was slightly more in the second picking (9.60%) than in first picking (9.34%), but decreased in the third picking (8.24%). Relatively, higher moisture content was seen in Jayadhar (12.17%) in the first picking, R-51 in the second (10.80%) and the third pickings (13.00%). However, there was no consistency in the decrease in seed moisture among the varieties with successive pickings.

In *G. arboreum*, the variation in seed moisture with successive pickings was considerable, reducing from 8.61% in the first picking to 5.15% in the third picking. In all the pickings, Lohit maintained relatively higher content (10.78, 7.91 and 7.00% , respectively).

v) Final seed moisture: The data on final seed moisture was also collected to verify whether the expected decreased seed moisture is related to enhanced germination. The data indicated a decrease in seed moisture to around 7–8 percent irrespective of initial seed moisture to attain 80 percent germination in all the three species studied.

Experiment II: Methods to break seed dormance in cv. LRA-5166.

Among the eight treatments tried in comparison to the untreated control, fuzzy seeds subjected to a temperature of 45°C for seven days showed a significant increase in the germination to 81.3 percent that is 44.0 per cent more than the seeds stored at room temperature. Even sun drying for two consecutive days, showed a 34.7 percent increased germination over the control. Although other treatments gave significantly higher germination, they were not on par with the above two treatments (Table 3).

Discussion

Initial germination, seed dormancy and seed characteristics in cotton.

Of the three cultivated species, only the varieties of *G. hirsutum*, showed considerable variation for period of dormancy; while in other species, it was either negligible or absent. The occurrence of seed dormancy in cotton, particularly in *G. hirsutum*, has also been previously reported (2, 3, 4, 7, 9). Christidis (4) was of the opinion that cotton seeds probably need several days of rest, depending upon the date of maturity and variety.

Reasons for differences in the initial seed germination in successive pickings among the species and varieties within the same species may be attributable to certain seed characteristics, be different for each species. Correlations worked out between initial germination and seed characters amply indicated this possibility (Table 4). One of the reasons for the extended period of seed dormancy in *G. hirsutum* may be ascribed to fuzziness as there was a significant negative relationship between initial germination and fuzz content ($r = -0.9603$). As far back as 1935, Simpson (9) and recently Bhagavandas (3) have shown that sulphuric acid treatment to freshly harvested seeds increased the germination percentage. Bailey (1) suspected that wax and lignin in conjunction with fuzz might play a part in restricting the

Table 4. Simple correlations between initial germination and seed characters (correlation co-efficient = r).

Species	Seed germination vs.			
	Initial moisture content	Fuzz content	Seed index	Hull to kernel ratio
G. hirsutum	-0.0597	-0.9603**	0.1817	-0.3682
G. herbaceum	0.0611	-0.2133	0.5277*	-0.3931
G. arboreum	-0.6128*	0.3702	0.1458	0.1479
Levels of significance			5% P	1% P
G. hirsutum (25 df)			0.381*	0.487**
G. herbaceum and *G. arboreum* (13 df)			0.514*	0.641**

absorption of moisture by seeds. Further, it has been observed that delayed germination due to fuzz on cotton seeds may exert a secondary inhibition (3). In *G. herbaceum*, the initial seed germination (although high) was positively related with seed index (r = 0.5277). The seed index in this species generally decreased, with progressive pickings, indicating a possibly poor development of embryo. It is reported that poor and delayed germination in seeds of certain varieties of cotton was due to poor embryo development (2). Further, it was also observed that embryo was not dormant but only weak. Regarding *G. arboreum*, in which no dormancy was observed, the initial seed moisture was correlated negatively with seed moisture (r = -0.6178). Paddy seeds having both high and low moisture content germinated late.

Those having low moisture content germinated late and also showed high germination (8). The final seed moisture data from the present studies have also amply proved this observation.

In none of the species did hull to kernel ratio have any relationship with initial germination, although considerable variation was observed among the species and among the pickings within a variety. Kempanna *et al.* (5) investigating the causes for poor germination in four cultivated species of cotton found that the germination in *G. arboreum* was not influenced by impermeable seed coat. According to them, germination in *G. hirsutum* was controlled by factors of an unknown physiological nature. However, the present studies clearly indicated that fuzziness in *G. hirsutum*, seed weight in *G. herbaceum* and seed moisture in *G. arboreum* influence initial seed germination.

Methods to break seed dormancy in cv. LRA-5166.

The data indicated that either sun-drying or heat treatment successfully breaks the seed dormancy in cv. LRA-5166. Similar results were also reported in paddy seeds by heat treatment (6, 8). High temperatures (40-50°C) breaks the seed dormancy in cotton by making the seed coat permeable (2). It may also possibly destroy certain growth inhibitors accumulated in the seed during its development. By removing fuzz by acid delinting, the germination increased only to a small extent, thereby indicating that fuzz forms only a part of the impediment affecting good germination. Probably some internal factor(s) may be responsible. However, it was also observed that none of the growth regulators used, which are reported to break dormancy, enhanced the germination to the extent of heat treatment. Thus, it may be surmised that heat treatment, not only enhanced the permeability of seeds, but also destroyed some interfering substances.

Summary

Germination of freshly harvested seeds at three successive pickings, and the extent of dormancy in a few genotypes of *G. hirsutum*, *G. herbaceum* and *G. arboreum*, in relation to some seed characters and also methods to break the seed dormancy, were studied. The initial germination was highest in *G. herbaceum* followed by *G. arboreum* and least in *G. hirsutum*. In *G. herbaceum*, the initial seed germination declined with successive pickings; while in *G. arboreum*, there was a tendency for enhanced germination. *G. hirsutum* showed significantly low germination in the first and third pickings. In general, the genotypes of *G. hirsutum* showed varying periods of seed dormancy, while the other two species remained non-dormant, in all the pickings. Cv. LRA-5166 (*G. hirsutum*) showed a relatively longer period of dormancy ranging from 24 to 36 days after harvest. A significant negative relation between initial germination and fuzz content in *G. hirsutum* (r = -0.9603), positive correlation with seed index in *G. herbaceum* (r = 0.5277) and negative relation with initial seed moisture in *G. arboreum* (r = -0.6128) were observed.

Heat treatment of fuzzy seeds (45°C) for seven days broke the dormancy effectively in the dormant variety LRA-5166. Sundrying for two consecutive days also increased germination significantly.

Literature cited

1. BAILEY, A. E. "Cotton seed and cotton seed products". Interscience Publishers, Inc. New York. 1948.

2. BALASUBRAMANIAM, R. and MUDALIAR, V. R. A physiological study of delayed germination in cotton. Madras Agricultural Journal 21:147-162. 1933.

3. BHAGAVANDASS, M. Preliminary investigations on the dormancy of cotton seeds. Madras Agricultural Journal 56:97-99. 1969.

4. CHRISTIDIS, B. G. Dormancy in cotton seed. Agronomy Journal 47:400-403. 1955.

5. KEMPANNA, C., PARAMESHWAR, N. S. and KHALLAK, A. Variations in seed germination of four *Gossypium* species. Mysore Journal Agricultural Science 1:71-80. 1967.

6. MISRO, B. and MISRA, P. K. Certain consideration on seed dormancy. Oryza 61:18-22. 1969.

7. PATIL, M. S. Seed dormancy and viability in some varieties of cotton. Current Research 5:1-2. 1976.

8. SIKDER, H. P. Dormancy of rice seeds in relation to different moisture contents of grains and dates of harvests. Journal Agricultural Science Cambridge 81:159-163. 1973.

9. SIMPSON, D. M. Dormancy and maturity of cotton seed. Journal Agricultural Research 50:429-434. 1935.

Notas y comentarios

Premio Nobel de Medicina y Biología de 1984

César Milstein, un argentino del Laboratorio de Biología Molecular de Cambridge, Inglaterra, Nils Jerne, un danés nacido en Londres, del Instituto de Inmunología de Basilea, y el intermediario entre los dos, George Köhler, de Alemania Occidental, fueron los escogidos para recibir el Premio Nobel de Biología y Medicina de 1984, por haber inventado los anticuerpos monoclonales, y haber abierto, con esta investigación, las puertas de la ingeniería genética.

Como muchos otros importantes logros científicos, el primer aviso se encuentra en una carta al editor que dirigieron Milstein y Köhler (quien trabajaba con Milstein en Cambridge) a la revista **Nature**, y publicada el 7 de agosto de 1975, en la que describían un método para producir grandes cantidades, muy puras y muy precisas, de anticuerpos, esas armas con que el sistema inmunológico del cuerpo lucha contra las enfermedades.

Los anticuerpos son muy útiles, no sólo para combatir enfermedades sino en otras esferas de la biomedicina. El problema reside en que el sistema de la inmunidad produce anticuerpos a toda clase de antígenos, lo que quiere decir que es muy difícil obtener anticuerpos puros. Una proteína simple puede tener varios puntos antigénicos y un solo patógeno puede llevar consigo muchas proteínas superficiales diferentes. El cuerpo responde con un coctel de anticuerpos mezclados; el purificar una sola especie de anticuerpo lleva al investigador contra los límites de los métodos fisicoquímicos.

Aquí entran los anticuerpos monoclonales. Estos son hechos por un cultivo de células genéticamente idénticas, o sea, un clon. Debido a que un solo linfocito produce un solo anticuerpo, un cultivo monoclonal secreta un producto puro. Pero las células normales del organismo tienen una vida muy limitada, consiguiéndose sólo unas siete u ocho divisiones antes de que ellas mueran. Un cultivo derivado de un solo linfocito sería de una vida demasiado corta para ser útil.

La hazaña de Milstein y Köhler fue fusionar células de un tumor canceroso, que son inmortales, con una

célula normal que producía un anticuerpo específico. Crearon así una línea celular inmortal que secretaba un anticuerpo puro. Estas células se llaman hibridomas.

Pronto se dieron cuenta de las implicaciones de su descubrimiento. Los anticuerpos monoclonales podrían ser utilizados para fabricar el interferón más puro. Pueden distinguir un tipo de célula de otro; un tipo de leucemia de otro. Y lo mejor de ellos es su confiabilidad; presentan siempre las mismas propiedades porque todas las células del clon son idénticas.

El gobierno británico declinó patentar el descubrimiento por lo que Milstein y Köhler decidieron redactar la comunicación que publicó **Nature**. Esto ha sido criticado en algunas revistas científicas, entre ellas **New Scientist**, que editorialmente señaló que algunos premios Nobel recibidos por científicos de instituciones británicas han sido explotados comercialmente por compañías de otros países (otro ejemplo es la penicilina). Pero, en realidad, es sólo en los últimos años en que las universidades y organismos estatales están patentando los avances que obtienen en la electrónica y la biotecnología. A partir de las investigaciones dirigidas por Milstein, la industria ha venido desarrollando usos comerciales a los anticuerpos monoclonales, principalmente 1) en procesos industriales, como en la producción de interferones, esas sustancias antivíricas y anticancerosas elaboradas por el cuerpo; 2) en diagnosis, como anticuerpos para tipificar tejidos, lo que es necesario para trasplantes; y 3) en terapia, como drogas citotóxicas que son llevadas por anticuerpos específicos a células cancerosas, dejando intactas las otras células. Y hay muchos ejemplos más.

Milstein, quien tiene 57 años, se graduó en química en la Universidad de Buenos Aires, en 1957. En 1958, estudió para su doctorado en el departamento de bioquímica de la Universidad de Cambridge. Trabajó por primera vez en el Consejo de Investigación Médica en 1960, y regresó a la Argentina en 1961. Por dos años fue jefe del Instituto Nacional de Microbiología en Buenos Aires, pero renunció y regresó a Inglaterra cuando cuatro miembros de su personal fueron despedidos por pertenecer a un sindicato.

Köhler, quien tiene 38 años, aparte de sus dos años con Milstein, ha realizado su labor en el Instituto de Inmunología de Basilea, Suiza, que fue fundado por Jerne. Se espera que asuma la dirección del nuevo Instituto Max Plank de Inmunología en Freiburg, Alemania Occidental.

Jerne, quien dice que para el trabajo teórico que él realiza, sólo necesita un pedazo de papel, fue incluido en el premio en razón de que sus teorías sobre la diversidad de los anticuerpos marcaron una nueva dirección en la ciencia de la inmunología. Cuando los químicos descubrieron que la conformación de las moléculas de proteína era determinada solamente por la secuencia de los aminoácidos que la componen, cayó en descrédito la teoría anterior, la hipótesis de instrucción, que sostenía que los anticuerpos eran como cadenas desdobladas que, en contacto con el antígeno, se envolvían sobre este antígeno para formar un anticuerpo a la medida. Jerne propuso entonces, en 1955, la teoría de la selección clonal de la formación de anticuerpos. Esta idea fue la base subsecuente de la inmunología. Esto llevó al trabajo de Gustav Nossal, en Australia, que mostró que cada linfocito producía sólo una clase de anticuerpo, un concepto que hizo posible la técnica de Milstein y Köhler para producir hibridomas. Su siguiente idea, dice Jerne, fue que la mutación somática para darle a los linfocitos su vasto repertorio de anticuerpos. Jerne es danés, aunque nacido en Londres. Hizo su trabajo doctoral en Copenhague y trabajó seis años en el Instituto Serum. Mientras dirigía el Instituto Paul Erlich, en Frankfurt, le fue propuesto, por la firma suiza Hoffman-La Roche, que iniciase el Instituto de Inmunología de Basilea. Adalberto Gorbitz.

INFLUENCE OF LIGHT INTENSITY ON CHLOROPHYLL DISTRIBUTION AND ANATOMICAL CHARACTERS OF CASSAVA LEAVES[1] /

*T RAMANUJAM**
*J S JOS**

Resumen

Se estudió la influencia de la intensidad luminica sobre las características morfológicas y anatómicas de hojas de cuatro cultivares de yuca promisorias. Con poca luminosidad, se observó una reducción significativa del peso específico de la hoja (SLW) y un aumento en la relación de área foliar (LTR). Las características anatómicas de una variedad de alta producción (H-165) se compararon con las de la variedad de baja producción (M-4), creciendo con poca luminosidad. El parénquima esponjoso fue más grueso en H-165 que en M-4 bajo condiciones normales de luz y con poca luz en ambos casos hubo una misma en el grosor de la capa del mesofilo. De la misma manera, las estomas en H-165 estaban más hundidas que en M-4 bajo condiciones normales de luz, pero al reducir la luminosidad en ambas variedades se encontraron en la superficie de la hoja. En las cuatro variedades estudiadas se experimentó una reducción en el número de estomas por unidad de superficie foliar bajo poca luminosidad. Asimismo, la concentración de clorofilato aumentó significativamente en relación a la clorofila a bajo condiciones de poca luz.

Introduction

In recent years, mixed cropping is gaining importance in the tropical agriculture in view of increasing the productivity per unit land area by making use of the solar radiation more effectively. Besides monocropping, cassava (*Manihot esculenta* Crantz) is traditionally grown as intercrop with coconut palms in the State of Kerala, which contributes about 80% of cassava production in India. The work conducted at CTCRI, Trivandrum suggested that the light intensity available for the growth of cassava plants in the established coconut plantation was 10 to 15% of the normal sunlight which resulted in significant yield reduction ranging from 65 to 94% depending upon the gentoypes (9). Further investigations were made to compare the anatomical and morphological characters of cassava leaves exposed to normal and shaded environments.

Materials and methods

Four promising varieties of cassava Viz., M-4, H-165, H-1687 and H-2304 were grown in open area (normal light) with a mean diurnal light intensity of 40 000 lux and in shaded area (low light) with a mean diurnal light intensity of 6 000 lux. The shade was created artificially by screening the sunlight. The experiment was conducted during 1982 cropping season. All the observations were made between 2nd and 3rd month stage and the experiment was terminated by 4th month. Anatomical studies were made using sections 10 to 15 thickness following the usual dehydration, embedding and staining procedures (6). Chlorophyll pigments (total, Chl. a and Chl. b) were estimated by the method of Arnon (1).

Results and discussion

The specific leaf weight (SLW) for the varieties grown under normal light ranged from 3.9 to 4.7 mg/cm^2 while under low light (shade) the SLW was reduced significantly in all the four varieties by 57 to 62% (Table 1). The leaves grown under shade were thinner and dark green in colour when compared to normal light. However the intensity of anthocyanin in the petioles of M-4 and H-2304 was

1 Received for publication in June 15, 1983
The authors are grateful to the Director, Central Tuber Crops Research Institute, Trivandrum for the facilities provided to carry out this piece of work. The help of Mr. C. S. Antoniswamy, Artist cum Photographer is also acknowledged

* Scientist S2 (Plant Physiology); Scientist S2 (Cytogenetics) CTCRI, Trivandrum 695 017, Kerala, India

very low in contrast to their characteristic dark red petioles found in normal light. The finding suggested that besides the quality of light (5), the quantum of light is also essential for the synthesis of the flavonoid pigments despite the exact role of anthocyanin in cassava is yet to be investigated.

Distribution of chlorophyll pigments also varied significantly in different treatments (Table 2). The leaves grown under low light (6 000 lux) recorded higher concentration of total chlorophyll per unit leaf weight (mean value = 3.72 mg/g fresh leaf) when compared to the leaves grown in normal light (mean value = 2.58 mg/g fresh leaf). However, the concentration of total chlorophyll per unit leaf area was higher under normal light (3.30 mg/dm^2 leaf) when compared to low light (2.82 mg/dm^2 leaf). The significant increase in chlorophyll concentration under low light on leaf weight basis was mainly due to increase in leaf area ratio (67% over control). As there was little difference in total leaf area between the two treatments, the chlorophyll content of the whole plant will be higher under normal light when compared to low light. Among the two fractions of chlorophyll, the low light favoured increase concentration of chlorophyll, the low light favoured increase concentration of chlorophyll-b, consequently the ratio of chlorophyll-a to chlorophyll-b was reduced significantly which may be also an important factor for the causes of low productivity of cassava under shade. Similar observations on increased concentration of chlorophyll-b under low light was already made (3) in other crops. The role of chlorophyll-b in the primary photosynthesis is mainly to trap the photon and transfer to chlorophyll-a where the process of energy conversion (photosynthetic phosphorylation) takes place. Hence any significant reduction in the ratio of chlorophyll-a to chlorophyll-b found in normal leaves may bring out reduction in the photosynthetic efficiency.

The cassava leaves grown under low light intensity were found to be thin and papery which may affect the leaf water potential and osmotic regulatory mechanism. Though the leaf blades were slightly broader under low light, the leaf area ratio (leaf area per unit leaf weight) was very high, which may affect the productivity as LTR was also considered an important physiological parameter in crop production (8).

Table 1. Effect of light intensity on certain leaf characteristics of cassava.

Variety	Specific leaf weight mg/cm^2		Leaf area ratio cm^2/g	
	Normal light	Low light	Normal light	Low light
M-4	3.9	1.6	84.0	135.7
H-165	4.7	1.9	71.1	118.4
H-1687	4.3	1.6	80.0	114.6
H-2304	4.2	1.8	79.9	128.0

Table 2. Effect of light intensity on distribution of chlorophyll pigments in cassava.

Characters	Leaf weight basis (mg/g fresh leaf)				Leaf area basis (mg/dm^2 fresh leaf)			
	M-4	H-165	H-1687	H-2304	H-4	H-165	H-1687	H-2304
1. Total Chlorophyll								
Normal light	2.18	2.74	2.54	2.86	2.60	3.85	3.18	3.58
Low light	3.30	3.36	3.94	4.26	2.43	2.84	2.72	3.32
2. Chlorophyll-a								
Normal light	1.04	1.34	1.22	1.42	1.24	1.88	1.53	1.78
Low light	1.48	1.50	1.74	1.90	1.09	1.27	1.20	1.48
3. Chlorophyll-b								
Normal light	1.16	1.40	1.30	1.46	1.38	1.97	1.63	1.83
Low light	1.82	1.86	2.22	2.36	1.34	1.57	1.54	1.84
4. Ratio of Chl-a/Chl-b								
Normal light	0.90	0.96	0.94	0.97	0.90	0.95	0.94	0.97
Low light	0.81	0.81	0.78	0.81	0.81	0.81	0.78	0.80

By evaluating a set of promising cultivars of cassava under uniform shade, Ramanujam *et al* (9), observed significantly higher yield in H-165 when compared to M-4. Under normal light also, the former is a higher yielder than the latter. The leaf anatomy of H-165 and M-4 under both the light intensities were compared. The results suggested that the leaves of H-165 were found to be thicker than M-4 under normal light (Table 3) and interestingly, the spongy parenchymatous layer was found to be characteristically thicker in H-165 than in M-4. The chloroplast was present in both palisade and spongy parenchyma of both the varieties. The different layers of the leaves grown under shade were distinctly thinner when compared to the leaves grown under normal light (Figures 1, 2). There was a reduction in the distribution of chloroplast in the palisade but the size appeared to be almost the same as in the control. The spongy parenchyma also showed a reduction in the chloroplast distribution, but there was an increase in the size of the chloroplast. Under shade (low light), the leaves of both the clones were almost of same thickness and similar in the distribution of different layers. The most significant reduction was noticed in the thickness of spongy parenchyma of H-165 (Table 3) where, it was 60.0 in the leaves grown in open while 25.6/u in the leaves grown in shaded conditions. Rarefication was noticed in palisade and spongy layers.

The size of the stomata and distribution was studied in detail for all the four varieties (Table 4). The stomata were found on the lower surface of the leaves for all the cultivars under both the treatments confirming the earlier report (10). Comparison was made between M-4 and H-165 for stomatal distribution which revealed that under normal light, they

Table 3. Leaf anatomy of M-4 and H-165grown under normal light and low light.

	Normal light (40 000 lux)		Low light (6 000 lux)	
	M-4	H-165	M-4	H-165
1 Thickness of Epidermis (μ)	20 0	20 0	14 0	13 2
2 Radial length of palisade (μ)	68 0	68 0	41 2	39 2
3 Thickness of spongy parenchyma lower epidermis (μ)	41.2	60 0	26 4	25 6
4 Thickness of leaf (μ)	129 2	148 0	81 6	78 0

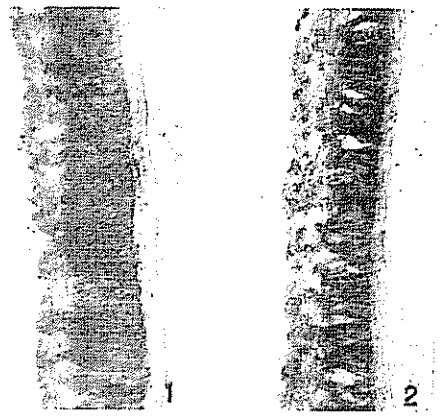

Fig. 1. T.S. of leaf of M-4 from normal light, x 150.

Fig. 2. T.S. of leaf of M-4 grown in shade, x 150.

were found on the surface in M-4 (Figure 3) while in H-165 they were slightly sunken with relation to other epidermal cells (Figure 4). However, under low light, the stomata were found on the surface in both the varieties. There was significant reduction in the number of stomata per unit leaf area due to low light (Figures 5, 6). The mean stomatal number per unit leaf area in M-4 was 56.3 and 48.7 under normal and low light respectively.

Plants adapted to bright sunlight are found to show different leaf morphology under low light (2, 3, 4, 7). Crockston *et al* (4), demonstrated that higher light intensity promoted the development of palisade and spongy mesophyll tissues resulting thicker leaves. Cassava being a sun loving crop, the associated anatomical and morphological changes are apparently brought out by low light.

Adaptation to lower light intensity appears to be a genetical character which would make effective use

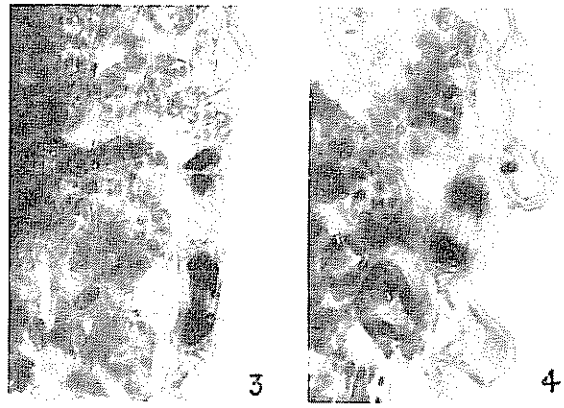

Fig. 3. Stomata on the surface in M-4, x 650.

Fig. 4. Sunken stomata in H-165, x 650.

Table 4. Influence of light intensity on distribution and size of stomata.

Variety	Treatment	Number of stomata*		Lenght of stomata (μ)	
		Range	Mean	Range	Mean
M-4	Normal light	55–59	56.3	20–24	21.6
	Low light	45–56	48.7	16–24	18.8
H-165	Normal light	45–59	50.2	16–24	22.0
	Low light	38–46	41.8	16–20	19.2
H-1687	Normal light	51–57	54.1	20	20
	Low light	45–56	50.2	16–20	18.4
H-2304	Normal light	47–53	50.9	20–24	23.2
	Low light	38–50	42.4	16–24	19.2

* Under the microscopic field of 40 x 10

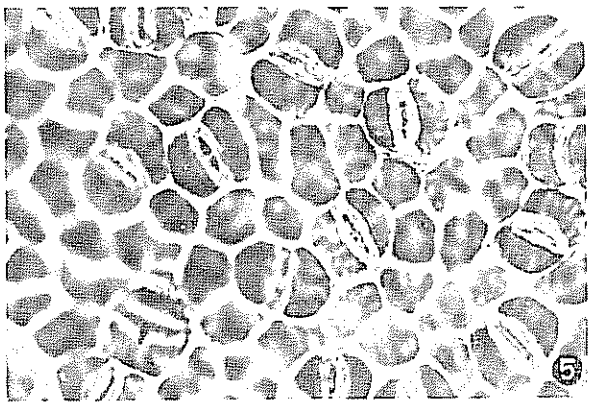

Fig 5. Stomatal distribution in normal leaves, x 500.

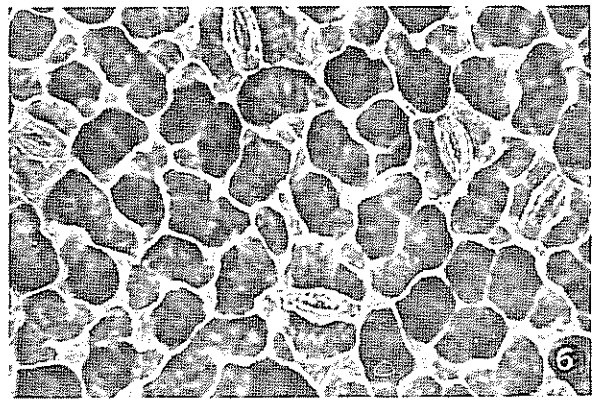

Fig 6. Stomatal distribution under shade, x 500

of available solar radiation. All the four varieties of cassava used in the present study showed definite anatomical changes under low light resulting in a significant reduction in photosynthetic efficiency, as the volume of the photosynthetic apparatus per unit leaf area was curtailed significantly under shade.

Crockston *et al.* (4) recorded 38% reduction in photosynthesis of bean leaves due to shading mainly because of increase in stomatal and mesophyll resistance to diffusion of CO_2. It was also demonstrated that the reduction in stomatal number would increase stomatal resistance. Besides, the reduction in the thickness of palisade and spongy parenchyma observed under low light may also result in increased mesophyll resistance to CO_2 uptake, ultimately the efficiency of the cassava leaves for CO_2 assimilation will be affected.

The present findings explain the causes for low yield of cassava under shaded environment. Inspite of its poor yield under mixed cropping system, large scale cultivation of cassava in coconut garden is prevalent in Kerala due to limited and holding. As none of the high yielding clones of cassava showed complete adaptation to uniform shade, further studies on this line is essential to find out the critical light intensity for cassava to achieve reasonable yield under mixed cropping system in addition to developing shade tolerant clones.

Summary

The influence of light intensity on morphological and anatomical characteristics of the leaves of four promising cultivars of cassava was investigated. Significant reduction in specific leaf weight (SLW) and increase in leaf area ratio (LTR) were observed under low light in all the cultivars. The anatomical features of the leaves of a high yielding variety (H-165) was compared with a low yielding variety (M-4) under two light intensities. The spongy parenchymatic layer

was thicker in H-165 than in M-4 under normal light while under low light both the varieties showed reduction in the thickness of mesophyll layers. Similarly the stomata in H-165 were found sunken when compared to that of M-4 under normal light but under low light they were found on the surface in both the varieties. There was a significant reduction in the number of stomata per unit leaf area under low light in all the four varieties studied presently. The concentration of chlorophyll-b increased significantly when compared to clorophyll-a under low light

Literature cited

1. ARNON, D. I. Cropper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24:1-15. 1959.

2. BJORKMAN, O. Carboxy dismutase activity in shade adapted and sun adapted species of higher plants. Physiologia Plantarum 21:1-10. 1968.

3. BJORKMAN, O. and HOLMGREN, P. Adaptability of the photosynthetic apparatus to light intensity in Ecotypes from exposed and shaded habitate. Physiologia Plantarum 16: 889-914. 1963.

4. CROOKSTON, R. K., TREHARNE, K. J. LUDFORD, P. and OZBUN, J. L. Response of beans to shading. Crop Science 15:412-416. 1975.

5. GRISEBACH, H. Biosynthesis of flavonoids. (**In** T. W. Goodwin ed. Chemistry and Biochemistry of Plant pigments. Academic Press New York) pp. 279-308. 1965.

6. JOHANSEN, D. A. Plant microtechnique. McGraw Hill, New York, 1940.

7. NOBLE, P. S. Photosynthetic rates of sun versus shade leaves of *Hyptisemoryi* Torr. Plant Physiology 58:218-223. 1976.

8. RADFORD, P. J. Growth analysis formula – their use and abuse. Crop Science 7:171-175. 1967.

9. RAMANUJAM, T., MURALEEDHARAN NAIR, G. and INDIRA, P. Growth and development of cassava (*Manihot esculenta* Crantz) under shade in coconut garden. Turrialba 34(3): 1984.

10. VIJABA BAI, K. and JOS, J. S. Distribution of stomata and trichomes in cassava leaves. Journal of Root Crops 7:11-14. 1981.

Reseña de libros

CROSSLEY, P., KILGOUR, J. and MORRIS, J. Small farm mechanization for developing countries. John Wiley, 1983. 253 p.

Uno de los problemas más apremiantes que encara la agricultura en los países tropicales en vías de desarrollo, es el de aumentar el rendimiento de los cultivos, sin que esto signifique un incremento significativo en el costo de producción. La tecnología moderna ha puesto a disposición de los agricultores una amplia gama de equipos y de maquinaria, para hacer más eficiente el proceso de producción. Sin embargo, una buena parte de estos equipos han sido diseñados para las regiones extratropicales, en donde las condiciones edáficas, topográficas, climáticas y económicas difieren mucho de las condiciones del trópico. Además, el precio de estos equipos es alto, en relación con las posibilidades financieras de la mayoría de los agricultores tropicales. Por otra parte, la experiencia ha mostrado que muchos de estos agricultores, sin una debida asesoría técnica, han adquirido equipos costosos y poco adaptados a sus necesidades, lo que ha acarreado pérdidas no sólo a sus economías particulares, sino también a la de sus países, crónicamente deficitarios en divisas extranjeras. A todo esto hay que agregar el fuerte aumento en el costo de los combustibles, con que funcionan la mayoría de estas máquinas. Todo esto plantea la necesidad de una selección muy rigurosa del equipo más apropiado para cada situación, de tal suerte que haya una buena relación entre costo y beneficio.

Por tal motivo considero que este libro puede ser un elemento importante para los técnicos en mecanización agrícola y para muchos agricultores, en el momento de escoger el equipo y la maquinaria más apropiados para determinada actividad. El libro refleja un conocimiento profundo de los autores sobre los problemas de la mecanización agrícola en los trópicos, el que logran comunicar de manera sencilla y clara. No se limitan sólo a discutir problemas de mecanización y de maquinaria agrícola, sino que también en algunos casos recomiendan como la tecnología más apropiada es el uso de fuerza animal o humana.

La obra está dividida en tres partes fundamentales y cada una de ellas a su vez subdividida en capítulos, según el siguiente desglose:

Parte I Necesidades de la agricultura

Capítulo 1. Condiciones y labores de campo
Capítulo 2. Labores en la finca.
Capítulo 3 Condiciones de transporte.

Parte II Equipo para la mecanización

Capítulo 4 Fuerza humana y animal.
Capítulo 5 Características de transmisiones y de máquinas pequeñas
Capítulo 6. Tracción, dirección y frenos
Capítulo 7 Características de los implementos

Parte III Escogencia del equipo según las diferentes labores.

Capítulo 8 Economía de la mecanización en pequeñas fincas
Capítulo 9 Funcionamiento del equipo de motor en el campo
Capítulo 10. Operación segura y eficaz del equipo.
Capítulo 11. Valoración y modificación de equipos.
Capítulo 12. Mantenimiento y producción local de equipo

El análisis del contenido del libro muestra que en su preparación, se le ha dado importancia no sólo a los problemas de escogencia de equipo, sino también a los detalles más importantes sobre el funcionamiento y mantenimiento de éste, aspectos que muchas veces no se les da la consideración que merecen. Otro aspecto que se considera en la obra, es el ambiente en que operará el equipo, información vital para su escogencia.

Por el contenido y por la claridad y sencillez con que está escrita esta obra considero que no debe faltar en la biblioteca de las escuelas de agricultura de los países tropicales, así como en las de los técnicos especializados en mecanización agrícola. También puede ser de interés para los agricultores que poseen empresas de tamaño mediano a grande.

LUIS A. FOURNIER
ESCUELA DE BIOLOGIA
UNIVERSIDAD DE COSTA RICA

DINÂMICA POPULACIONAL DO *Cosmopolites sordidus* (Germ., 1824) (Col.: Curculionidae) EM BANANAIS DA cv. PRATA (GRUPO AAB), EM ALFREDO CHAVES, ESPÍRITO SANTO[1]

*R. J. ARLEU**
*S. S. NETO***
*J. A. GOMES**
*A. C. NOBREGA**
*D. M. SCARDINI**

Summary

This experiment was carried out in Alfredo Chaves, Espirito Santo State, Brazil, with the cultivar Prata, from 1977 to 1982, in order to study the effect of climatic elements on the movement of the adult Cosmopolites sordidus *(Germ., 1824) and its population tendency in a banana plantation. Adult population was evaluated utilizing pseudostem baits from the meter of pseudostem. Conclusions were.* C. sordidus *adults had a constant movement throughout the year, climatic elements had no influence on the population fluctuation, there was a decrescent population tendency during the time of the experiment, perhaps due to the utilized practice.*

Introdução

Na região produtora do Estado do Espirito Santo, Brasil, em 1981, foram produzidas 90 000 toneladas de banana, em uma área de 22 000 hectares, com um valor de produção de Cr$ 771 390 000.00 (4).

Dentre os vários problemas da bananicultura brasileira a broca *Cosmopolites sordidus* (germ., 1824) tem contribuído de forma significativa para a baixa produtividade. O *C. sordidus* é nativo da Asia e seu centro de origem encontra-se provavelmente na região Malásia-Java-Bornéu (12). Ressalta-se que o material classificado por Germar em 1824 era proveniente de Java (8). Quanto à sua distribuição no globo, Montellano (10), Simmonds (12), Instituto Agronomico Per l'Oltremare (6), Beccari (1) e Feakin (5) relataram que o inseto encontra-se distribuído nas Américas, África, Austrália, Ásia e Oceânia. Sua ocorrência no Brasil foi assinalada por Chevrolat em 1885 (3) e em 1915, Costa Lima o encontrou em Campos, no Rio de Janeiro (2).

Não obstante a importância da cultura e da praga, poucos são os trabalhos existentes, na literatura, relativos à movimentação do inseto, nos diferentes meses do ano, para se tentar estabelecer um calendário de controle e mesmo para verificar a influência dos elementos climáticos na movimentação.

Lara (7) relatou que, na Costa Rica, a maior movimentação do inseto ocorre no período de novembro a janeiro e que a população é maior no 2° e 3° ano de idade do bananal, quando, então, ela começa a decrescer até tornar-se mais ou menos estável. Esta estabilidade é atribuída aos efeitos da umidade e da drenagem, do controle de ervas daninhas e da decomposição da matéria orgânica.

Martinez (9) verificou que, em bananais da cv. Nanicão, no Vale do Ribeira, em São Paulo, a maior movimentação ocorreu no período de novembro abril, e Oliveira *et al.* (11) verificaram que, em Angra dos Reis, no Rio de Janeiro, o pico da população em bananal da cultivar Prata, localizado na várzea, ocorreu no mês de setembro; no localizado na encosta, deu-se nos meses de agosto-setembro; e no bananal da cultivar Nanicão, o pico ocorreu no mês de maio. Relatou, ainda, que não houve influência dos elementos climáticos na flutuação da população.

1 Recebido para publicação em 29 agosto 1983
Parte da dissertação submetida pelo primeiro autor, à Escola Superior de Agricultura Luiz de Queiroz-ESALQ/USP, para obtenção do título de mestre em Entomologia. Trabalho desenvolvido com recursos EMBRAPA/EMCAPA.

* Empresa Capixaba de Pesquisa Agropecuária – EMCAPA – Caixa Postal 391, CEP. 29.000 – Vitória/ES

** Professor titular do Departamento de Entomologia – ESALQ/USP.

Zem e Alves (16) constaram que, na Bahia, a maior movimentação dos adultos ocorreu no período de março a maio, e que houve influência negativa dos meses chuvosos. Veiga *et al.* (15) observaram que, em Pernambuco, a maior movimentação ocorreu no período de setembro a março.

Na presente pesquisa, estudou-se a flutuação populacional da praga no período de 1977/1982, correlacionando-a com os elementos climáticos e, também a tendência da população da praga em bananal da cultivar Prata estabelecido, com modificação do manejo aplicado pelo produtor.

Material e métodos

A presente pesquisa foi realizada numa área de 5 000 m^2, plantada com a cultivar Prata, com 8 anos de idade, contendo, aproximadamente, 700 touceiras e localizada no distrito de Barra do Batatal, no município de Alfredo Chaves, Estado do Espírito Santo. Foi iniciada em setembro de 1977, tendo sido concluída em agosto de 1982, totalizando 5 anos.

A área experimental recebeu inicialmente os seguintes tratos culturais: desbaste, formando a touceira com 3 plantas; desfolha; capina; retirada dos restos de pseudocaule e, finalmente, a adubação mineral e correção em função da análise química do solo. Isto feito, a área continuou a receber os mesmos tratos culturais, sempre que necessário. Ressalta-se que o controle ao mal de Sigatoka não foi realizado, por não ser prática comum na região, não sendo feito, também, o controle da broca.

Para avaliar a população de adultos de *C. sordidus* utilizaram-se iscas de pseudocaules, segundo Simmonds e Simmonds (13), obtidas do primeiro metro da planta colhida, a partir do nível do solo.

As iscas, com 0,50 m de comprimento, eram divididas ao meio, longitudinalmente, e a face cortada era colocada em contato com o solo, ao lado de plantas que haviam emitido inflorescência, sendo o local em torno da touceira previamente limpo. As contagens dos insetos foram semanais, sendo as iscas mudadas de local a cada coleta e substituídas quinzenalmente. No primeiro ano, utilizaram-se 40 iscas por hectare com remoção dos insetos da área; e, nos demais, 60 iscas, com devolução dos insetos às touceiras.

Para registro dos elementos climáticos, instalou-se, na área, um termohigrógrafo de rotação semanal Lambrecht, um pluviógrafo IH, um pluviômetro IH, um termômetro de máxima, um termômetro de mínima, um termômetro de bulbo seco e um termômetro de bulbo úmido.

Investigaram-se as correlações simples entre as médias mensais de inseto por isca e as médias dos elementos climáticos (temperatura média, mínima e máxima; umidade média, mínima e máxima; e precipitação pluvial) e correlação múltipla entre as médias mensais de adultos por isca e as médias mensais de temperatura, umidade relativa e precipitação pluvial.

Verificou-se, também, a tendência da população através de correlação simples entre o mês da coleta e a média mensal de insetos por isca, obtendo-se o índice de tendência segundo Sounis (14).

Resultados e discussão

Os dados relativos à flutuação populacional de *C. sordidus* e ao nível de equilíbrio encontram-se no Quadro 1. Nos Quadros 2 e 3, encontram-se os dados relativos aos elementos climáticos e no 4 os coeficientes de correlação simples (r) e múltipla (R).

Os dados referentes à flutuação populacional e aos elementos climáticos bem como o nível de equilíbrio são representados, na Figura 1. Por estes resultados, observa-se, através de uma análise anual, que os maiores picos ocorreram em outubro e fevereiro, no período de 77/78, maio e agosto, no período de 78/79; setembro e agosto, no período 79/80; outubro, no período 80/81; e outubro e agosto, no período 81/82. As épocas de menor ocorrência foram setembro e dezembro, no período 77/78; dezembro e fevereiro, no período 78/79; dezembro, abril e maio, no período 79/80; março e julho, no período 80/81; e fevereiro e maio, no período 81/82 (Quadro 1 e Figura 1).

A análise anual revela grande variação na ocorrência dos valores máximos e mínimos, na flutuação da praga, o que caracteriza flutuações de curto período. Observando-se as médias mensais dos 5 anos, constata-se, que o acme ocorreu em outubro, sendo os demais picos obtidos no período julho, setembro e no mês de maio, sendo, também, os meses cujos valores estiveram acima do nível de equilíbrio (NE) da população (Quadro 1).

Nota-se, também, que os picos ocorreram nos meses de menor precipitação pluvial, e o acme no início da estação chuvosa (Figura 1). Esta ocorrência deve-se ao fato do inseto ser higrófilo e a um período de baixas precipitações pluviais, que acarretou diminuição da disponibilidade de locais úmidos propícios aos insetos. Neste caso, as iscas passaram a ser os locais preferidos pelos insetos por se apresentarem mais úmidas que outros pontos da área. Entretanto, ressalta-se que houve uma movimentação uniforme, durante todo o período de observação, sendo este um aspecto importante para o controle microbiano, sendo necessário

Quadro 1. Média mensais de adultos de *C. sordidus* por isca, coletados de setembro de 1977 a agosto de 1982, em bananal de encosta da cv. Prata, em Alfredo Chaves, Espírito Santo.

	Cosmopolites sordidus					
Mês	77/78*	78/79	79/80	80/81	81/82	Média
Setembro	3.77	4.20	5.15	4.91	3.17	4.24
Outubro	5.98	3.05	4.57	6.13	4.08	4.76
Novembro	4.41	3.95	3.64	3.82	2.98	3.76
Dezembro	3.65	2.79	3.37	3.77	3.53	3.42
Janeiro	3.97	2.79	3.72	3.12	3.07	3.33
Fevereiro	6.42	2.73	3.72	3.32	2.61	3.76
Março	5.50	3.30	3.75	2.42	3.04	3.60
Abril	4.33	3.30	3.36	4.35	3.38	3.74
Maio	5.66	5.13	3.29	3.31	2.74	4.03
Junho	4.66	3.61	4.35	3.58	3.18	3.88
Julho	5.45	4.79	4.64	2.77	3.89	4.31
Agosto	4.38	5.40	5.49	3.75	4.09	4.62
Média	4.85	3.75	4.10	3.77	3.31	3.96

* 20 iscas/0.5 ha, com remoção dos insetos da área, e, nas demais, 30 iscas/0.5 ha com devolução dos insetos às touceiras.

** Nível de equilíbrio (NE).

que as aplicações sejam feitas quando as condições ambientais forem favoráveis ao agente

Correlacionando as médias mensais de adultos por isca com os meses de coleta e com os elementos climáticos, verificou-se que houve uma correlação negativa ao nível de 5% de probabilidade, para o mês da coleta e a precipitação pluviométrica, não havendo significância para os demais (Quadro 4). Estes resultados não concordam com os observados por Oliveira *et al.* (11), no Rio de Janeiro, para um período de 12 meses.

Verificou-se, também, uma tendência decrescente da população (Quadro 1 e Figura 1), sendo explicada pelo manejo aplicado à cultura durante a condução do ensaio.

Com relação à regressão linear múltipla, o coeficiente (R) obtido indica que os elementos climáticos exerceram uma pequena influência na flutuação populacional da espécie (Quadro 4).

Conclusões

Pelos resultados obtidos no presente trabalho, pode-se, concluir que:

Os adultos de *C. sordidus* têm uma movimentação uniforme durante todo o ano.

Os elementos climáticos exercem pouca influência na flutuação populacional da espécie.

Houve uma tendência decrescente da população de *C. sordidus*, durante o período, em função do manejo da cultura empregado.

Resumo

O presente trabalho foi realizado em Alfredo Chaves, Estado do Espírito Santo, Brasil, em Bananal da

Quadro 2. Médias mensais de temperatura (°C), de setembro de 1977 a junho de 1982, obtidas em bananal de encosta da cv. Prata, em Alfredo Chaves, Espírito Santo.

Mês	Temperatura média (°C)					Temperatura mínima (°C)					Temperatura máxima (°C)				
	Período														
	77/78	78/79	79/80	80/81	81/82	77/78	78/79	79/80	80/81	81/82	77/78	78/79	79/80	80/81	81/82
Setembro	21.7	20.5	21.5	21.7	23.4	18.0	16.7	17.4	16.4	17.0	26.8	25.5	26.4	26.9	92.8
Outubro	23.2	22.9	24.2	23.9	22.2	19.4	18.3	19.2	18.8	17.9	28.3	28.2	29.1	29.1	26.0
Novembro	24.0	24.0	24.5	23.9	25.1	21.0	19.8	20.0	19.2	20.5	28.3	29.2	29.4	28.6	29.8
Dezembro	23.8	24.7	25.6	26.1	25.6	20.4	20.1	20.9	21.3	20.6	28.5	30.0	29.5	31.3	30.6
Janeiro	26.0	22.8	25.3	27.1	24.4	21.4	19.7	20.9	21.6	20.3	31.5	27.0	29.8	32.7	28.4
Fevereiro	25.1	24.7	25.9	26.2	26.5	21.5	20.6	21.0	20.5	20.4	30.0	29.9	30.8	31.7	32.5
Março	24.8	23.2	26.0	25.0	22.1	20.2	19.2	20.3	20.9	15.2	30.7	28.4	31.7	30.4	29.0
Abril	22.6	22.2	23.6	22.7	22.0	19.1	18.3	19.9	18.2	17.6	27.6	27.3	27.4	27.3	26.3
Maio	20.8	22.0	22.2	21.6	20.6	17.3	18.1	17.4	16.8	16.3	25.4	27.4	27.1	26.4	24.9
Junho	19.0	18.5	20.3	20.3	21.3	15.5	14.6	16.0	16.0	15.7	23.8	24.3	24.6	24.6	26.8
Julho	19.9	19.0	20.6	19.6	–	16.3	15.0	15.5	14.7	–	24.8	24.1	25.8	24.7	–
Agosto	19.8	21.2	21.7	21.1	–	15.4	16.3	16.6	15.6	–	25.8	26.9	26.7	26.6	–

Quadro 3. Precipitação pluvial (mm) e médias mensais de umidade relativa (%), no periodo de setembro 1977 a junho de 1982, obtidas em bananal de encosta da cv. Prata, em Alfredo Chaves, Espírito Santo.

Mês	U. R. média (%)					U. R. mínima (%)					U. R. máxima (%)					Precipitação (mm)				
	Período																			
	77/78	78/79	79/80	80/81	81/82	77/78	78/79	79/80	80/81	81/82	77/78	78/79	79/80	80/81	81/82	77/78	78/79	79&80	80/81	81/82
Setembro	75.8	79.9	75.9	79.6	85.3	52.4	57.5	51.7	53.3	43.5	93.0	95.3	93.6	94.1	91.1	121.7	165.2	67.1	39.6	45.2
Outubro	76.5	75.2	76.3	76.2	86.4	53.7	56.0	52.9	49.1	58.7	93.7	94.2	91.9	92.5	94.0	152.0	135.0	134.9	131.1	176.7
Novembro	83.0	74.9	78.9	77.8	82.2	62.2	52.7	55.3	53.6	56.4	93.8	93.6	94.1	93.8	92.6	326.2	139.8	186.1	184.6	277.7
Dezembro	81.0	77.4	79.1	78.4	88.5	56.1	52.8	58.8	52.5	46.3	94.4	95.0	94.4	94.1	92.4	307.3	199.8	304.7	162.5	127.4
Janeiro	72.2	87.1	81.6	74.0	80.8	47.5	67.9	60.3	47.5	–	92.7	95.7	95.4	92.1	–	113.6	472.5	218.2	112.4	299.6
Fevereiro	78.5	78.8	78.2	80.4	69.8	56.5	57.5	50.7	48.8	–	94.0	94.0	94.5	92.5	–	193.2	162.4	127.4	76.7	72.0
Março	76.5	82.2	73.7	83.5	82.8	49.0	56.5	42.1	57.9	–	94.9	95.2	92.4	93.6	–	67.1	244.3	68.1	244.0	261.6
Abril	82.6	82.4	85.7	81.9	82.4	56.2	58.4	63.4	55.5	–	94.9	95.5	94.6	94.1	–	105.0	94.9	414.3	193.4	214.1
Maio	84.5	80.7	82.4	86.1	85.6	59.4	54.8	57.3	59.4	–	95.7	95.3	95.1	94.2	–	96.1	115.8	26.8	108.2	86.4
Junho	86.2	85.3	85.1	83.8	82.5	61.1	56.4	58.6	62.1	–	95.5	95.6	94.0	94.3	–	56.5	50.6	10.3	50.6	6.7
Julho	86.6	82.9	80.8	83.2	–	60.8	56.5	51.5	51.0	–	95.2	95.6	94.6	93.6	–	203.3	41.0	68.0	75.5	–
Agosto	80.0	75.4	81.9	82.4	–	51.8	47.8	53.8	48.6	–	95.1	96.0	94.1	93.7	–	94.9	27.6	95.4	75.2	–

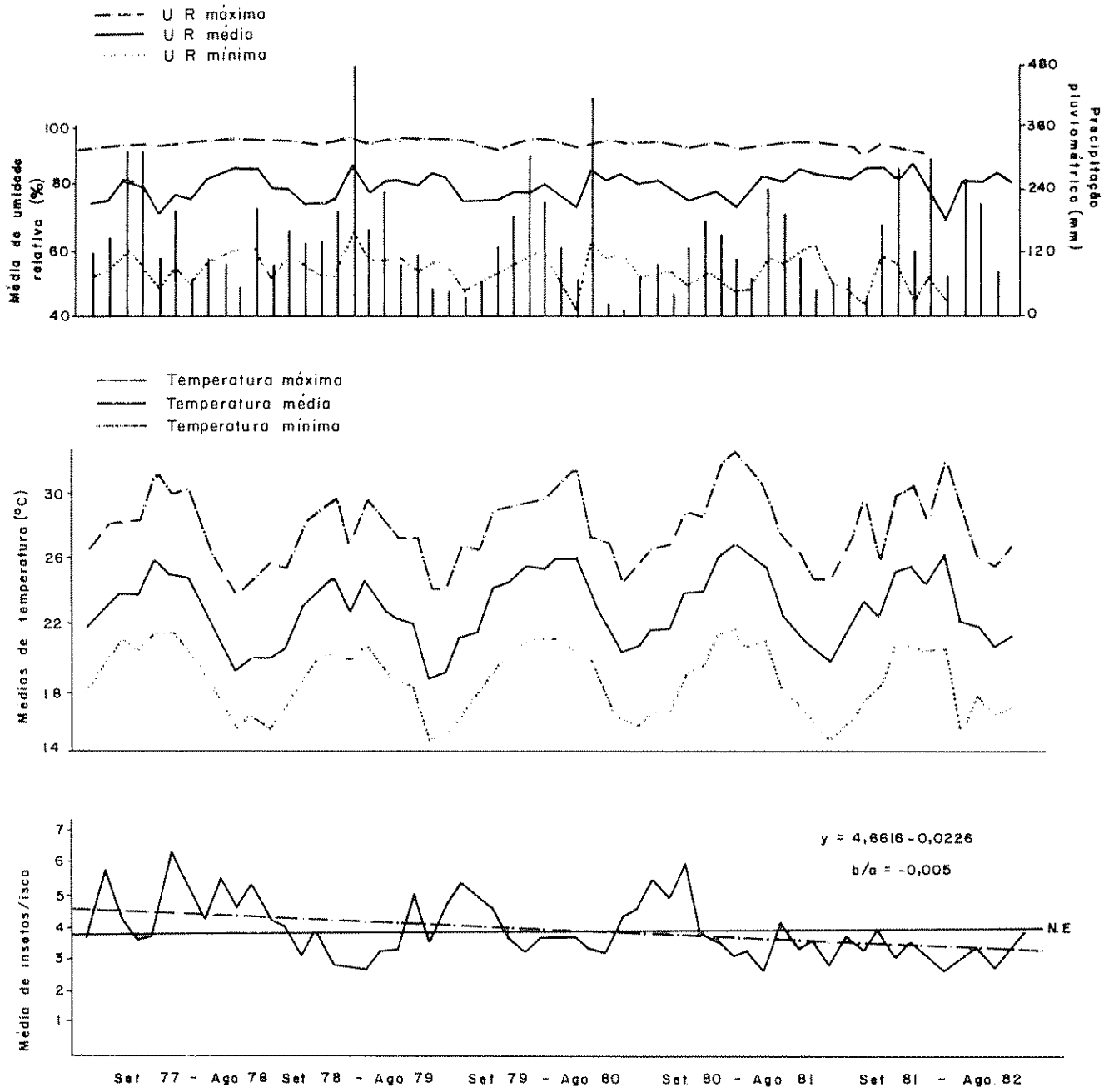

Fig. 1 Flutuação populacional de *C. sordidus*, tendência da população, nível de equilíbrio (N.E.) e elementos climáticos. Alfredo Chaves, ES.

cv. Prata, no período de 1977 a 1982, com o objetivo de estudar a influência dos elementos climáticos na movimentação do adulto de *Cosmopolites sordidus* (Germ., 1824) e a tendência da população em bananal onde o manejo foi modificado. A população de adultos foi avaliada através de iscas de pseudocaule, obtidas do primeiro metro da planta colhida, a partir do nível do solo. Pelos resultados obtidos, concluiu-se que os adultos de *C. sordidus* têm uma movimentação uniforme durante todo o ano, sendo que os elementos climáticos pouco influenciaram a flutuação da população e, que houve uma tendência decrescente da população da espécie, durante o período, em função do manejo da cultura empregado.

Quadro 4. Coeficientes de correlação simples (r) e múltiplo (R) e equações de regressão calculados a partir das médias de adultos de *C. sordidus*, coletados de setembro de 1977 a agosto de 1982, em bananal de encosta da cv. Prata, em Alfredo Chaves, Espírito Santo.

Parâmetros	*Cosmopolites sordidus*	
	Equação de Regressão	r
• Meses de coleta	Y = 4.6616 – 0.0226 X	– 0.3538*
• Temperatura média °C	Y = 6.7651 – 0.1177 X	– 0.2690
• Temperatura mínima °C	Y = 5.9046 – 0.0994 X	– 0.2159
• Temperatura máxima °C	Y = 6.9730 – 0.1040 X	– 0.2458
• Umidade relativa média %	Y = 7.4431 – 0.0419 X	– 0.1724
• Umidade relativa mínima %	Y = 4.9429 – 0.0161 X	– 0.0861
• Umidade relativa máxima %	Y = -16.6914 + 0.2204 X	+ 0.2491
• Precipitação	Y = 4.4615 – 0.0028 X	– 0.2782*
	Regressão linear múltipla	
Cosmopolites sordidus –	U = 14.35 – 0.17 x – 0.08 y – 7.90 z	
	R = 0.18	

* Significativo ao nível de 5%.

x e y = médias mensais de temperatura e umidade relativa.
z = precipitação pluviométrica

Literatura citada

1 BECCARI, F. Contributo alla conoscenza del *Cosmopolites sordidus* (Germar) (Coleoptera-Curculionidae). Rivista de Agricoltura Subtropicale e Tropicale 61(4/6):131-150. 1976.

2 COSTA LIMA, A. da. Insetos do Brasil. Coleopteros. Rio de Janeiro, 1956. Vol. 10 (Série Didática, 12).

3 CUILLE, J. Recherches sur le charançon du bananier *Cosmopolites sordidus*, Germ. Monographic de l'insecte et recherches de ses Chimiotropismes. Paris, Institut Des Fruits er Agrumes Coloniaux, 1950. 225 p. (Ser. Tech, 4).

4 ESPÍRITO SANTO. Secretaria de Estado da Agricultura. 3° ano de atividade no setor agrícola. Vitória-ES, 1982. 13 p.

5 FEAKIN, S. B. Control de las plagas de los bananos. Londres, Center for Overseas Pest Research, 1975. 147 p. (Pans Manual, 1).

6 INSTITUTO AGRONOMICO PER L'OLTREMARE. Scheda entomologica di *Cosmopolites* (Calandra) *sordidus* (Germar, 1824). Rivista de Agricoltura Subtropicale e Tropicale 60 (1/3):4. 1966.

7 LARA, E. F. Problemas y procedimientos bananeros en la zona atlántica de Costa Rica. San José – Costa Rica, s e , 1970. 278 p.

8 MARQUES, L. A. de. A praga da bananeira no Rio de Janeiro (Biologia do *Cosmopolites sordidus* – Germar). Boletim da Sociedade Entomológica do Brasil (3):24-32. 1922.

9 MARTINEZ, J. A. Flutuações da população da broca da bananeira "moleque" (*Cosmopolites sordidus* Germar). In Congresso Brasileiro

de Fruticultura, 1, Campinas-SP, 1971. Anais Campinas-SP, 1971. pp. 187-194.

10. MONTELLANO, O. B. Estudios biológicos del *Cosmopolites sordidus*, que infesta el rizoma de abacá. Tese de Mestrado. Turrialba, IICA, 1954. 27 p.

11. OLIVEIRA, A. M. *et al.* Flutuação da população de *Cosmopolites sordidus* e *Metamasius* spp., em bananais de Angra dos Reis, Estado do Rio de Janeiro. Pesquisa Agropecuária Brasileira, Série Agronomia 11(2):37-41. 1976.

12. SIMMONDS, N. W. Bananas. London, Longmans, 1966. 512 p.

13. SIMMONDS, N. W. e SIMMONDS, F. J. Experiments on the banana borer, *Cosmopolites sordidus*, in Trinidad, B. W. I. Tropical Agriculture 30(10/12):217-223. 1953.

14. SOUNIS, E. Bioestatística: princípios fundamentais, metodologia estatística, aplicação às ciências biológicas. São Paulo, McGraw-Hill, 1979. 230 p.

15. VEIGA, A. F. S. *et al.* Dinâmica populacional de *Cosmopolites sordidus* (Germar, 1824) e *Metamasius hemipterus*, e ocorrência da epizootias por *Beauveria bassiana* em plantios da bananeira 'Prata' situados em topografia de várzea e serra, no Estado de Pernambuco. **In** Congresso Brasileiro de Fruticultura, 6, Recife-PE, 1981. Anais Recife-PE, 1981. pp. 252-268.

16. ZEM, A. C. e ALVES, E. J. A broca da bananeira *Cosmopolites sordidus* (Germar, 1824) no Estado da Bahia – 1 – Incidência e movimentação. **In**: Congresso Brasileiro de Fruticultura, 5, Pelotas-RS, 1969. Anais Pelotas-RS, 1969. pp. 284-289.

IMPROVED ROOTING OF DIFFERENTIATED SHOOTS FROM SUGARCANE CALLUS TISSUE[1] /

*T S LEE**
*O. O BACCHI**

Resumen

*Se describe un método rápido, sencillo, económico y confiable para enraizar plántulas de caña de azúcar (*Saccharum *spp.) producidas a partir de cultivo de tejido transferidas directamente a un medio de vermiculita sin esterilizar. Después del trasplante las hojas son removidas y los potes se mantienen en el invernadero sin ninguna covertura a una temperatura entre 24-32°C y una humedad relativamente 70-90%. Con este método la sobrevivencia de las plántulas es excelente así como la formación de raíces.*

Introduction

Research on tissue culture of sugarcane was initiated in Hawaii in 1961 by Nickell (4). In general, callus can be obtained from almost any sugarcane tissue, and the callus in turn can be made to differentiate into shoots without much difficulty. However, the rooting of these differentiated shoots is generally considered a more difficult task (2, 4, 5, 6). The Hawaii group has suggested several methods for encouraging root production, such as culturing the shoots in water, storing them at 15°C, trimming the leaves, adding a-naphthaleneacetic acid (NAA) or dalapon to the medium, and transferring to a medium consisting of 1% agar and 7% sucrose (2, 6). Liu (4), using a modified Schenk and Hildebrandt (SH) medium for such a purpose suggested, with two basic requirements, a sufficient quantity of medium, and the maintenance of the culture under diffuse sunlight conditions.

The IAA/PLANALSUCAR laboratory at Araras–SP has been using some of these methods, but the performance was considered unsatisfactory for mass production of plants from sugarcane callus. Results of an improved method of rooting are reported here.

Materials and methods

Three sugarcane varieties of commercial interest were cultured from the spindle leaf of young plants grown in vermiculite from single bud cuttings in a growth chamber at 30°C. The culture medium (CM) for callus formation and proliferation was that of Murashige-Skoog, with modifications (4). Shoot differentiation was initiated in a culture room (26°C) on a medium (PM) similar to that described above, but with 1 mg NAA and 1 mg of kinetin instead of 4 mg of 2.4-D per liter. Root formation was induced either in a modified SH medium (RM), directly in vermiculite, or in a soil mixture (soil : sand : filtercake = 2:1:2).

Rooting conditions were introduced when the differentiated shoots in PM were about 8 to 10 cm in length. One group (120 plantlets) of differentiated shoots was transferred to test tubes containing RM. Each test tube received only one plantlet. The second group (120 plantlets) was transferred directly to a disposable plastic cup containing nonsterilized vermiculite, pre-moistened with tap water. Leaves were them trimmed. Each plastic cup also contained only one plantlet. The group of plantlets in vermiculite, together with 30 plantlets in RM, were then transferred to a greenhouse where the temperature varied from 24 to 32°C and the relative humidity,

1 Received for publication in October 13, 1983.
We wish to express our thanks to Dr. L. Sodek, Dr. S. Matsuoka, Dr. Y. Masuda and Dr. N. Macedo for their suggestions and critique of this work. Thanks are also due to Mr. C. de C. Amaral and Miss S. Perissatto for their technical assistance.

* Researchers, IAA/PLANALSUCAR, Southern Regional Coordination Service, Araras, SP, Brasil

from 70 to 90%. The remaining 90 plantlets were kept in the culture room. Another test was conducted by transferring shoots from PM directly to either vermiculite or a soil mixture. About half of each treatment was then transferred to the greenhouse and the other half maintained in the laboratory. The plastic cups, containing a single plantlet in vermiculite or soil, were irrigated inmediately after they had been moved to the greenhouse, but no covering was used. The plantlets in vermiculite or soil received a nutrient solution once a week (either a commercial leaf fertilizer or Hoagland solution). Survival rate and root formation were recorded after two or four weeks of treatment.

Results and discussion

Sugarcane callus normally gives rise to shoots and roots independently. Some varieties develop only shoots from their callus. In such cases, a special effort has to be made to stimulate root production (4). Several alternative methods are now available to improve development of roots in sugarcane, such as those mentioned above. In some situations (e.g. rapessed), the shoots can be directly transferred to vermiculite in a pot and covered with plastic bags to maintain humidity until the plant is well established (3). The results reported in other laboratories, and our own experience with these methods, were generally unsatisfactory for mass production of plants, since the roots are slow to form and are few in number, with little more than 50% of the plants rooted within one month of development in the sterile culture medium (6). The MS_5 medium used by Nadar and Heinz (6) seems good for root initiation, but no data were shown concerning root proliferation. The root medium consisting of 1% agar and 7% sucrose as reported by Hiraki and Maretzki (2) was very satisfactory for root formation. However, as can be seen in Figures 1 and 2, the present method produces considerably better results for root formation and proliferation. The sugarcane shoots can be easily rooted in non-sterilized vermiculite without any covering, thus obviating the sophisticated sterile culture technique. When shoots are transferred to the greenhouse, leaves must be trimmed to prevent wilting.

Greenhouse conditions were better than the culture room for rooting of sugarcane (Tables 1, 2). Under greenhouse conditions, the temperature varied between 24°C and 32°C, and the light intensity was higher (4-14 mW). Such conditions were more favourable for the sugarcane plant than those used in the culture room (constant temperature, 26°C with artificial light, 1.5-2.0 mW). Light appears to be important for rooting (1). If plantlets are kept under poorly lighted conditions, such as in the laboratory, the mortality rate increased. Perhaps light is important for an adequate production of the hormones necessary for root initiation; alternatively, an adequate supply of photosynthates may be necessary at the sites of root initiation. In any case, some light-dependent process would appear to be more important for rooting than the actual medium used.

Table 1 shows that differentiated shoots rooted better in vermiculite than in culture medium (RM), in terms of both the number of rooted plants, and the size of the root. The plantlets maintained in RM in the culture room have a good survival rate, but root formation is poor after 30 days. The ambient conditions apper to be more important than the rooting medium, because shoots rooted in the greenhouse, whether in RM or vermiculite, were always stronger, greener and presented a much more highly developed root system than those in the culture room (Figures 1, 2). Rooting in vermiculite in the greenhouse is the best choice since this combination gives very good results, in terms root formation, proliferation and survival rate. The formation of a root system can be easily noted after one week in vermiculite in greenhouse, while in RM in culture room roots can barely be seen after two weeks. Root initiation in RM in the greenhouse is faster than that in RM in the culture room.

Covering the test tube or flask containing the plantlets in order to maintain humidity or better aseptic conditions is not recommended for the greenhouse, since it inhibits air exchange and causes an undesirable rise in temperature (reaching 38°C at times), which burns the plantlets. A soil mixture is not a good rooting medium (Tables 2 and 3) because of a high mortality rate of the plants caused by microorganism growth on the soil surface and less favourable drainage. Nevertheless, plants that survived did grow better than those in vermiculite, perhaps

Table 1. Total number of plantlets and percent of rooted plantlets in response to the culture medium (RM) and vermiculite for root formation under (culture room and greenhouse) conditions.

Treatment	Total plantlets	Rooted plantlets (%)	Survival rate (%)	Fresh weight per plant (g)
RM – Culture room	90	50	96	0.068
RM – Greenhouse	30	77	77	0.332
Vermiculite-Greenhouse	120	96	96	1.224

* Plantlets were regenerated from tissue culture of var CB40-13. Data were obtained 30 days after treatment.

Table 2. Total number of plantlets and survival rate in response to two different potting media for root formation (vermiculite and soil mixture) under two different conditions (culture room and greenhouse).

Treatment	Total plantlets	Rooted plantlets (%)	Survival rate (%)	Fresh weight per plant (g)	Dry weight per plant (g)
Vermiculite-Culture room	192	90	90	0.148	0.018
Soil mixture-Culture room	189	23	23	0.099	0.017
Vermiculite-Greenhouse	179	99	99	3.439	0.469
Soil mixture-Greenhouse	189	86	86	5.962	1.079

* Plantlets were regenerated from tissue culture of var. Co740. Data were obtained 25 days after treatment.

of Ca concentration of tops (data not shown) and because of a better nutrient supply. The survival rate of plantlets of IAC48-65 in the soil mixture is especially low, since this variety regenerates very weak plantlets even after they have been transferred to RM before being potted in the soil mixture (Table 3).

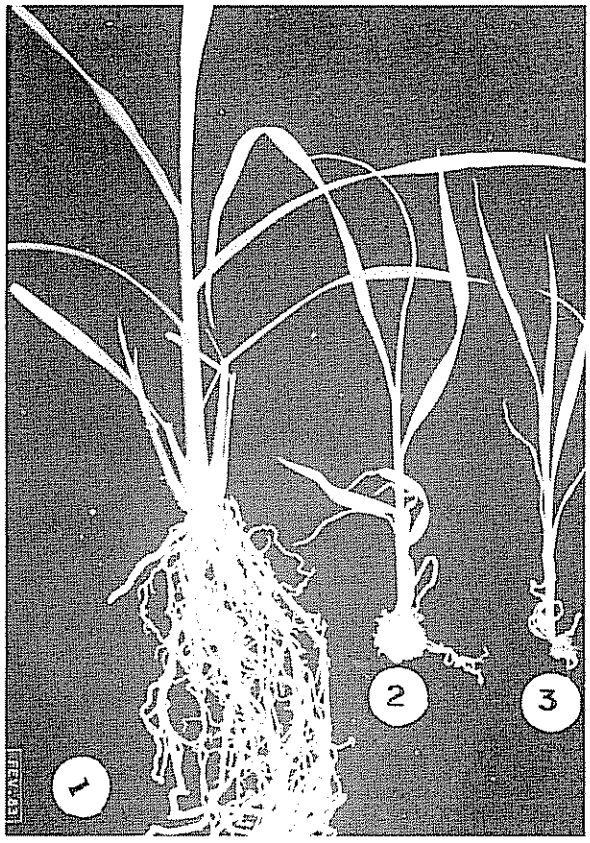

Fig. 1. Root formation (30 days after treatment) on plantlets of variety CB40-13 redifferentiated from callus tissue using

(1) vermiculite in greenhouse
(2) culture medium (RM) in greenhouse
(3) culture medium (RM) in culture room

Rooting sugarcane plantlets in greenhouse in vermiculite without any covering is simple, low-cost, rapid and very reliable. Thus the common procedure of transferring the differentiated shoot of sugarcane first, to a rooting medium in sterile culture and then, to sterilized vermiculite in a greenhouse, can be replaced by the direct transfer of plantlets to unsterilized vermiculite without any covering in greenhouse.

Summary

A rapid, simple, economical and reliable method of rooting the differentiated shoots from sugarcane (*Saccharum* spp.) callus tissue was achieved by the direct transfer of plantlets to moist unsterilized vermiculite. After transplanting, the leaves of the plantlets were trimmed, and the pots maintained in a greenhouse without any form of cover. The greenhouse conditions were: temperature 24-32°C and relative humidity 70-90%. With this method, the survival rate of the plantlets is very high and root formation is excellent.

Table 3. Survival rate of plantlets in response to vermiculite and soil mixture.

Parameters evaluated	Treatment	
	Verm-greenhouse	Soil mixture – greenhouse
Total plantlets	48	48
No. of plantlets dead		
14 days	1	25
15 days	3	1
Total	4	26
Survival rate	92	46

* Plantlets were regenerated from tissue culture of var. IAC48-65. In this case differentiated shoot had been transfered to RM for 40 days and only rooted plantlets were chosen for survival test.

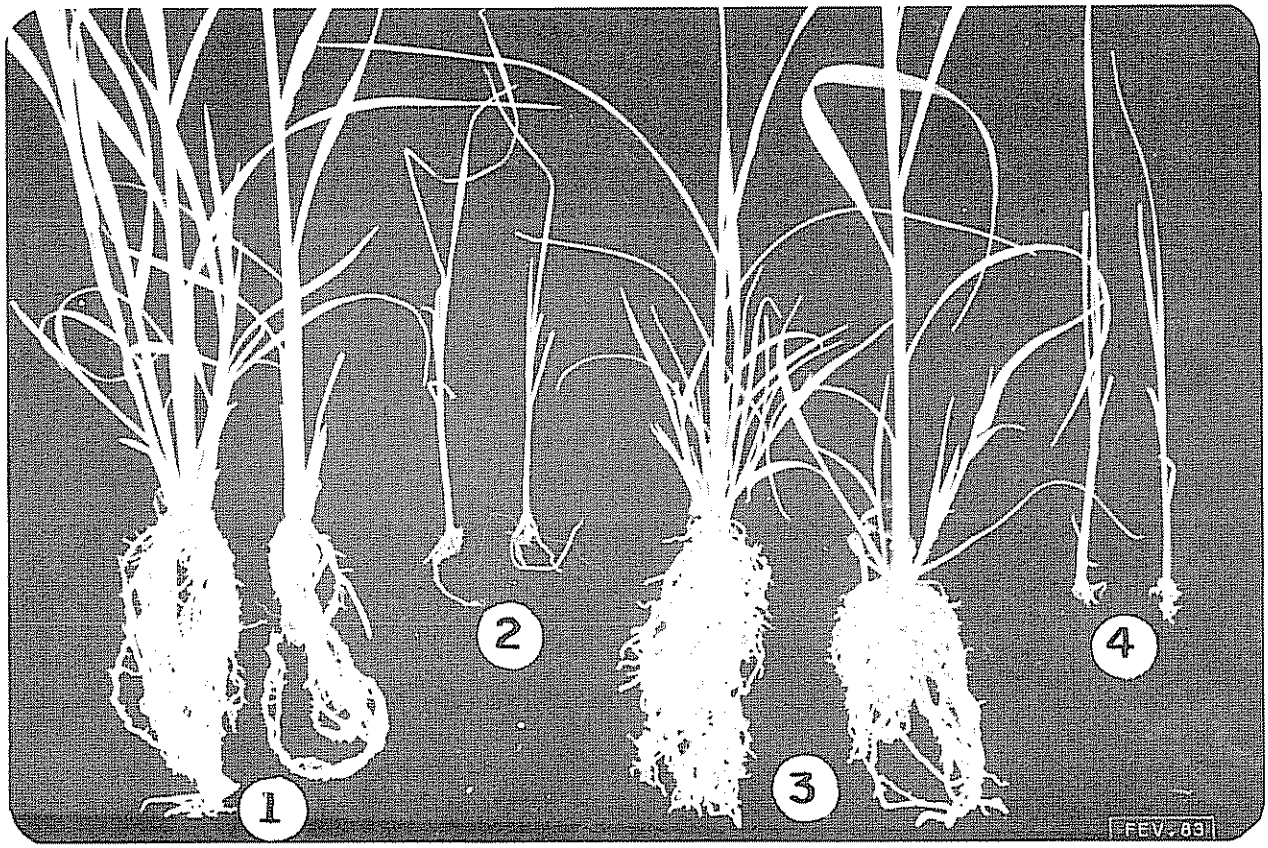

Fig. 2 Root formation (25 days after treatment) on plantlets of variety Co740 redifferentiated from callus tissue using

(1) soil mixture in greenhouse
(2) soil mixture in culture room
(3) vermiculite in greenhouse
(4) vermiculite in culture room

Literature cited

1 BARBA, R. C.; ZAMORA, A. B.; MALLION, A. K. and LINGA, C. K. Sugarcane tissue culture research. Proceeding Congress International of Society Sugar Cane Technology (São Paulo) 16:1 843-1 863. 1977.

2. HIRAKI, P. A. and MARETZKI, A. Rooting of plantlets regenerated from callus tissue. HSPA Expt. Sta. Annual Report 1977 p. 9.

3. KARTHA, K. K. Organogenesis and embryogenesis. **In** Gamborg, O. L.; Wetter, L. R. ed. Plant Tissue Culture Methods. National Research Council of Canada, Prairie Regional Laboratory, Soskatoon. 1975. pp. 44-49.

4 LIU, M. C. **In vitro** methods applied to sugarcane improvement. **In** Thorpe, T. A. ed. Plant Tissue Culture Methods and Application in Agriculture. Academic Press, New York. 1981. pp. 299-323.

5. LIU, M. C.; HUANG, X. J.; SHIH, S. C. The **in vitro** production of plants from several tissues of *Saccharum* species. Journal Agricultural Association China 77:52-58. 1972.

6 NADAR, H. M.; and HEINZ, D. J. Root and shoot development from sugarcane callus tissue. Crop Science 17:814-816. 1977.

GERMINATION AND SEEDLING GROWTH OF *Pithecellobium arboreum* Urban[1]

*E. M. FLORES**
*B. MORA**

Resumen

La testa es negra, suave y delgada. El micropilo es una estructura no especializada; el hilo es lateral. El embrión es de color verde, recto, con un eje embrional bien desarrollado. Los cotiledones son carnosos y de textura suave. No se observó endosperma ni perisperma en las semillas maduras. Las semillas sembradas en cámaras de germinación alcanzaron mayor crecimiento que las sembradas en cajas de madera o en placas de Petri

La germinación de las semillas es hipógea, las plántulas son criptocotilares. Pueden germinar 24 ó 72 horas después de sembradas, aunque algunas son muy precoces y lo hacen en pocas horas. Después de 48 horas de sequía pierden la viabilidad. Se observó ausencia de dormancia y corta viabilidad. Las semillas pequeñas son lentas en germinar o no lo hacen.

Introduction

Pithecellobium arboreum (Mimosoideae) is a tropical rain forest tree distributed from Mexico to Ecuador. It is a branched tree, 10 to 30 m high, with gray bark and dark green foliage. The leaves are alternate and bipinnate; the leaflets entire and asymmetrical. Flowers are bisexual, actinomorphic, and congested in dense heads. Typically, the five sepals of a single flower are triangular, green, short and glabrous while the white corolla is tubular, five lobed, valvate and glabrous. Stamens are numerous and basally connate. The ovary is superior, unilocular, with several ovules on a parietal placenta. The fruit is a twisted and red colored legume; seeds are elliptic shiny black, and are shed from April to October (6, 12, 14).

This species exhibits precocious germination, as do some tropical rain forest trees. The phenomenon of absence of dormancy is obvious as well as the short viability of the seeds. The purpose of this paper is to describe the germination and early growth in *P. arboreum* seeds under field and greenhouse conditions.

Materials and methods

Observations and experiments were made from January 1983 to January 1984, in the Escuela de Biología, Universidad de Costa Rica and a neighboring small secondary forest of recent regeneration. This area receives more than 2 000 mm during the rainy season and has an average temperature of 23°C.

Seed shedding, germination and seedling growth were observed in several trees growing in the immediate vicinity of the building and in the small forest. In the greenhouse, germination was carried out in wooden trays measuring 30 x 15 x 5 inches lined with a transparent polythene sheet and filled with sterilized soil and rice hulls. The seeds were sown one every 3 inches at a depth of one inch in 5 rows, 5 inches apart. The sowing medium was thoroughly watered before the seeds were sown and thereafter as the moisture status of the medium required. Other germination tests were made in Petri dishes with two layers of Wathman filter paper moistened with distilled water and in greenhouse germination beds 90 x 40 x 10 inches filled with a mixture of sand, soil and rice hulls. Seeds were sown the day of collection, in 4 rows 8 inches apart (10 seeds, 8 inches apart in each

1 Received for publication on April 6, 1984.
This work was supported by Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT). We are grateful to Luis A. Fournier and Clark Cowan for their valuable comments on earlier drafts of this paper.

* Escuela de Biología, Universidad de Costa Rica, San José, Costa Rica

row). Only seeds 1.5 to 2.0 cm long were used. Additional observations were made with smaller seeds, as well as with seeds collected one, two or three days before sowing, following the same procedure as above. There were 4 replicates for each treatment and germination was recorded every day for 30 days. Seedling development was measured every 7 days for 60 days.

Results and discussion

In January, most tree stands around the University of Costa Rica are almost completely leafless. At the end of February, the tree produces a dense crop of green leaves. Evidence of inflorescence development is observed in March. From late March to May the tree produces dense heads of flowers. There are one to 3 flower clusters at base of a leaf or at a node lacking leaves, on stalks 2 to 3 inches long, containing numerous sessile flowers. By June, most of the flowers have fallen and the first large pods, slightly roughened, finely pubescent, twisted and red colored are observed. In early July, the pods expand to full size, 3 to 4 inches in length, and some begin to ripen and dehisce, exposing the several black elliptic seeds that hang from short whitish funiculi (Figure 1a). Seeds turn from shiny to dull black color and fall to the ground. By the end of October, all of the seeds as well as some of the pods have fallen and by November, the last of the pods fall.

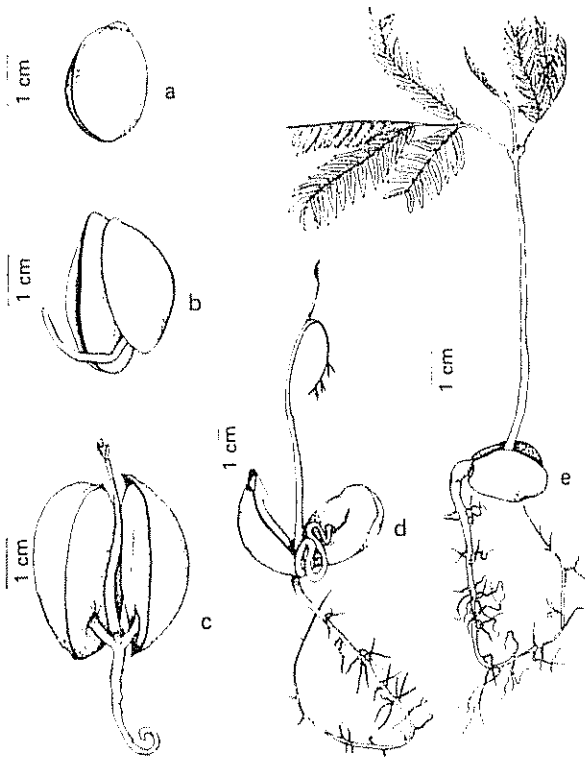

Fig. 1. Different states of seedling development.

The black seed coat is thin, smooth and soft. It is interrupted by the unspecialized micropyle at one end of the ellipse, contiguous to the radicle, while the chalaza is lateral. The embryo is green, stright, with a well developed embryonic axis; the radicle is small. Cotyledons are green and bulky. The smallest seeds exhibit light greenish embryos. No traces of endosperm nor perisperm were found in the mature seeds.

Germination is hypogeal and may be precocious; seedlings are cryptocotylar. Sometimes, one to several seeds begin to germinate inside the pod before shedding. In the ground, the seeds germinate 24 to 72 hours after shedding if there is enough rain to maintain the soil at field capacity. When the soil moisture regime is not adequate, the seeds do not germinate and lose viability after 48 hours. In the forest, the production of litter provides seed cover and 85 percent of germination is obtained. In December 1983 and January 1984 most of the seedlings growing in the ground died.

At the end of the greenhouse experiments (30 days after sowing), seeds sown in germination beds achieved 80 percent germination compared to 52 percent for seeds sown in wooden trays. In the Petri dishes, only a 25 percent germination was obtained.

During the period of inhibition, the seed produces an irritating and foul smell. Germination commences after one or two days later, with a longitudinal rupture of the seed coat (Figure 1b). Within 4 days, the cotyledons open slightly exposing the radicle while the seed coat remains attached to the abaxial surface of the cotyledons (Figure 1c). On the seventh day, the epicotyl with a pair of leaf primordia is observed. Each cotyledon is attached to a thick petiole (Figure 1c). Well developed axillary buds are seen in the cotyledonar node (Figure 1d).

Within twelve days, the compound leaf blades begin to extend from the first pair of leaf primordia. The axomorphic radical system is well developed. After 22 days, the compound leaves adopt a horizontal position. The first pair of leaves are opposite while the following are alternate. The main axis has 8 to 16 pairs of pinnae, with a gland at the base of each pair, each pinna bearing 20 to 40 pairs of sessile leaflets. The leaflets are oblong, with a short mucro, oblique at base, thin, glabrous and paler beneath. At the end of 25 days, the first pair of bipinnate leaves are completely extended (Figure 1e).

The cummulative growth of seedlings in the three different assays is presented in Figure 2. The figure illustrates that seeds sown in germination beds attained the greatest height followed in order by seeds sown in wooden trays and Petri dishes.

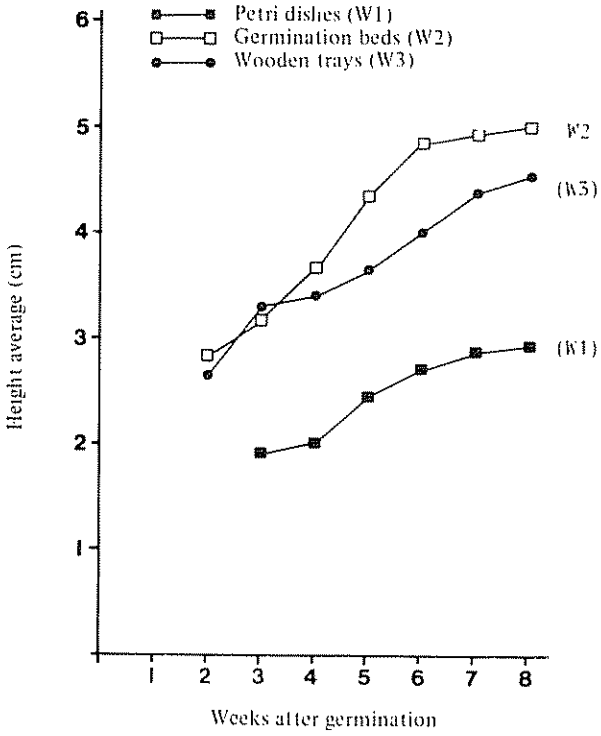

Fig 2 Cummulative height of seedlings growing in different substrates

Discussion

Most woody leguminous trees are characterized by having longlived seeds with hard seed coats which are mechanically resistant and impermeable (1, 2, 11, 15, 16) Usually those seeds are able to dry down to as low as 4 percent moisture content and do not regain moisture until the seed coat is penetrated by abrasion, preheating or microbial action (3) The hard seed coat and a naturally low moisture content permit a long period of dormancy and viability On the contrary, mature seeds of *P. arboreum* have a thin and soft seed coat, a high misture content and a very well developed embryo ready to grow immediately These characteristics do not permit a delay in germination nor seed storage

It is well known that tropical trees have short-lived seeds (4, 13) We have observed in other genera of the Momosoideae as **Inga**, the precocious germination and short viability exhibited by *P. arboreum* In all cases, the seed coat is soft and easily removed Gutterman (5) proposed that the environmental conditions under which the mother plants grow have a far-reaching influence on the germinability of the seeds and therefore, on the optimum germination requirements Seeds of tropical trees growing in zones of high precipitation and humid soils require an adequate soil moisture and temperature

One peculiarity was the discovery of a green embryo We agree with Jansen's assumption (9) that the chlorophyll content exhibited by developing embryos among perennial plants in tropical forests plays a role in embryo growth before the seed is shed This assumption is reinforced in *P. arboreum* by the loss of chlorophyll in sown seeds We found that seeds germinating in the ground, exposed to diurnal light, keep the green color indicative of chlorophyll However, these seeds do not successfully develop vigorous seedlings Most of them die after a few days

Seedlings grown in the greenhouse produced taller plants than those grown in the small forest Although water is the most critical factor in germination, seedling establishment and later development seem to depend on the amount of light available For this reason, the majority of seedlings grown in the small forest never survive past the seedling stage

The irritating and foul smell liberated by the seeds during the period of inhibition suggests production of substances that prevent animal predation (7, 8) In contrast to other leguminous trees, in which the pods remain small and dormant throughout the rainy season (10), *P. arboreum* develops and matures its fruits in a few months, without a period of dormancy

Summary

The seed coat is black, thin and soft The micropyle is an unspecialized structure; the chalaza is lateral The embryo is green, straight, with a well developed embryonic axis Cotyledons are bulky No traces of endosperm nor perisperm were found in the mature seeds Seeds sown in germination beds attained the greatest height followed in order by seeds sown in wooden trays and Petri dishes

Germination of *P. arboreum* seeds is hypogeal; seedlings are cryptocotylar Seeds germinate 24 to 72 hours after being shed although sometimes may germinate precociously After 48 hours of water stress, the seeds lose viability Seed storage is difficult because of rapid dehydration and subsequent loss of viability During the period of inhibition the seed liberates an irritating and foul smell

Literature cited

1 CLEMENS, J., JONES, P G and GILBERT, N H Effect of seed treatments on germination in **Acacia** Australian Journal of Botany 25:269-276 1977

2. FORDHAM, A. J. Germination of woody legume seeds with impermeable seed coat. Arnoldia 25:1-8. 1965.

3. GERLMOND, H. Physiological aspects of seed germination. Seed Science and Technology 6:625-639. 1978.

4. GOMEZ-POMPA, A., VASQUEZ-YANES, C., del AMO, S. and BUTANDA, A. Regeneración de selvas. Comp. Edit. Continental, S.A., México. 1976.

5. GUTTERMAN, I. Differences in the progeny due to day length and hormone treatment of the peanut mother plant. **In:** Seed ecology. W. Heydecker, ed., Butterworths, London. 1973.

6. HOLDRIDGE, L. R. and POVEDA, L. J. Arboles de Costa Rica. I. Centro Científico Tropical, San José, Costa Rica. 1975.

7. JANZEN, D. H. Seed eaters versus seed size, number, toxicity and dispersal. Evolution 23:1-27. 1969.

8. JANZEN, D. H. Digestive seed predation by a Costa Rican Baird's tapir. Biotropica, (suppl.) 13:59-63. 1981.

9. JANZEN, D. H. Ecological distribution of chlorophyllous developing embryos among perennial plants in a tropical deciduous forest. 14:232-236. 1982.

10. JANZEN, D. H. Cenizaro tree (Leguminosae, *Pithecellobium saman*) delayed fruit development in Costa Rican deciduous forests. American Journal of Botany 69:1269-1276. 1982b.

11. JANZEN, D. H. and HIGGINS, M. L. How hard are *Enterolobium cyclocarpum* seeds. Brenesia 16:61-67. 1979.

12. LITTLE, E. L. and WADSWORTH, F. H. Common trees of Puerto Rico and the Virgin Islands. Agriculture Handbook No. 249. U.S. Dept. of Agriculture. Forest Service. Washington, D.C. 1964.

13. MORENO, P. Estudios sobre viabilidad y latencia de semillas tropicales. Tesis Facultad de Ciencias. U.N.A.M., México. 1973.

14. PENNINGTON, I. D. and SARUKHAN, J. Arboles tropicales de México. Instituto Nacional de Investigaciones Forestales, México. 1968.

15. QUINLIVAN, B. J. The effect of constant and fluctuating temperatures on the permeability of the hard seeds of some legume species. Australian Journal of Agricultural Research 12:1009-1022. 1961.

16. VASQUEZ-YANES, C. Notas sobre la morfología, la anatomía de la testa y la fisiología de las semillas de *Enterolobium cyclocarpum*. Turrialba 27:427-430. 1977.

ISOLATION, IDENTIFICATION AND ROLES OF FUNGI FROM CREOSOTE TREATED PINE POLES[1] /

*J. CARRANZA**

Resumen

El presente trabajo se realizó en la ciudad de Syracuse, Nueva York. La muestra consistió de 46 postes de pino tratados con creosota y de diferentes años de servicio (10-50 años). De dichos postes se aislaron 71 hongos pertenecientes a 12 géneros y 8 especies, incluyendo 11 hongos que no pudieron ser identificados.

Se llevaron a cabo pruebas de podredumbre y de tolerancia al preservante para determinar la función que pueden desempeñar dichos hongos en su invasión a la madera. Dicho estudio se completó con observación de la madera atacada al microscopio de luz y de barrida, para relacionar los cambios anatómicos de la madera con la actividad de los hongos.

Se concluye que tres grupos de hongos interactúan en estos postes. El primer grupo incluye a los llamados "invasores primarios", los cuales son muy tolerantes a la creosota y pueden actuar destoxificando el sustrato; el segundo grupo incluye a los "oportunistas", los cuales aprovechan el sustrato modificado y pueden ejercer reacciones antagonistas contra los otros hongos, sin producir podredumbre; el tercer grupo lo constituyen los hongos que producen "podredumbre", los cuales son algunas veces tolerantes a los preservantes y causan podredumbre. En este último grupo se incluyen a los himenomicetes y a los hongos causantes de podredumbre suave (ascomicetes).

Introduction

Wood is a renewable natural resource of major importance for fuel, construction, and as a chemical raw material. Despite the advantages wood has over many other construction materials, it has several disadvantages which limit its usefulness. A major one is that it is biodegradable under some conditions of use.

Although wood degradation by microorganisms has been studied extensively, additional research is needed to understand more fully the microorganisms involved, how they invade the wood, and the interactions among them that lead to complete breakdown of wood. Added information on these processes may lead to more economical and effective ways to control decay.

This research deals mainly with the identification, anatomical effects on wood structure, relative tolerance to a preservative, and elucidation of the roles and interactions among fungi associated with decay development in creosoted pine poles from utility lines.

Literature review

A thorough review of the current literature on decay in wood products has been assembled by Scheffer (42). Important contributions and methodologies directly pertaining to the research topic are briefly reviewed here.

Primarily it is fungi (Basidiomycetes and Ascomycetes) that invade, digest, and thereby reduce the advantageous properties of wood (14, 23, 38, 41).

1 Received for publication in March 30, 1983.
This research was part of a thesis completed for an MS degree at the State University of New York, College of Environmental Science and Forestry, at Syracuse, N. Y., under the direction of Dr. R. A. Zabel. Thanks are due to Dr. Chunk Wang for assistance in identifying many of the fungi reported.

* Tucson, Arizona, 3334 E. Presidio Rd. 85716, USA

If wood is properly handled and used, decay can be greatly minimized. Where the wood product is in constant contact with soil, or in other uses where the moisture content of the wood frequently rises above the fiber saturation point, preservatives are needed to minimize decay losses and to insure an economical service life. Although the use of wood preservatives has reduced decay significantly, losses still occur. Some wood-inhabiting organisms not necessarily responsible for major decay (ascomycetes and fungi imperfecti) are capable of modifying these poisonous substances to less toxic forms, thus rendering it less effective in protecting wood from decay by basidiomycetes wood destroyers (8, 30).

Some of these fungi are now known also to be soft rotters (10, 15, 33, 36).

Tolerance to certain preservatives occurs also among many of the wood-decay fungi (12, 13, 14, 26, 55); therefore, it is important in the selection of preservatives to consider the major decay fungi of a product and their tolerances to toxicants.

In some laboratory experiments the tolerance of fungi to preservatives increases with the time of incubation, and the same may occur in nature where, after a period of time, the substrate is detoxified (5).

The organisms associated with decay development in living trees, in fence posts and untreated stakes have been studied and a predominance of typical rot fungi are found in the interior and ascomycetes, fungi imperfecti, and bacteria are commonly found in the outer zone (2).

Such studies have also been carried out for treated wood and recently in chip piles (24, 30).

Data on the organisms involved assist in the selection of test fungi from knowledge of the most susceptible species in the system, and also develop a deeper insight into the decay process.

Eslyn (19) in a study of the decay of utility poles treated with creosote, found no evidence that the preservative influenced the species of fungi invading the central or untreated interior of the poles. He believed that decay fungi gain entry primarily through seasoning checks or, perhaps to a lesser extent, through zones of inadequately treated wood.

Studies on the interactions and related roles of the organisms which degrade wood have many complications, such as time of sampling, sampling techniques, isolation difficulties, and identification of the many associated organisms (24, 32, 44).

Dwyer and Levy (16) developed an objective analysis approach by investigating methods to process in a statistical way the data obtained from organisms colonizing wood. Previous studies of organisms invading at various times wood can be described as subjective analyses of objectively obtained data; these studies included those by Corbett and Levy (11), Merrill and French (36), Käärik (28, 29), and Banerjee and Levy (2). Butcher (6) was the first to attempt to carry out an objective analysis based on the methods commonly applied to higher plant communities, using frequency of isolation as a measure of abundance.

Selective media for separating the different groups of wood-inhabiting fungi have been used by many investigators (9, 13, 24).

A complete review of selective media for the isolation of basidiomycetes from wood is given by Hale and Savory (25).

Many researchers have suggested the possibility of succession and related interactions among organisms associated in the process of discoloration and decay of treated and untreated wood (22, 31, 35, 37, 46, 47, 48, 49, 51).

Some recent investigations have been concerned with the development and use of natural preservatives to minimize problems in the environment or to nontarget organisms. Antibiotic toxicants have been suggested as possible preservatives (40, 53).

Biological control, the employment of biological antagonists, has been proposed as a feasible alternative to the use of toxic chemicals for prevention of wood decay (27, 45).

This research deals mainly with the identification, anatomical effects on wood structure, relative tolerance to a preservative, and elucidation of the roles and interactions among fungi associated with decay development in creosote pine poles from utility lines.

Materials and methods

The general design of this study was to select randomly from creosoted pine poles in utility lines, four groups of poles representing service ages of 20, 30, 40, and 50 years. A total of 46 southern yellow pine poles were made available for the study by the New York Bell Telephone Co. in Syracuse, N. Y.

Sampling technique

Core samples were collected with an increment borer during the period January-August 1978. Ap-

proximately 10 poles in each age group were sampled at the ground line (pH of the soil varied from 4.5–5).

Media

Since some fungi grow better on certain media, ten media were tested initially to determine which would yield the largest number of organisms from the cores (Table 3), at pH values varying from 5.7 to 6.2 as follows:

2% malt agar: malt extract 25 g; agar 15 g; water 1000 ml.

3% malt agar: malt extract 30 g; agar 15 g; water 1000 ml.

5% malt agar: malt extract 50 g; agar 15 g; water 1000 ml.

Nutrient agar: nutrient broth 8 g; agar 15 g; water 1000 ml

Duncan's modified media: NH_4NO_3 6 g; K_2HPO_4 5 g; KH_2PO_4 5 g; $MgSO_4 \cdot 7H_2O$ 4 g; agar 15 g; water 1000 ml

Benomyl agar: malt extract 12.5 g; benomyl 0.05 g streptomycin sulphate 30 μg/ml; penicillin G 40 units per ml; agar 15 g; water 100 ml.

Copper sulphate: malt extract 25 g; agar 15 g; copper sulphate hydrated 10 g; water 1000 ml.

2% malt agar–0.5 malic acid: malt extract 20 g; agar 15 g; 0.5 g malic acid; water 1000 ml

2% malt agar-0.06 g o-phenyl phenol: malt extract 20 g; agar 15 g; 0.06 o-phenyl phenol; water 1000 ml.

Sodium caseinate: sodium caseinate 2 g; glucose 1 g; K_2PO_4 0.2 g; $MgSO_4 \cdot 7H_2O$ 0.2 g; $FeSO_4$ 0.01 g; agar 16 g; water 1000 ml

Several chips were placed per plate per core position. The cultures were incubated at 28°C.

Isolation and identification of organisms

Identification and frequencies of the major microorganisms were determined by isolations from outer, middle, and inner core positions. Identification of the fungi was made mostly by cultural and microscopic means using general keys (1, 3, 4, 17, 18).

Tests

The roles and interactions of the major isolates, grouped by radial position in the core and in service age classes, were determined by decay and preservative tolerance tests, and anatomical study

Preservative tolerance test

For the preservative tolerance test, crude creosote previously sterilized (United States Steel Corporation; Clairton Coal, Coke, and Chemical Works, Clairton, Pa.), was added aseptically to sterilized 2.5% malt extract agar in the following quantities:

Concentration selected	Creosote added
%	g
0.05	0.25
0.10	0.50
0.50	2.50
1.00	5.00
5.00	25.00
10.00	50.00

The plates were inoculated with 14 major representative fungi, and incubated for two weeks at 28°C. Radial measurements of growth were made daily during the period of incubation. Plates without preservatives were also included as controls. Dosage response curves were plotted on semi-log graph paper and 50% inhibition values determined by extrapolation.

Wood decayers test

The fungi with clamps connections and putative decayers (the ones that appeared to be decayers but lacked clamps) were selected for the decay test. A well-known decayer [*Poria placenta* (Fr.) Cke.], commonly isolated from poles, was included in the test for comparison.

The procedure used was the agar type decay chamber with malt extract agar and wood blocks (3 x 2 x 1.5 cm). The inoculated French square bottles were incubated for one week at 28°C.

After one week when the mycelium had spread over the surface, sterilized glass V-support rods were placed aseptically at the center of the mycelium, and small sapwood blocks of southern yellow pine (3 x 2 x 1.5 cm) (previously oven-dried, water-satured and surface steam-sterilized) were introduced with the cross section surface down. The oven-dried weight for each block was recorded for determination of weight loss at the end of the test.

Chambers with blocks and no inoculum were assembled as controls. The decay chambers were incubated at 28°C in the dark for four months.

After two months of exposure, blocks for decay-rate determinations and sectioning were removed from some chambers. Sections from fixed (FAA)

blocks were cut (20-30 microns thick) from radial face of the block with a sliding microtome and stained using the picroaniline blue procedure (7). The blocks for weight loss determinations were oven-dried 24 hours at 105°C and reweighed.

After four months of exposure, the rest of the blocks were removed from the chambers. The same procedures were followed as in the two-month exposure.

Soft-rotters test

A modification of the Nilsson (38) method was used for the soft-rot test on the major microfungi isolated.

Two southern yellow pine sapwood blocks (3 x 2 x 1.5 cm) treated as previously described and with filter paper squares at their bases (to stimulate fungal growth), were buried in 10 g of vermiculite contained in eight-ounce French square bottles fitted with cotton filter caps. The blocks were oven-dried and weighed before introducing them into the bottles.

Thirty milliliters of the following nutrient solution were added aseptically to the bottles after introducing the blocks:

NH_4NO_3	6.0 g
K_2HPO_4	4.0 g
KH_2PO_4	5.0 g
$MgSO_4 \cdot 7H_2O$	4.0 g
Glucose	2.5 g
Water	1000 ml

After the addition of the nutrient solution, the bottles were sterilized 30 minutes at 121°C.

Inoculations were made with suspensions of isolate mycelium incubated in 5 ml of malt extract solution for 48 hours at 28°C. Bottles without inocula were prepared for checks. All the chambers were incubated in the dark at 32°C for four months.

Microscopic studies

After two and four months of exposure to different fungi, representative blocks from the decay and soft-rot tests were removed for anatomical studies. After four months, oven-dry weights for the test blocks were determined as described previously.

Permanent slides were prepared and studied under the light microscope for evidence of decay, such as bore holes, cell wall erosion, cell wall thinning of channeling on the inner lumen wall, and the dissociation or destruction of parenchyma cells. Special attention was directed to detect the two types of attack caused by soft rotters, i.e., type 1) longitudinal cavity formation and type 2) cell wall erosion (34).

Small sections were cut from the blocks with a sharp razor blade for scanning electron microscope observations. They were fixed in 2% glutaraldehyde or 2% OSO_4, rinsed in sodium cacodylated buffer or distilled water and dehydrated in a graded ethanol series, and then dried by the method of critical-point drying with CO_2, and sputter-coated with gold palladium, and observed under the scanning electron microscope Model ETEC Autoscan for evidence of decay.

Results

Isolation and identification of fungi

Seventy-one fungi assignable to 12 genera and including also 11 unknows were isolated from the 46 poles. The genera and species are listed in Table 1.

Table 1. Isolation frequency of the fungi obtained from 33 poles with service ages ranging from 10 to 50 years.

Genus and/or Species	Isolation frequency	Pole service age (10-50 years)
Frequent (Isolated 7-20 times)		
Cladosporium resinae	21	10-40
Exophiala jeanselmei	8	10 and 30
Hyalodendron sp	7	10, 30, and 40
Acremonium spp	7	20, 30, and 50
Infrequent (Isolated 2-6 times)		
Phialophora spp	5	10-30
Unknown decayers	4	30 and 40
Phialophora lagerbergii (Melin & Nannf.) Conant	2	20 and 30
Phialophora heteromorpha (Nannf.) Wang	2	30
Sporothrix sp.	2	30
Scytalidium album Pesante	2	10 and 30
Rare (Isolated only once)		
Scytalidium lignicola Pesante	1	10
Paecilomyces varioti Bainer	1	50
Penicillium sp.	1	20
Talaromyces sp	1	40
Rhinocladiella atrovirens Nannf.	1	30
Ramichloridium sp	1	30
Unknowns	7	10, 20 and 30

Table 2. Identity, location, and frequency of the fungi obtained from cores of 33 creosoted pine poles.

Outer position	Frecuency
Putative decayers	2
Phialophora sp	1
Sporothrix sp	1
Phialophora heteromorpha	1
Cladosporium resinae	12
Hyalodendron sp	4
Exophiala jeanselmei	4
Talaromyces sp	1
Acremonium spp	3
Phialophora spp	2
Paecilomyces varioti	1
Penicillium sp	1
Middle position	
Wood decayers	
Unknown decayer	1
Soft rotters	
Phialophora sp	1
Non-decayers	
Sporothrix sp	1
Phialophora heteromorpha	1
Scytalidium album	1
Phialophora spp	4
Cladosporium resinae	14
Hyalodendron sp	3
Exophiala *jeanselmei*	2
Phialophora lagerbergii	2
Acremonium spp	5
Paecilomyces varioti	1
Scytalidium lignicola	1
Unknown	1
Center position	1
Putative decayers	1
Acremonium sp	1
Unknowns	2

Cont.

Outer position	Frecuency
Sporothrix sp	1
Scytalidium album	1
Phialophora spp	4
Cladosporium resinae	12
Hyalodendron sp	2
Exophiala jeanselmei	5
Phialophora lagerbergii	1
Acremonium spp	4
Talaromyces sp	1
Rhinocladiella atrovirens	1
Scytalidium lignicola	1
Ramichloridium sp	1
Unknown	4

according to the frequency of isolation. The most common fungus was *Cladosporium resinae*, followed by *Exophiala jeanselmei*, *Phialophora* spp., *Hyalodendron* sp., and *Acremonium* spp. Four isolates of unknown (clamps) or putative decayers were obtained.

The majority of the fungi were isolated from the center (42) and middle portions (38) of the three core positions and the lowest number from the outer core position (33) (Table 2). The poles in 10 to 30 years of service range had the greater number of fungi (60); few fungi were isolated from 50-year-service poles (10) (Table 1).

Media

The majority of the fungi were isolated on the malt extract agar (112) and sodium caseinate media (47). Some of the fungi isolated grew in all media except the copper sulphate medium where no growth was observed. Benomyl medium reduced the growth of the microfungi and often growth was restricted to the surface of the wood chip. Some fungi grew on 2% malt agar ortho-phenyl phenol and 2% malt agar malic acid media. No specificity of media for a particular fungus was observed, eg., *C. resinae* grew on all media except benomyl, where the growth was reduced (Table 3).

Creosote tolerance test

The test fungi showed appreciable variation in resistance to creosote at the one week exposure (Table 4). *Poria placenta*, a very sensitive fungus and *Lentinus lepideus* Fries, a very tolerant fungus, used as controls, grew on 0.05% (1.0 cm/week) and 0.1% (0.5 cm/week) (Controls 3 cm/week). Some growth was observed in the case of *Lentinus lepideus*

Table 3. Types and frequency of microorganisms isolated from creosoted pine poles by using various selective media.

Media	Ascomycetes[a]			Basidio-mycetes[a]	Bacteria[a]		
	F	I	R	R	F	I	R
					Rod Coccus P	Rod Coccus P	P
					+ – + –	+ – + –	
Malt agar 2%	0	16	2	–	0 0 0 0 0	2 7 0 2 3	–
Malt agar 3%	45	0	0	–	4 1 0 1 1 8	0 0 0 0 0	–
Malt agar 5%	49	0	0	–	0 0 0 0	4 5 0 1 1 4	–
Nutrient agar	0	0	3	–	0 0 0 0	3 4 0 1 4	–
Malt agar 2% –0.5 malic acid	0	22	0	–	– – – – –	– – – – –	8
Malt agar 2% –0.06 g 0-phenyl phenol	0	23	0	–	– – – – –	– – – – 14	–
Sodium caseinate	46	0	0	1	8 6 3 5 1 4 –	– – – – –	–
Benomyl agar	–	24	–	3	– – – – – –	6 4 2 2 7	–
Copper sulphate	0	0	0	–	0 0 0 0 0 –	0 0 0 0 0	–

a/ F = frequent (30-50); I = infrequent (10-29); R = rare (1-9); P = non-identified

Table 4. Tolerances to creosote concentrations after one week of exposure.

Fungi	Concentration (%)		
	Growth[a] Reduction 50% (LD-50)	Threshold Toxicity	Minimum for Growth
Hyalodendron sp	4.50	15.0	10.0
Cladosporium resinae	0.90	15.0	10.0
Acremonium sp	0.05	1.0	0.5
325-3E	0.04	0.5	0.1
Exophiala jeanselmei	0.04	1.0	0.5
Lentinus lepideus	0.04	0.5	0.1
334-1B	0.03	5.0	1.0
119-1B	0.03	0.5	0.1
Poria placenta	0.03	0.5	0.1
328-3E	0.03	0.5	0.1
334-2E	0.03	0.5	0.1
326-3B	0.02	0.5	0.1
394-3M	0.02	0.1	0.05
Phialophora sp		0.05	

a Values extrapolated from the dosage response curves

on 0.5% after the seventh day (0.2 cm); in the case of *Poria placenta* only a trace of growth was observed on 0.5% (0.1 cm).

The unknown decayer and the putative decayers were resistant to the preservative and grew on 0.05% (0.5 cm/week) and 1.0% (0.2 cm) after the fifth day.

Decoloring of the media was observed with some fungi at the high concentrations of creosote previous to any fungal growth on the media.

Phialophora sp., a vigorous soft rotter, was very susceptible and did not grow on any of the creosote concentrations. The other soft rotters grew on 0.05% (1.0 cm/week) (Controls 2.0 cm/week). *Acremonium* sp., a weak soft rotter, grew on 0.1% (0.6 cm/week), on 0.5% some growth was observed on the eight day (0.1 cm) but did not continue (Controls 2.0 cm/week). Another soft rotter (325-3E) also grew on 0.5% (0.5 cm/week) but the growth did not increase after the sixth day (Controls 3 cm/week).

Exophiala jeanselmei and 328-3E, non-decay fungi, grew on 0.05% (0.4 cm/week) and 0.1%

(0.2 cm/week) (Controls 1 cm/week). *E. jeanselmei* also grew on 0.5% (0.1 cm/week) but no increase in growth was observed after the sixth day. Some growth started on 1% on the tenth day of exposure (0.1 cm). Two non-decay fungi, *Cladosporium resinae* and *Hyalodendron* sp., were highly tolerant to creosote. They grew on all media and abundant sporulation was observed after the fourth day (Controls 2.0 cm/week).

Decay studies

Substantial weight loss of the wood test blocks was obtained with three of the four putative decayers after a four-month exposure. The weight losses ranged from 20.74 to 27.99%. One of the suspect decay fungi showed less ability to decay wood, and the weight losses ranged from 3.51% to 17.95%. Four microfungi which caused more than 5% weight loss are grouped tentatively as soft rotters. A high weight loss (13.91-15.31%) was obtained with one of the soft rotters (*Phialophora* sp.). The weight loss for the other fungi ranged from 3.30% to 11.15%.

The remaining fungi tested were judged to be non-decayers when weight losses were less than 5% (Table 5).

Some of the wood decayers and soft rotters showed great variability as indicated by the high values

Table 5. Weight losses[a] of southern yellow pine blocks exposed to fungal isolates for four months.

Fungus	Weight loss percent	Standard deviation	
Wood decayers[b]	Mean	Range	
119–1B	27.99	23.70–31.15	2.767
334–2E	25.43	12.00–34.70	8.156
326–3B	20.74	14.25–26.45	3.903
334–1B	12.74	3.51–17.95	7.081
Poria placenta	59.28	27.70–65.75	13.970
Soft rotters			
Phialophora sp. (334–2A)	15.31	10.00–21.25	4.313
Phialophora sp. (334–1E)	14.55	7.55–21.00	4.847
Phialophora sp. (334–2D)	13.91	10.80–20.15	3.412
394–3M	9.78	9.00–11.15	0.701
325–3E	6.65	4.85–9.05	1.261
Acremonium sp. (335–3D)	5.15	3.30–7.70	1.756
Non–decayers			
Acremonium sp. (325–3D)	3.90	2.65–5.20	0.792
328–3E	3.84	3.40–4.70	0.418
Phialophora lagerbergii	3.48	1.90–4.55	0.992
Phialophora sp. (338–2A)	2.95	1.65–4.15	0.894
Exophiala jeanselmei	2.91	1.35–4.00	0.918
Phialophora sp. (335–2A)	2.71	1.60–3.95	0.830
Cladosporium resinae	2.70	1.60–3.45	0.543
Hyalodendron sp.	2.60	1.70–4.15	0.808
Rhinocladiella atrovirens	2.46	1.75–3.65	0.704
Acremonium sp. (371)	2.11	1.75–3.05	0.419
Phialophora heteromorpha	1.91	1.20–3.35	0.698
Sporothrix sp.	1.68	0.05–3.20	0.980

a Values for individual block weight losses were corrected by reference blocks subjected to all aspects of the test other than exposure to a fungus. These adjustments include a 2% correction in weight loss for all blocks due to loss of water solubles during water soaking to adjust moisture contents.

b Decayers: fungi with clamps, formed bore holes as large or larger than the hyphae and / or causing weight loss in a block exceeding 12%. Soft rotters: fungi which produce longitudinal bore holes and with weight losses exceeding 5%. Non-decayers: less than 5% weight loss.

obtained in the standard deviation (Table 5). These were associated with appressed mycelial mats and the difficulty in achieving uniform inoculation of the blocks

Microscopic studies of wood decayers

In some cases initially hyphae were concentrated in ray parenchyma which were later obliterated (334-1B; 119-1B; 334-2E; 326-3B), and ray tracheids, also in springwood tracheids (119-1B); or heavily and uniformly distributed in the wood (334-2E; 326-3B).

Hyphae were frequently or infrequently branched, with clamps (medalin type 334-2E) or without clamps (119-1B; 326-3B; 334-1B) and with a general diameter of 1-4 μ.

Small bore holes (119-1B) or some larger than the diameter of the hyphae were commonly observed (326-3B; 334-2E). Pit passages were very common (119-1B; 334-2E) (Figure 1). Longitudinally oriented erosion channels (334-2E; 334-1B) followed the fibril angle (Figure 2) and cell wall thinning were very common (334-2E). Pit apertures were eroded (326-3B).

Microscopic studies of soft rotters

Hyphae were abundant in wood (*Phialophora* sp 334-1E, 2A, 2D); concentrated in ray parenchyma

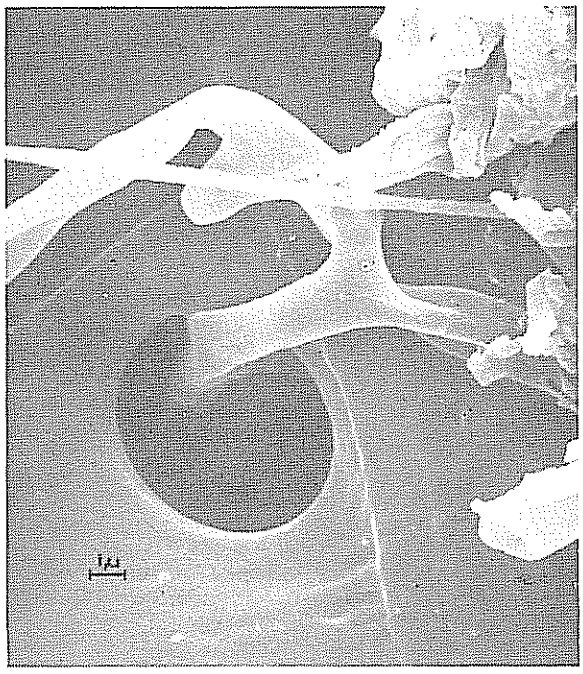

Fig 1 A radial longisection of decay fungi (119-1B) growing in southern yello pine sapwood showing a bordered pit penetration. SEM Mag 4400 X

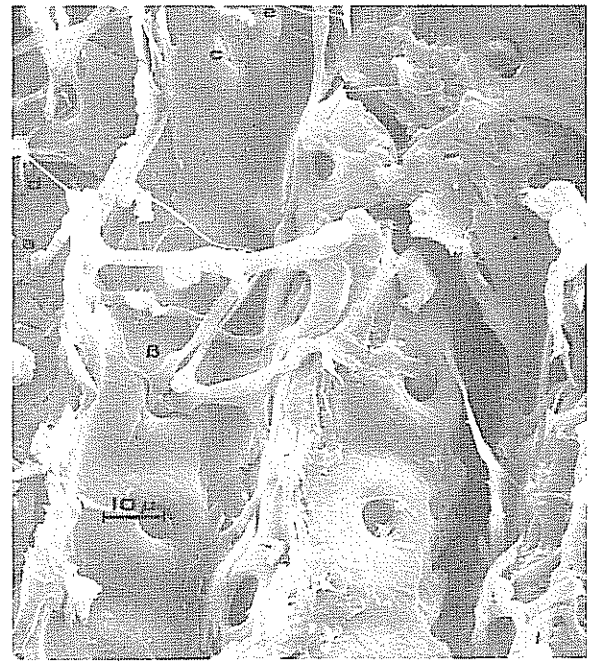

Fig 2 A radial longisection of decay fungus (334-2E) growing southern yellow pine sapwood, after a 4 month exposure in an agar wood-block decay test. Special features are designated by letter as follows: a) clamp connection, b) bordered pit penetration, c) differential cell wall erosion, d) desicatted slime tendrils, and e) possible microphyphae. SEM Mag. 925 X

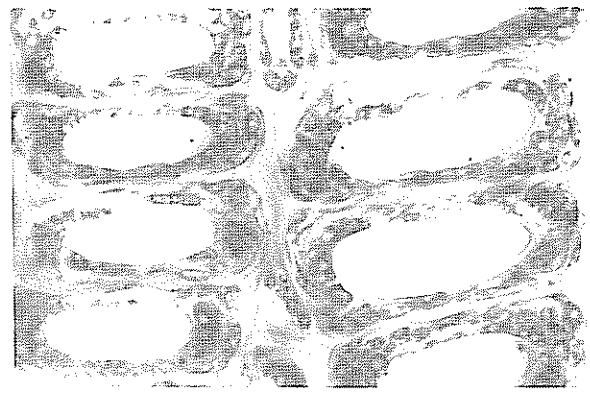

Fig 3 A transverse section of a soft-rot fungus (*Phialophora* sp.) growing in southern yellow pine sapwood after a 4 month-exposure in a decay chamber (Nilsson 1973) showing several states in the severity of secondary wall invasion by the fungus. LM Mag. 72 X.

and springwood tracheids (394-3M; 325-3E; 355-3D); or concentrated in the secondary wall of summerwood cells (*Acremonium* sp. 335-3D). The secondary wall was removed in several springwood cells (334-1E); in others, the secondary wall had numerous small cavities (334-1E; 325-3E; 394-3M).

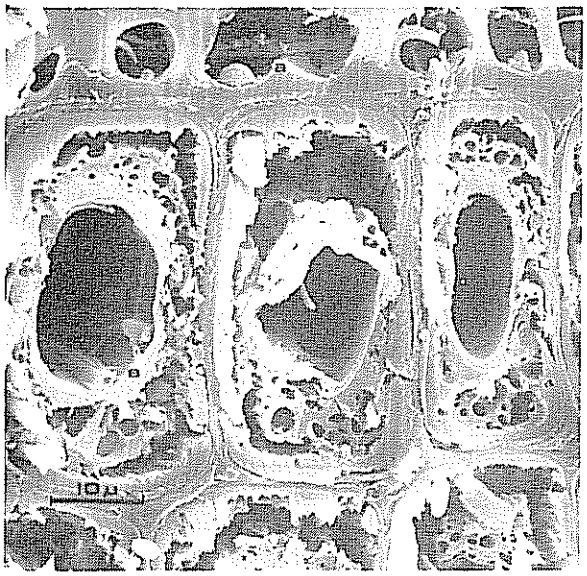

Fig 4 An enlarged cross section of southern yellow pine sapwood invaded by a soft-rot fungus (*Phialophora* sp.) showing a hypha transversely penetrating the cell wall (a) and hyphal filaments lining the inner lumen wall (b) SEM Mag 1950 X

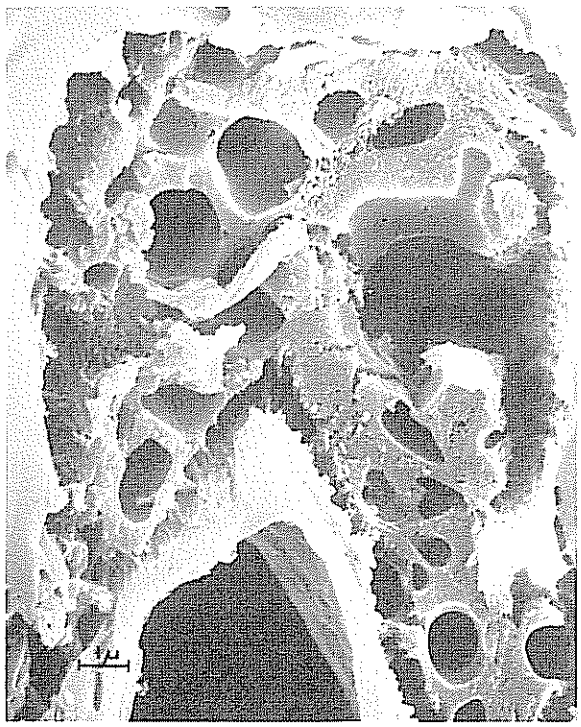

Fig 5 An enlarged section of a tracheid showing substantial cell wall erosion associated with the longitudinal bore holes SEM Mag 6250 X

The summerwood cells were heavily attacked and in some cells the secondary wall had been removed or appeared swollen (334-1E). Small cavities were observed in the tangential sections parallel to the microfibrills (Figures 3-5). Cell wall erosion (*Acremonium* sp. 335-3D) and pit passages were common (394-3M; 325-3E).

Hyphae were frequently or infrequently branched, without clamps, and with a general diameter of 1-4-3 μ

Pictures were taken with light microscope and scanning electron microscope of some of the wood decayers and one of the soft rot fungi (Figures 1-5).

Discussion

The 71 isolates totaling 8 species and 12 genera suggest a rather restricted range of fungi inhabiting creosote-treated wood. Although many researchers have reported difficulty in isolating wood decayers from creosoted wood, these techniques using selective media and daily subculturing obtained four decayers, representing approximately 6% of all isolations. Daily subculturing was judged to be as important as the selective media in obtaining pure cultures of the wood decay fungi from creosoted pine.

The isolation of fungi from only 33 poles, of the 46 sampled, demonstrated that the conventional creosote preservative treatment frequently protects poles effectively up to 40-50 years. There was no clear relationship between increasing pole age and the numbers of fungi isolated. This suggests a wide variation in the treatment effectiveness of poles and the probable concentration of the best treatments (by replacements) in older age classes. The small number of isolates obtained may reflect in part also the difficulty encountered in finding suitable media for the isolation of the fungi present. Also some slow-growing fungi may be missed by the growth-retardation effect of fast growing fungi which quickly cover the plates and dominate the media.

The kinds and numbers of fungi isolated were closely related to the external conditions of the poles as mechanical injuries or deep checks.

Based on these studies the fungi found in creosote-treated poles may represent a specialized fungi that can be divided into three groups.

The first are those "toxicant tolerant primary invaders" which include the fungi able to tolerate high levels of creosote in the wood and that primarily utilize wood components of low molecular weight

for their energy requirements. *Cladosporium resinae* is in this group. It has been reported as a fungus very tolerant to creosote by several investigators (8). *Hyalodendron* sp., *Acremonium* sp., and *Exophiala jeanselmei* were demonstrated also in this study to be tolerant to creosote. They did not produce visible cell wall erosion or appreciable weight losses. This suggests a possible role in the wood as detoxifiers or wood modifiers, facilitating the more creosote-susceptible fungi to invade the wood later. Most of them were found in the middle position of the cores.

A second group are those fungi that can be called "non-tolerant opportunists" because they follow and appear to invade the wood when the preservative level has been diminished by the detoxifiers. As a group, they were sensitive to creosote. They did not cause any decay as indicated by the very low weight losses obtained with these fungi (Table 5). They were commonly isolated from the middle and center position of the cores.

A third group the "wood decayers" include both hymenomycetes and soft-rot-type decayers. This group showed very special characteristics. Some of the decay fungi were highly tolerant to creosote and were fast growing fungi, which retard the growth of the non-decayers; others were more sensitive to creosote. One of the more aggresive soft rotters (*Phialophora* sp.) was totally inhibited by low concentrations of creosote. This fungus might be called a non-tolerant opportunist because its attack is delayed until the substrate is detoxified.

Some of the decayers produced substantial weight losses, copious bore holes, cell wall erosion, and cell wall thinning, and in the case of the soft rotter *Phialophora* sp., the secondary wall was completely destroyed.

The interaction roles of the soft rotters with the hymenomycetous decayers is unknown. In this study, the aggresive soft rotter (*Phialophora* sp.) was retarded by the fast growing decayers, but no visible distortion of the hyphae was observed and the fungus sporulated abundantly.

Association of bacteria with the fungi was observed frequently but studies correlating their processes in the decay of wood were not carried out.

A succession of fungi in the invasion and decay of wood has been suggested by many investigators (31, 37, 46, 47, 48, 49, 50, 51).

From this limited study, successional sequences can be postulated as the fungi from the first and second group were commonly isolated from the same position and from poles with the same service life. Also, the wood decayers which were very tolerant to creosote can appear early in the pole and not until the substrate is modified to start the invasion. A clear interaction between the three groups can be postulated on the basis that the "opportunists" were commonly found first on positions with low preservative concentrations and the detoxifiers on the highly concentrated; wood decayers were isolated from the outer portions of poles where preservatives were absent or in low concentrations.

In studies of wood preservative effectiveness and in the determination of the treatments to be used, it may be important to consider the possible toxicant modifying roles of the tolerant fungi.

The possible damaging roles of soft rot fungi in poles may be important and needs additional study.

Summary

Forty-six creosoted pine poles representing four ages of service (10-50 years) were sampled in the Syracuse area for wood-inhabiting fungi.

Seventy-one fungi assignable to 12 genera and 8 species including 11 unknowns were isolated from the poles.

Creosote tolerance and decay tests were carried out to determine the possible roles and interactions of the fungus. Anatomical studies with light and scanning electron microscopes were related to changes in wood by the decay fungi.

Three groups of fungi appeared to interact in creosoted pine poles. The first group includes the so-called "tolerant primary invaders" which are highly tolerant to creosote and may serve as detoxifiers. the second group include the "non-tolerant opportunists" which take advantage of the modification of the wood and may exert antagonistic reactions to other fungi, but do not cause decay. The third group were the "conventional decayers" which sometimes are tolerant to preservatives, and cause extensive decay. This group included both hymenomycetes and soft-rot fungi.

Literature cited

1. ARX, J. A. von. The genera of fungi sporulating in pure culture. Germany J. Cramer 1970 288 p.

2. BANERJEE, A. K. and LEVY, J. F. Fungal succession in wooden fence posts. Mat. und Org. 6(1):1-25. 1971.

3. BARNETT, H. L. and HUNTER, B. B. Illustrated genera of Imperfect Fungi. Third Edition. Minnesota. Burgess Publishing Co. 1972. 241 p.

4. BARRON, G. L. The genera of Hyphomycetes from soil. Baltimore. Williams and Wilkins Co. 1968. 364 p.

5. BRAVERY, A. F. Determining the tolerance of soft-rot fungi to wood preservatives: A comparison of test methods. Mat. und Org. 3:213-227. 1968.

6. BUTCHER, J. A. Techniques for the analysis of fungal floras in wood. Mat. und Org. 6:209-232. 1970.

7. CARTWRIGHT, K. ST. G. A satisfactory method of staining fungal mycelium in wood sections. Annals of Botany London. 43:412-413. 1929.

8. CHRISTENSEN, C. M. *et al. Hormodendrum resinae* (L.) an inhabitant of wood impregnated with creosote and coal tar. American Journal of Botany 29:552-558. 1942.

9. CLUBBE, M. and LEVY, J. F. Isolation and Identification of fungal flora in treated wood. Devised technique. The International Res. Group on Wood Preserv. Princes Risborough Lab. England. 1977. Doc. No IRG/WP/159. 3 p.

10. CORBETT, N. H. Micro-morphology studies on the degradation of lignified cell walls by Ascomycetes and Fungi Imperfecti. Journal Institute of Wood Science 14:18-29. 1965.

11. CORBETT, N. H. and LEVY J. F. Ecological studies on fungi associated with wooden fence posts. I. Brit. Wood Press. Ass. News Sheet No. 27. 1963.

12. COWLING, E. B. The relative preservative tolerance of 18 wood destroying fungi. Forest Products Journal 7(10):355-359. 1957.

13. DUNCAN, C. G. and DEVERALL, F. Degradation of wood preservatives by fungi. For. Prod. Lab. For. Service USDA. Applied and Environmental Microbiology 12(1):57-62. 1964.

14. DUNCAN, C. G. and LOMBARD, F. Fungi associated with principal decays in wood products in the United States. US. For. Service Research Paper WO-4 DA. 1965. pp. 1-13.

15. DUNCAN, C. G. and ESLYN, W. Wood-decaying Ascomycetes and Fungi Imperfecti. Mycologia 58:642-645. 1966.

16. DWYER, G. and LEVY, J. F. The colonization of wood by microorganisms: An objective approach. Beihefte zu Mat. und Org. 3:13-20. 1976.

17. ELLIS, M. B. Dematiaceous Hyphomycetes. England. Commonwealth Mycological Institute. 1971. 608 p.

18. ELLIS, M. B. More Dematiaceous Hyphomycetes. England. Commonweath Mycological Institute. 1976. 507 p.

19. ESLYN, W. E. Utility pole decay. Part II. Basidiomycetes associated with decay in poles. Wood Science Technical 4:97-103. 1970.

20. GAMS, W. *Cephalosporium*-artige Schimmelpilze (Hyphomycetes) Urgarn: Gustav Fischer Verlag. 1971. 262 p.

21. GAMS, W. and HOLUBOVA-JECHOVA, V. *Chloridium* and some other dematiaceous hyphomycetes growing on decaying wood. Studies in Mycology 13:97. 1976.

22. GREAVES, H. Microbial ecology of untreated and copper-chrome-arsenic treated stakes exposed in a tropical soil. I. The initial invaders. Canadian Journal of Microbiology 18:1923-1931. 1972.

23. GREAVES, H. Influence of a mixed microbial population on basidiomycetes decay. Mat. und Org. 7(1):11-25. 1972b.

24. GREAVES, H. and SAVORY, J. G. Studies of the microfungi attacking preservative-treated timber, with particular reference to methods for their isolation. Journal Institute of Wood Science 15:45-50. 1965.

25. HALE, M. D. C. and SAVORY, J. G. Selective agar media for the isolation of basidiomycetes from wood. A review. International Biodetn. Bulletin 12(4):112-115. 1976.

26. HENNINGSSON, B. and NILSSON, T. Some aspects on microflora and the decomposition of preservative-treated wood in ground contact. Beihefte zu Mat. und Org. 3:307-318. 1976.

27. HULME, M. A. and SHIELDS, J. K. Biological control of decay fungi in wood by competition for non-structural carbohydrates Nature 227:300-301. 1970.

28. KAARIK, A. Colonization of pine and spruce poles by soil fungi after six months. Mat. und. Org. 2:97-108. 1967.

29. KAARIK, A. Colonization of pine and spruce poles by soil fungi after 12 and 18 months. Mat. und Org. 3:185-198. 1968.

30. KERNER-GANG, W. Effect of microorganisms on creosote. Beihefte zu Mat. und Org. 3:319-330. 1976.

31. LAFLAMME, G. and LORTIE, M. Microorganisms in discoloured and decayed wood of *Populus tremuloides*. Canadian Journal and Forestry Research 3(2):155-160. 1973.

32. LEIGHTLEY, L. E. and EATON, R. A. A method for studying the growth of soft-rot fungi in wood veneers. Int. Biodetn. Bulletin 12(2):44-48. 1976.

33. LEVY, J. F. The soft-rot fungi. Their mode of action and significance in the degradation of wood. **In** R.D. Preston Advances Botany Research 2:323-357. 1965.

34. LEVY, J. F. and STEVENS, M. G. The initiation of attack by soft-rot fungi in wood. Journal Institute of Wood Science 16:49-55. 1966.

35. LEVY, M. P. The effects of microorganisms on chromated-copper-arsenate preservatives in wood. Beihefte zu Mat. und Org. 3:297-306. 1976.

36. MERRILL, W. and FRENCH, D. W. Colonization of wood by soil fungi. Phytopathologist 56:301-303. 1966.

37. MORTON, L. H. G. and EGGINS, H. O. W. Studies of interactions between wood-inhabiting microfungi. Mat. und Org. 11(3): 197-214. 1976.

38. NILSSON, T. Studies on wood degradation and cellulolytic activity of microfungi. Studia Forestalia Suecica Nr. 104. Sweden. 1973. 40 p.

39. NOBLES, M. K. Studies in Forest Pathology VI. Identification of cultures of wood-rotting fungi. Canadian Journal Research 26:281-431. 1948.

40. RICHARD, J. L. Biological control of decay in standing creosote-treated poles. Journal Institute Wood Science 7(4):6-9. 1976.

41. SAVORY, J. G. Breakddown of timber by Ascomycetes and Fungi Imperfecti. Annals of Applied Biology 41:336-347. 1954.

42. SCHEFFER, T. C. Microbiological degradation and the causal organisms. In Wood Deterioration and its Prevention by Preservative Treatments. Vol. 1. Degradation and Protection of Wood. Syracuse: Syracuse University Press. 1973. pp. 31-106.

43. SCHOL-SCHWARZ, M. B. Revision of the genus *Phiolophora* (Moniliales) Persoonia 6(1):59-94. 1970.

44. SHARP, R. F. and EGGINS, H. O. W. A perfusion technique for culturing fungi in wood. Institute Wood Science Journal 4(4): 22, 24-31. 1969.

45. SHIELDS, J. K. and ATWELL, E. A. Effect of a mold *Trichoderma viride*, on decay of birch by four storage-rot fungi. Forestry Products Journal 13:262-265. 1953.

46. SHIGO, A. L. Succession of organisms in discoloration and decay of wood. International Review Forestry Research 2:237-285. 1967.

47. SHIGO, A. L. Interaction of organisms in the process of decay in forest trees. Bulletin Fonds. Rech. For. University Laval 13:43. 1970.

48. SHIGO, A. L. Succession of microorganisms and patterns of discoloration and decay after wouding in red oak and white oak. Phytopathology 62(2):256-259. 1972.

49. SHIGO, A. L. Biology of decay and wood quality. Biological transformation of wood by microorganisms. Proceedings Sessions Wood Prod. Path. Second International Congress Plant Pathology, Sept. 10-12. Minn. USA. 1973.

50. SHIGO, A. L. Compartmentalization of discolored and decayed wood in trees. Mat. und Org. Beihefte 3:221-226. 1976.

51. SHIGO, A. L. and HILLIS, W. E. Heartwood, discolored wood and microorganisms in living trees. Annual Review of Phytopathology 11:197-222. 1973.

52. SIGLER, L. and CARMICHAEL, J. W. Taxonomy of Malbranchea and some other Hyphomycetes with arthroconidia. Mycotaxon 4(2):349-488. 1976.

53. STILLWELL, M. A. *et al.* Production, isolation and antifungal activity of scytalidin, a metabolite of *Scytalidium* species. Canadian Journal of Microbiology 19:597-602. 1973.

54. VAN BEYMA THOE KINGMA, F. H. Beschreibung der im Centraal-bureau voor Schimelcultures vorhandenen arten der gattungen *Phialophora* Thaxter und *Margarinomyces* Laxa, Nebst schussel zu Ihrer Bestimmung. Antonie von Leenwenhoek. Journal Microbiology of Serology 9:51-76. 1943.

55. ZABEL, R. A. Variations in preservative tolerance of wood-destroying fungi. Journal Forestry of Production Research Society 4:166-169. 1954.

Reseña de libros

FRENEY, J. R y SIMPSON, J. R. ed. Gaseous los of nitrogen from plant-soil systems. M. Nijhoff y Dr. W. Junk Publication. La Hoja. 1983. 317 p.

Este volumen altamente especializado es el No. 9 en la serie de este editorial en el campo del desarrollo en ciencias de suelo y de plantas. Lo escribieron 21 especialistas escogidos de Europa, USA, Nueva Zelandia y Australia, de donde proceden los dos editores. Ellos han tratado de resumir los estudios voluminosos sobre las emisiones gaseosas de diferentes sistemas suelo-planta y las maneras de como influirlos.

El material se presenta en 12 capítulos con bibliografía amplia que llega incluso al año previo a la publicación de la obra.

El primer capítulo discute la volatilización del amoniaco que es importante a partir de abonos y de excrementos de animales. El proceso lo influyen una serie de factores biológicos, químicos y físicos. Aunque varios de estos factores son bien estudiados, falta información sobre las pérdidas de muchos sistemas naturales o artificiales.

La bibliografía se presentó con 188 referencias sobre este tópico.

El Dr. Fillery del IRRI resume la información sobre denitrificación biológica particularmente importante en suelos inundados.

Más de 200 referencias resumen la información hasta 1982. Se discuten aquí los microorganismos involucrados en el proceso, la bioquímica del mismo y la biología de la denitrificación en el suelo.

La denitrificación química es el tópico del tercer capítulo. El material es presentado más que todo con base en experimentos de laboratorio. Se discute el efecto de diferentes condiciones en el suelo y de los inhibidores de nitrificación. La bibliografía de 92 trabajos ilustra el progreso en este campo.

El cuarto capítulo se dedica a las difíciles mediciones de la denitrificación. Se discuten aquí técnicas con trazadores, cromatografía de gases, análisis infrarojo y la técnica de inhibición de formación de acetileno. 189 citas que llegan hasta 1982 y son dominantemente de los últimos años, documentan el progreso en esta área.

En el quinto capítulo, uno de los más cortos, se presentan los métodos micrometológicos para medir las pérdidas de N en el campo. La bibliografía de 44 trabajos resume lo conocido en este campo. El capítulo es presentado con un enfoque matemático que permite una generalización de los conceptos presentados y asume conocimientos de matemática avanzada.

La pérdida de nitrógeno gaseoso de plantas es el tópico del sexto capítulo, otro de los relativamente cortos. La magnitud de este proceso se discute, algunos autores indican hasta 38 mg para un período de 10 semanas de una cosecha con gran superficie foliar. La bibliografía de 97 trabajos permite a los interesados profundizarse en este tópico poco explorado.

El séptimo capítulo, el más corto, presenta la información sobre la pérdida de nitrógeno de excrementos de animales y residuos municipales aplicados a tierras agrícolas. A pesar de la importancia de estas prácticas, solamente 29 referencias amplían la información, ya que existen pocos trabajos que en forma precisa informan sobre el proceso y los factores que lo determinan.

La pérdida del amoniaco de abonos aplicados a pastos tropicales es el tópico del octavo capítulo. La información usa ampliamente datos de Australia tratando de explicar las pérdidas de 20 a 80% de N que se aplicó. El crecimiento de pastos y las lluvias parecen ser algunos de los factores principales que influyen aquí. La información se completa con 55 referencias.

En el noveno capítulo se presenta los adelantos sobre el intercambio gaseoso del nitrógeno para potreros en uso. El cuadro de este ecosistema complejo influye muchos aspectos poco entendidos, particularmente lo que se refiere a los de contaminación ambiental.

Por el momento queda mucho para determinar en lo referente a la importancia y significancia agronómica de este proceso, sobre el cual se citan 99 referencias.

La suerte del nitrógeno que se aplica al arroz inundado es el tópico del décimo capítulo, uno de los más largos del volumen y las 136 referencias correspondientes al capítulo son de las más completas de la obra. A pesar de los amplios trabajos, parece que mientras no existan experimentos de campo donde se midan directamente la denitrificación y la volatilización de NH_3, no se podrán determinar cuáles son las pérdidas importantes de N de abonos aplicados a suelos inundados.

El onceavo capítulo discute la suerte de los compuestos de nitrógeno en la atmósfera. Se presenta un balance de estos compuestos aunque para partes del sistema la información es muy escasa. La bibliografía del capítulo llega a 100 referencias.

En el doceavo capítulo se discute los enfoques tecnológicos y agronómicos para reducir al mínimo las pérdidas de N gaseoso de tierras agrícolas. Esta parte del volumen es hasta cierto grado un resumen donde se refleja la aplicación de los procesos antes expuestos, indicando en la bibliografía las 108 referencias más pertinentes con base al excelente conocimiento de la bibliografía que usualmente caracterizan las publicaciones del Dr. Hauck de la TVA, autor del capítulo.

El volumen tiene un breve índice de materia a su final y contribuye en forma significativa a un mejor conocimiento de las pérdidas de N del ecosistema suelo-planta. El libro es útil para las bibliotecas agrícolas de centros de conservación de recursos y dedicados a la ecología.

ELEMER BORNEMISZA
FACULTAD DE AGRONOMIA
UNIVERSIDAD DE COSTA RICA

MODELO MATEMATICO DEL BALANCE HIDRICO[1] /

*J P LHOMME**
*L GOMEZ***
*A JARAMILLO***

Summary

An agroclimatic model which simulates the evolution of the soil water balance under perennial crops is proposed The model is recurrent, operates with a time step of one day and utilizes daily rainfall data, potential evapotranspiration values and a given value of the soil maximum water holding capacity It allows to determine water storage (RH), drainage (DR) and water deficit (DH) on a daily basis Each parameter appears in the form of a matrix which have the same dimensions as the daily rainfall matrix Practically the model can be used to characterize drought or water excess risks and duration of dry periods

Introducción

En el campo de la agroclimatología, cuando se analizan las condiciones de abastecimiento hídrico de las plantas, se considera generalmente el balance potencial (lluvia – evapotranspiración potencial), sobre una base de tiempo que puede variar desde una semana hasta un mes, y se estudia estadísticamente su variabilidad interanual. Este balance potencial, constituye una manera simple de representar el balance hídrico real y no toma en cuenta el papel de reserva que desempeña el suelo, almacenando y restituyendo el agua de lluvia.

El modelo que se presenta trata de caracterizar mejor las condiciones de abastecimiento hídrico de las plantas a través de una simulación de la evolución de la reserva hídrica del suelo (1). Los datos climáticos que sirven de entrada al modelo son las precipitaciones diarias que aparecen en forma de una matriz PJ (m, 365), representando m el número de años de registro y los valores promedios interanuales de la evapotranspiración potencial colocados en la forma de un vector de valores diarios EPJ (365). Al lado de estos datos climáticos aparecen ciertos parámetros de ajuste a las condiciones edáficas consideradas

Presentación del modelo

La reserva útil del suelo

La reserva hídrica del suelo, representada por RH, constituye la cantidad de agua almacenada en el suelo y disponible para las plantas. Es un número positivo, expresado en milímetros, que varía entre 0 y la reserva útil RU. La reserva útil representa la diferencia entre las cantidades de agua almacenadas a la capacidad de campo y al punto de marchitez permanente dentro de una capa de suelo de profundidad igual a la profundidad media de las raíces. Esta reserva útil evoluciona en función de la profundidad de las raíces, es decir, en función del estadío de desarrollo del cultivo. Para simplificar el problema considérese un cultivo perenne cuyo arraigamiento es aproximadamente constante en el transcurso del tiempo. De esta manera se puede suponer que la reserva útil es constante.

La lluvia eficaz

Admítase que una lluvia demasiado débil ($P < P_n$) se evapora inmediatamente sin que logre contribuir a la reconstitución de la reserva hídrica. Si P_i designa

1 Recibido para publicación el 3 de mayo, 1984

* IICA, Sede Central, Coronado, San José, Costa Rica

** Sección de Agroclimatología, CENICAFE, Chinchiná, Caldas, Colombia.

la lluvia registrada del día j y PE_j la lluvia eficaz, entonces:

$$PE_j = \begin{cases} P_j, \text{ si } P_j \geq P_n \\ 0, \text{ si } P_j < P_n \end{cases} \quad [1]$$

Para anular la contribución de la escorrentía, debe considerarse el suelo como horizontal

De este modo, si la intensidad de la lluvia es superior a la capacidad de infiltración del suelo, el agua que no se infiltra inmediatamente se quedará en el mismo lugar al estado libre hasta el momento que se evapore o se infiltre. Tampoco habrá transferencias laterales de agua dentro del suelo.

El drenaje

Si la cantidad de lluvia caída el día j es superior a la capacidad de retención del suelo, habría drenaje, es decir una pérdida de agua por salida subterránea. La capacidad de retención del suelo el día j, representada por CR_j, corresponde a la diferencia entre la reserva útil RU y la reserva hídrica del día anterior RH_j-1

$$CR_j = RU - RH_{j-1} \quad [2]$$

La cantidad de agua perdida por drenaje se escribe:

$$DR_j = \begin{cases} P_j - CR_j, \text{ si } P_j > CR_j \\ 0 \quad\quad\quad , \text{ si } P_j \leq CR_j \end{cases} \quad [3]$$

La evapotranspiración real

La evapotranspiración máxima (ETM) evoluciona, respecto a la ETP, en función del estado de recubrimiento del suelo por el follaje. En el caso de un cultivo cuyo follaje cubre totalmente el suelo, se puede admitir que la ETM iguala a la ETP. Así, siempre para simplificar el problema, debe considerarse un cultivo perenne que cubre totalmente el suelo.

La evapotranspiración real (ETR) evoluciona, respecto a la ETM, en función del estado de la reserva hídrica del suelo. Los conceptos clásicos sobre la utilización del agua del suelo por las plantas tienen en cuenta una reserva fácilmente utilizable (RFU), que representa la fracción de la reserva útil para las plantas sin dificultad, es decir, sin que eso provoque un cierre de los estomas, y así una reducción de la evapotranspiración respecto a la ETM. Abajo de este umbral, la ETR decrece conforme el agua disponible disminuye hasta el punto de marchitez permanente donde se anula. El punto crítico, que constituye el umbral de regulación, varía según el tipo de suelo y el desarrollo de las raíces. Si RFU = c. RU, siendo c un coeficiente de ajuste ($0 < c < 1$), se encuentra definida también una reserva difícilmente utilizable (RDU) que es el complementario de la RFU respecto a la RU: RU = RFU + RDU.

Asumiendo que el cociente ETR/ETM aumenta linealmente de 0 a 1 en función de la reserva hídrica RH del suelo, cuando ella pasa del valor 0 al valor RDU, debe mantenerse en este valor cuando la reserva fluctúe entre RDU y RU. Representando RH_{j-1} el estado de la reserva hídrica al fin del día j −1 y por consiguiente al inicio del día j:

$$ETR_j/ETP_j = \begin{cases} 1 \quad\quad\quad\quad , \text{ si } RH_{j-1} \geq RDU \\ RH_{j-1}/RDU, \text{ si } RH_{j-1} < RDU \end{cases} \quad [4]$$

El déficit hídrico diario a nivel del cultivo se encuentra definido por la relación:

$$DH_j = ETP_j - ETR_j \quad [5]$$

A partir de eso se definirá un déficit hídrico acumulado sobre el período de cultivo o sobre una fase particular del ciclo (estadío crítico por ejemplo):

$$DHC = \sum_{j=J1}^{J2} DH_j \quad [6]$$

La ecuación del balance hídrico

La ecuación que traduce el balance hídrico se escribe sobre una base diaria:

$$RH_j = RH_{j-1} + PE_j - ETR_j - D_j \quad [7]$$

Esta ecuación va a servir como relación recurrente para calcular las reservas hídricas diarias sucesivas. A menos que se tenga una manera particular de conocer la reserva hídrica al inicio del proceso recurrente (RH_0), se puede basar en las consideraciones siguientes para iniciar el proceso: si el día 1 se encuentra en plena estación seca, se escoge $RH_0 = 0$, y si se encuentra en plena estación lluviosa, $RH_0 = RU$; de lo contrario, se toma un valor promedio $RH_0 = RU/2$.

Aplicaciones del modelo

Utilización práctica del modelo

Se puede utilizar el modelo en tiempo "real" para simular y seguir la evolución de la reserva hídrica del

suelo. Para eso hay que ajustar el modelo a las condiciones específicas del caso considerado, escogiendo juiciosamente los parámetros (RU, RFU). Pero el modelo propuesto debe servir en primer lugar al análisis agroclimático, basado sobre la explotación estadística de las series de datos climáticos.

El modelo se ha programado en FORTRAN IV en forma de una subrutina llamada SBH (Simulación de Balance Hídrico), cuya lista de entrada comprende dos grupos de datos:

- datos climáticos: PJ, EPM, IJ. PJ representa la matriz de las precipitaciones diarias, de dimensión 365 x IJ, siendo IJ el número de años de registro. EPM representa el vector de las evapotranspiraciones potenciales mensuales (dimensión 12) que se transformará en un vector EPJ (365) de valores diarios.
- Características edáficas: RU, C. RU es la reserva útil del suelo considerado expresada en milímetros y C el coeficiente que define la RFU. El parámetro PN que define el límite de eficacia de la lluvia varía poco. Se lo considerará como interno al modelo y no figurará en la lista de entrada.

El proceso recurrente se inicia el primero de enero del primer año de registro de la pluviosidad PJ (1,1) con un valor de la reserva hídrica del día anterior RH_0 escogido en función de su posición frente a los períodos secos y lluviosos. Los años siguientes se encadenan regularmente: el 31 de diciembre del año n con el 1° de enero del año n + 1. La matriz QJ que figura en la lista representa los "output" del modelo. Tiene las mismas dimensiones que la matriz de las lluvias PJ. Su contenido varía según el valor del indicador IN. Si IN vale 1, QJ contiene las reservas hídricas diarias. Con IN = 2, QJ contiene los déficit hídricos diarios y con IN = 3, los valores diarios del drenaje. En el Anexo se presenta el programa que fue enfocado sobre una computadora IBM 360.

Estudio de los riesgos de sequía y de exceso de agua

Para estudiar los riesgos de sequía con el modelo es conveniente utilizar como salida los valores diarios del déficit hídrico, ya que representan la falta de agua a nivel del cultivo. La Figura 1 da un ejemplo. La estación escogida es la de Pueblo Bello (altitud: 1000 m, latitud: 10°22'N y longitud: 73°38'W), ubicada en la parte norte de la zona cafetera de Colombia (2), en la región de la Sierra Nevada. Cuenta con 20 años de registro de las precipitaciones (1961-80). Se consideró una reserva útil (RU) de 120 mm y una RFU de 60 mm. Se ha dividido el año en períodos elementales de 10 días y para cada período elemental se ha calculado, año tras año, el déficit hídrico acumulado DH_{10}, es decir la suma de los déficit diarios

$$(DH_{10} = \sum_{j=1}^{10} DH_j)$$

Después se ha analizado estadísticamente la repartición interanual de los déficit DH_{10} por clasificación frecuencial para calcular la mediana y el cuartil superior correspondientes a cada período elemental.

Si se quieren estudiar los riesgos de exceso de agua con el modelo, hay que utilizar como salida los valores del drenaje (IN = 3 en el programa), y después se puede estudiar este parámetro de la misma manera que el déficit hídrico. La Figura 2 muestra para la misma estación de Pueblo Bello y según el mismo procedimiento, la evolución en el transcurso del año de los valores del drenaje acumulados sobre 10 días y sobrepasados con las probabilidades 0.50 y 0.25.

Estudio de la duración de un período seco o lluvioso

Para estudiar con el modelo la duración de un período seco, veranillo por ejemplo, se puede proceder de la manera siguiente. Definiendo un día seco como un día con una reserva hídrica inferior a la reserva difícilmente utilizable (RDU) y la duración de un período seco como el número de días consecutivos secos, se calcula, año por año, durante los meses en que suele ocurrir el veranillo, el número de días consecutivos con una reserva inferior a la RDU. Para la estación de CENICAFE (altitud: 1310 m) en la zona cafetera de Colombia, que cuenta con veinte años de registro de la lluvia, los valores siguientes se refieren a la duración del veranillo de los meses de julio-agosto, calculada según lo indicado (RU = 120 mm, RFU = 60 mm):

17, 8, 11, 5, 6, 17, 37, 0, 26, 2,
0, 21, 10, 1, 0, 30, 22, 3, 9, 17.

A partir de esta muestra se puede calcular la frecuencia correspondiente a una duración dada. La frecuencia de un veranillo de más de 15 días es 0.40, de más de 20 días 0.25 y de más de 25 días, 0.15.

Conclusión

El modelo presentado es simple y su programación fácil. No pretende ser una simulación exacta de la evolución de la reserva hídrica del suelo pues habría que utilizar una escala de tiempo aún más pequeña, del orden de una hora.

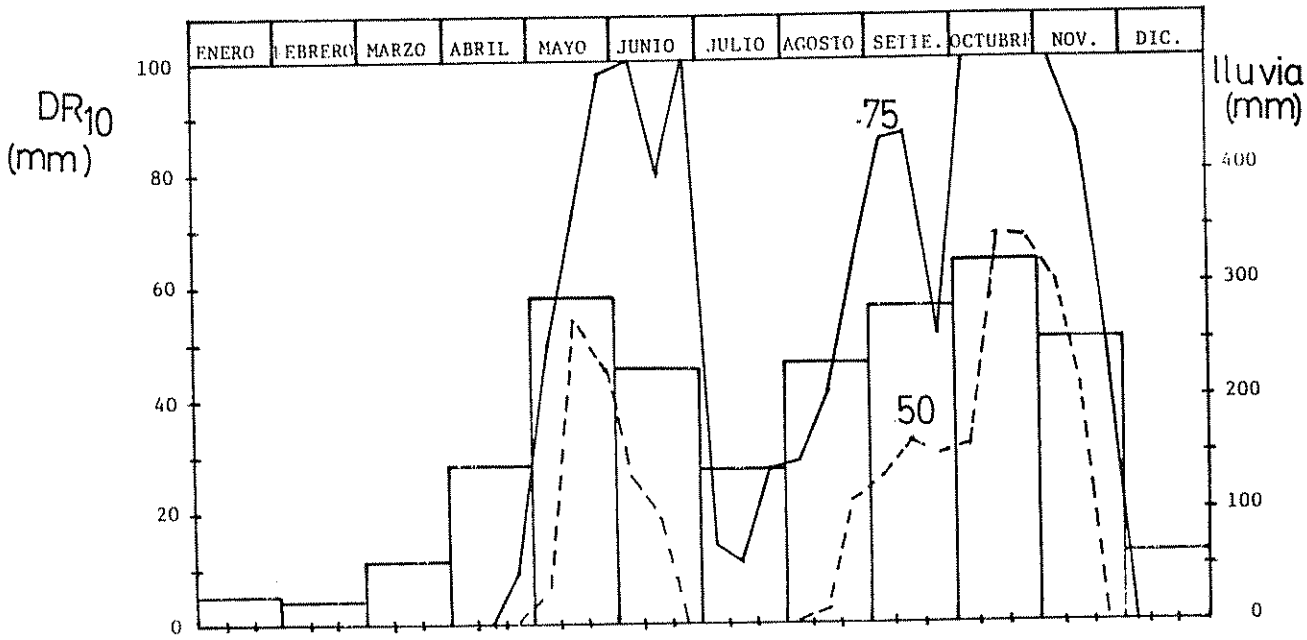

Fig 1 Estación de Pueblo Bello, Colombia. Evolución a lo largo del año de los valores del déficit hídrico acumulado sobre 10 días (DH = ETM – ETR) que son sobrepasados 1 año cada 4 y 1 año cada 2. Se indica también la precipitación media mensual en forma de histograma.

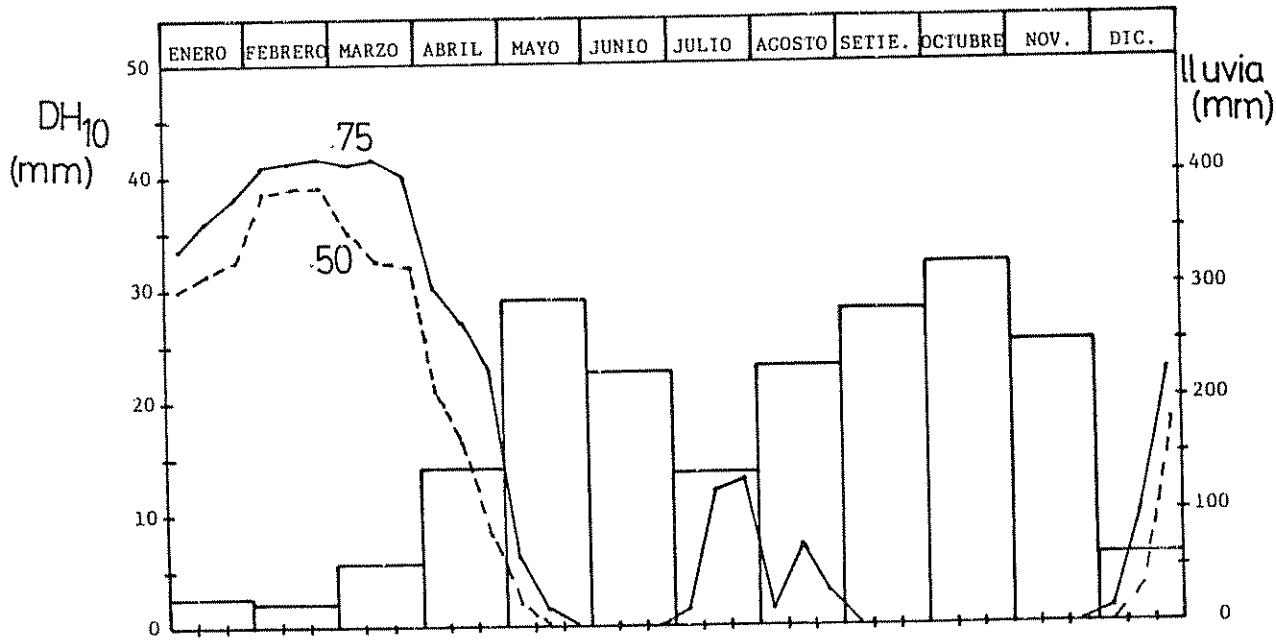

Fig 2. Estación de Pueblo Bello, Colombia. Evolución a lo largo del año de los valores del drenaje acumulado sobre 10 días que son sobrepasados 1 año cada 4 y 1 año cada 2. Precipitación media mensual en forma de histograma.

El modelo proporciona solamente un instrumento de análisis agroclimático que es más preciso y mejor adaptado a la realidad agronómica que el balance potencial (lluvia-ETP). Los datos climáticos necesarios son los mismos (precipitaciones diarias y valores promedios de la ETP). Además intervienen dos parámetros que definen la reserva útil y la reserva fácilmente utilizable del suelo.

Resumen

Se presenta un modelo agroclimático que simula la evolución del balance hídrico del suelo bajo un cultivo perenne. El modelo es recurrente y funciona con un paso de tiempo de un día. Los datos climáticos que sirven de entrada al modelo son los datos diarios de la lluvia y los valores de la evapotranspiración potencial. Además intervienen los valores de la reserva útil (RU) y de la reserva fácilmente utilizable (RFU). Este modelo programado permite determinar la reserva hídrica del suelo (RH), el drenaje (DR) y el déficit hídrico (DH) en una base diaria, apareciendo cada uno de estos tres parámetros en forma de una matriz que tiene las mismas dimensiones que la de la lluvia. El modelo puede ser utilizado para caracterizar estadísticamente los riesgos de sequía o de exceso de agua y la duración de un período seco, tal como el veranillo.

Literatura citada

1. FRANQUIN, P. et FOREST, F. Des programmes pour l'évaluation et l'analyse fréquentielle des termes du bilan hydrique. Agronomie Tropicale, 32(1):7-11. 1977.

2. JARAMILLO, A. Balance hídrico de la zona cafetera colombiana. CENICAFE 33(1):15-34. 1982

Anexo

Subrutina SBH

```
      SUBROUTINE SBH (PJ, EPM, Ij. RUX, C, QJ, IN)
      PN = 3.
      CRHO = .5
      DIMENSION PJ (30, 366), QJ (30, 366), RU
     (366)
      DIMENSION EPM (12), EPJ (366), EMJ (366)
      INTEGER*2 PJ, QJ
      CALL AAA (EPM, EPJ)
      DO 5 J = 1,365
      RU (J) = RUX
    5 EMJ (J) = EPJ (J)
      RH = RU (1)*CRHO
      DO 10 I = 1, IJ
      DO 10 J = 1,365
      PR = PJ (I, J)* .1
      ETM = EMJ (J)
      RDU = RU (J)* (1.–C)
      CR = RU (J) – RH
      PE = PR
      DR = 0
      IF (PR. LT. PN) PE = 0.
      IF (PR. GT. CR) DR = PR–CR
      IF (RH. GE. RDU) ETR = ETM
      IF (RH. LT. RDU) ETR = ETM*RH/RDU
      RH = RH +PE–ETR–DR
      DH = ETM–ETR
      IF (IN. EQ. 1) QJ (I,J) = INT (RH/.1)
      IF (IN. EQ. 2) QJ (I,J) = INT (DH/.1)
      IF (IN. EQ. 3) QJ (I,J) = INT (DR/.1)
   10 CONTINUE
      RETURN
      END

      SUBROUTINE AAA (EPM, EPJ)
      DIMENSION EPM (12), EPJ (366), LX(12), LY
     (12)
      DATA LX/31, 28, 31, 30, 31, 30, 31, 31, 30, 31.
     30, 31/
      DATA LY/0, 31, 59. 90, 120, 151, 181, 212,
     243, 273, 304, 334/
      DO 10 K=1, 12
      L1=LX(K)
      L2=(LY(K)
      DO 10 L = 1, L1
      LL=L2+L
   10 EPJ(LL) = EPM(K)
      RETURN
      END
```

Reseña de libros

VIZIER, J. F. Etude de phénomènes d'hydromorphie dans les sols des régions tropicales a saisons contrastées Dynamique du fer et différenciation du profile. Travaux et Documents de l'ORSTOM No. 165. ORSTOM, Paris, 1983 294 p

El trabajo de Vizier versa sobre catenas en regiones del Trópico Africano con estación seca contrastante, con énfasis en la dinámica del hierro y la diferenciación de perfiles de suelos. El estudio de catenas es tan antiguo como la cartografía de suelos; sin embargo, no es sino en textos recientes en los que se enfatiza en el uso de este concepto, a veces para explicar la presencia de ciertos suelos en diferentes latitudes (Bridges Turrialba 29(4):322 1979), otras para explicar la influencia del clima en la formación de los suelos (Gerrard Turrialba 33(2):142 1983)

Quizá para ubicar a los pedólogos en el contexto del estudio, sea conveniente nombrar los principales suelos de las dos secuencias estudiadas En el caso de Chad, la secuencia se divide en sus partes alta, media y baja; en la parte alta dominan suelos hidromórficos de profundidad, en la media solonetz solodizados y gley y en la parte baja, hidromórficos y vertisoles En Madagascar la secuencia se divide en un plateau con suelos ferralíticos profundos, el flanco de la pendiente con suelos ferralíticos poco desarrollados, el pie de monte con suelos coluviales y la zona baja con suelos hidromórficos orgánicos o de pradera flotante

La obra está dividida en cuatro partes que incluyen diez capítulos El inicio incluye dos capítulos, en los que se discute la importancia del hidromorfismo como factor formador de suelos y su empleo en diferentes sistemas de clasificación de suelos (p. ej. hidromórficos, Aquepts, etc.).

La segunda parte incluye los capítulos III a V en los que se describe el medio ambiente y edáfico en que se realizó el estudio en Chad y Madagascar Siempre se insiste en el hidromorfismo como factor dominante en ambos casos

En los siguientes tres capítulos se describe la variación del contenido y las formas del hierro en las secuencias En primer término se discute la metodología empleada, en seguida se presenta la información de los estudios *in situ*, y se termina con resultados experimentales sobre migración de hierro.

La cuarta sección se dedica a interpretaciones generales con una explicación fisicoquímica del fenómeno estudiado y un resumen de las consecuencias de los procesos que intervienen en la dinámica del hierro y su efecto sobre la diferenciación de los suelos que sufren un exceso de humedad Se incluyen conclusiones generales y 197 referencias sobre el tema

El aporte de Vizier es importante en tanto que se trata de un estudio de campo, con comprobaciones de laboratorio, tan escasos en la literatura moderna En este caso cabe mencionar que la calidad del estudio es proporcional al tiempo que se invirtió en él, y a fe que con creces Las secuencias estudiadas en Africa son comunes en el Trópico Americano de régimen ústico, en donde no conozco ningún estudio similar al presente. El documento puede adquirirse en el SERVICE DES PUBLICATIONS D L'ORSTOM, 70-74 route d'Aubray, 93140 Bondy, France.

ALFREDO ALVARADO
FACULTAD DE AGRONOMIA
UNIVERSIDAD DE COSTA RICA

EFFECTS OF N–CARRIERS AND Al–LEVELS ON DRY MATTER PRODUCTION AND NUTRIENT CONTENT OF TWO PASTURE GRASSES[1] /

*M. L. R. ARRUDA**
*M. S. FERNANDES***
*R. O. P. ROSSIELLO****

Resumo

*Em casa de vegetação, e sob solução nutritiva, foi estudada a resposta de duas gramíneas forrageiras (*Cenchrus ciliaris *e* Brachiaria decumbens*) a doses crescentes de Al (0 – 0.75 – 1.5 – 3.0 – 6.0 ppm), usando-se NO_3 ou NH_4 como fonte de N.*

Ambas as gramíneas sofreram redução de peso seco com doses crescentes de Al. Em termos absolutos, Brachiaria *apresenta maior produção de matéria seca sob NH_4 do que sob NO_3, acontecendo o inverso com* Cenchrus. *Quando entretanto a redução do peso com doses crescentes de Al é tomado em termos relativos (% do máximo),* Brachiaria *sob NO_3 mostra maior estabilidade, enquanto que* Cenchrus *é mais estável quando sob NH_4. A fonte de N afetou diferencialmente a acumulação de Al, e N na raiz e parte aérea das plantas estudadas. O maior acúmulo de Al ocorre nas raízes, sendo maior nas plantas sob NO_3 do que nas NH_4. Em ambas as espécies houve maior acúmulo de P na parte aérea das plantas sob NH_4 do que sob NO_3, enquanto que nas raízes foi observada uma tendência inversa. Acúmulos de N e K nos tecidos das plantas parecem estar relacionados com a tolerância à toxidez de Al*

Introduction

In acid soils, high levels of exchangeable aluminum is one of the principal factors limiting plant growth. This condition is usually associated with low fertility levels (15) in such a way that the direct and indirect effects of Al on root growth and nutrient absorption are referred as "Al toxicity".

Aluminum appears to affect root growth more closely than top growth. Interferences in the process of cell division due to high Al concentrations result in abnormal development of root tissue (5). In the tops the symptoms are less characterized and can be mistaken for P or Ca deficiency (11, 18).

The effects of high Al levels on the solubility of P in the growth medium and its absorption are well known (8). Clarkson (5) suggests that there are two reactions between Al and P; one at the cell surface or in the free space and the other within the cell, possibly within the mithocondria. Soluble Al may reduce the uptake of other plant nutrients. In rice, Al toxicity is associated with lower concentrations of Ca, Mg, K and Si and higher concentrations of N and P in plant tops (16). Reductions in the concentration of K in tops of leguminous plants are associated with Al sensibility (2). High Al concentration in the nutrient solution decreased the concentrations of P, K, Ca, Mg and increased Fe and Al concentrations in roots as well as in top of oats (1). Zn, Fe, Mn an Cu concentrations were affected in rye, barley and wheat according to age and cultivar sensibility to increasing levels of Al (13).

Since Al tolerance and nutrient utilization and absorption are controlled by genetic factors (5), an association between Al tolerance and nutrient utilization efficiency seems probable. However, both processes (Al exclusion and nutrient absorption) are conditioned to pH fluctuations in the medium. Since

1 Received for publication in April 6, 1983.
Contribution from the Department of Soil Science U.F.R.R.J. km 47 Rod. Rio-São Paulo. Seropédica, 23460, Rio de Janeiro-RJ

* Agronomist from EPAMIG, MG-Brasil

** Associate professor and Assistent professor respectively UFRRJ km 47 Rod. Rio-São Paulo, 23460, Rio de Janeiro-RJ.

N is the nutrient of highest metabolic demand in plants, its rate of uptake greatly effects pH changes in the root-solution interface (7, 11, 19).

In this work we examined the effects of the two ionic forms of nitrogen absorption (NH_4^+ or NO_3^-) on dry matter production and Al, N, P, and K absorption by *B. decumbens* and *Cenchrus ciliaris*. These two species are known to differ in their Al tolerance (20).

Material and methods

The experiment was conducted in a greenhouse at mean air temperature of 24°C and 70% relative humidity. A fatorial design of two species, two nitrogen sources and five Al concentration replicated three times was used. The nutritive solution used was similar to that described by Andrew *et al.* (2). The main modifications were that $(NH_4)\ Mo_7O_{24}\ 4H_2O$ was substituted by H_2MoO_4. Ca was supplied as $CaCl_2$ and plants supplied amonium received N as $(NH_4)\ SO_4$ (Table 1).

The species studied were *C. ciliaris* obtained from the Centro Nacional de Gado de Leite – EMBRAPA collections, and *B. decumbens* obtained from commercial sources.

Seeds were germinated in plastic plates containing sand and vermiculite and irrigated when necessary with nutrient solution. After 15 days 300 plants from each species were selected and transferred to 60, 8-liter plastic pots, whose outside were painted silver to prevent light penetration. Seedlings were supported in holes in the lids of the pots by rubber foam. Plastic perforated tubing was attached to the base of each solution container to provide an aeration system operating at a frequency of 3 periods (60 minutes) each 24 hours. Each pot was thinned to 6 seedlings 15 days after germination. The culture medium was similar to that described above but containing in addition either 0; 0.75; 1.5; 3.0 or 6.0 ppm Al as $Al_2\ (SO_4)_3 \cdot 18H_2O$. The solutions were adjusted to pH 4.2 with 0.1 N NaOH or 0.1 N H_2SO_4. The P and N concentrations were determined each 2 days. The plants were exposed to the treatments solutions for 4 weeks and then harvested.

Roots were separated from tops and washed with deionized water. Plant tops and roots were dried at 70°C in a forced-air oven for at least 36 hours. Dried tops and roots were ground in a Wiley mill to pass a 40 mesh screen and analysed for N, P, Ca, K and Al.

After dry digestion at 500°C, P was determined as described by Leece and Short (12); Al by the method of Otomo (17); K by flame photometry and Ca by complexometry with EDTA. Nitrogen was determined by destillation (3).

Table 1. Nutrient solution composition prepared from modified Andrew's solution.

Elements	Concentrations	Salt
K	1.0 mM	K_2SO_4
Cl	0.5 mM	NaCl
Mg	0.5 mM	$MgSO_4 \cdot 7H_2O$
Cu	0.02 ppm	$CuSO_4 \cdot 5H_2O$
Zn	0.05 ppm	$ZnSO_4 \cdot 7H_2O$
Mn	0.25 ppm	$MnSO_4 \cdot H_2O$
B	0.5 ppm	H_3BO_3
Mo	0.01 ppm	H_2MoO_4
Fe	1 ppm	Fe-citrate
P	2 ppm	KH_2PO_4
Ca* e N*	1 mM	$Ca(NO_3)_2 \cdot 4H_2O$
Ca**	1 mM	$CaCl_2$
N**	1 mM	$(NH_4)_2\ SO_4$

* Plants receiving N-nitrate

** Plants receiving N-ammonium

Results and discussion

Dry matter production. There was a significant reduction in dry matter accumulation for both plant species as Al in solution increased (Table 2). N-form also affected plant dry weight. Plants under nitrate had higher dry weight than plants under ammonium. There was no difference in dry matter accumulation by the roots of the two species, but *Brachiaria* plants accumulated more dry matter in the tops than did *Cenchrus*.

There was a significant N x genotype interaction. *Cenchrus* plants accumulated more top and root dry matter when under NO_3 –N, while *Brachiaria* plants showed no response to N–form.

When the total dry matter production was examined in terms of percentage reduction to the treatment of maximum production (0 ppm Al = 100%), it was verified that the production of *B. decumbens* was more stable when receiving nitrate than when treated with ammonium (Figure 1). In the first case, the production decreased 15% up to 3.0 ppm of Al and decreased 67% at the highest level the production was 60% of the maximum whereas for *C. ciliaris* the relative production was more stable when ammonium was the source of N. The yields relative to the highest level of Al were 67.0 and 48.3% of the 0 level for plants treated with ammonium and nitrate respectively. These results

Table 2. Effects of Al and N source on root and top dry weights (g/pot) of *Brachiaria decumbens* and *Cenchrus ciliaris*

	Mean Al effect	
Al (ppm)	Roots	Tops
0.00	2.74 a *	22.56 a
0.75	2.35 ab	19.61 b
1.50	2.31 ab	16.84 b
3.00	2.16 b	16.00 bc
6.00	1.60 c	13.38 c

	Mean N effect	
N-carrier	Roots	Tops
NO_3	2.80 a	18.91 a
NH_4	1.66 b	16.18 b

	Mean genotype effect	
	Roots	Tops
Brachiaria	2.12 a	16.37 b
Cenchrus	2.34 a	18.71 a

	Mean N x genotype effect			
	Roots		Tops	
	Brach.	*Cench.*	*Brach.*	*Cench.*
NO_3	2.28 a	3.32 a	15.5 a	22.3 a
NH_4	1.96 a	1.36 b	17.2 a	15.1 b

* Means in a column group (tops or roots) not followed by the same letter differ significantly at the 5% level according to Tukey's test.

stress the metabolic interrelationship of Al tolerance and the mechanism that regulates nitrogen absorption by plants. It must be noted, however, that the growth period (pre-stress) of 4 weeks must reduce the toxic effects of Al for *C. ciliaris*, since the effects are more drastic at the seedling stage after the radicle emergence (11). In another experiment, it was observed (data not shown) that *C. ciliaris* exposed to soil Al saturation levels higher than 10% does not grow beyond the seedling stage, whereas *B. decumbens* grows satisfactorily.

Aluminium concentration

There is a significant increase in Al concentration in roots, but not in tops as Al concentration increases in the nutrient solution (Table 3). Accumulation of Al in roots of both species was higher in plants supplied nitrate than in plants supplied ammonium (Table 3). For both species the concentration of Al was higher in roots than in tops. This fact has been previously documented by Foy and Brown (9) and Nunns (14). The major part or this Al in roots must be absorbed in the cellular wall or precipitated in the free space of the roots, specially in the epidermis (2, 5, 11, 18).

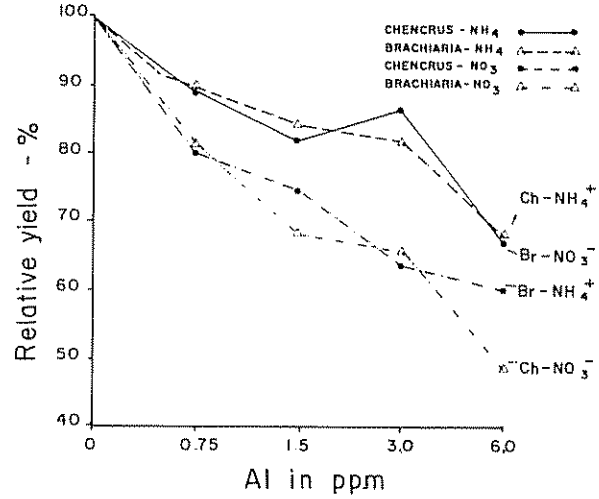

Fig. 1. Effects of Al levels and N source on relative growth of *Cenchrus ciliaris* and *Brachiaria decumbens*.

Regardless of N source *B. decumbens* accumulated significantly more Al than *C. ciliaris* suggesting that root Al accumulation might be associated with higher Al tolerance.

Phosphorus concentration

Phosphorus concentration of plant roots was significantly increased at or above 1.5 ppm of Al in solution (Table 4). For the two sets of treatments and for both species, plants receiving N as ammonium accumulated more P in the tops than plants under nitrate (0.183 x 0.296) whereas in the roots an inverse relationship was obtained ($N-NH_4 = 0.326$; $N-NO_3 = 0.375$). The higher concentration of P in the roots than in the tops must be due to P precipitation at the surface or internally within the roots (14). The effects of Al on root P content differ between the two species and N sources. With either nitrate or ammonium nutrition the P concentration of roots for *B. decumbens* was increased at or above 1.5 ppm of Al. Regardless of N source, the P concentration of roots for *C. ciliaris* was not affected by Al concentration in solution (Table 4).

Table 3. Effects of Al and N source on Al concentration of roots and tops (µg/g of dry matter) of *Brachiaria decumbens* and *Cenchrus ciliaris*

	Mean Al effect	
Al (ppm)	Roots	Tops
0.00	690 d*	198 a
0.75	1 854 cd	223 a
1.75	3 184 c	238 a
3.00	5 366 b	228 a
6.00	7 281 a	270 a

	Mean N effect	
N-carrier	Roots	Tops
NO_3	6 186 a	242 a
NH_4	1 292 b	221 a

	Mean genotype effect	
	Roots	Tops
Brachiaria	4 715 a	254 a
Cenchrus	2 762 b	208 b

	Mean Al x genotype effect			
	Roots		Tops	
Al (ppm)	*Brach*	*Cench*	*Brach*	*Cench*
0.00	1 145 d	1 140 c	200 b	198 a
0.75	2 186 cd	1 523 bc	228 b	219 a
1.50	4 058 c	2 309 bc	237 b	240 a
3.00	6 867 b	3 595 ab	245 ab	210 a
6.00	9 319 a	5 244 a	360 a	180 a

	Mean N x genotype effect			
	Roots		Tops	
N-carrier	*Brach*	*Cench*	*Brach*	*Cench*
NO_3	7 916 a	4 456 a	285 a	200 a
NH_4	1 514 b	1 069 b	223 b	220 a

* Means within a colum not followed by the same letter differ significantly at the 5% level according to Tukey's test

Potassium concentration

Table 5 shows the effects of Al on K concentration of roots and tops. Increasing Al levels affected K-content of roots but not of tops. Response of roots K to Al levels had a quadratic fit with a maximum at 3.0 pp. Al.

When nitrate was the source of N the K concentration in plant tops was significantly affected by Al concentration in solution, but each specie showed differential responses (Table 5). K concentration of tops for *B. decumbens* with nitrate as the source of N increased with increasing Al levels, whereas the K content of the tops for *C. ciliaris* decreased as the Al in solution increased. K content of roots for *B. decumbens* and *C. ciliaris* was also affected by Al concentration and the effects of the two forms of N were similar to those observed on tops (Table 5).

The effects of higher levels of Al on K concentration of plant tops and roots of *B. decumbens* have been reported for other vegetable species (2, 5) and suggest that Al tolerance might be related to the root capacity to absorb K. The results of this experiment (Table 6) show that increasing levels of Al in solution favors an increase in K concentration of tops and roots for species less sensible to Al (*B. decumbens*). Andrew *et al.* (2) observed that in tolerant species the reduction of Ca concentration of tops due to an excess of Al in solution was balanced by an increase of K and Mg. In this experiment it was observed a marked effect of Al upon the reduction of Ca concentration of tops (data not shown) and the increase in K content of tops and roots (Table 5), suggesting that this effect represents a way by which the plant preserve its ionic equilibrium.

The reduction in K content of plant tops for *C. ciliaris* as a result of increasing levels of Al can be associated with the reductions of dry matter (Table 2 and 5). Andrew *et al.* (2) observed marked reductions in K concentrations of species more sensible to Al in solution.

Nitrogen concentration

There was an increase in N-concentration of roots and tops as Al in solution increased. Maximum N concentration was reached at the 3.0 ppm level. However, responses of plant-N to Al was affected by N-carrier and plant genotype. Plants under NH_4 –N had significantly higher N than those under NO_3 –N, and *B. decumbens* accumulated more N than did *C. ciliaris* (Table 6). A significant interaction was found between Al-levels and N-carriers.

Brachiaria decumbens showed an increase in the absorption and translocation of nitrogen as the level

of Al in solution was increased, whereas the nitrogen content for *C. ciliaris* was decreased whenever Al was added to the solution. An increase in N concentration for *B. decumbens* with increasing Al levels in solution could result from the reduction in plant dry weight. However, under the same experimental conditions the

Table 4. Effects of Al and N source on phosphorus concentration of roots and tops (% dry matter) of *Brachiaria decumbens* and *Cenchrus ciliaris*

	Mean Al effect	
Al (ppm)	Roots	Tops
0 00	0 256 b*	0 254 a
0 75	0 295 b	0 227 a
1 50	0 376 a	0 209 a
3 00	0.414 a	0 244 a
6 00	0 403 a	0 260 a

	Mean N effect	
N-carrier	Roots	Tops
NO_3	0 375 a	0 183 b
NH_4	0 326 b	0 296 a

	Mean Al x genotype effect			
	Roots		Tops	
Al (ppm)	*Brach*	*Cench*	*Brach*	*Cench*
0 00	0 268 b	0 309 a	0 221 a	0 287 a
0 75	0 297 b	0 294 a	0 241 a	0 213 a
1 50	0 417 a	0 336 a	0 209 a	0 209 a
3 00	0 439 a	0 394 a	0 236 a	0 254 a
6 00	0 353 a	0 459 a	0 270 a	0 250 a

	Mean N x genotype effect			
	Roots		Tops	
N-carrier	*Brach*	*Cench*	*Brach*	*Cench*
NO_3	0 429 a	0 320 a	0 214 b	0 152 b
NH_4	0 297 b	0 354 a	0 257 a	0 334 a

* Means in a column group (tops or roots) not followed by the same letter differ significantly at the 5% level according to Tukey's test.

Table 5. Effects of Al and N source on potassium concentration of roots and tops (% dry matter) of *Brachiaria decumbens* and *Cenchrus ciliaris*

	Mean Al effect	
Al (ppm)	Roots	Tops
0 00	3 10 c*	5 28 a
0 75	3 81 ab	5 00 a
1 50	4 07 a	5 33 a
3 00	4 14 a	5 07 a
6 00	3 35 bc	5 01 a

	Mean N effect	
N-carrier	Roots	Tops
NO_3	3 57 b	5 17 a
NH_4	3 82 a	5 11 a

	Mean genotype effect	
	Roots	Tops
Brachiaria	3 39 b	4 44 b
Cenchrus	4 00 a	5 11 a

	Mean Al x genotype effect			
	Roots		Tops	
Al (ppm)	*Brach*	*Cench*	*Brach*	*Cench*
0 00	2 48 b	3 72 ab	4 13 b	6 43 a
0 75	3 36 a	4 26 a	3 97 b	6 04 ab
1 50	3 78 a	4 37 a	4 58 ab	6 08 ab
3 00	3 93 a	4 35 a	4 57 ab	5 58 b
6 00	3 41 a	3 29 b	4 98 a	5 04 c

	Mean N x genotype effect			
	Roots		Tops	
N-carrier	*Brach*	*Cench*	*Brach*	*Cench*
NO_3	3 40 a	3 74 b	4 70 a	5 64 b
NH_4	3 38 a	4 26 a	4 19 b	6 02 a

* Means within a column not followed by the same letter differ significantly at the 5% level according to Tukey's test.

Table 6. Effects of Al and N source on nitrogen concentrations of roots and tops (% dry matter) of *Brachiaria decumbens* and *Cenchrus ciliaris*

	Mean Al effect	
Al (ppm)	Roots	Tops
0.00	2.32 b*	2.07 bc
0.75	2.50 ab	2.13 ab
1.50	2.57 ab	2.21 ab
3.00	2.60 a	2.28 a
6.00	2.31 b	1.96 c

	Mean N effect	
N-carrier	Roots	Tops
NO_3	2.31 b	2.07 b
NH_4	2.61 a	2.19 a

	Mean genotype effect	
	Roots	Tops
Brachiaria	2.58 a	2.31 a
Cenchrus	2.34 b	1.96 b

	Mean Al x N effect			
	Roots		Tops	
Al (ppm)	NO_3	NH_4	NO_3	NH_4
0.00	2.42 ab	2.22 b	1.97 bc	2.19 bc
0.75	2.29 ab	2.71 a	2.22 a	2.05 bc
1.50	2.48 a	2.68 a	2.17 ab	2.27 ab
3.00	2.36 ab	2.85 a	2.12 abc	2.44 a
6.00	2.04 b	2.60 ab	1.90 c	2.04 c

	Mean Al x genotype effect			
	Roots		Tops	
Al (ppm)	*Brach*	*Cench*	*Brach*	*Cench*
0.00	2.29 b	2.35 ab	2.14 b	2.02 a
0.75	2.65 ab	2.36 ab	2.24 ab	2.03 a
1.50	2.66 ab	2.49 a	2.42 a	2.00 a
3.00	2.69 a	1.53 a	2.37 ab	2.19 a
6.00	2.63 ab	1.98 b	2.36 ab	1.58 b

* Means within a column group (tops or roots) not followed by the same letter differ significantly at the 5% level according to Tukey's test

Al treatments reduced the dry weight of *C. ciliaris* that had also less N concentration suggesting that N accumulation is a characteristic of Al tolerant species.

The results suggest that tolerance to Al-toxicity is related to the patterns of ion uptake. The effects of N-carriers on Al-toxicity are thus mainly due to its influence on the ion uptake patterns of the plants.

Summary

To study the effects of various Al levels and the possible role of nitrate or ammonium nutrition on the growth and nutrient accumulation of two gramineous species (*Brachiaria decumbens* and *Cenchrus ciliaris*) several rates of Al (0, 0.75, 1.5, 3.0 and 6.0 ppm) were added to nutrient solutions in a greenhouse experiment utilizing NH_4 or NO_3 as source of nitrogen.

For both species, dry weights decreased as the Al concentration was increased. In absolute terms, *B. decumbens* produced more dry matter when relying on NH_4 than when utiliizing NO_3, the inverse happened with *C. ciliaris*. However, when the reduction in dry weight with increasing Al levels is taken in relative term (% of the maximum) *B. decumbens* receiving NO_3 showed more stability whereas *C. ciliaris* was more stable when treated with NH_4. The Al and N concentrations of roots and tops of plants treated were differentialy affected by the form of nitrogen used. Plants receiving nitrate as the source of nitrogen, accumulated more Al in roots than those receiving ammonium. For both species, plants receiving nitrogen as NH_4 had higher P concentrations in tops than did nitrate plants, whereas in the roots an inverse relationship was observed. Nitrogen and potassium accumulation in plant tissue appear to be related to Al tolerance.

Literature cited

1. ALAM, S. M. and ADAMS, W. A. Effects of aluminium on nutrient composition and yield of oats. Journal of Plant Nutrition 1:365-375. 1979.

2. ANDREW, C. S., JOHNSON, A. D. and SANDLAND, R. L. Effect of aluminium on the growth and chemical composition of some tropical and temperate pasture legumes. Australian Journal of Agriculture Research 24:325-339. 1973.

3. BREMNER, I. M. and KEENEY, D. R. Steam distilation methods for determination of am-

monium, nitrate and nitrite. Analytica Chimica Acta 32:485-495. 1965.

4. CARVALHO, M. M. de. A comparative study of the responses of six stylosanthes species to acid soil factors with particular reference to aluminium. Ph.D. Thesis. University of Queensand Australia. 1978.

5. CLARKSON, D. T. Effects of aluminium on the uptake and metabolism of phosphorus by barley seedlings. Plant Physiology 41:165-172. 1966.

6. CLARKSON, D. T. Interactions between aluminium and phosphorus on root surfaces and cell wall material. Plant Soil 23:347-356. 1967.

7. FERNANDES, M. S. and ROSSIELLO, R. O. P. Effects of NH_4 and a nitrification inhibitor on soil pH and phosphorus uptake by corn (*Zea mays* L.). Cereal Research Commonwealth 6:183-191. 1978.

8. FOY, C. D. Effects of aluminium on plant growth. **In** The plant root and its environment. E. W. Carson ed. University Press Virginia. 1974. pp. 601-642.

9. FOY, C. D. and BROW, J. C. Toxic factors in acid soils: II. Differential aluminium tolerance of plant species. Soil Science Society of American Proceedings 28:27-32. 1964.

10. FOY, C. D. CHANEY, R. L., WHITE, M. C. The physiology of metal toxicity in plants. Annual Review of Plant Physiology 29:511-566. 1978.

11. HELYAR, K. R. Effects of aluminium and manganese toxicities on legume growth. **In** Mineral nutrition of legumes in tropical and subtropical soils. C. S. Andrew and E. J. Kamprath eds. CSIRO, Melbourne, Australia. 1978. pp. 207-232.

12. LEECE, D. R. and SHORT, C. C. A. A routine procedure for the nutrient element analysis of peach leaves utilizing atomic absorption spectroscopy. New South Wales Dept. of Agriculture Divsion of Scientific Services. Chemistry Branch Bulletin, 1967.

13. MUGWIRA, L. M. Aluminium effects on the growth and mineral levels of triticale, wheat and rye. Journal of Plant Nutrition 1:219-240. 1979.

14. MUNNS, D. N. Soil acidity and growth of a legume. II. Reactions of aluminium and phosphate in solution and effects of aluminium, phosphate, calcium and pH on *Medicago sativa* L. and *Trifolium subterraneum* L. in solution culture. Australian Journal of Agricultural Research 16:743-755. 1965.

15. OLMOS, J. I. L. and CAMARGO, M. N. Ocorrência de alumínio tóxico nos solos do Brasil, sua caracterizacão e distribuição. Ciência e Cultura 28:171-180. 1976.

16. OTA, Y. Studies on the occurrence of the physiological disease of rice called "bronzing". Bulletin Nat. Institute Science Nishigahara, Tokyo, Japan, ser. D, n^{o}. 18 (Japan). 1968.

17. OTOMO, M. The spectrophotometric determination of aluminium with xylenol orange. Bulletin Chemical Society Japan 36:809-813. 1963.

18. RHUE, R. D. Differential aluminium tolerance in crop plants. **In** Stress physiology in crop plants. (Mussell, H. and R. C. Staples Ed.). John Wiley and Sons, New York, 1979. pp. 61-80.

19. RILEY, D. and BARBER, S. A. Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root-induced pH changes at the root-soil interface. Soil Science Society of American Proceedings 35:301-306. 1971.

20. SPAIN, J. M. and ANDREW, C. S. CSIRO. Division of Tropical Crops and Pastures. Annual Report 1975-1976. 50 p.

Reseña de libros

KOOLEN, A. J. y KUIPERS, H. Agricultural soil mechanics. Springer-Verlag, New York. 1983. 241 p.

Este volumen preparado por dos prestigiosos colaboradores de la bien conocida Universidad Agrícola de Wageningen, Holanda, presenta una discusión original de tres campos de la Ingeniería Agrícola, dedicando a cada uno una parte del libro.

Las condiciones generales del suelo con el tópico de la primera parte, mucho más corta que las otras. Se enfoca aquí el suelo como un sistema que presenta oposición a fuerzas aplicadas a la misma.

La segunda parte que incluye un poco más que un tercio del volumen analiza el comportamiento mecánico de los elementos del suelo. Esta parte consiste en cinco subdivisiones que se refieren a:

a) Los aspectos generales del comportamiento mecánico de las unidades elementales del suelo.

b) Un tratamiento básico de la compactación.

c) Un análisis de la deformación del suelo.

d) Un estudio del rompimiento del suelo. y

e) Un estudio básico de la adherencia y fricción entre suelo y otros materiales.

La tercera parte se dedica al estudio de los procesos referentes a la capacidad del suelo de llevar cargas y de su desintegración. Esta parte es la más larga al incluir más de la mitad del volumen, tiene siete subdivisiones que son:

a) Aspectos generales de los procesos de arado.

b) Ruedas, llantas y rodillos.

c) Elementos que penetran al suelo (Cuñas, Conos, Placas, Alambre y Esferas).

d) Cuerpos cortantes

e) Orugas

f) Dientes

g) Cuerpos de arado

En todos los capítulos se discute los principios que se aplican en esta parte y la aplicación de los principios en las operaciones pertinentes. Tiene 231 referencias, y la mayoría de ellas son inglés con unas pocas en holandés y alemán, permiten al interesado a profundizarse en los diferentes tópicos, a los cuales se localiza fácilmente por medio de un buen índice de materiales al final.

El volumen es sin duda una adición útil a la no muy amplia bibliografía en Ingeniería Agrícola y por esto se le recomienda a todos los que se interesan en mecánica de suelos. El inglés de los autores es claro y la mayor parte del volumen es comprensible aún para no ingenieros agrícolas, siempre que tengan buenas bases en mecánica.

ELEMER BORNEMISZA S
FACULTAD DE AGRONOMIA
UNIVERSIDAD DE COSTA RICA

CONSERVAÇÃO E ARMAZENAMENTO DO MARACUJÁ AMARELO *Passiflora edulis f. flavicarpa* Deg. III – VARIAÇÕES NO TEOR DE ÁCIDO ASCORBICO[1]

*E. CEREDA**
*U.A. LIMA***
*R.J.P. CUNHA**
*M.P. CEREDA****

Summary

*The value of the fruits juice is frequentely expressed by his ascorbic acid content In this work, yellow passion fruit (*Passiflora edulis f. flavicarpa*) was used. Ripe and partially ripe passion fruits were stored, in both, temperature controlled rooms (5, 6, 7, 2°C), with relative humidity ranging from 85 to 90% and under environmental conditions. The fruits were submitted to treatments with skin-coating, germicide products and wrappers. During the storage period, samples of fruits were taken periodically for analysis. The effect of the treatments on ascorbic acid content, was determinated for two months. The results showed that there is a progressive decrease in ascorbic acid content, independent of the treatments, maturation stage, and storage conditions. It was observed that the treatments that propitiated better conservation, caused the lower decrease in the ascorbic acid content.*

Introdução

O maracujazeiro (*Passiflora edulis f. flavicarpa* Deg.) conhecido como peroba, amarelo, é o mais cultivado comercialmente. É valioso pelas suas características nutricionais e pela sua grande aplicação (9). Pode ser consumido **in natura** ou na forma de suco, que adicionado de água, resulta em refresco de agradável sabor (8).

A comercialização do fruto é prejudicada devido a ocorrência de rápido murchamento, conferindo má aparência à casca em apenas três dias (4). Uma vez resolvido o problema do murchamento através do uso de produtos que diminuam as trocas gasosas com o ambiente, resta saber o que ocorre com o teor de ácido ascórbico do suco, pois geralmente, é a presença deste, o principal indicador do valor nutritivo de um suco.

O teor de ácido ascórbico nos frutos é muito variável segundo o local de produção, estádio de desenvolvimento, amadurecimento, temperatura de armazenamento, fotoperiodismo e outros. Essa variação tem oscilado de 7 a 20 mg por 100 g de suco (1, 6, 10). Segundo alguns autores, o teor de ácido ascórbico do suco diminue com a maturação (12). O presente trabalho tem por objetivo conhecer a variação no teor de ácido ascórbico do suco de maracujá durante a conservação e armazenamento do fruto.

Material e métodos

O experimento foi conduzido na Faculdade de Ciências Agronômicas "Campus" de Botucatu, UNESP. Trabalhou-se com 3 168 frutos de maracujá amarelo, colhidos em dois estádios de maturação ou seja: 1 584 frutos maduros (M) e 1 584 frutos de maturação incompleta (V) de acordo com a coloração da casca, após rigorosa seleção com relação à sanidade e tamanho.

Os frutos receberam os seguintes tratamentos: A-testemunha; B-imersão em parafina de ponto de fu-

1 Recebido para publicação el 22 de julho, 1982

* Professor Assistente Doutor do Departamento de Horticultura, Faculdade de Ciências Agronômicas do "Campus" de Botucatu, UNESP

** Professor Titular do Departamento de Tecnologia Rural, Escola Superior de Agricultura "Luiz de Queiroz", USP.

*** Professora Assistente Doutora do Departamento de Tecnologia dos Produtos Agropecuários, Faculdade de Ciências Agronômicas do "Campus" de Botucatu, UNESP.

são 56-58°C; C-sacos de polietileno; D-imersão em ortofenilfenato de sódio; E-imersão em ortofenilfenato de sódio e em seguida em parafina; F-imersão em ortofenilfenato de sódio e ensacados em sacos de polietileno; G-embalados em papel de seda tratado com difenil

Após os tratamentos, os frutos foram colocados em bandejas superpostas e armazenados em câmaras sob condições controladas com a temperatura de 5.6 a 7.2°C e a umidade relativa do ar de 85 a 90% e em condições ambientes com à temperatura variando de 18 a 30°C e a umidade relativa de 55 a 90%

No mesmo dia em que foram realizados os tratamentos, analisaram-se amostras de frutos nos dois estádios de maturação Durante o armazenamento, a intervalos de 15 dias, analisavam-se amostras para determinar o teor de ácido ascórbico do suco Essas determinações foram feitas pelo método de Tillmans modificado por Arzolla (2) e expressos em mg/100 g de suco As amostras foram constituidas por quatro frutos escolhidos ao acaso, fazendo-se duas repetições.

Convencionou-se chamar CO (zero), o ciclo inicial ou seja, o dia em que se realizaram os tratamentos, seguida pela análise do teor de ácido ascórbico A cada 15 dias se seguiram os ciclos CI, CII, CIII e CIV

O delineamento estatistico foi em blocos ao acaso segundo o fatorial 7 x 4 x 2 (tratamentos, ciclos e estádios de maturação) para os ensaios em condições controladas e fatorial 7 x 2 x 2 para os ensaios em condições ambientes. Os resultados foram analisados e comparados pelo este de Tukey segundo Gomes (7).

Antes das análises estatísticas, os dados foram preparados de modo a se chegar a uma distribuição normal dos erros, conforme Steel e Torrie (13). Deste modo, as porcentagens foram transformadas em arco seno $\sqrt{\%}$

Resultados e discussão

Os resultados obtidos nas condições de câmara com temperatura e umidade controladas, estão relacionados no Quadro 1 e nas condições ambientes, estão no Quadro 7

No Quadro 2, a análise de variância indica que todos os fatores interagiram entre si, mostrando que o estádio de maturação, os tratamentos e o periodo de armazenamento influiram conjuntamente sobre a conservação dos frutos

Pelo Quadro 3, nota-se no ciclo I que o teor de ácido ascórbico foi maior nos tratamentos F seguido pelos tratamentos G e E que não diferiram entre si e de-

Quadro 1. Valores de ácido ascórbico em mg/100 g no suco do maracujá amarelo sob efeito de 7 tratamentos, em 2 estádios de maturação, nos 4 ciclos de armazenamento sob condições de câmara fria.

Tratamentos	Estádios	Ciclos			
		I	II	III	IV
A Testemunha	M	22.75	23.25	16.00	9.00
	V	18.50	21.50	17.00	16.00
B Parafina	M	25.00	29.00	17.50	15.25
	V	20.00	14.00	17.00	13.50
C Sacos de polietileno	M	24.00	23.00	16.00	16.00
	V	24.25	20.50	17.50	17.00
D Ortofenilfenato de sódio	M	26.50	24.50	15.50	9.75
	V	25.00	20.00	16.00	16.50
E Ortofenilfenato de sódio + parafina	M	28.50	26.75	22.00	9.25
	V	24.50	19.00	14.00	14.50
F Ortofenilfenato de sódio + saco de poliet	M	29.00	25.00	21.00	21.00
	V	29.00	18.50	17.50	20.00
G Papel de seda + Difenil	M	25.00	20.75	13.00	12.75
	V	29.00	23.00	20.50	15.25

Quadro 2. Análise de variância para dados de teores de ácido ascórbico no suco do maracujá amarelo, mantido em condições de câmara fria.

C. Variação	G.L.	F
Estádios de maturação (E)	1	4.53*
Ciclos (C)	3	171.68**
Interação (E x C)	3	22.52**
Tratamentos (T)	6	9.35**
Interação (E x T)	6	11.23**
Interação (C x T)	18	3.96**
Interação (E x C x T)	18	3.59**

C.V. = 5.28%

* Significativo ao nível de 5% de probabilidade.

** Significativo ao nível de 1% de probabilidade.

cresceram dos tratamentos D até A. Já no ciclo teores não diferiram entre si ao nível de 5% pelo teste de Tukey enquanto que no ciclo III pode ser observado o efeito dos tratamentos na conservação do fruto pois o teor de ácido ascórbico do maracujá com o tratamento F permaneceu alto e continuou constante no ciclo IV enquanto que nos demais tratamentos, os teores sofreram quedas sensíveis.

Com relação aos ciclos dentro do tratamento, nota-se uma queda nos teores no tratamento A, D, E e G enquanto que no tratamento B, C e F as quedas foram menores. De forma geral, os resultados são concordantes com aqueles obtidos no maracujá roxo por Pruthi e Lal (11), que obtiveram nos frutos armazenados a 6.5°C, a queda gradativa de 30.4 mg/100 g até 12.5 mg/100 g de ácido ascórbico ao fim de 8 semanas.

Pelo Quadro 4 observa-se que para os frutos maduros o tratamento F foi o mais eficiente seguidos pelos tratamentos E, B e C que diferiram entre si pelo teste de Tukey, enquanto que para os frutos de maturação incompleta, os tratamentos G, F e D foram os mais eficientes.

Quadro 3. Valores médios dos tratamentos, e o valor obtido para a d.m.s. pelo teste de Tukey, para o teor de ácido ascórbico no suco do maracujá amarelo mantido em condições de câmara fria durante os 4 ciclos de avaliação (mg/100 g).

Tratamentos	Ciclos			
	I	II	III	IV
A	20.62 a C	22.37 a C	16.50 abB	12.50 a A
B	22.50 ab B	21.50 a B	17.25 abA	14.25 ab A
C	24.37 b B	21.75 a B	16.75 abA	16.50 b A
D	25.75 b C	22.25 a B	15.75 a A	13.20 a A
E	26.50 bcD	22.87 a C	18.00 abB	11.87 a A
F	29.00 cB	21.75 a A	19.25 bA	20.75 cA
G	27.00 bcD	21.87 a B	16.75 abA	14.00 ab A

d.m.s. (Tukey) à 5% = 3.00

Médias com letras iguais não diferem entre si.

Letras maiúsculas referem-se a ciclos dentro de tratamentos (linhas).

Letras minúsculas referem-se a tratamentos dentro de ciclos (colunas).

Quadro 4. Valores médios dos tratamentos, e o valor obtido para a d.m.s. pelo teste de Tukey, para o teor de ácido ascórbico no suco do maracujá amarelo nos estádios maduro e de maturação incompleta, mantido em condições de câmara fria (mg/100 g).

Tratamentos	Estádios	
	Maduro	Maturação incompleta
A	17 75 a A	18 25 b A
B	21 69 b B	16 12 a A
C	19 75 ab A	19 94 bcA
D	19 06 a A	19 37 bcA
E	21 62 b B	18 00 ab A
F	24 00 cB	21 25 cA
G	17 87 a A	21 94 cB

d m s (Tukey) a 5% = 2 12

Médias com letras iguais não diferem entre si

Letras maiusculas referem-se a estádios dentro de tratamentos (linhas)

Letras minusculas referem-se a tratamentos dentro de estádios (colunas)

Com relação aos estádios de maturação dentro do tratamento, observa-se que os frutos maduros, na maioria dos tratamentos, apresentou maiores porcentagens que nos frutos de maturação incompleta

No Quadro 5 observa-se que os teores de ácido ascórbico diminuiram durante o armazenamento tanto para os frutos maduros como para os maturação incompleta e que no ciclo I e III os teores não diferiram para os estádios de maturação enquanto que no ciclo II, os frutos maduros apresentaram teores mais altos mas no ciclo IV houve uma inversão provavelmente por falha de amostragem na maturação dos frutos.

No Quadro 6, a análise de variância mostra que os tratamentos, os estádios de maturação e os ciclos apresentaram diferenças significativas ao nivel de 1% de probabilidade bem como a interação estádio e tratamento com diferenças ao nivel de 5% de probabilidade para os frutos armazenados sob condições ambientes

Quadro 5. Valores médios dos dois estádios de maturação, para o teor de ácido ascórbico no suco do maracujá amarelo mantido em condições de câmara fria durante os 4 ciclos de avaliação (mg/100 g).

Ciclos	Estádios	
	Maduro	Maturação incompleta
I	25 82 cA	24.39 cA
II	24.61 cB	19 50 b A
III	17 28 b A	17 07 a A
IV	13 28 a A	16.11 a B

d m s (Tukey) a 5% = 1 39

Médias com letras iguais não diferem entre si

Letras maiusculas referem-se a estádios dentro de ciclos (linhas)

Letras minusculas referem-se a ciclos dentro de estádios (colunas)

Quadro 6. Análise de variância para dados de teores de ácido ascórbico no suco do maracujá amarelo, mantido em condições ambientes.

C. Variação	G.L.	F
Estádios de maturação (E)	1	89 16**
Ciclos (C)	1	8 85**
Interação (E x C)	1	1.37 n s
Tratamentos (T)	6	3 71**
Interação (E x T)	6	3 18*
T em EM	6	5.83**
T em EV	6	1 05n s
Interação (C x T)	6	1.06n s
Interação (E x C x T)	6	1 06n s

C.V = 7 18%

* Significativo ao nivel de 5% de probabilidade

** Significativo ao nivel de 1% de probabilidade

n s não significativo

Pelo Quadro 8, nota-se que os tratamentos B, E e D foram os que apresentaram os mais altos teores de ácido ascórbico, enquanto a testemunha A apresentou o mais baixo teor para o ciclo I enquanto que no ciclo II ainda o tratamento B foi o mais eficiente. Nas condições ambientes, o tempo de armazenamento foi a metade do tempo de armazenamento sob condições controladas ou seja, um mês já que cada ciclo corresponde a 15 dias. Como era esperado, a maioria dos tratamentos apresentou um teor mais alto no início e queda no teor durante o período.

No Quadro 9 ve-se que os ciclos não diferiram em relação ao estádio maduro enquanto que o teor de ácido ascórbico diminuiu durante o armazenamento para os frutos de maturação incompleta. Os frutos apresentaram em ambos os ciclos, valores mais altos que os de maturação incompleta.

Pelo Quadro 10, observa-se que o tratamento B foi o que melhor conservou o teor de ácido ascórbico do fruto maduro e que somente o tratamento C e A não foram eficientes, A por ser a testemunha e C porque a formação de água no interior do invólucro propiciou o desenvolvimento de microorganismos que aceleraram a maturação.

A queda no teor de ácido ascórbico parece ser devida a respiração. Loewus *et al* citado por Biale (3) postulou que o último precursor do ácido ascórbico é a glicose – 6 – fosfato. Duckworth (5) considera que durante a respiração, a glicose – 6 – fosfato é degradada no processo Embden-Meyerhof-Parnas (E.M.P.) seguida pelo ciclo de Krebs, daí a causa da diminuição no teor de ácido ascórbico dos frutos armazenados.

Segundo Pruthi (10), o teor de ácido ascórbico do maracujá varia até com o tamanho do fruto, por isso torna-se difícil interpretar o efeito dos tratamentos.

Conclusões

Dos resultados obtidos pode-se concluir o seguinte:

1. O teor de ácido ascórbico do suco do maracujá na forma "in natura" diminui gradativamente durante o armazenamento.

2. A redução no teor do ácido ascórbico é menor para os tratamentos que induzem a conservação por maior período de tempo como no caso da parafina e saco de polietileno.

Quadro 7. Valores de ácido ascórbico em mg/100 g no suco do maracujá amarelo sob efeito de 7 tratamentos em 2 estádios de maturação, nos 2 ciclos de armazenamento sob condições ambientes.

Tratamentos		Estádios	Ciclos	
			I	II
A	Testemunha	M	20.50	26.00
		V	17.50	15.50
B	Parafina	M	33.00	29.50
		V	19.00	19.50
C	Sacos de polietileno	M	20.50	19.50
		V	22.50	17.50
D	Ortofenilfenato de sódio	M	26.50	26.00
		V	22.00	17.25
E	Ortofenilfenato de sódio + parafina	M	29.00	27.25
		V	21.50	16.50
F	Ortofenilfenato de sódio + saco de poliet	M	28.50	25.00
		V	17.50	17.00
G	Papel de seda + Difenil	M	27.50	21.50
		V	19.25	15.00

Quadro 8. Valores médios dos tratamentos e o valor obtidos para a d.m.s. pelo teste de Tukey, para o teor de ácido ascórbico no suco do maracujá amarelo mantido em condições ambientes durante 2 ciclos de avaliação (mg/100 g).

Tratamentos	Ciclos	
	I	II
A	19.00 a	20.75 b
B	26.00 d	24.50 c
C	21.50 b	18.50 a
D	24.25 cd	21.62 b
E	25.25 d	21.87 b
F	23.00 bc	21.00 b
G	23.37 c	18.25 a

d.m.s. (Tukey) a 5% = 1.70

Médias com letras iguais não diferem entre si

Letras maiusculas referem-se a ciclos dentro de tratamentos (linhas)

Letras minusculas referem-se a tratamentos dentro de ciclos (colunas).

Quadro 9. Valores médios dos dois estádios de maturação para o teor de ácido ascórbico no suco do maracujá amarelo mantido em condições de ambientes durante 2 ciclos de avaliação (mg/100 g).

Ciclos	Estádios	
	Maduro	Maturação incompleta
I	26.50 a B	19.89 bA
II	24.96 a B	16.89 a A

d.m.s. (Tukey) a 5% = 2.06.

Médias com letras iguais não diferem entre si.

Letras maiusculas referem-se a estádios dentro de ciclos (linhas).

Letras minusculas referem-se a ciclos dentro de estádios (colunas)

Quadro 10. Valores médios dos tratamentos e o valor obtido para a d.m.s., pelo teste de Tukey, para o teor de ácido ascórbico no suco do maracujá amarelo no estado maduro mantido em condições ambientes. (mg/100 g).

Tratamentos	Estádio maduro
A	23.25 a
B	31.25 c
C	20.00 a
D	26.25 b
E	28.12 b
F	26.75 b
G	24.50 b

d.m.s. (Tukey) a 5% = 4.49.

Médias com letras iguais não diferem entre si.

3 Em câmaras sob condições de temperatura e umidade sob controle, a conservação é maior que nas condições ambientes.

4. Em ambas as condições de armazenamento, os frutos maduros apresentaram maior teor de ácido ascórbico.

Resumo

O valor de um suco de fruta, quase sempre é expresso pelo seu teor em vitamina C. No presente trabalho, frutos de maracujá amarelo, maduro e parcialmente maduro, foram conservados através do uso de germicidas, impermeabilizantes e envoltorios. Em seguida foram armazenados em câmaras com temperatura e umidade relativa do ar sob controle e também sob as condições ambientes. Semanalmente determinou-se o teor de ácido ascórbico, durante o período de dois meses. Os resultados revelaram que houve um decrescimo progressivo no teor de ácido ascórbico dos frutos, independente dos tratamentos utilizados, estádios de maturação e condições de armazenamento. Entretanto, constatou-se que, nos tratamentos que propiciaram maior conservação, o decrescimo no teor de ácido ascórbico foi menor.

Literatura citada

1 ABDULLAH, F e RAGAB, M. H. H. Vitamina C content, pH value and titratable acidity of 26 malayan fresh fruits grown in Selangor. Malaysia Agriculture Journal Kuala Lumpur, 47 416-428, 1970

2. ARZOLLA, J. D. P. Guia de trabalhos práticos. Piracicaba, Escola Superior de Agricultura "Luiz de Queiroz", 1969. 832 p

3 BIALE, J. B. The post harvest biochemistry of tropical and subtropical fruits. Advances Food Research, New York, 10:293-354, 1960

4 COOPER, B. e BROSTOWICZ, R. Estudo econômico da cultura do maracujá no Estado do Pará. Belém, SUDAM, Div. de Documentação, 1971. 119 p

5. DUCKWORTH, R. B. Frutas y verduras. 1 ed. Editorial Acribia, Zaragoza, 1968. 304 p

6 FONSECA, H. *et al.* Teor de ácido ascórbico e beta caroteno em frutas e hortaliças brasileiras. Archivos Latinoamericanos de Nutrición, Caracas, 19:9-16, 1969.

7. GOMES, F. P. Curso de estatistica experimental. 3 ed. Piracicaba, Escola Superior de Agricultura "Luiz de Queiroz", 1966. 404 p

8 HUET, R. Perspectivas d'industrialization des pays en voie de devélopment offertés par las production d'extraits aromatiques associée aux cultures fruitiéres. In: Expert Group Meeting on Processing Selected Tropical Fruits and Vegetables for Export to Premium Markets, Salvador, 1971. pp. 1-27

9 MARTIN, F. W. e NAKASONE, H. Y. The edible species of *Passiflora*. Economic Botany, Lancaster 24:333-343, 1970.

10 PRUTHI, J. S. Physiology, chemistry, and technology of passion fruit. Advances Food Research, New York, 12:203-282, 1963

11 PRUTHI, J. S. e LAL, G. Studies on the nutritive value and utilization of Purple Passion Fruits (*Passiflora edulis Sims*). Indian Journal of Horticultural, Sabour, 12:204-209, 1955

12 PRUTHI, J. S. e LAL, G. Chemical composition of passion fruit (*Passiflora edulis sims*). Journal of the Science of Food and Agriculture London 10:188-192, 1959.

13 STEEL, R. G. D. e TORRIE, J. H. Principles and procedures of statistics. New York, Mc Graw – Hill, 1960. 481 p.

Reseña de libros

HAINES, M. An introduction to farming systems. Longman, 1982. 214 p

A pesar del avance impresionante que la aplicación de la tecnología moderna ha logrado en el terreno industrial, la agricultura continúa siendo, en su contexto más amplio, la actividad más importante del hombre moderno

En los países económicamente desarrollados, la práctica de la agricultura afecta significativamente al ambiente ecológico y a la actividad social, política y económica. La necesidad de conservar el ambiente y la creciente conciencia política de los pueblos más avanzados, se han unido para cuestionar seriamente el papel que juega la agricultura en su forma actual de la vida y más importante aún, su papel como modeladora del mundo del futuro

El autor de este libro sustenta la tesis de que en el sector no agrícola de la población, existe un gran desconocimiento de lo que realmente es la agricultura y ello es una de las principales razones por las que se cuestiona su papel en la sociedad moderna. La obra entonces se escribió con el propósito de describir en forma integrada la actividad agrícola, de tal forma que sea comprensible por el público culto que no está directamente relacionada con la agricultura. Se espera que los conocimientos aportados a través del libro, contribuyan a reducir la brecha de entendimiento que existe entre el sector agrícola y el resto de la sociedad de los países avanzados, principalmente europeos.

Los capítulos introductorios explican la importancia de los factores socio-económicos y físico-biológicos como determinantes del tipo de sistema de producción agrícola existente en un lugar dado dentro de un país. Estos capítulos son generales y sus conocimientos aplicables al entendimiento de cualquier sistema de producción.

Los capítulos restantes son más específicos y en cada uno de ellos se trata un sistema de producción diferente. Entre estos sistemas tratados se encuentran: sistemas de producción de cultivos con labranza; sistemas de producción de leche; sistemas de producción animal basados en praderas de ladera; sistemas de producción mixtos cultivo/animal y por último sistemas intensivos de producción ganadera.

Además de estos capítulos, se pueden citar como partes importantes en esta obra, algunos comentarios acerca de la producción agrícola usando sólo insumos de tipo orgánico, que ha adquirido reciente popularidad en ciertos países y otra parte que dice relación con la comercialización de productos, siempre en el contexto de Europa.

Todos los ejemplos prácticos que aporta la obra han sido tomados de la agricultura actual en la Gran Bretaña, que guarda un alto grado de similitud, al menos físico-biológica, con otros países europeos. Para el estudioso de los problemas de la agricultura en los países subdesarrollados, esta obra sólo adquiere un carácter de referencia ocasional como punto de comparación

Indudablemente el autor cumple con su propósito de ilustrar en forma sencilla, pero al mismo tiempo completa, el conjunto de problemas de tipo ecológico y económico que enfrenta la agricultura actual en Gran Bretaña. A través de la obra, se percibe con claridad cómo la política agrícola común que siguen los países que pertenecen a la Comunidad Económica de Naciones, afecta no sólo la agricultura europea, sino a las posibilidades de exportación de los países del tercer mundo.

RAUL A. MORENO
CENTRO AGRONOMICO TROPICAL
DE INVESTIGACION Y ENSEÑANZA (CATIE)
TURRIALBA

COMUNICACIONES

Identificación de especies y biotipos de *Brucella* aisladas en Costa Rica.

Summary. This is the first report of the presence and identification of *Brucella* in Costa Rica *Brucella abortus* was isolated from eight bovine aborted fetus and from the blood of one human case All the bovine isolates belonged to *B. abortus* biotype 1 *Brucella suis* biotype 1 was isolated from one case of a suine aborted fetus An unknown variant of smooth *Brucella* was isolated from the testis of an infected dog The importance of these findings is discussed in relation to the animal and human brucellosis in the region

La brucelosis es una de las enfermedades bacterianas más importantes en veterinaria ya que afecta tanto al ganado de leche como al de carne (3, 7, 9), y es quizás la zoonosis de tipo ocupacional más frecuente (3, 10) Aunque usualmente no es una enfermedad letal, causa trastornos físicos y psíquicos en el hombre enfermo, lo que conduce a una disminución de su rendimiento laboral. Recientemente en Centroamérica, se han iniciado programas que tienen como fin controlar y erradicar la brucelosis (5, 9). El aislamiento e identificación de las especies y biotipos de brucelas es de vital importancia para comprobar el diagnóstico serológico y tener una idea clara del origen y distribución de la infección (3, 7). En este trabajo reportamos por primera vez en Costa Rica el aislamiento de dos especies de *Brucella* y de sus respectivos biotipos.

Materiales y métodos

Las muestras utilizadas para el aislamiento de las brucelas fueron el contenido abomasal de fetos de bovinos o suinos, el testículo de un perro adulto y la sangre de un hombre adulto con historia clínica de brucelosis. Los ocho fetos abortados de bovinos fueron recogidos en San Rafael y San Isidro de Heredia, Escazú, provincia de San José y en las faldas del Volcán Irazú de Cartago El perro infectado provino de la ciudad de Heredia y el hombre con historia clínica de brucelosis reside en Sarchí, provincia de Alajuela.

El aislamiento primario de las brucelas obtenidas de animales, se llevó a cabo en platos de agar sangre incubados en una atmósfera de CO_2 al 5% . El aislamiento en el caso humano se realizó en el medio difásico de Castañeda (2) Todas las brucelas se mantuvieron en agar tripticasa soya con 1% de suero humano o alternativamente se liofilizaron.

La producción de sueros inmunes monoespecíficos en conejos, el diagnóstico de los animales y humanos infectados y la identificación y biotipificación de brucelas por medio de pruebas bioquímicas y serológicas, se llevó a cabo según los métodos convencionales (1).

Resultados y discusión

Las aglutinaciones de los sueros provenientes del hombre y de los animales analizados presentaron títulos mayores de 500, por lo que serológicamente los individuos se consideraron como infectados (1). Las reacciones cruzadas con otras bacterias Gram negativas, especialmente con *Yersinia enterocolitica* tipo IX, rara vez alcanzan títulos superiores al de 200 (1, 5). La serología y la clínica se confirmaron con el aislamiento de la bacteria en los diferentes casos.

De los ocho fetos bovinos, se identificó *Brucella abortus* biotipo 1; del feto de suino abortado se aisló *Brucella suis* biotipo 1 y de la sangre del hombre con historia clínica de brucelosis se identificó *B abortus*, sin embargo, debido a que se perdió el aislamiento, no fue posible establecer el biotipo a que

pertenecia esta bacteria. Del testículo de un perro infectado, se aisló una especie lisa de *Brucella* la cual aglutinaba con suero monoespecífico contra *B. abortus*, pero que bioquímicamente presentaba características similares a *Brucella canis*

Este trabajo preliminar, establece por primera vez en Costa Rica la presencia de dos especies diferentes de *Brucella*. Los resultados obtenidos sugieren que el biotipo 1 de *B. abortus* es el más abundante en el Valle Central ya que fue el único identificado de los ocho aislamientos; lo anterior está de acuerdo con lo encontrado en otros países del Continente Americano (3, 7, 9). Actualmente, no se sabe si el biotipo 1 es el que predomina en el resto del país; la confirmación de esta suposición así como la posible identificación de nuevas especies y biotipos de *Brucella* requerirá de un muestreo extensivo de bovinos, suinos, caprinos, ovinos, caninos y humanos en diferentes regiones.

El aislamiento de *B. abortus* en un caso humano, complementa los estudios serológicos de Williams Tassara (10) y los casos clínicos mencionado por el Ministerio de Salud de Costa Rica (8), que indican que la brucelosis es una zoonosis frecuente en el país. Solamente durante el año 1983, el Ministerio de Salud identificó serológicamente 8 casos de brucelosis en individuos del sexo masculino. Es posible, que debido a la falta de un registro y reconocimiento adecuados de la enfermedad, esta cifra sea superior a la mencionada actualmente. Es importante señalar, que las dos especies aisladas (*B. abortus* y *B. suis*) son patógenas para el hombre. El aislamiento de una variante lisa de *Brucella* con características bioquímicas de *B. canis* obtenida del testículo de un perro infectado, presenta un problema interesante, ya que *B. canis* solamente se ha descrito en fase rugosa (6). En estos momentos se analizan las características del lipopolisacárido y de las proteínas de esta bacteria para determinar exactamente la especie de *Brucella* a que pertenece. El aislamiento de *B. suis* biotipo 1 confirma estudios serológicos (Moreno, E., resultados no publicados) en los que se sugiere la presencia de esta bacteria en cerdos de Costa Rica.

El reconocimiento de las especies y biotipos de *Brucella* es fundamental para establecer la distribución y el movimiento de la enfermedad en la región, así como en la evaluación del problema de zoonosis. Para poder determinar el riesgo biológico es importante identificar las especies y biotipos de brucelas que se encuentran en el país, ya que no todas son igualmente patógenas.

Los resultados obtenidos en este trabajo justifican un estudio intensivo de aislamiento y caracterización de las brucelas en Costa Rica lo que eventualmente servirá de apoyo a los programas nacionales de Control y Erradicación de la Brucelosis que han empezado a funcionar en Centro América y el Caribe (5, 9).

Agradecimientos

Se agradece al Dr. Manuel Padilla de la Escuela de Medicina Veterinaria de la Universidad Nacional por proporcionarnos dos muestras de sueros bovinos y al Dr. Ortiz Borbón por facilitar las muestras de porcinos. También se agradece la asistencia técnica de la Srta. Luz Marina Sánchez. Edgardo Moreno fue becario del Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT) de Costa Rica durante esta investigación.

Resumen

Este es el primer informe sobre la presencia e identificación de *Brucella* en Costa Rica. *Brucella abortus* se aisló de ocho fetos de bovinos que abortaron y de la sangre de un caso humano. Todos los casos aislados en bovinos pertenecían a *B. abortus* biotipo 1. El biotipo 1 de *Brucella suis* se aisló de un feto abortado de cerdo. Una variedad desconocida de *Brucella* se aisló de los testículos de un perro infectado. Se discute la importancia de estos hallazgos en relación con la brucelosis animal y humana en la región.

23 de diciembre de 1983

A. SEQUEIRA*
E. CAMPOS**
L. MENDOZA***
M. DE LOS A. SAN ROMAN****
E. MORENO*

* Escuela de Medicina Veterinaria UNA, Heredia, y C. I. B.C.M., Universidad de Costa Rica, San José Costa Rica.

** INCIENSA, Tres Ríos, Costa Rica

*** Escuela de Medicina Veterinaria, UNA, Heredia, Costa Rica

**** Hospital San Juan de Dios, CCSS, San José, Costa Rica.

Literatura citada

1. ALTON, G., JONES, L. M. y PIETZ, D. E. Laboratory techniques in brucellosis. World Health Organization Monograph Series No. 55 WHO/PAO. 2nd. edition V. Geneva, 1975.

2. CASTAÑEDA, R. M. Practical method for routine blood cultures in brucellosis. Proceedings of the Society for Experimental Biology and Medicine 64:114-115. 1947.

3. CENTER FOR DISEASE CONTROL, BRUCELLOSIS SURVEILLANCE, ANNUAL SUMMARY. Publication No (CDC) 76-8181. U.S., Department of Health Education and Welfare, Public Health Service, Atlanta, 1976.

4. DIAZ, R., BOSSERAY, N. Estudio de las relaciones antigénicas entre *Yersinia enterocolitica* serotipo 9 y otras especies bacterianas Gram negativas. Microbiología Española 27:1-4. 1974.

5. LEY No. 6243. Ley General de Salud. Reglamento sobre el control de la brucelosis en los animales. Ministerio de Agricultura y Ganadería de Costa Rica. Ciencia Veterinaria 1:64-74. 1979.

6. MORENO, E. Características y actividades biológicas del lipopolisacárido de *Brucella*. Adel. Microbiol. Enferm. Infec. 2:21-65. 1983.

7. NICOLETTI, P. The epidemiology of bovine brucellosis. C. A. Brandly y Charles E. Cornelius (eds). **In:** "Advances in Veterinary Science and Comparative Medicine" 24. 1980.

8. REPORTES DE ZOONOSIS EN COSTA RICA. Archivos, Ministerio de Salud, 1983.

9. VICENTE, G. Consideraciones sobre la vacunación antibrucelosa del ganado bovino en Costa Rica. Tesis para optar el grado de Magister en Salud Pública. Escuela de Salud Pública. Facultad de Medicina. Universidad de Chile, 1982.

10. WILLIAMS-TASSARA, O. La brucelosis bovina y humana en Costa Rica. Estudio preliminar. Tesis de grado. Universidad de Costa Rica. Facultad de Ciencias. Sección Microbiología, 1951.

Aplicación de N y de estiércol en la lechuga (*Lactuca sativa* L.).

Summary. The objective of this study was to know the amount and the application form of nitrogen and the levels of poultry manure (P.M.) more efficients in the lettuce (*Lactuca sativa* L. cv. Great Lakes, crisp head salad) production. We tested 3 levels of N (0, 100 and 200 kg/ha); three levels of P. M. (0 20 and 40 m^3/ha); and 3 application forms of nitrogen (total amount at transplanting time, on half at transplanting time, on half at transplanting and 1/2 20 days after, and one third at transplanting 1/3 20 days after and 1/3 40 days after transplanting). A factorial arrangement of treatments was used, in a randomized complete blocks desing, with three replications, at Santa Rosa Exp. Stn., Mérida, Venezuela, on a typic Humitropepts soil. The use of N increased the lettuce yield, however, there was not significative differences between 100 and 200 kg/ha. Poultry manuer also influenced the crop yield but we did not detect significative differences between 20 and 40 m^3/ha. We did not find significative interactions between N and P. M. applied. When N was used in fractions had effect neither on the crop yield nor on the loose heads percentage.

Las plantas de lechuga poseen un sistema radicular superficial y pequeño que requieren un suelo bien provisto de nutrimentos y de materia orgánica. Las aplicaciones de 20 a 40 toneladas de estiércol curado con 50 kg de N, 50 kg de K y 180 kg de P/ha, deben producir buenos resultados incluso en suelos pobres (1, 3, 10, 13, 16). La lechuga absorbe el 70% de sus nutrimentos durante el último 30% de su ciclo y el 80% del N en las 4 semanas anteriores a la cosecha; por tal motivo, necesita altos niveles de fertilidad en el suelo especialmente de N muy cerca de la cosecha (6, 8, 15). Debe tenerse cuidado al aplicar el N en la mitad o tercio final de su ciclo a las variedades que forman cabezas, porque un exceso de NO_3^- en esa etapa, tiende a promover crecimiento y a producir cabezas flojas no deseables comercialmente (9).

Las diferencias de N en lechuga, en medios carentes del elemento, ocurren alrededor de una semana después de la emergencia y aparecen cuando los niveles de NO_3^- N en las nervaduras principales de las hojas son menores de 5 000 ppm, en todo caso se retarda la formación de las cabezas y los rendimientos se reducen (7, 11, 12). Se ha señalado la fertilización nitrogenada a pH bajos, no aumenta los rendimientos de lechuga y algunas veces los reduce; sin embargo, el rendimiento es óptimo y los rendimientos son máximos a pH entre 6 y 7 (4, 14), el crecimiento es óptimo y los rendimientos son máximos.

En los Andes venezolanos, algunos productores de lechuga aplican estiércol bovino y la fórmula 10–10–15 a razón de 11 000 y 250 kg/ha respectivamente (5). El presente trabajo tiene como objetivos, i) conocer dosis N para el desarrollo y producción de lechuga, así como la forma de aplicación más adecuada para la formación de cabezas comercialmente deseable, ii) medir los beneficios de la aplicación de estiércol al suelo y la cantidad más efectiva para el logro de altos rendimientos en lechuga y iii) disponer de información confiable, que pueda ser recomendada a los productores de lechuga de la región.

Materiales y métodos

Una siembra de lechuga variedad Great Lakes, tipo cabeza rizada, fue sometida a diferentes niveles y formas de fertilización nitrogenada y a la aplicación de varias dosis de estiércol en un campo de la Estación Experimental "Santa Rosa", del Instituto de Investigaciones Agropecuarias, Universidad de Los Andes, Distrito Libertador, Edo. Mérida (08° 35' 30" N, 71° 08' 30" W), altitud 1 920 msnm, precipitación y temperatura anuales promedios de 12 años (1968-1979) de 2 072, 2 mm y 16, 9°C respectivamente

El suelo ha sido clasificado taxonómicamente como Humitropepts típico franco grueso/esquelético/franco, vermiculítico, isomésico (2) Se tomaron muestras del horizonte superficial (0–20 cm), revelando los análisis de laboratorio las características siguientes: textura franco arcilloso, pH 6.2, carbón orgánico 6.65% N total 0.402% c/N 16 5, P (Olsen) 44 ppm, K asimilable 120 ppm y mg asimilable 110 ppm

Durante el estudio se usó una población de 83 333 plantas/ha (0.4 m x 0 3 m de distancias de siembra). La preparación del suelo se hizo con bueyes, las parcelas se emparejaron y terminaron de acondicionar con escardilla. El 7–05–79 se sembraron dos semilleros de 10 m^2 cada uno; la germinación ocurrió a los 7 días. La aplicación de fertilizantes se hizo a mano durante el trasplante el 19–06–79 sobre parcelas individuales de 1.92 m^2, sobre 4 hileras de 1.2 m de largo, con un total de 16 plantas de lechuga por tratamiento. Las fuentes de fertilizantes fueron: estiércol de gallina (gallinaza) densidad: 0.52 ton/m^3, con una composición promedio de pH: 6.77, C. O.: 10.52% , N total: 0.67% , C/N: 15.7, P soluble: 0 011% , K soluble: 1 77% y mg soluble: 0 26% , urea con 46% de N, superfosfato triple con 46% de P_2O_5 y cloruro de potasio con 60% de K_2O

El diseño experimental usado fue un arreglo factorial 3^3, tres factores a tres niveles cada uno. Se apoyó 0.20 y 40 m^3/ha de estiércol de gallina (E) 0, 100 y 200 kg/ha de nitrógeno (N), todo el N al momento del trasplante, 1/2 N al trasplantar y 1/2 N 20 días después, 1/3 N al trasplantar, 1/3 N a los 20 días y 1/3 N 40 días después del trasplante. El diseño empleado fue de bloques al azar con 3 repeticiones y los 27 tratamientos que hacen las combinaciones 3 x 3 x 3. Todas las parcelas llevaron una fertilización complementaria consistente de 100 kg de P_2O_5 y 100 kg de K_2O por hectárea, aplicados al momento del trasplante.

Durante el ciclo del cultivo se hizo el control de malezas con escardilla, riego por aspersión para complementar los requerimientos hídricos de la lechuga, y fumigaciones periódicas de fungicidas y aplicaciones de insecticidas.

De la cosecha, realizada a mano el 20–08-79 sobre un área de 0.48 m^2, se estimó el rendimiento en kg/0 48 m^2, el peso promedio por planta y la conformación y dureza de las cosechas.

Sólo los datos de peso promedio por planta y conformación y dureza de las cabezas, se analizan en el presente trabajo.

Resultados y discusión

Las aplicaciones de N y de estiércol, aún cuando actuaron independientemente ejercieron su acción significativa sobre la producción de la lechuga. El fraccionamiento del N no tuvo ningún efecto sobre la misma (Cuadro 1).

La dosis más alta de estiércol (40 m^3/ha) produjo los mayores efectos sobre los rendimientos de lechuga, aunque no mostró diferencias significativas con la aplicación de 20 m^3/ha (0.66, 0.88 y 0.90 kg lechuga/planta cuando se aplicó 0.20 y 40 m^2/ha de estiércol) En la Figura 1 se muestra la respuesta de la lechuga a la aplicación de estiércol.

El suministro de 20 m^3/ha de estiércol, con una densidad de 0.52 ton/m^2 equivale a incorporar al suelo 10.4 ton/ha, concordando con las cantidades reportadas por Faillace *et al.* (5), para el cultivo de la lechuga en Los Andes.

La acción independiente del estiércol sobre el rendimiento de la lechuga, el poco desarrollo de su sistema radicular y el bajo contenido de mg en los suelos del estudio, nos mueve a considerar que el efecto principal del estiércol fue como acondicionador del horizonte superficial del suelo y como suplidor de mg y otros elementos menores deficientes coincidiendo con Añez (1) Añez y Pereyra (3) y con Thompson y Kelly (13)

El uso de 100 y 200 kg/ha de N tuvo un efecto significativo, sobre el rendimiento de lechuga, aunque no hubo diferencias significativas entre ambas dosis (0 65, 0.89 y 0.90 kg lechuga/planta cuando se aplicó 0, 100 y 200 kg N/ha).

El análisis de regresión muestra que el componente lineal fue el mayor responsable en la variabilidad de los rendimientos de lechuga a las diferentes dosis de nitrógeno empleadas (Figura 2). El análisis del porcentaje de cabezas abiertas, mal conformadas o poco compactas, no reveló diferencias significativas entre los tratamientos.

Cuadro 1. Análisis de varianza del peso de lechuga en kg/planta bajo diferentes dosis de N y de estiércol de gallina.

Fuente de Variación	Grados de Libertad	Suma de Cuadrados	F Calculada
Bloques	2	1 14	19 **
Tratamientos	26	2 56	3 28**
E	2	0 92	15 33**
Comp. Lineal	1	0 74	29 15**
Desv Comp Lineal	1	0 18	7 09**
N	2	1 03	17 17**
Comp. Lineal	1	0 80	26 67**
Desv Comp Lineal	1	0 23	7 67**
F	2	0 11	1 83
E x N	4	0 20	1 67
E x F	4	0 06	0 50
N x F	4	0 12	1 00
E x N x F	8	0 12	0 50
Error	52	1 32	--
Total	80	5 02	--

$\bar{Y}$ = 0 81 kg/planta de lechuga CV = 21 38%

Resumen

El objetivo de este estudio fue determinar la cantidad y forma de aplicaciones de N y la dosis de estiércol de gallina (Gallinaza), más efectiva en la producción de lechuga (*Lactuca sativa* L. var. Great Lakes). Se probaron dosis de (0. 100 y 200), dosis de (0 20 y 40 m^3 gallinaza/ha) y 3 formas de aplicación del N (todo al trasplantar, la mitad al trasplante y la otra mitad 20 días después y el tercer tratamiento consistió en añadir tercios al trasplante 20 y 40 días después del trasplante). El trabajo de campo fue realizado en un suelo Humitropepts típico, franco-arenoso de la Est. Exp Santa Rosa, Mérida, Venezuela.

Los rendimientos aumentaron con la aplicación de N, aunque sin diferencias significativas entre 100 y 200 kg/ha. El estiércol influyó también los rendimientos sin detectarse diferencias significativas entre 20 y 40 m^3/ha. No se consiguió interacciones significativas entre el N y el estiércol aplicados. El fraccionamiento del N no tuvo efecto sobre los rendimientos ni sobre el porcentaje de cabezas abiertas o mal conformadas.

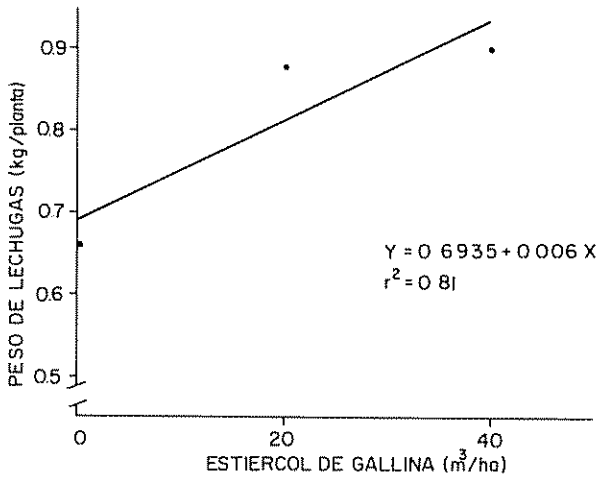

Fig 1 Relación dosis de estiércol y el rendimiento por planta

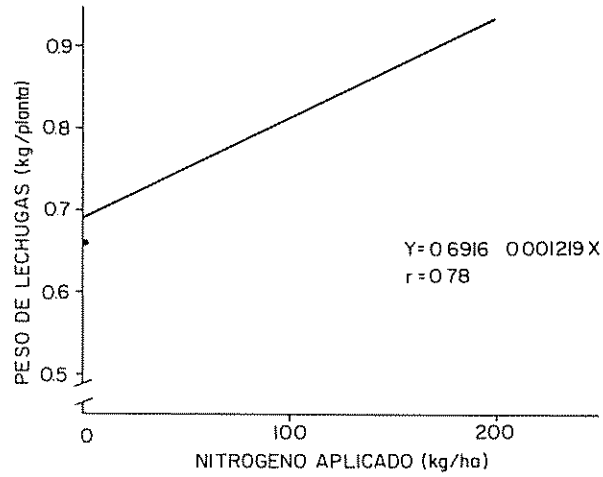

Fig 2. Relación dosis de nitrógeno y el rendimiento por planta

Agradecimiento

Los autores expresan su agradecimiento a Manuel Briceño y a María Marlene Aranguren, por su ayuda en el dibujo de las figuras y el trabajo de mecanografía respectivamente.

5 de julio de 1983

B. AÑEZ*
E. M. TAVIRA*

* Instituto de Investigaciones Agropecuarias (IIAP), Facultad de Ciencias Forestales U. L. A. Apdo 220, Mérida, Cod Postal 5101, Venezuela

Literatura citada

1 AÑEZ, B. El uso del estiércol en Los Andes. I.I.A.P., F.C.F., U.L.A., Mérida, Venezuela. 1979. 14 p.

2. AÑEZ, B. Fertilización química y orgánica en zanahoria (*Daucus carota* L.) Anexo. pp. 57-61. Trabajo de ascenso I.I.A.P., F.C.F., U.L.A., Mérida, Venezuela 1980. 61 p.

3. AÑEZ, B. y PEREYRA, J. Investigaciones agrícolas usando diseños experimentales de funciones continuas. Aplicación de estiércol en papa (*Solanum tuberosum* L.), en Cacute, Mérida. III Congreso Venezolano de la Ciencia del Suelo. Mérida, Venezuela. 1974. 15 p.

4. BORKOWSKI, J. Effect of calcium, nitrogen and potassium fertilizing on physiological diseases and on growth and yield of lettuce. Biuletyn Warzywniczy No. 22 pp. 345-359. 1977. Soils and Fertilizers 42(12):8 403. 1979.

5. FAILLACE, P. *et al.* Diagnóstico olericola de la región de Los Andes. Tomo II. Corpoandes. Mérida, Venezuela. Publicación No. 11, 1977. 457 p.

6. FERNANDEZ, P., de OLIVEIRA, G. y HAAG, H. Mineral nutrition of vegetable crops. XIV. Major nutrient absorption by a lettuce crop. O Solo 63(2):7-10. 1971. Horticulture Abstracts 43(9):5 911. 1973.

7. GARDNER, B. y PEW, W. Response of fall grown head lettuce to nitrogen fertilization. Arizona Agricultural Experimentation. Stn. Technical Bulletin. No. 199, 1972. 8 p. Horticulture Abstracts 44(4):2 358. 1974.

8. GARDNER, B. y PEW, W. Comparison of varius nitrogen sources for the fertilization of winter-grown head letucce. Journal of the American Society for Horticultural Science 103(4):534-536. 1979. Horticulture Abstracts 50(1):286. 1980.

9. Mac GILLIVRAY, J. Vegetable production. Mc Graw-Hill Book Co. Inc. New York. 1961.

10. MALAVOLTA, E. Manual de química agrícola. Adubos e adubação. Editora Agronómica "Ceres" Ltda. São Paulo. 1959. 398 p.

11. MARLATT, R. Nonpathogenic diseases of lettuce their identification and control. University of Florida. Technical Bulletin 721 A. 1974.

12. PEREZ, M. G., ESCALONA, L. y STEINER, A. Leaf analysis as a diagnosis of nutritional deficiency or excess in the soil less culture of lettuce. Plant and Soil 48(2):259-267. 1977. Soils and Fertilizers 41(5):2 977. 1978.

13. THOMPSON, H., y KELLY, W. Vegetable crops. 5th edition. Mc Graw-Hill, Co., New York. 1957.

14. WEIR, B., PAULSON, K. y LORENZ, O. The effect of ammoniacal nitrogen on lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) plants. Soil Science Society of American Proceeding 36(3):462-465. 1972.

15. WELCH, N. TYLER, K., RIRIE, D. Nitrogen stabilization in the Pajaro Valley in lettuce, celery and strawberries. California Agriculture 33(9):12-13. 1979.

16. WORK, P. y CAREW, J. Vegetable production and marketing. 2d. edition. John Wiley and Sons, Inc. New York. 1955.

Padrão de crescimento de raízes e parte aérea de mandioca (*Manihot esculenta*, Crantz), em condições de Cerrados do Distrito Federal.

Summary. To evaluate the growth of the roots and vegetative part (leaves + stems) on cassava (*Manihot esculenta*, Crantz) an experiment was carried out in the Cerrados National Research Center, Planaltina, Federal District, Brazil, from 1979 to 1982.

Stem cuttings, 20 cm long, of the cultivars IAC 352-7 (Jaçanã) Cavalo, Cacau-Vermelho and Sonora were planted equally spaced by 1.0 m. Six plants per cultivar were harvested every two months, from the sixth to the twenty-fourth month.

Two patterns of root growth were identified. One showed an increasing root weight up to the eighteenth month while the other showed an increase of root weight up to the twenty-fourth month. Maximum root yield for cultivars Jaçana, Cacau-Vermelho and Cavalo was obtained eighteen months after plantig, while for 'Sonora' maximum root yield was obtained twenty-four months after planting.

A mandioca assume posição destacada na conjuntura mundial. O Brasil, embora seja o maior produtor (9), investia pouca na sua pesquisa, em comparação com outras culturas (4), dada a sua pequena expressão econômica como cultura de subsistência. Um dos

aspectos pouco estudados na cultura da mandioca é o padrão de crescimento de raízes e parte aérea (2, 3, 5, 10).

As informações disponíveis sobre comportamento e crescimento de cultivares desta espécie nas condições de Cerrado são escassas. Baseando-se nesses fatos, foi conduzido um experimento, com objetivo de avaliar o crescimento das raízes e parte aérea, bem como determinar a época de maior produção de raízes de quatro cultivares de mandioca em um solo anteriormente sob vegetação de Cerrados.

Material e métodos

O experimento foi conduzido no período de 1979 a 1982, no Centro de Pesquisa Agropecuária dos Cerrados-CPAC, Planaltina-DF-Brasil, em um Latossolo Vermelho-Escuro argiloso (7). A análise química desse solo revelou pH 5.93; 0.03 meq de Al/100 g; 4.92 meq de Ca + Mg/100 g; 5.1 ppm de P e 27.1 ppm de K. Os métodos de análise química foram descritos por EMBRAPA (8).

A adubação foi realizada no sulco, antes do plantio e a 15 cm de profundidade, com 60 kg/ha de P_2O_5, 30 kg/ha de K_2O e 4.5 kg/ha de Zn. O nitrogênio foi aplicado em cobertura, na dose de 30 kg/ha, 45 dias após o plantio.

As manivas-sementes com 20 cm de comprimento, das cultivares IAC 352-7 (Jaçanã), Cavalo, Cacau-Vermelho e Sonora, foram plantadas a 10 cm de profundidade, no sentido horizontal, e com espaçamento de 1.0 x 1.0 m. O plantio foi feito em dois anos consecutivos: na segunda semana de outubro de 1979 e na primeira semana de novembro de 1980. A produção de raízes e parte aérea (folhas + ramas) foi avaliada de dois em dois meses, do 6º ao 24º mês após o plantio, através da colheita e pesagem de seis plantas de cada cultivar.

Resultados e discussão

Na Figura 1 pode ser observada a curva de produção de raízes e de parte aérea das quatro cultivares de mandiocas estudadas.

As cultivares Cavalo, IAC 352-7 (Jaçanã) e Cacau-Vermelho mostraram rápido e contínuo aumento de peso das raízes tuberosas, do 6º ao 18º mês. O incremento foi de aproximadamente 191% para a cultivar Cavalo, 302% para IAC 352-7 (Jaçanã) e 542% para Cacau-Vermelho (Quadro 1). Aparentemente não houve queda na produção de raízes durante este período, que abrangeu uma estação chuvosa (outubro-abril) e uma estação seca (maio-setembro), segundo EMBRAPA (6).

Conforme se pode observar na Figura 1, a produção de raízes decresceu do 18º ao 22º mês nas cultivares Jaçanã e Cacau-Vermelho, e até o 24º mês na cultivar Cavalo. A redução em peso foi de 41.36 e 18% , respectivamente (Quadro 1). O decréscimo da produção de raízes dessas cultivares ocorreu no segundo ciclo da cultura, durante a estação seca, quando a precipitação total registrada foi de 95.2 mm e a temperatura média foi de 21.0°C (Quadro 2). Esses

Quadro 1. Produção de raízes e de parte aérea (kg/planta) das cultivares de mandioca IAC 352-7 (Jaçanã), Cacau-Vermelho, Cavalo e Sonora, em colheitas do 6º ao 24º mês. CPAC, Planaltina-DF, 1979-1982.

Cultivar	Mês / kg/planta	6º	8º	10º	12º	14º	16º	18º	20º	22º	24º
IAC 352-7 (Jaçanã)	Raiz	0.49	0.50	0.78	0.90	1.49	1.57	1.97	1.48	1.17	1.74
	Parte aérea	0.59	0.47	0.73	0.82	1.34	1.24	1.20	0.80	0.75	0.78
Cacau-Vermelho	Raiz	0.26	0.76	0.57	0.97	1.02	1.39	1.67	1.17	1.07	1.31
	Parte aérea	0.50	1.82	0.81	1.46	1.50	1.87	1.52	0.82	0.77	1.06
Cavalo	Raiz	0.78	0.49	0.56	0.74	1.33	1.65	2.27	2.16	1.97	1.87
	Parte aérea	1.04	0.57	0.57	0.90	1.18	1.03	1.24	0.84	0.74	0.58
Sonora	Raiz	0.76	0.89	0.86	1.42	0.92	1.21	1.35	1.67	1.90	2.17
	Parte aérea	0.62	0.88	0.68	0.93	0.73	0.82	0.50	0.60	0.81	0.92

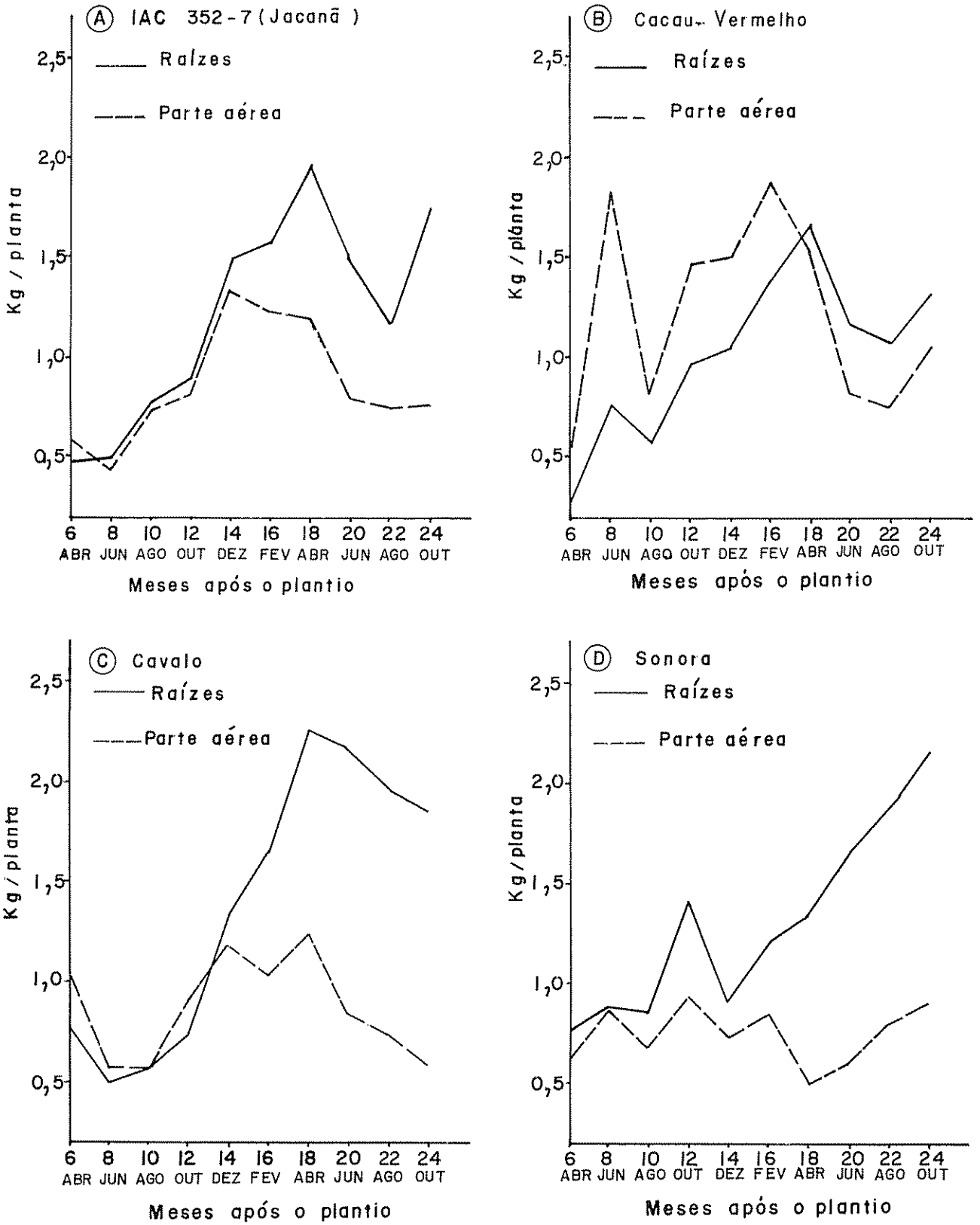

Fig. 1. Curvas de produção de raizes e de parte aérea das cultivares IAC 352-7 (Jaçanã), Cacau-vermelho, Cavalo e Sonora, resultantes das colheitas do 6º ao 24º mês. CPAC, Planaltina-DF.

Quadro 2. Distribuição mensal da precipitação, temperatura e umidade relativa do ar no período de outubro de 1979 a setembro de 1982. CPAC, Planaltina-DF.

Meses	Precipitação (mm)	Temperatura (°C)	Umidade relativa (%)
Out	188 3	23 1	67
Nov	155 1	22 3	76
Dez	230.0	22 0	77
Jan	339 0	21 7	81
Fev	182 3	22.3	75
Mar	226 1	22 5	76
Abr	73 2	21 7	74
Mai	23 5	20 7	70
Jun	11 9	20 7	68
Jul	6 5	19 1	63
Ago	15 6	21 3	60
Set	37 7	23 1	56

dados concordam com Correa e Andrade (2), que estudaram o crescimento de outras cultivares, em condições de Cerrado, o que pode ser atribuído a características genéticas das cultivares

As curvas de produção de raízes e de parte aérea tendem ao paralelismo (Figura 1), e isso é particularmente evidenciado na cultivar Cacau-Vermelho Essa tendência também foi observada por Corea e Andrade (2) com as cultivares Arrebenta-Burro, SF-2473 e Sertaneja. Portanto, o crescimento da parte aérea da mandioca pode ser um indicador da produção de raízes para as cultivares citadas, embora o ponto de máximo crescimento não coincida com o máximo rendimiento de raízes

A curva de produção da cultivar Sonora difere das demais estudadas por mostrar um aumento contínuo na produção de raízes durante o segundo ciclo, sem decréscimo após o 18º mês (Figura 1). Carvalho *et al.* (1), trabalhando com a cultivar Sonora em condições de solo e clima diferentes da região dos Cerrados, não verificaram aumento na produção de raízes do 20º ao 24º mês Presume-se, portanto, que esta discordância seja devido às diferentes condições em que foram conduzidos os experimentos.

Dos resultados obtidos podemos identificar dois padrões de crescimento de raízes durante os dois ciclos da cultura da mandioca, em condições de Cerrados. O primeiro padrão mostra um aumento na produção até o 18º mês, seguido por uma queda no peso das raízes, conforme pode ser observado na Figura 1, para as cultivares IAC 352-7 (Jaçanã), Cacau-Vermelho e Cavalo O segundo padrão, exemplificado pela cultivar Sonora, caracteriza-se por crescimento de menor intensidade no primeiro ciclo, seguido por aumento contínuo no peso das raízes até o 24º mês

A diferença nos padrões de crescimento das cultivares estudadas evidencia diferentes reações dos genótipos da espécie *Manihot esculenta* às condições de Cerrados. Esse aspecto tem grande importância agronômica, pois o fato de certas cultivares chegarem ao seu máximo de produção em um período mais curto possibilita o melhor atendimento a diferentes sistemas de produção.

Conclusões

1. Sob condições de Cerrados e em dois ciclos da cultura de mandioca foi possível identificar dois padrões de crescimento de raízes: um com aumento progressivo até o 18º mês, seguido de redução, e outro sem decréscimo no segundo ciclo.

2. As cultivares Cavalo, IAC 352-7 (Jaçanã) e Cacau-Vermelho mostraram incremento na produção de raízes de 191, 302 e 542%, respectivamente, no período do 6º ao 18º mês de plantio.

3. A producão de raízes decresceu do 18º ao 22º mês para cultivar IAC 352-7 (Jaçanã) e Cacau-Vermelho, e até o 24º mês para a cultivar Cavalo A cultivar Sonora mostrou aumento contínuo durante o 2º ciclo da cultura

Resumo

Com o objetivo de avaliar o crescimento de raízes e parte aérea (folhas + ramas) e determinar a época de maior produção de raízes de quatro cultivares de mandioca (*Manihot esculenta*, Crantz), foi conduzido um experimento no CPAC, Planaltina, DF, no período de 1979 a 1982. Manivas-sementes das cultivares IAC 352-7 (Jaçanã), Cacau-Vermelho, Cavalo e Sonora, com 20 cm de comprimento, foram plantadas com espaçamento de 1.0 x 1.0 m. A colheita foi realizada de dois em dois meses, do 6º ao 24º mês.

Dois padrões de produção de raízes foram identificados. O primeiro é caracterizado pelo aumento no peso das raízes até o 18º mês, e o segundo ate o 24º. A máxima produção de raízes das cultivares IAC 352-7 (Jaçanã), Cacau-Vermelho e Cavalo foi obtida no 18º mês após o plantio e da cultivar Sonora no 24º mês.

Agradecimentos

Os autores agradecem ao Técnico Agrícola Wilmar Lacerda pela ajuda na condução do experimento e a Antônio de Pádua Carneiro pela revisão do texto original.

13 de Julho, 1983

I. R. COSTA*
N. M. A. NASSAR**
S. PERIM*

* Pesquisadores do Centro de Pesquisa Agropecuária dos Cerrados - CPAC. BR 20 - km 18 - Caixa Postal 70-0023 - CEP 73.300 - Planaltina-DI Brasil

** Professor de melhoramento de plantas do Dept^{O} de Agronomia da Universidade de Brasília, UnB, Brasília-DI

Literatura citada

1. CARVALHO, V. D de; CHALFOUN, S. M.; TANAKA, M. A de S.; MORAES, A. R. de e CARDOSO, D. A. M. Influência da época de colheita sobre a produtividade e composição química de cultivares de mandioca. **In**: Empresa de Pesquisa Agropecuaria de Minas Gerais. Belo Horizonte. Projeto mandioca: relatório 76/79. Belo Horizonte, 1982. pp. 25-82.

2. CORREA, H. e ANDRADE, A. M. de S. Introdução de clones e cultivares de mandioca. **In**: Empresa de Pesquisa Agropecuaria de Minas Gerais. Belo Horizonte. Projeto mandioca: relatório 76/79. Belo Horizonte, 1982. pp. 91-114.

3. EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA. Centro Nacional de Pesquisa de Mandioca e Fruticultura. Relatório Técnico Anual do Centro Nacional de Pesquisa de Mandioca e Fruticultura 1977. Cruz das Almas, 1979. 125 p.

4. EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA. Centro Nacional de Pesquisa de Mandioca e Fruticultura. Relatório Técnico Anual do Centro Nacional de Pesquisa de Mandioca e Fruticultura 1979. Cruz das Almas, 1980. 183 p.

5. EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA. Centro Nacional de Pesquisa de Mandioca e Fruticultura. Relatório Técnico Anual do Centro Nacional de Pesquisa de Mandioca e Fruticultura 1981. Cruz das Almas, 1982. 209 p.

6. EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA. Centro de Pesquisa Agropecuária dos Cerrados. Relatório Técnico Anual do Centro de Pesquisa Agropecuária dos Cerrados 1975/76. Brasília, 1976. 154 p.

7. EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA. Serviço Nacional de Levantamento e Conservação de Solos. Levantamento de reconhecimento dos solos do Distrito Federal. Rio de Janeiro, 1978. 455 p. (Boletim Técnico, 53).

8. EMPRESA BRASILEIRA DE PESQUISA AGROPECUARIA. Serviço Nacional de Levantamento e Conservação de Solos. Rio de Janeiro, RJ. Manual de métodos de análise de solo. Rio de Janeiro, 1979.

9. FAO. Roma. Production yearbook. Roma, 1980. v. 34.

10. MENDES, C. T. O cyclo vegetativo na mandioca. Revista de Agricultura, Piracicaba, 4(11-12): 471-490. 1929.

Isolation of mesophyll protoplasts of the genus *Coffea*.

Resumen. Se describe un método rápido para el aislamiento de protoplastos a partir de hojas de café, probándose diferentes enzimas en varias combinaciones. Es posible liberar abundante cantidad de protoplastos a partir de hojas jóvenes, provenientes de varias líneas y cruzamientos, de las especies *Coffea arabica* y *C. canephora*, mediante su incubación durante 4 horas en celulasa (3%), pectoliasa (0.5%) y manitol (0.6 molal) a pH 5.8. Los protoplastos se filtraron y lavaron varias veces en agua de mar (85%), y fue necesario resuspenderlos en percoll (70%) debido a su densidad. Los protoplastos sobreviven varias semanas, y regeneran pared celular. En uno de los medios (A 43), después de 2 semanas, se observan algunas divisiones. Se están probando varios medios de cultivo con diferentes concentraciones de hormonas.

The potential of protoplast culture and fusion in plant breeding is well known (8, 9) and this is not an exception for the genus *Coffea*. In this genus several genetic and chromosomic barriers exists between the

cultivated tetraploid species *Coffea arabica* and the diploid wild type species. Therefore it is difficult to transfer desirable genetic traits from the wild type species to the cultivated *Coffea arabica*. Somatic hybridization via protoplast fusion could be an excellent complement to conventional coffea breeding programs.

Only a few attempts have been made in coffee to isolate and culture protoplasts, and this with only little success. Söndahl *et al.* (6), reported protoplast liberation and possible callus formation of coffee protoplasts derived from callus tissue. In another short communication, the isolation of few mesophyll protoplasts after a long period (13-16 h) of enzyme treatment was reported (7). It is obvious that it could be of great advantage to have also for coffee methods which allow the isolation of mesophyll protoplasts in large numbers during a relative short time of enzyme incubation. The present technical note describes a method which leads in a short time to large numbers of mesophyll protoplasts of several coffee lines and species.

Leaf protoplasts in general have the advantage that they possess a defined chromosome number in comparison to callus or cell suspension derived protoplasts which make them more useful for somatic hybridization experiments.

Material and methods

Lines of the following species were used for the experiments: *Coffea arabica*, *Coffea canephora*, a sexual hybrid line between *Coffea arabica* and *Coffea canephora*, all obtained from Cenicafé (Colombia). These lines and species were cultivated in the greenhouse. Another sexual hybrid between *Coffea arabica* and *Coffea canephora* (Arabusta) which were obtained from the Laboratoire de Culture in Vitro, GERDAT (France) were cultivated as aseptic shoot cultures on MS agar medium (3), supplemented with 40 g/l sucrose and 1 mg/l BAP (6-benzylaminopurine). Before use, leaves from greenhouse plant material were surface sterilized by 7% sodium hypochlorite for 10 min. and subsequently washed 3 times with autoclaved tap water. For protoplast isolation leaves of different developmental stages were cut with a razor blade into small pieces (2-3 mm^2) in the presence of 0.3 M mannitol and were subsequently transferred into the various enzyme mixtures. The following enzymes dissolved in 0.6 M mannitol (ca. 730 mOsm), pH 5.8, were tested in various concentrations and combinations: Cellulase "Onozuka" R 10 and macerozyme R 10 (Kinki Yakult, Japan), cellulase 2230A (Röhm, FRG) pectolyase Y-23 (Seishin Pharmaceutical Co., Japan), driselase (Kyowa Hakko Kogy, Japan) and lysozyme (Worthington Biochem. Corp., USA).

The enzyme incubation was carried out for 4-24 h on a roller (2 rpm) at 25°C. After incubation the protoplast suspensions were sieved to remove the undigested leaf material and washed two times with seawater (ca. 730 mOsm) by centrifugation. After washing the protoplasts were resuspended in 0.6 M sucrose or 70% percoll dissolved in 0.6 M mannitol and centrifuged for 10 min. The protoplast containing supernatant was diluted with 85% seawater (1:5) and recentrifuged to remove percoll. The pellet containing the protoplasts was finally suspended in the protoplast regeneration media V 47 according to Binding (1) or A 43, according to Poirier-Hamon *et al.* (5).

Results and discussion

The enzyme combinations and concentrations tested for protoplast isolation of coffee are listed in Table 1. No protoplast release could be obtained after enzyme treatment up to 24 h of old and fully expanded leaves with all enzyme mixtures used. However, by 5 h treatment of young leaves with cellulase

Table 1. Treatment of fully expanded old and of young leaves from coffee plants with various enzyme combinations for protoplast release.

Enzyme	Old leaves	Young leaves
Driselase (2.5%) Macerozyme R 10 (2%)	–	–
Lysozyme (2%) Macerozyme R 10 (1%)	–	–
Cellulase R 10 (3%) Macerozyme R 10 (1%)	–	+
Cellulase 2230 (3%) Macerozyme R 10 (1%)	–	+
Driselase (2.5%) Pectolyase Y-23 (0.3%)	–	–
Lysozyme (2%) Pectolyase Y-23 (0.3%)	–	–
Cellulase R 10 (3%) Pectolyase Y-23 (0.5%)	–	+++
Cellulase 2230 (3%) Pectolyase Y-23 (0.5%)	–	+++

+ = few, +++ = satisfactory

R 10 or cellulase 2230 in combination with macerosyme R 10, a few protoplasts could be obtained Longer treatment, however, gave no higher yield of protoplasts. Much higher yields of protoplasts could be obtained with all lines and species tested if young leaves, less than one month old, were treated for 4 h with cellulase R 10 or cellulase 2230 in combination with pectolyase Y-23. Short time (25 min) treatments with these enzyme mixtures were not successful as it is for example the case for tobacco (4), *Datura* and *Petunia* (Schieder, unpublished)

After sieving and washing the protoplasts, the suspensions contained still broken protoplasts and undigested cells. To separate them from the protoplasts, the pellets were resuspended in 0.6 M sucrose and centrifuged. However, the protoplasts did not float in the supernatant as is observed for protoplasts of most other species (2). Much better results could be obtained if the protoplasts were suspended in 70% percoll dissolved in 0 6 M mannitol The protoplasts of coffee, though relatively small seem to be more dense than protoplasts of other species which makes it necessary to centrifuge them for floating in a solution with a higher density

The washed protoplasts suspended and cultured in the V 47 or A 43 medium survived for more than 3 weeks. They showed cell wall resynthesis and changes in their shape In the A 43 medium after 3 weeks some divisions could already be observed. Further cultivation experiments with different hormone concentrations and combinations and also with other protoplast regeneration media are under way.

Summary

A quick test for coffee mesophyll protoplasts isolation is described. By using combinations of enzymes it was possible to isolate numerous protoplasts of young leaves of hybrids of *Coffea arabica* and *C canephora* Treatments were incubated for four hours with 3% cellulase. 0.5% prectolyase and 0 6 molal manitol, at pH 5.8 Protoplasts were filtered, washed with 85% see water and resuspended in 70% percoll due to this density The protoplasts were able to survive for a few weeks and regenerated the cell wall. In media A 43 the protoplasts with some sell division was observed. New analysis are in the way trying different hormons concentration in the medio.

Acknowledgement

For F J Orozco. this research was partially supported by the Deutsche Stiftung für Internationale Entwicklung.

July 7, 1983

F J. OROZCO*
O. SCHIEDER**

* On leave from Cenicafé, Chinchiná, Colombia

** Max-Planck-Institut für Züchtungsforschung (Erwin-Baur-Institut) 5000 Köln 30, FRG

Literature cited

1. BINDING, H. Regeneration von haploiden und diploiden Pflanzen aus Protoplasten von *Petunia hybrida* L. Z. Pflanzenphysiol. 74:327-356 1974.

2. DAVEY, M. R., BUSH, E and POWER, J. B. Cultural studies of a dividing legume leaf protoplast system. Plant Science Letters 3:127-133 1974.

3. MURASHIGE, T. and SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15:473-497. 1962

4. NAGATA, T. and ISHII, S. A rapid method for isolation of mesophyll protoplasts. Canadian Journal Botany 57:1 820-1 823. 1979.

5. POIRIER-HAMON, S., RAO, P. S. and HARADA, H. Culture of mesophyll protoplasts and stem segments of *Antirrhinum magus* (Snapdragon): Growth and organization of embryoids. Journal of Experimental Botany 25(87):752-760. 1974.

6. SÖNDAHL, M. R., CHAPMAN, M. S. and SHARP, W. R. Protoplast liberation cell wall reconstruction, and callus proliferation in *Coffea arabica L. callus tissues*. Turrialba 30(2):161-165. 1980

7. SÖNDAHL, M. R. and MARTINS, J. S. Isolamento e cultura de protoplastos de folhas de *Coffea arabica* e *Nicotiana tabacum*. **In:** Congreso Brasileiro de pesquisas cafeiras 8a. Campos de Jardao, Brasil. Resumo I.B.C. 1980, p. 220

8. SCHIEDER, O Somatic hybridization: a new method for plant improvement. XIII International Botanical Congress. **In:** Plant improvement and somatic cell genetics (eds. I Vasil and W. R Scowcroft) V 13:206-220. 1982

9. VASIL, I. K. and VASIL, V. Isolation and culture of protoplasts. **In:** International review of cytology Supplement 11B 1980. pp. 1-19

Agriculture sur brûlis en forêt tropicale humide. A propos du rôle du feu dans la compétition entre espèces cultivées et espèces pionnières

Resumen. El análisis de los conocimientos actuales sobre la regeneración natural en la selva tropical húmeda y el impacto del fuego sobre dicha regeneración, permiten explicar la reducción de la competencia entre las plantas cultivadas y las plantas pioneras durante las primeras etapas del cultivo en el sistema de rozatumba – quema. La ventaja competitiva temporal de las plantas cultivadas se considera aquí como un resultado de la eliminación por el fuego de la mayor parte de las semillas y plántulas que normalmente aseguran una colonización rápida y vigorosa de los "chablises"

Rôles du feu dans l'agriculture sur brûlis traditionnelle

L'agriculture sur brûlis ou agriculture itinérante (agriculture migratoria, shifting cultivation) est, à l'heure actuelle, l'un des systèmes agricoles les plus importants, sinon le plus important, de la zone tropicale humide. D'après une estimation récente, ce systhème serait pratiqué par environ 140 millions de personnes, sur une surface de 2 millions de km^2, soit $1/5^{ème}$ du biome forêt tropicale humide (35).

On peut distinguer, avec Watters (45), une agriculture itinérante traditionnelle, pratiquée de longue date par des populations vivant en équilibre avec le milieu forestier, d'une "agriculture itinérante imposée par la nécessité", résultat de conditions socio-politiques relativement récentes, dans laquelle "l'agriculteur est un vrai colon, à la recherche de terres nouvelles à cultiver", qu'il utilisera "jusqu'à épuisement complet du sol" (45).

L'agriculture itinérante traditionnelle peut être caractérisée, pour une parcelle de forêt donnée, par un défrichement "doux" – simple abattage des arbres – suivi d'un brûlage, par la plantation quasi simultanée d'espèces cultivées variées dont la récolte s'étale sur une périodo de deux oux trois ans, et par une jachère jorestière de durée relativement longue.

Dans ce type d'agriculture, dont on ne peut nier l'efficacité dans le cas de populations à faible densité en équilibre démographique, trois types d'effets positifs du feu ont été reconnus:

- Après l'abattage des arbres, la parcelle défrichée est encombrée de troncs, branches et lianes entremêlés, offrant au regard l'image d'un paysage chaotique décourageant toute volonté de plantation; le feu a donc un rôle évident de **dégagement** et de **nettoyage** de l'abattis (34, 36).
- Le rôle **fertilisant** du feu est unanimement reconnu; il consiste en un enrichissement temporaire du sol en éléments minéraux, accompagné néanmoins de pertes importantes en azote et en soufre. On trouvera une revue détaillée de l'effet du feu sur les caractéristiques physiques et chimiques du sol dans, notament, Nye et Greenland (36), Watters (45), Fontaine *et al* (11).
- Enfin, on reconnaît au feu un rôle **phytosanitaire** important, les fortes températures ayant pour effet la destruction des populations de phytophages et de parasites (2, 23, 32, 34).

Ce sont là les raisons invoquées habituellement pour expliquer la croissance plus vigoureuse des plantes sur les sols préalablement brûlés (27, 36).

A ces trois effets positifs du feu, je voudrais ajouter ici, à la lumière des connaissances actuelles sur les premiers temps de la régénération naturelle, l'impact du feu sur la compétition entre plantes cultivées et plantes pionnières. Cet impact, s'il apparaît de manière implicite dans certaines études (6, 7, 23, 36), n'a jamais été clairement explicité; or, il semble qu'il s'agisse là, pour l'agriculture sur brûlis traditionnelle, d'un rôle positif du feu au moins aussi important que l'effect fertilisant ou phytosanitaire.

Ecologie des premiers temps de la régénération naturelle en forêt tropicale humide

Il s'agit ici de dresser un bilan schématique des connaissances actuelles sur la question, bilan qui permet d expliquer la colonisation rapide et vigoureuse des défrichements non brûlés par des espèces pionnières issues de la forêt naturelle avoisinante.

Lors d'un chablis, trouée forestière causée par la chute d'un ou plusieurs arbres (37), ou d'un défrichement, l'instant initial de la régénération que constitue l'ouverture du milieu est absolument fondamental à considérer, puisque c'est à ce moment que se fixe le potential floristique (1), qui va assurer la colonisation du milieu ouvert. Au cours de cette phase, l'avantage compétitif des premiers occupants doit être souligné (1, 12, 30), dû en majeure partie à la capture d'une fraction disproportionnée des ressources de l'environnement par les individus qui émergent rapidement (20).

On peut distinguer, au sein des forces floristiques en présence lors de l'ouverture du milieu forestier, trois ensembles (la terminologie employée est celle d'Alexandre (3) choisie ici pour son caractère concis et expressif):

- Le **potentiel végétatif** est formé par l'ensemble des individus survivant à l'ouverture du milieu; il est constitué des plantules au sens large, "seedling" et "sapling", ainsi que des rejets et drageons

- Le **potentiel extérieur** est constitué par l'ensemble des diaspores susceptibles d'envahir le site; il faut souligner la lenteur relative de l'établissement de cet ensemble floristique (8, 38, 41), mais aussi sa constance temporelle, dépendant de la phénologie et des caractéristiques de la dissémination des espèces extérieures à la zone ouverte

- Le **potentiel séminal édaphique**, enfin, constitue ce qu'on appelle habituellement le réservoir de graines du sol. Il est composé, d'une part de graines d'espèces forestières, d'autre part de graines dormantes d'espèces pionnières

  Les espèces forestières, c'est-à-dire, les espèces qui germent et se développent généralement au sein du sous-bois, possèdent le plus souvent des graines à durée de vie courte et germination rapide, indépendante des conditions lumineuses.

  Les espèces pionnières sont typiquement héliophiles et ne trouvent les conditions favorables à leur germination et à leur développement qu'au niveau de trouées suffisamment grandes pour provoquer un bouleversement microclimatique à la surface du soil (10, 18, 21, 22).

  L'existence et l'omniprésence d'un important stock de graines dormantes d'espèces pionnières, en attente dans le sol des forêts tropicales humides, sont largement démontrées que ce soit en Afrique (2, 4, 17, 28), dans le Sud-Est Asiatique (8, 31, 40), ou en Amérique (16, 24, 38, 41)

En forêt naturelle, le chablis constitue le moteur de la dynamique sylvigénétique, entraînant un rajeunissement ponctuel lorsque les dimensions de la trouée sont suffisantes. Dans la compétition opposant les ensembles floristiques, il semble qu'il existe une balance entre potentiel végétatif et potentiel séminal édaphique, dont le bilan est fonction de la taille des trouées (3, 9, 18, 21, 37, 39, 47), lorsque la perturbation est faible, la cicatrisation de la trouée est assurée par la stimulation du développement des plantules et des arbres préexistants. Passé un certain seuil, la perturbation favorise la germination et le développement des espèces pionnières, du moins dans les zones les plus éclairées. Enfin, dans le cas d'un défrichement de taille comparable à celle d'un abattis (de l'ordre de l'hectare), ce sont les espèces pionnières, issues principalement du potentiel séminal édaphique, qui assurent la domination pendant la première phase de la régénération.

Pendant cette phase, le potentiel extérieur n'a qu'une importance minime, en raison de la lenteur relative de l'apport de graines d'une part, et de l'avantage compétitif des premiers occupants, d'autre part; ainsi, les éléments de ce potentiel en sont le plus souvent réduits à l'occupation de sites particuliers, tels que souches et troncs à terre

Impact du feu sur la régénération naturelle implications pour l'agriculture sur brûlis traditionnelle

Dans une parcelle de forêt défrichée et brûlée, les différences locales de l'intensité du feu (température et durée) ont pour effet la création d'un paysage en mosaïque de troncs et souches plus ou moins calcinés de sol plus ou moins recouvert de cendres, et de zones visiblement peu brûlées (5, 12, 14, 41). Dans les partties fortement brûlées, les températures peuvent atteindre, d'après UHL *et al* (41), 593°C à 7.5 cm au-dessus du sol, 310°C à la surface du sol et 199°C à 1 cm au-dessous de cette surface. Brinkman et Vieira (7) notent que la température peut osciller entre 70°C et 100°C pendant plusieurs heures dans la couche supérieure du sol (0 – 5 cm), ceci en raison du "tapis" racinaire forestier qui se comporte comme un réseau de diffusion de chaleur particulièrement efficace

Au moment du brûlage, les plantules, rejets et drageons présentent des organes en activité, particulièrement sensibles à une exposition au feu; d'après Hare (19), la plupart des tissus végétaux sont endommagés ou détruits s'ils sont soumis à des températures supérieures à 54°C pendant plusieurs minutes. Quant aux graines formant encore le potentiel séminal édaphique, Brinkman et Vieira (7) pour les espèces forestières, ainsi que Vasquez-Yanes (43, 44) et Uhl *et al* (41) pour les espèces pionnières, ont montré que la très grande majorité de ces graines sont détruites par les hautes températures associées au feu, jusqu'à 5 cm de profondeur

Dans ces conditions, la colonisation des zones fortement brûlées repose principalement sur la dissémination des graines postérieure au feu; cette colonisation paraît, par ailleurs, affectée par l'hétérogénéité de l'abattis (41, 48). La comparaison de relevés effectués dans la région de la piste de St. Elie, en Guyane Française, pour des végétations de cinq mois, montre clairement les différences de colonisation (vitesse et composition floristique) entre défrichement non brûlé et abattis expérimental brûlé, ainsi que les variations dues à l'hétérogénéité de l'abattis (Tableau 1).

La régénération naturelle est donc considérablement perturbée par le feu: la destruction de la majeure partie des deux ensembles floristiques principaux, potentiel végétatif et potentiel séminal édaphique, a

Tableau 1. Nombre de plantules et rejets sur des parcelles élémentaires de 10 m^2, pour des végétations pionnières âgées de 5 mois. (Piste de S^t Elie, Guyane française).

Espèce	Type biologique écologie	Défrichement non brûlé	Abattis expérimental brûlé		
			Pas de tronc peu brûlé	Sur un tronc ouvert, calciné	Pas de tronc très brûlé
Cecropia obtusa Tréc		106	42	96	7
Cecropia sciadophylla Mart		41	8	5	0
Goupia glabra Aubl		68	3	2	1
Xylopia nitida Dun		31	5	3	0
Laetia procera (P et E) Eichl.		12	0	2	0
Annona sericea Dun	Arbres pionniers	6	0	0	0
Melastomataceae spp		22	6	1	0
Vismia spp		6	17	4	0
Isertia sp		1	4	0	0
Fagara pentandra Aubl		2	3	0	0
Jacaranda copaia (Aubl.) D. Don		0	1	0	0
Inga spp		0	0	1	0
Dolliocarpus guyanensis (Aubl.) Gilg.		23	3	9	1
Passiflora coccinea Aubl.	Lianes	0	1	0	0
Lianes indéterminées		3	0	0	0
Renealmia guyanensis Maas.	Herbacée pionnière	5	5	1	0
Herbacées indéterminées	rudérales	6	2	0	4
Epiphytes intéderminées	rudérales	2	0	1	2
Espèces forestières indéterminées	Arbres	71	1	1	2
Espèces pionnières indéterminées	Arbres et arbustes	12	1	0	0
Solanum spp	Arbustes rudéraux	2	20	17	11
Pityrogramma calomelanos (L.) Link.	Herbacée rudérale	0	6	14	4
Rejets	Arbres forestiers	24	10	6	2
Total		443	138	163	34

pour effet de libérer l'espace, donnant ainsi libre cours à l'envahissement par le potentiel extérieur

Traditionnellement, le défrichement des abattis a lieu en début de saison sèche; le brûlage intervient en fin de saison sèche, généralement deux à trois mois plus tard, et se déroule le plus souvent en deux étapes: un premier feu, détruisant la majeure partie de la matière végétale, est suivi d'un deuxième, qui porte sur les branchages insuffisamment brûlés, préalablement rassemblés en tas (15, 32, 33). Enfin, la plantation, suivant de près le brûlage, commence avec les premières pluies. Il faut noter que les zones peu brûlées sont généralement laissées de côté, la préférence des agriculteurs pour la plantation allant le plus souvent aux parties fortement brûlées (15, 25, 32, 34). Or, on a vu que le feu avait pour effet, dans ces zones, la destruction des plantules, rejets et drageons, d'une part, des graines du sol, d'autre part.

Dans l'étude des successions, on peut dissocier le processus de compétition entre espèces colonisatrices en deux phases (46):

– "Competition to reach (or to be at) a site first and preempt space.

– Interactive competition"

Par rapport à une grande trouée forestière ou à un défridhement non brûlé, le feu décale dans le temps l'instant initial de la régénération et introduit une donnée nouvelle fondamentale par l'intermédiaire d'un bouleversement du potentiel floristique, élimination presque totale du potentiel végétatif et du potentiel séminal édaphique (Figure 1)

Le feu agit donc sur la première phase de la compétition, libérant l'abattis de la grande majorité des

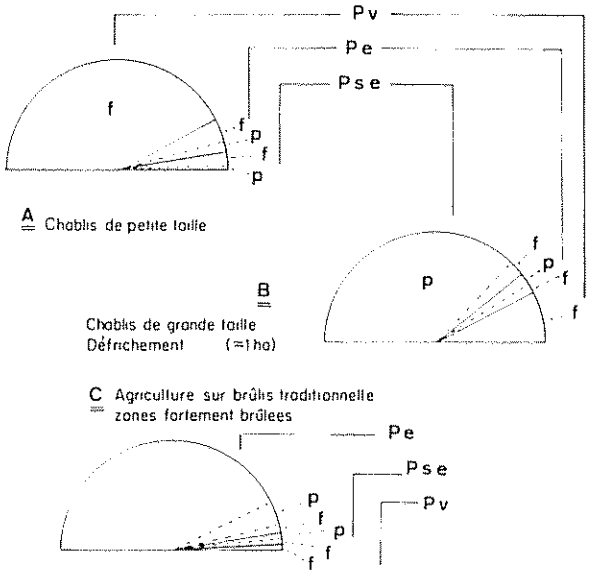

Fig 1 Importance compétitive schématique des différentes ensembles floristiques en présence lors de l'ouverture du milieu forestier

f : espèces forestières
p : espèces pionnières
PV : potentiel végétatif
Pe : potentiel extérieur
Pse : potentiel séminal édaphique

compétiteurs au moment de la plantation. Les plantes cultivées – étouffées très rapidement par le développement de la régénération naturelle en l'absence de feu préalable – peuvent être alors assimilées à un sous-ensemble favorisé (plantation) du potentiel extérieur, et, bénéficiant de l'avantage des premiers occupants, peuvent se développer jusqu'à leur maturité

La compétition avec les espèces pionnières, indéniablement avantagées par leurs caractéristiques de croissance lors de la deuxième phase ("interactive competition") prend une importance croissante avec le temps, en relation avec l'envahissement progressif du potentiel extérieur, allant jusqu'à provoquer l'abandon de la parcelle exploitée, au même titre que la baisse de fertilité du sol (1, 25, 26, 32, 36, 45)

Conclusion

Dans l'agriculture sur brûlis traditionnelle, la compétition entre plantes cultivées et plantes pionnières, est, dans les premiers temps, nettement à l'avantage des premières. La raison principale de cet avantage, décisif pour l'intérêt de la récolte, paraît être la destruction par le feu de la majeure partie des ensembles floristiques qui assurent normalement la cicatrisation rapide des trouées forestières

Dans le cas de l'agriculture itinérante imposée par la nécessité (45), cet effet positif tend à se diluer. En effet, la forte pression anthropique entraîne un raccourcissement de la durée des jachères et une augmentation de la fréquence des feux, favorisant la sélection d'espèces pionnières à graines dormantes résistantes au feu: *Trema guineensis* en Afrique de l'Ouest (2), et *Ochroma lagopus* en Amérique centrale (42), ainsi que l'expansion rapide d'espèces à forte capacité d'invasion telles que *Imperata cylindrica*, dans le Sud-Est Asiatique (29). Dans ce cas, il apparaît que l'agriculture sur brûlis traditionnelle perd sa justification scientifique autant que son efficacité (1), et doit être remplacée au plus vite par des systèmes de production adaptés, permettant de soutenir de plus fortes densités de population

Résumé

L'analyse des connaissances actuelles concernant la régénération naturelle en forêt tropicale humide et l'impact du feu sur cette régénération, permet d'expliquer l'absence de compétition entre plantes cultivées et plantes pionnières dans les premiers temps de culture sur les abattis traditionnels. L'avantage compétitif temporaire des plantes cultivées, en partie garant du succès de l'abattis, est dû à la destruction par le feu de la très grande majorité des graines et plantules d'espèces pionnières assurant normalement la colonisation rapide et vigoureuse des trouées forestières

Summary

The analysis of current knowledge concerning natural regeneration in tropical rainforest and the effects of fire on this regeneration allows us to explain the absence of competition between cultivated and pioneer plants during the beginning of cultivation in the slash and burn traditional system. The momentary competitive advantage of cultivated plants is considered here as a result of the elimination by fire of nearly all the seeds and seedlings of pioneer species which normally ensure a fast and vigorous colonization in forest openings.

30 Mars, 1983

H. de FORESTA*

* Laboratoire de Botanique 163, rue A. Broussonet, 34000 – Montpellier – France

Literature citée

1 ALEXANDRE, D Y Essai d'approche schématique des phénomènes de compétition au cours de la régénération, et son application au problème des adventices dans le système agricole traditionnel Miméogr ORSTOM 1977 15 p

2 ALEXANDRE, D Y Observations sur l'écologie de *Trema guineensis* en basse Côte d'Ivoire Cah ORSTOM sér Biol XIII(3):261-266 1978

3 ALEXANDRE D Y Aspects de la régénération naturelle en forêt dense de Côte d'Ivoire Candollea 37 579-588 1982

4 AUBREVILLE, A Les brousses secondaires en Afrique équatoriale Bois et Forêts des Tropiques 2:21-35 1947

5 BENDELL, J F Effects of fire on birds and animals **In:** Fire and Ecosystems T T Kozlowski et C E Ahlgren eds 1974 pp 73-138

6 BOALER, S B et SCIWALE, K C Ecology of a Miombo site, Lupa north forest reserve Tanzania III Effects on the vegetation of local cultivation practices Journal Ecologyque 54(3):577-587 1966

7 BRINKMAN, W L F et VIEIRA, A N The effect of burning on germination of seeds at different soil depths of various tropical tree species Turrialba 21(1):77-82 1971

8 CHEKE, A ; NANAKORN, W et YANKOSES, C Dormancy and dispersal of seeds of secondary forest species, under the canopy of a primary tropical rainforest in Northern Thailand Biotropica 11(2):88-95 1979

9 DAWKINS, H C The management of natural tropical high forest with special reference to Uganda Commonwealth Forestry Institute 1958 Paper 34

10 FLORENCE, J Chablis et sylvigenèse dans une forêt dense humide sempervirente du Gabon Thèse de 3ème cycle Univ L Pasteur STRASBOURG 1981 261 p

11 FONTAINE, R G, GOMEZ-POMPA, A et LUDLOW, B Successions secondaires **In:** Ecosystèmes forestiers tropicaux UNESCO-PNUE 1979 pp 233-251

12 FORESTA, H de Premier temps de la régénération naturelle après exploitation papetière en forêt tropicale humide ARBOCEL – Guyane française Thèse de 3ème cycle USTL MONTPELLIER 1981 114 p

13 FORESTA, H de Hétérogénéité de la végétation pionnière en forêt tropicale humide; exemple d'une coupe papetière en forêt guyanaise Oecol Applic 4(3):221-235 1983

14 GASCHE, J Le système cultural Witoto **In:** Culture sur brûlis et évolution du milieu forestier en Amazonie du Nord-Ouest Bull de la Société Suisse d'Ethnologie 1975 pp 111-128

15 GRENAND, F et HAXAIRE, C Monographie d'un abattis Wayapi JATBA XXIV(4):285-310 1977

16 GUEVARA-SADA, S et GOMEZ-POMPA, A Seeds from surface soils in a tropical region of Veracruz, Mexico Journal Arnold Arb 53(3):312-325 1972

17 HALL, J B et SWAINE, M D Seed stocks in Ghanaian forest soils Biotropica 12(4):256-263 1980

18 HALLE, F, OLDEMAN, R A A et TOMLINSON, P B Tropical trees and forests An architectural analysis Springer-Verlag Berlin – Heidelberg – New York 1978 441 p

19 HARE, R C Heat effects on living plants U S forest Service Occasional paper 1961 183 p

20 HARPER, J L Population biology of plants Academy Press London – New York – San Francisco 1977 892 p

21 HARTSHORN, G S Tree falls in tropical forest dynamics **In:** Tropical trees as living systems P B Tomlison et M H Zimmerman eds 1978 pp 617-638

22 HARTSHORN, G S Neotropical forest dynamics Biotropica 12(2):23-30 1980

23 HATCH, T C Shifting cultivation in Sarawak: past, present and future **In:** Proceedings of the Vth International Symposium of Tropical Ecology Furtado, J I ed 1980 pp 483-496

24. HOLTHUIJZEN, A. M A et BOERBOOM, J H A. Experiments on the Cecropia seedbank of the Suriname lowland rainforest Biotropica 14(1):62-67 1982

25 HURAULT, J La vie matérielle des noirs réfugiés Boni et des indiens Wayana du Haut Maroni (Guyane française) Agriculture, économie et habitat Mémoires ORSTOM no 3 – Paris 1965. 142 p

26 JOACHIM, A. W R. et KANDIAH, J The effect of shifting cultivation ("chena") and subsequent regeneration of vegetation on soil composition and structure Tropical Agriculturist 104:3-11 1948

27 KARTAWINATA, K , RISWAN, I et SOEDITO, H The floristic changes after disturbances in lowland Dipterocarp forest in East Kalimantan, Indonesia In: Proceedings of the Vth International Symposium of Tropical Ecology Furtado, J I ed 1980 pp 47-54.

28 KEAY, R W. J Seeds in forest soils Nigerian forestry information bulletin 4:1-12 1960

29. KELLMAN, M C Geographic patterning in tropical weed communities and early secondary succession Biotropica 12(2):34-39. 1980

30. KRAMER, F De natuurlijke verjonging in het Goenoeng Gedeh complex. Tectona 26:156-185 1933

31. LIEW, T. C Occurence of seeds in virgin forest top soil with particular reference to secondary species in Sabah. Malay Forest 36(3):185-193 1973

32 LIZOT, J La Agricultura Yanomami Antropologica 53 3-93- 1980

33 LYMAN, T A Miao (Meo) slash and burn agriculture JATBA XVI(6-7-8):251-283 1969,

34 MOUTON, J et SILLANS, R Les cultures indigènes dans la région forestière de l'Oubangui-Chari. Ann. du Musée colonial de Marseille VII(2):114 1954

35 MYERS, N. Conversion of tropical moist forests National Acad of Sciences – Washington. 1980 205 p

36. NYE, P H et GREENLAND, D J The soil under shifting cultivation Commonwealth Agricultural Bureaux Farmham Royal – Bucks – England 1960 156 p

37. OLDEMAN, R A. A. L'architecture de la Forêt guyanaise Mémoires ORSTOM no 73 – Paris 1974. 204 p.

38 PREVOST, M F Mise en évidence de graines d'espèces pionnières dans le sol de forêt primaire en Guyane Turrialba 31(2):121-127 1981.

39 SCHULZ, J. P Ecological studies on Rain Forest in Northern Suriname North Holland Publishing Company – Amsterdam 1960 267 p

40 SYMINGTON, C F The study of secondary growth on rain forest sites in Malaya Malay. Forest 2:107-117 1933

41 UHL, C , CLARK, K , CLARK, H et MURPHY, P Early plant succession after cutting and burning in the Upper Rio Negro region of the Amazon basin Journal of Ecology 69:631-649 1981.

42 VASQUEZ-YANES, C Studies on the germination of seeds of *Ochroma lagopus* Swartz Turrialba 24(2):176-179. 1974

43 VASQUEZ-YANES, C Seed dormancy and germination in secondary vegetation tropical plants. the role of light Compar Physiology and Ecology 1(1):30-34 1976

44. VASQUEZ-YANES, C Estudio sobre la ecofisiologia de la germinación en una zona callido-húmeda de MEXICO In: Regeneración de Selvas GOMEZ-POMPA, A. *et al* eds. 1976. pp 278-387

45. WATTERS, R F. L'agriculture itinérante en Amérique latine. Mise en valeur des forêts no 17. FAO. 1971. 354 p

46 WERNER, P A Ecology of plant populations in successional environments. Systematic Botany 1(3):246-268. 1976

47 WHITMORE, T. C Tropical Rain Forests of the Far East Oxford University Press 1975. 282 p

48 ZWETSLOOT, H Forest succession on a deforested area in Suriname Turrialba 31(4): 369-379 1981.