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DETERMINACION DEL NIVEL DE INOCULO PRIMARIO DE *Pseudomonas solanacearum* EN SUELOS CON INFESTACION NATURAL¹*/

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Abstract

Several methods were tested for detecting the residual inoculum of *Pseudomonas solanacearum*, Race 1, in inceptisols at Turrialba, Costa Rica, where the bacterium is apparently endemic. Planting healthy potato tubers in soil samples in the greenhouse, then using plant wilt as indicator of the pathogen's presence, was qualitatively the most efficient method; however, it was not possible to quantify precisely the level of primary inoculum in several soils. A selective culture medium containing tetrazolium chloride plus antibiotics allowed detection of *P. solanacearum* at concentrations near 25 000 cells/gram of dry soil, even though a large number of antagonistic bacteria predominated in many samples, completely inhibiting *P. solanacearum*.

Both methods were used to detect *P. solanacearum* in samples from soils subjected for nearly four years to three systems of minimum tillage or one of traditional mechanical tillage, populations were consistently larger in mechanically-tilled soil. This was confirmed by planting potato and tomato directly in the field, where significant contrasts in percentage of bacterial wilt resulted. The decrease of the bacterium in minimum-tilled soils was attributed to an increase in organic matter and thus an increase in antagonistic microorganisms.

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Introducción

La raza I de la bacteria *Pseudomonas solanacearum* E. F. Smith, cuyo ámbito de hospedantes incluye tomate, papa, chile, tabaco, berenjena y numerosas especies no cultivadas, es un habitante de los suelos de muchas regiones tropicales; generalmente causa síntomas típicos de marchitez vascular, pero también infecciones sin síntomas (1). En Costa Rica se encuentra en las zonas medias y bajas, por debajo de los 1 200 msnm aproximadamente (2), atacando principalmente tomate y tabaco; la papa se siembra poco en tales zonas, en gran medida debido a su susceptibilidad a esta enfermedad. La frecuente aparición de marchitez bacteriana en papa-

les y tomatales sembrados en terrenos previamente libres de cultivos susceptibles (2, 3, 12) hace presumir que la bacteria puede permanecer en el suelo por periodos largos, ya sea alojada en el xilema de hospedantes que no dan síntomas (1, 7), en la rizosfera de los mismos o aún de especies no hospedantes (13), o bien en niveles relativamente profundos del suelo (4, 18).

En Costa Rica y otros países se han hecho intentos de disminuir experimentalmente la población de la Raza 1 de *P. solanacearum* en el suelo mediante rotaciones con hospedantes no susceptibles, como maíz, frijol y camote (3, 7). En ningún caso se ha podido disminuir el inóculo con esas rotaciones, si bien se observó que en terrenos donde las malezas fueron controladas con herbicidas, el ataque posterior de marchitez bacteriana en papa fue menor que donde se practicó labranza de suelo para el control de malezas (7).

Se considera importante seleccionar un método confiable pero simple, que no requiera instalaciones especiales, para detectar la presencia de la Raza 1 de *P. solanacearum* en los suelos y que también sirva, de ser posible, para cuantificar el nivel de inóculo. Esto permitiría evitar la siembra comercial de hospedantes susceptibles en terrenos infestados o, por el contrario, programar la siembra experimental de variedades cuya reacción a ese patógeno se desea evaluar. Algunos autores (1, 13) han intentado este reconocimiento mediante el aislamiento de la bacteria de presuntas malezas hospedantes sin síntomas. Otros han hecho siembras de plantas indicadoras directamente en el campo (8, 12, 16), o en muestras de suelo llevadas del campo al invernadero (4, 9, 16, 18); en la mayoría de los casos se ha usado tomate o tabaco, que indican, mediante su reacción de marchitez, la presencia de *P. solanacearum* en las muestras; sin embargo, esto toma varias semanas y requiere considerable espacio y material de invernadero.

También se han desarrollado medios selectivos de cultivo, que se espera puedan detectar, directamente y en pocos días, las poblaciones de *P. solanacearum* en determinado terreno (5, 6, 9, 15). La mayoría de estos medios selectivos, empero, no han sido probados con suelos tropicales, y en general sólo han detectado la bacteria en suelos deliberadamente infestados con suspensiones del patógeno poco tiempo antes del muestreo, y no en terrenos agrícolas con infestaciones naturales.

Esta investigación se hizo con la finalidad de evaluar algunos de esos métodos de detección de la Raza 1 de *P. solanacearum*, en terrenos de zonas bajas tropicales, así como determinar la forma en que ésta

bacteria sobrevive en estas zonas y el efecto del manejo del terreno sobre la misma. El estudio se desarrolló en el Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) en Turrialba, Costa Rica, durante el año de 1980.

Materiales y métodos

A. Determinación de *P. solanacearum* en malezas sin síntomas

Se trató de determinar si la bacteria podía sobrevivir en hospedantes alternos sin causar síntomas. Se utilizaron malezas que invadieron un terreno donde, desde 1976 hasta 1980, hubo siembras de diferentes cultivos en rotación con el cultivar de papa 'Atzimba', que es muy susceptible a la marchitez bacteriana, y con el híbrido MS 35-22, que es tolerante (7). La selección del terreno se hizo bajo la suposición de que, donde estuvo el cultivar 'Atzimba', el nivel de inóculo sería mayor que donde hubo MS 35-22. De cada una de las 12 subparcelas muestreadas (6 de cada cultivar) se recolectaron 10 ejemplares de las malezas más importantes que tenían leño en el tallo: *Melampodium perfoliatum* H. B. K., *Bidens pilosa* L. y *Galinsoga ciliata* (Raf) Blake, las tres susceptibles a *P. solanacearum*, ninguna de las plantas recolectadas mostraba síntomas de marchitez.

Por otro lado, se determinó si en plantas de *M. perfoliatum* sin síntomas, pero contiguas a plantas marchitas, se podía detectar la presencia de esta bacteria. Esto se hizo en un terreno recién arado, sin historial reciente de solanáceas, donde había *M. perfoliatum* en diferentes situaciones, así: a—jóvenes marchitas; b—adultas marchitas; c—jóvenes sin síntomas; d—adultas sin síntomas; e—jóvenes sin síntomas pero contiguas a plantas marchitas; f—con los primeros síntomas de marchitez y contiguas a plantas marchitas.

El tallo de cada planta muestreada se pasó a agua estéril tras desinfección superficial y se hizo un estrizado en el medio TZC (11); como control se hizo estrizado de *P. solanacearum* puro, aislado de *M. perfoliatum*.

B. Determinación de nivel de *P. solanacearum* en el suelo por medio de plantas indicadoras

En una primera prueba se utilizaron como indicadoras la papa (cv. 'Atzimba'), el tomate (cv. Tropic), y la maleza *Melampodium perfoliatum*. De cada una de las 12 subparcelas utilizadas para la búsqueda de la bacteria en malezas se obtuvieron 10 submuestras de

suelo a 10-20 cm de profundidad, que se combinaron en una sola muestra por subparcela; la mitad de cada muestra se distribuyó de inmediato en seis potes en el invernadero (4-5 kg de suelo por pote), sembrándose cada especie indicadora en dos potes. Cuando las raíces llenaron el pote, se cortaron verticalmente a 8 cm del tallo en cuatro costados. La segunda mitad de cada muestra se pasó a cajas grandes, donde se sembró maíz para mantener una condición similar a la del campo

La reacción de las plantas indicadoras se calificó un mes después de la siembra, de acuerdo con la siguiente escala; Grado 1 = plantas sanas al llegar a la madurez; Grado 2 = plantas con síntomas de marchitez sólo después de cortar raíces; Grado 3 = plantas con síntomas de marchitez aún antes de cortar raíces. En los potes donde no hubo marchitez se arrancó la planta indicadora adulta y se sembró una nueva, para verificar la ausencia de la bacteria

Tres meses después se repitió la misma prueba, utilizando el suelo mantenido con plantas de maíz.

Una segunda prueba se realizó utilizando como indicadoras plántulas de berenjena (*Solanum melongena*), plántulas de *Nicotiana glutinosa*, tubérculos pequeños de papa, cv. 'Atzimba'; y brotes de cuatro semanas, separados de tubérculos de papa. Se utilizaron dos tipos de suelo: uno provino de un terreno donde se acababa de cosechar un papal que sufrió ataque severo de *P. solanacearum* ("suelo infestado"); el otro fue de un terreno vecino al infestado, pero donde no se había sembrado papa ("suelo testigo"). Cada uno de estos tipos de suelo se evaluó dos veces con cada planta indicadora, excepto los tubérculos de papa, con los que se evaluó cada suelo en seis oportunidades. El corte de raíces y la calificación en tres grados de reacción se efectuaron al igual que en la primera prueba.

C. Detección de la bacteria en medios de cultivo selectivos

Se utilizaron cuatro medios de cultivo selectivos, desarrollados por Granada y Sequeira (5) para determinar niveles de *P. solanacearum* en el suelo, que contienen complementos crecientes de antibióticos.

Fórmula A = Glucosa (5 g/l), Peptona (10 g/l), Caseína hidrolizada (1 g/l), Cloruro de tetrazolio (50 ppm), Agar (18 g/l), Cristal violeta (50 ppm), sulfato de Polimixina B (100 ppm). Fórmula B = fórmula A + Mertiolato (0.05 ppm)

Fórmula C = fórmula B + tirotricina (20 ppm) y Cloromicetina (5 ppm).

Fórmula D = fórmula C + Vancomicina (10 ppm) y Bacitracina (50 ppm).

Se probaron dichas fórmulas con cultivos puros de *P. solanacearum*, con suelos de campo conocidos como naturalmente infestados con *P. solanacearum* y con suelos de infestación desconocida, sin antecedentes de siembras de solanáceas. El suelo se muestreó a 10-30 cm de profundidad, se homogenizó y se suspendió mediante agitación lenta en agua estéril; se probaron diluciones de 1:10, 1:100 y 1:1 000 g:ml. De estas se esparcieron alícuotas de 1.0, 0.5 y 0.1 ml por plato petri de 9 cm diámetro, con el medio de cultivo solidificado.

D. Predicción del nivel de inóculo en suelos con diferentes labranzas

Para comprobar su eficacia, los métodos seleccionados de las etapas anteriores se pusieron a prueba mediante la evaluación de suelos con nivel de infestación desconocido, pero manejados (labrados) de diferentes maneras, de tal forma que fuera de esperar diferencias microbiológicas entre ellos. Para ese fin se utilizó un terreno sin historial reciente de solanáceas y sometido, durante los tres años y medio anteriores, a los siguientes cuatro diferentes sistemas de labranza, todos dentro del sistema de cultivo maíz-frijol (14):

- a. Suelo preparado en forma tradicional (TA): terreno roturado dos veces al año con arado y desmenuzado con rotavator; los desechos de cada cosecha anual de maíz y frijol, así como las malezas, se arrancaron de raíz y se sacaron del terreno, dejando el suelo limpio.
- b. Cobertura vegetal mezclada con tierra (CMMT); el terreno no se trabajó con implementos; las cañas de maíz de cada cosecha anterior se arrancaron manualmente, se trozaron junto con las malezas y se mezclaron levemente con tierra de la misma parcela, quedando en una proporción de 1:1. Inmediatamente después de cada siembra se aplicó Gramoxone (Paraquat 0.5 kg/ha i.a.).
- c. Mantenimiento de cobertura vegetal de residuos de cosecha sobre el suelo (CMSS); los desechos de maíz y las malezas más sobresalientes se trozaron y se dejaron sobre el suelo. Este tratamiento es similar al anterior, pero no se realizó ninguna mezcla de los residuos de cosecha con suelo; se usó Gramoxone contra las malezas.
- d. Suelo no alterado (CMSR); cada año las cañas de maíz del cultivo anterior quedaron en pie, dobladas a la mitad. Se realizó únicamente el trabajo de

preparación de suelo necesario para la siembra y la fertilización; se aplicó Gramoxone para controlar las malezas.

El ensayo estaba sembrado en un diseño experimental de parcelas divididas con cuatro repeticiones, como parte de un experimento del CATIE a largo plazo (14). Para la presente prueba, se tomaron muestras de suelo a lo largo de una banda central en cada una de las 16 parcelas, a una profundidad de 10-30 cm. De cada parcela se tomaron 16 muestras, que se mezclaron; luego se distribuyó la mayor parte de este suelo en el invernadero en ocho potes de 24 x 20 cm, en seis de los cuales se sembró un tubérculo de papa (cv. 'Atzimba'); en los otros dos se sembraron seis plantas de *Nicotiana glutinosa*. Estas fueron las dos mejores especies indicadoras en ese orden encontradas en anteriores ensayos de invernadero.

El resto del suelo se utilizó para aislamientos con medios selectivos en el laboratorio, se usaron tres platos petri con medio de la fórmula C por cada una de las 16 muestras de suelo del experimento; la dilución del suelo en agua fue de 1:300 y la alícuota de 0.1 ml.

Para conocer el nivel real de infestación se sembró papa (cv. 'Atzimba') y tomate (cv. 'Tropic') directamente en el campo, y se correlacionaron los datos de predicción en invernadero y laboratorio con los obtenidos en el campo. En éste el parámetro medido fue el porcentaje de plantas marchitas, que se tomó a intervalos semanales; de todas estas lecturas se obtuvo el porcentaje promedio de marchitez (PPM).

Resultados y discusión

A. Determinación de *P. solanacearum* en malezas sin síntomas

No se observó exudado bacteriano, ni fue posible aislar la bacteria, de ninguna de las 360 plantas examinadas de las malezas *Melampodium perfoliatum*, *Bidens pilosa* y *Galinsoga ciliata*. El terreno muestreado estaba altamente infestado con *P. solanacearum* a juzgar por el alto porcentaje de marchitez en las siembras de papa anteriores y posteriores al muestreo (7); aún así, ni siquiera se presentaron malezas con síntomas de marchitez durante el reconocimiento.

En plantas de *M. perfoliatum* con síntomas externos de marchitez, de un terreno recién arado, así fue posible detectar la bacteria mediante aislamientos en medios de cultivo. Se obtuvo mayor densidad de población bacteriana en el medio cuando se utiliza-

ron plantas, jóvenes o adultas, con síntomas avanzados de marchitez que cuando se utilizaron plantas con síntomas incipientes. Por el contrario, no se obtuvo ninguna colonia en los platos correspondientes a plantas sin síntomas, aún aquellas que crecieron a sólo 5 cm de plantas totalmente marchitas.

M. perfoliatum se ha reportado como hospedante con síntomas de la bacteria, tanto en combinación como en ausencia de cultivos susceptibles, y así se observó durante el transcurso de esta investigación, en diversos terrenos cercanos. Puede considerarse una planta indicadora de la presencia de *P. solanacearum* en determinadas áreas, cuando invade el terreno después de la labranza mecánica (arados o rastreadas). Sin embargo, en los terrenos sin labranza colonizados por esta y otras malezas, al terminar el cultivo de papa, *M. perfoliatum* no mostró marchitez; es difícil que la bacteria pueda haber estado infectando estas plantas sin causar síntomas, puesto que hubiese sido detectada aunque fuera en unas pocas de las plantas examinadas en esta prueba. El hecho de que haya habido gran infestación en ese suelo y, a pesar de ello, no se hayan encontrado malezas marchitas, posiblemente se deba a la no labranza del suelo después de la cosecha de papa; es de esperar que, si estos terrenos hubieran sido rastreados luego de la cosecha, permitiendo la invasión de *M. perfoliatum*, sí se hubiesen encontrado plantas marchitas en el campo (7). Corrobora lo anterior el hecho de que se aisló consistentemente la bacteria de las malezas que invadieron un terreno que fue arado y rastreado, aún cuando tuvo una siembra previa con un cultivo no susceptible (maíz).

Los resultados sugieren que esta raza de *P. solanacearum* es un componente normal de la microflora de estos suelos, persistiendo al menos temporalmente como saprófita o en asociación no patogénica en la rizosfera de especies hospedantes o no hospedantes (13, 17). No está claro por qué sólo infecta al hospedante susceptible tras la labranza del suelo, y no cuando éste se mantiene en barbecho con una población heterogénea de malezas.

B. Determinación del nivel de *P. solanacearum* en el suelo por medio de plantas indicadoras

La siembra de tubérculos de papa (cv. 'Atzimba') resultó el mejor método de invernadero para detectar la presencia de *P. solanacearum* en el suelo. En todas las pruebas la papa tuvo un grado promedio de detección superior al resto de los indicadores utilizados (Cuadros 1 y 2).

Nicotiana glutinosa (Cuadro 2) fue algo menos eficiente en detectar la presencia de la bacteria, además

Cuadro 1. Detección de *P. solanacearum* en invernadero por medio de plantas indicadoras, en muestras de suelo de un terreno con diferentes porcentajes previos de marchitez bacteriana.

Siembra previa		Reacción de plantas indicadoras ^b					
Cultivar	PPM ^a	Papa		Tomate		<i>Melampodium</i>	
		Rep 1	Rep 2 ^c	Rp 1	Rp 2	Rp 1	Rp 2
'Atzimba'	36	1.0	1.0	1.0	1.0	1.0	1.0
'Atzimba'	28	1.5	2.5	1.0	1.0	1.0	1.0
'Atzimba'	43	1.0	1.0	1.0	1.0	1.0	1.0
'Atzimba'	55	2.5	3.0	1.0	1.0	1.0	1.0
'Atzimba'	62	1.5	3.0	1.0	1.0	1.0	1.0
'Atzimba'	46	1.0	1.0	1.0	1.0	1.0	1.0
MS 35-22	6	3.0	2.0	1.0	1.0	1.0	1.0
MS 35-22	10	2.5	2.0	1.0	1.0	2.0	1.0
MS 35-22	11	1.0	2.0	1.0	1.0	1.0	1.0
MS 35-22	23	2.5	3.0	1.0	1.0	1.0	1.0
MS 35-22	24	2.0	2.5	1.0	1.0	1.0	1.0
MS 35-22	20	2.0	1.0	1.0	1.0	1.0	1.0

a PPM: porcentaje promedio de marchitez en la siembra previa de papa en el campo (7).

b Escala 1 = sin síntomas; 2 = síntomas después de cortar raíces; 3 = síntomas antes de cortar raíces.

c Hecha 3 meses después de la repetición 1, con suelo mantenido bajo cultivo de maíz en el invernadero.

Cuadro 2. Detección de *P. solanacearum* en invernadero por medio de plantas indicadoras, en dos suelos contiguos, con y sin incidencia previa de marchitez bacteriana ("infestado" y "testigo", respectivamente).

Suelo	PPM ^a	Reacción de plantas indicadoras ^b			
		Papa (de tubérculos)	<i>Nicotiana glutinosa</i>	Berenjena	Papa (de brotes derraigados)
Infestado	59	1.8	1.4	1.5	1.0
Testigo	—	2.4	2.0	1.0	1.0

a Porcentaje promedio de marchitez en siembra previa de papa.

b Escala: 1 = sin síntomas; 2 = síntomas después de cortar raíces; 3 = síntomas antes de cortar raíces.

de mostrar ciertas desventajas prácticas (dificultad de germinación y crecimiento lento; sin embargo, en ciertas circunstancias podría ser un indicador útil, si se considera que su semilla se puede guardar en refrigeración por mucho tiempo, ocupa poco espacio y puede ser utilizada en cualquier momento. La berenjena fue aún menos eficiente. Con la maleza susceptible *Melampodium perfoliatum* se obtuvieron síntomas de marchitez en un solo caso, mientras que en tomate nunca se presentaron síntomas (Cuadro 1). Con brotes de tubérculos de papa (Cuadro 2) no se pudo detectar en el invernadero la presencia de la bacteria, debido a que tuvieron un crecimiento pobre al

ser transplantados al suelo en prueba, lo que aparentemente les hizo poco susceptibles a la infección por *P. solanacearum*.

En las verificaciones de ambas repeticiones, con tomate y *M. perfoliatum* en suelos que dieron reacción negativa, sólo se logró detectar la presencia de la bacteria en tres plantas de tomate y en ninguna de *M. perfoliatum*. esto confirmó la ineficacia de ambos como indicadores de invernadero. Además, nunca hubo evidencia de la bacteria en sus heces vasculares (prueba del exudado), por lo que aparentemente ni siquiera hubo penetración en estas plantas.

En cuanto a los niveles de inóculo de los suelos evaluados, los resultados fueron diferentes de lo que se esperaba obtener. Así, en suelo de subparcelas donde hubo 'Atzimba' se esperaba el nivel de inóculo más alto, y por lo tanto un grado mayor de reacción en el invernadero, que en el de las subparcelas de MS 35-22; sin embargo, ocurrió a la inversa (Cuadro 1). Lo anterior podría deberse a que, en las parcelas donde hubo Atzimba, la salida masiva de exudado bacteriano de las plantas hacia el suelo (9, 16) estimuló a organismos antagónicos a *P. solanacearum* a aumentar su población y por ende bajar la de *P. solanacearum* (6). En cambio, donde hubo MS 35-22 la población primaria de *P. solanacearum*, si bien menor, podría haber estado en cierto equilibrio con la flora microbiana y sobrevivir mejor en el suelo (6, 18). Esto en parte fue confirmado por siembras de papa posteriores en las mismas parcelas de campo (7), donde hubo alta incidencia de marchitez bacteriana, pero de nuevo sin correlación con los niveles de incidencia de la siembra anterior al muestreo.

Se trató de determinar si existía asociación entre los datos de invernadero y campo. Se encontró que no existía tal correlación entre los grados obtenidos con papa en el invernadero y los porcentajes promedios de marchitez previos y posteriores (7) al muestreo, en ninguna de las dos repeticiones.

Un resultado similar, más inesperado aún, ocurrió cuando se compararon un suelo con alta infestación natural conocida y ("suelo infectado") y el suelo vecino al anterior, sin siembras recientes de hospedantes susceptibles ("suelo testigo"). El nivel de inóculo detectado por la papa y *N. glutinosa* fue más alto en el segundo caso (Cuadro 2). De nuevo, esto podría obedecer a la acción de un mecanismo de control biológico en el terreno donde recientemente hubo alta incidencia de marchitez bacteriana. Casos similares han sido señalados por Jaworski y Morton en Georgia (8) y Martin *et al.* en el Perú (12).

C. Detección de la bacteria en medios de cultivo selectivos

En una primera prueba se esparcieron en platos petri con medios de las fórmulas A, B y D, alícuotas de 0.5 ml de diluciones 1:100 (g:ml) del suelo "infestado" y del suelo "testigo", descritos en la segunda prueba de detección por plantas indicadoras. Tras 3 días de incubación a 28°C, solamente se reconoció una colonia de *P. solanacearum* en un total de 112 platos; crecieron colonias de muchas otras bacterias no identificadas, con un promedio por plato de 1679 en A, 1306 en B y 870 en D. No hubo crecimiento de hongos en ningún plato. En los platos testigo, donde se esparcieron suspensiones de suelo mezclado con cultivos puros de *P. solanacearum*, las colonias de esta bacteria fueron reconocidas fácilmente, si bien su crecimiento se redujo ligeramente en el medio D. Al cabo de 3 días de incubación las colonias de *P. solanacearum* eran fluidas, color crema, de 2-3 mm de diámetro y pulvinadas.

En la segunda prueba se usó solamente la fórmula C, con muestras de un suelo sin siembras previas de solanáceas, pero tomadas a 2 m de plantas jóvenes de papa que empezaban a mostrar marchitez bacteriana (la que luego alcanzó 100% de incidencia). El suelo se suspendió 1:10 en agua; la suspensión se agitó por 30 minutos y se diluyó 1:100 y 1:1000; se esparcieron alícuotas de 0.1 ml por plato. Esta vez se obtuvieron cinco colonias de *P. solanacearum*, cuatro de ellas a la mayor dilución (Cuadro 3). Su crecimiento fue similar al de cultivos puros estriados en los medios B y C.

Con la dilución 1:100, el número de colonias de otras bacterias fue comparable al obtenido con el medio D en la primera prueba, pero hubo mucha inhibición mutua a dilución 1:10, lo que evidentemente imposibilitó también el desarrollo de *P. solanacearum*.

Cuadro 3. Colonias de *P. solanacearum* y otras bacterias obtenidas con tres diluciones de la suspensión de un suelo altamente infestado, en el medio de cultivo selectivo C.

Dilución (g:ml)	<i>P. solanacearum</i>			Otras bacterias		
	Plato 1	2	3	Plato 1	2	3
	Número de colonias					
1:10	0	0	0	1 590	684	827
1:100	1	0	0	254	239	455
1:1000	1	2	1	22	40	34

En vista de estos resultados, que sugieren la presencia en estos suelos de muy altas concentraciones de bacterias que compiten con *P. solanacearum* en el medio selectivo, se decidió utilizar, en adelante, una suspensión de suelo de 1:300 (g:ml) intermedia entre las dos que dieron resultado en la segunda prueba. Si bien esta dilución evidentemente sólo permitiría detectar poblaciones bastante altas de *P. solanacearum* (10^3 células/gramo o más), tales poblaciones no parecen excepcionales, en vista de las frecuentes epifitias severas de marchitez bacteriana en suelos como los estudiados (7, 8, 13).

D. Predicción del nivel de inóculo en suelos con diferentes labranzas

Cuando se compararon los métodos de detección de *P. solanacearum* en suelos sometidos a cuatro diferentes sistemas de labranza por tres años y medio, la papa en invernadero fue la que predijo con mayor

acierto el nivel de inóculo de las 16 parcelas, nivel determinado luego mediante el desarrollo de marchitez en siembras de papa y tomate directamente en el campo. *Nicotiana glutinosa* y los medios selectivos también detectaron la infestación existente, pero no predijeron con tanto acierto el nivel de inóculo. Hubo un mayor nivel de inóculo en el suelo sometido al método tradicional de labranza (TA) que en los otros tres, manejados con labranza reducida, donde el inóculo generalmente disminuyó.

I. Detección a través de plantas indicadoras de invernadero

La papa, utilizada como indicadora de invernadero, encontró diferencias significativas solamente entre TA (promedio 2.29 en la escala de 1 a 3), y CMMT (promedio 1.75) según la prueba de Duncan al 0.05% (Cuadro 4); no hubo diferencias significati-

Cuadro 4. Reacción de plantas indicadoras en invernadero como medio de predecir la presencia de la raza 1 de *P. solanacearum* en terrenos con cuatro diferentes labranzas de suelo.

Tratamiento	Repetición	Detección por plantas indicadoras		
		Papa (tubérculos)	<i>N. glutinosa</i>	Porcentaje marchitez en papa, campo (PRM)
		Grado promedio de la escala ¹		
TA	I	2.2	1.4	49.9
	II	2.2	1.5	61.3
	III	2.7	1.7	77.9
	IV	2.2	2.2	68.6
	Promedio	2.29 ^{a2}	1.70 ^a	64.40 ^a
CMMT	I	1.7	1.0	22.8
	II	1.8	2.2	33.5
	III	1.7	1.2	21.9
	IV	1.8	1.0	8.8
	Promedio	1.75 ^b	1.35 ^a	21.75 ^b
CMSS	I	1.3	1.0	8.6
	II	2.0	1.0	4.2
	III	2.5	1.9	54.0
	IV	2.2	1.9	38.4
	Promedio	2.00 ^{ab}	1.45 ^a	26.31 ^b
CMSR	I	2.0	1.2	4.5
	II	1.5	1.3	15.6
	III	2.3	1.8	54.6
	IV	1.8	1.0	23.6
	Promedio	1.92 ^{ab}	1.33 ^a	24.56 ^b
	C. V.	14.26	30.92	29.32

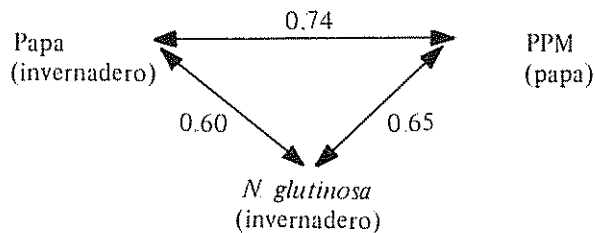
1 Escala: Grado 1 = plantas sanas al llegar a la madurez; Grado 2 = plantas con síntomas de marchitez solo después de cortar raíces; Grado 3 = plantas con síntomas de marchitez aún antes de cortar raíces

2 Promedios de la misma columna seguidos de la misma letra no difieren significativamente entre sí, de acuerdo a la prueba de Duncan, al 0.05%

vas entre estos y los otros dos tratamientos. A nivel de campo, sin embargo, el tratamiento TA produjo un porcentaje de marchitez (PPM) significativamente superior a los otros tres (Cuadro 4). Es de suponer que en el invernadero no se detectaron el resto de las diferencias entre tratamientos debido a la cantidad limitada de plantas indicadoras utilizadas (seis plantas por repetición por tratamiento).

Cuando se utilizó *Nicotiana glutinosa* como indicador, no se detectaron diferencias significativas al 0.05% entre tratamientos, si bien la tendencia fue similar a la de la papa.

Para determinar si existía asociación entre los valores de campo (PPM) y los datos de invernadero, se calculó el coeficiente de correlación entre los tres parámetros, el cual indicó la siguiente relación:



Esto sugiere la utilidad de la papa como medio para predecir la presencia de la Raza I de *P. solanacearum* en un terreno del que se desea saber si está infestado o no, a pesar del tiempo requerido (cerca de tres semanas). En cuanto a *Nicotiana glutinosa*, el coeficiente de correlación obtenido indica que se puede confiar en su uso como medio de predecir la presencia de esta bacteria en un suelo, si bien es menos sensible que la papa a la hora de cuantificar el nivel de inóculo.

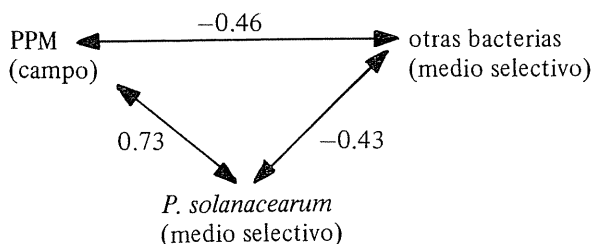
2. Aislamiento en medios selectivos

En este intento de predicción del nivel de inóculo sólo se logró detectar a *P. solanacearum*, mediante cultivo en medios selectivos, en aquellas parcelas que posteriormente mostraron más del 81% de marchitez de plantas de papa en la última semana de evaluación, o un porcentaje promedio de marchitez superior a 38% (Cuadro 5). La fórmula utilizada (C), a pesar de su alta concentración de antibióticos, aparentemente no inhibió el crecimiento de *P. solanacearum*, como lo indica el hecho de que cuando se suspendió 1 g de suelo en 300 ml de estéril y se le agregó 10^3 cel/ml (población estimada) de *P. solanacearum*, se desarrollaron 1 145 colonias por plato

Cuadro 5. Cuantificación de *P. solanacearum* de 16 parcelas de un suelo naturalmente infestado, por un medio selectivo de cultivo.

Tratamiento	Repetición	Aislamientos de suelo, medio selectivo		marchitez en papa %	
		<i>P. solanacearum</i> Prom. 3 platos	Otras bacterias Prom. 3 platos	PPM	Ult. semana
Número promedio de colonias					
TA	I	13	123	49.9	100.0
	II	0.3	76.7	61.3	100.0
	III	1.3	27.3	77.9	100.0
	IV	1.3	53.3	68.6	100.0
CMMI	I	0	102	22.8	53.8
	II	0	291.3	33.5	76.5
	III	0	38	21.9	45.6
	IV	0	323	8.8	28.6
CMSS	I	0	180	8.6	26.7
	II	0	164	4.2	19.0
	III	0	172.7	54.0	93.7
	IV	0.66	79	38.4	81.5
CMSR	I	0	100.7	4.5	19.5
	II	0	84.3	15.6	43.0
	III	0.33	27.7	54.6	100.0
	IV	0	125.3	23.6	53.1
Suelo (1:300) + <i>P. solanacearum</i> (aprox 10^3)		1 145	381		

de esta y 381 colonias de otras bacterias (Cuadro 5). Se notó que los platos en donde se detectó *P. solanacearum* tenían en general una población más baja de otras bacterias (72 colonias por plato en promedio), mientras que en platos sin *P. solanacearum* la población de tales bacterias fue generalmente elevada (promedio 140 colonias por plato). Esto pudiera indicar un efecto sobre *Pseudomonas*. Podría entonces ser la baja población de otras bacterias lo que permitió detectar a *Pseudomonas* en parcelas como las de TA. Para verificar lo anterior, se determinaron los coeficientes de correlación entre los siguientes parámetros:



Los resultados indican que existe asociación entre el número de colonias de *P. solanacearum* obtenidas en el laboratorio y los valores de PPM obtenidos en el campo. En los dos casos en que no se obtuvo correlación significativa, el signo sugiere que existe una asociación inversa, es decir, que a medida que aumenta el número de otras bacterias (posiblemente antagónicas a *P. solanacearum*) en el medio selectivo, disminuye el número de colonias de *P. solanacearum* y el porcentaje promedio de marchitez en el campo y viceversa. En Japón (18), en Kenia (6) y en Australia (4) se ha determinado que en suelos donde se incrementa la materia orgánica la población de *P. solanacearum* baja rápidamente, probablemente debido a una mayor actividad microbiana. Todos estos resultados permiten suponer que la posibilidad de detectar la presencia de *P. solanacearum* en medios selectivos de laboratorio depende del efecto que bacterias antagónicas ejerzan sobre su desarrollo en estos medios (6). Aún si el tipo de labranza no influyera directamente sobre *P. solanacearum* pero sí sobre sus organismos antagónicos, a través del incremento en materia orgánica, habría aumento o no de la población de la primera en la medida que quiebre o se guarde esa relación.

En las muestras donde se detectó la bacteria, su población varió de 2.5 a 5.0×10^4 células por gramo de suelo seco. Esta población resulta elevada si se considera que es natural, no inducida, puesto que se encontró en un terreno donde aún no había indicación alguna de marchitez bacteriana, ni hubo siembras de solanáceas por muchos años. Otros autores (9, 15, 16), han encontrado poblaciones similares

o mayores, pero en suelos directa o indirectamente infestados artificialmente; en suelos naturalmente infestados, Jenkins, Morton y Dukes (9) no lograron detectar a *P. solanacearum*, aún con métodos serológicos muy eficientes. En las tierras altas de Kenia, Harris (6) pudo detectar de 10^3 a 10^4 células/gramo de suelo naturalmente infestado, mediante aislamiento en un medio selectivo. Sin embargo, no se conoce información sobre el nivel de inóculo primario de *P. solanacearum*, Raza 1, en tierras bajas tropicales, por lo que el presente trabajo pareciera ser la primera indicación al respecto.

3. Desarrollo de la enfermedad en el campo

La marchitez bacteriana atacó con severidad la papa sembrada en las parcelas sujetas a labranza tradicional (tratamiento TA), donde se alcanzó el 100% de marchitez entre la quinta y la décima semanas. En contraste, en los suelos con los tres tratamientos de labranza reducida, el desarrollo de la marchitez fue más lento y apenas afectó, en promedio, cerca de la mitad de las plantas (Figura 1). No hubo diferencias entre estos tratamientos pero sí la hubo (al 0.01%) entre ellos y el laboreo tradicional (Cuadro 4).

En las parcelas manejadas con labranza reducida durante más de tres años, el tratamiento sin remoción de las malezas, controladas periódicamente con herbicidas, así como la ausencia de aradas, hicieron que se mantuviera una capa de mantillo sobre el suelo, donde, la elevada actividad biológica presumiblemente redujo las poblaciones de *P. solanacearum*, como lo sugieren los aislamientos en medio selectivo (Cuadro 5) y en concordancia con los resultados de otros autores (4, 6, 18). Probablemente la falta de diferencias significativas entre los PPM de

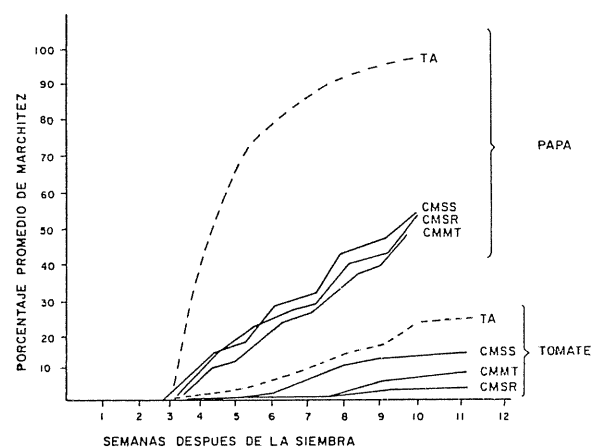


Fig. 1. Determinación del nivel de inóculo primario de *Pseudomonas solanacearum* en suelos con infestación natural.

los tratamientos de labranza reducida se debe, en parte, a una aparente recontaminación de las parcelas ubicadas en un borde del experimento; ahí el drenaje superficial, proveniente de terrenos vecinos arados regularmente, puede haber introducido cantidades importantes de inóculo (Cuadro 4: rep. II, trat. CMMT; rep. III y IV, trat. CMSS).

Donde el suelo se había arado y rastreado dos veces al año hubo repetidas brotaciones de malezas anuales, que eran controladas manualmente (14); entre ellas predominó *M. perfoliatum*, especie de la que algunas plantas jóvenes se marchitaron; no se llegó nunca, entonces, a la invasión y desarrollo sucesivo de una población diversa y estable de malezas, de manera que no se pudo verificar si se hubiera repetido aquí la situación descrita en el terreno donde se buscó sin éxito la bacteria en malezas sin síntomas. Es aparente que son las malezas jóvenes, que invaden los terrenos recién arados, las que al infectarse incrementan el inóculo de *P. solanacearum* en el suelo; en esta etapa, *M. perfoliatum* podría constituir un indicador de campo de la presencia de la bacteria, aunque sólo sea transitoriamente.

El tomate fue también afectado por marchitez bacteriana, pero más tarde y en menor grado que la papa (Figura 1); además, no indicó diferencias significativas al 0.05% entre tratamientos, si bien mostró correlación con la severidad de marchitez en papa. En una parcela TA el 76% del tomate se marchitó al cabo de 12 semanas; casos similares han ocurrido en terrenos vecinos. El nivel consistentemente bajo de marchitez en tomate en los suelos con labranza reducida (promedio 8.7% a las 12 semanas, nunca mayor de 28%) sugiere que este método de manejo podría ser una alternativa viable para el control de la marchitez bacteriana en suelos de regiones cálidas tropicales infestadas con la Raza I de *P. solanacearum*.

Resumen

Se evaluaron varios métodos de detectar el inóculo residual de *Pseudomonas solanacearum*, Raza I, en inceptisoles de Turrialba, Costa Rica, donde dicha bacteria es aparentemente endémica. El más eficaz cualitativamente fue la siembra de tubérculos sanos de papa en muestras de suelo llevadas al invernadero, utilizándose la marchitez como indicador de la bacteria; sin embargo, no fue posible cuantificar con exactitud el nivel de inóculo primario en varios suelos. Un medio de cultivo selectivo, conteniendo cloruro de tetrazolio más antibióticos, permitió detectar *P. solanacearum* a concentraciones de cerca de 25 000 células/gramo de suelo seco, si bien en

muchas muestras predominaron diversas bacterias antagonicas que inhibieron totalmente a *P. solanacearum*.

En muestras de suelos sometidas por casi cuatro años a tres sistemas de mínimo laboreo se detectaron, por ambos métodos, poblaciones menores de *P. solanacearum* que en las de suelos sometidas al laboreo mecánico tradicional. Esto se confirmó mediante siembra de papa y tomate directamente en el campo, determinándose contrastes significativos en el porcentaje de marchitez bacteriana obtenido. La disminución de la bacteria en suelos con mínimo laboreo se atribuyó al incremento en la materia orgánica y, por ende, en microorganismos antagonistas.

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Reseña de libros

FRISSEL, M. J. y J. A. VAN VEEN (eds) 1981.

Simulation of nitrogen behaviour of soil-plant systems. Centre for Agricultural - Publishing and Documentation, Wageningen. 277 p.

El nitrógeno es el nutriente que más limita, con excepción del agua, la producción de los cultivos en la mayoría de los suelos del mundo. Así para aumentar la producción agrícola en los próximos 20 años no sólo se debe aumentar la utilización de fertilizantes nitrogenados sino también, por razones de poca disponibilidad y alto precio, la eficiencia de su utilización por los cultivos. Para lograr este objetivo es necesario comprender con mayor profundidad la dinámica del nitrógeno en el suelo sobre todo en lo relativo a su disponibilidad y absorción por las plantas así como los procesos que conducen a la pérdida del mismo (nitrificación, desnitrificación, volatilización de NH_3).

Desgraciadamente la falta de información experimental sobre estos procesos impide la postulación de generalizaciones válidas que permitan predecir el comportamiento del nitrógeno en el suelo bajo la influencia de un número de factores ambientales dados. Sin embargo, con la información ya disponible es posible desarrollar modelos matemáticos que permitan un acercamiento al problema. Este fue el motivo de la reunión de trabajo del panel de expertos que se llevó a cabo en Wageningen a principios de 1980 y cuyas ponencias, discusiones y conclusiones recoge esta pu-

blicación. La reunión puso en relieve las grandes dificultades teóricas y prácticas en el campo.

La ventaja de los modelos es el de dar un asidero conceptual sobre cada proceso en particular, en tal forma que se puede comprender las posibles relaciones, causa-efecto de importancia.

En el desarrollo de un modelo se define muy bien los parámetros, así como su mutua interrelación. Desgraciadamente a veces la importancia relativa de cada factor es muy arbitraria y depende del criterio del autor; peor aún, raras veces este énfasis es verificable experimentalmente. Este es el mayor defecto de los modelos presentados, aparte de algunos errores de enfoque pues por ejemplo, para un modelo de desnitrificación de suelos bien estructurados se deja por fuera el papel de la rizosfera.

La gran contribución de esta publicación es precisamente poner en evidencia la enorme brecha de información que existe entre los modelos conceptuales y la información experimental disponible sobre los principales fenómenos responsables de los cambios del nitrógeno del suelo, al mismo tiempo señala aquellas áreas que necesitan atención experimental urgente.

De esta manera se aportan nuevas ideas que dan luz al camino a seguir en las investigaciones relacionadas con el nitrógeno del suelo.

Se recomienda este libro para todos aquellos investigadores dedicados al estudio del nitrógeno en el suelo, así como también para los agrónomos interesados en el tema.

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SHOOT REGENERATION FROM CALLUS DERIVED FROM EMBRYO AXIS CULTURES OF *Theobroma cacao in vitro*¹

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Resumen

Se indujo la producción de callo de secciones de la plúmula de semillas maduras de cacao cuando se cultivó embriones axiales en el medio básico compuesto de Murashige y Skoog, suplementado con nitrato de calcio, sucrosa, peptona bacteriológica, ácido neftalín acético, quitina e inositol. Subcultivos del callo después de 50, 60, 70, 80 y 90 días en el medio básico modificado con vitaminas blancas, diferenciaron gemas adventicias in vitro.

Introduction

In Nigeria, the cacao industry is currently faced with a prominent horticultural problem. This is the lack of an adequate rapid vegetative propagation method for cacao which is capable of making suppliers meet planting material demand pressures. This can be attributed to the rapidly expanding cacao growing areas and the giant rehabilitation scheme designed for improving old moribund farms. Accordingly, the tissue culture method is being explored with this problem in view. In recent years the application of tissue culture techniques has been extended to several plant breeding programmes. The ultimate aim has been to produce and rapidly propagate superior, uniform genotypes within a short time. To attain this, the induction and growth of organised structures have been attempted in somatic callus cultures of several tree species (5, 10, 13). Commercial feasibilities have been attained in the rapid clonal propagation of several herbaceous ornamental plants through the

application of plant tissue culture technique (13). However, in many tree species, callus subculture and recultures have formed either shoots or roots and only occasionally both organs are differentiated in succession (2, 14). Nevertheless, comparatively fewer tropical fruit trees have been successfully induced, reared and maintained under tissue culture conditions. In this paper, a report is made of an attempt at vegetatively propagating cacao through *in vitro* method.

Materials and methods

Mature unripe fruits (pods) of *Theobroma cacao* L. were randomly collected from trees located at the Gambari Experimental Station of the Cocoa Research Institute of Nigeria, Ibadan. The pods were first surface sterilised by washing in lukewarm water at 35°C to which had been added some drops of 'Dettol' and antiseptic liquid and a few drops of 'Teepol' liquid detergent. These pods were further immersed in 0.5% sodium hypochlorite solution (10% Clorox) for 15 minutes; without further rinsing, each of the pods were cut open. The pod husk was separated from the mucilage placenta and seeds. The embryo axis of each mature bean (seed) were excised carefully and aseptically, using mounted blades on metal scalpels. Each embryo axis (explant) was quickly transferred on to a sterilised 10 ml modified Murashige and Skoog (10) basal medium

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contained in 30 ml McCartney specimen bottles. The modifying addenda of the medium used for callus induction (callogenesis) and regeneration are presented on Table 1. The cultures were stored under 14 hour diffuse daylight condition provided by Phillips fluorescent lamps of 150 – 250 lux at a temperature of $26 \pm 2^\circ\text{C}$. The optimum auxin concentration (naphthalene acetic acid, NAA) for callus induction was determined in a preliminary trial (7). Eight concentrations examined were 0.0, 0.1, 1.0, 2.0, 3.0, 5.0, 10.5, 15.0 mg/l. A level of 2.0 mg/l concentration was chosen as the most satisfactory for callus induction from the plumule. Using this preliminary result as a guide, calli thus induced in this experiment were subcultured on the 50th, 60th, 70th, 80th and 90th days respectively. These subcultured calli were examined for possible incipient or latent organogenesis and/or embryogenesis microscopically. Callus originating from the plumule, the hypocotyl as well as the radicle ends were subcultured separately so as to compare and contrast their morphogenetic properties

Result

Callus was visible after 30 days in only those cultures that contained 2.0 or more mg/l of NAA. For concentrations of auxins lower than 2.0 mg/l of NAA, the embryo axis explant mostly germinated with or without production of callus. Callus induction occurred from three identifiable regions of the explants namely the root apex or radicle, the plumule and the hypocotyl regions. Callus induction was often earliest in the radicle region relative to the plumule

and hypocotyl regions. Most callus was induced when NAA concentrations were above 5.0 mg/l. Accordingly, these showed rapid necrosis (browning and progressive death) earliest. Callus produced from tissues cultured on media that contained concentrations between 2.0 and 5.0 mg/l were most friable and showed occasional nodulation. This was particularly most characteristic of callus induced from the plumule region.

All ages of subcultures made at 50, 60, 70, 80 and 90 days respectively showed a high degree of variability both within and between age treatments, especially with respect to the number of adventitious buds and shoots, the time required for their development, and the number of leaves eventually produced (Figures 1, 2, 3 and 4).

Adventitious roots were not observed in any of the cultures. Shoots were produced in all ages of subcultures (plumule callus) except those subcultured from the 90-day-old callus. The observation made is represented on Figures 2 and 3.

Organogenesis was not observed in all ages of callus induced from both the radicle as well as the hypocotyl regions of the explant. Differentiated adventitious buds and shoots were not always similar to those characteristic of cacao grown in nature. They often appeared vestigial. The number of leaves and leaf-like structures produced per shoot varied with treatments and ranged from 1 to 5. The highest number of these leaves and leaf-like structures occurred in subcultures made from the 70-day-old callus (Figure 4). No roots were produced in any of the regenerated adventitious shoots (Figures 5, 6 and 7).

Table 1. Media used for callus formation and shoot regeneration. M-S salts modified to contain calcium nitrate* and organic fraction made to contain bacteriological peptone.

Addenda to Murashige-Skoog (13) salts formulations	Callogenesis (ppm)	Shoot regeneration (ppm)
Ca(NO ₃) ₂ *	300.00	300.00
Sucrose	50.00	20.00
Bacteriological Peptone*	100.00	10.00
Naphthalene acetic acid	2.0	0.01
Kinetin	0.01	1.0
Bacto Agar	8 000.00	8 000.00
Inositol	100.00	400.00
Nicotinic acid	—	1.00
Glycine	—	2.00
Pyridoxine HCL	—	1.00
Thiamine HCL	—	4.00
pH Adjusted with 1N NaOH	6.1	6.1

* Ca (NO₃)₂ and bacteriological peptone were supplemented to M-S formulation.

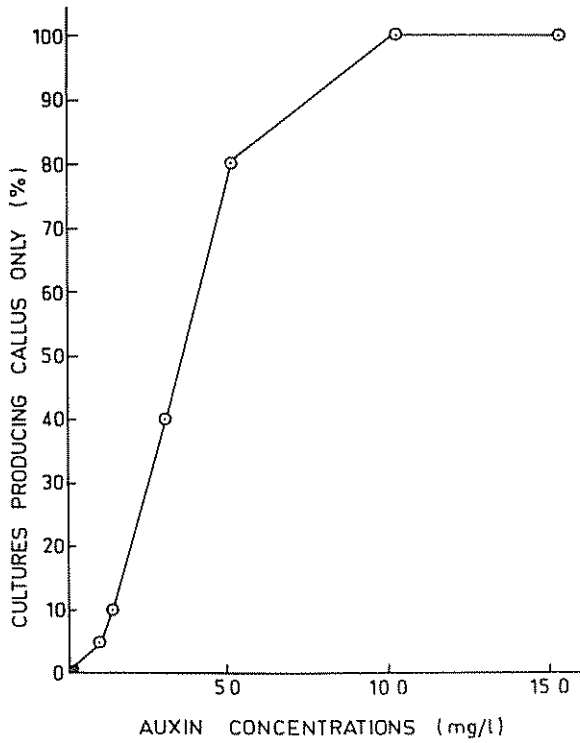


Fig 1. Effect of naphthalene acetic acid concentrations on callus induction in embryo axis explants.

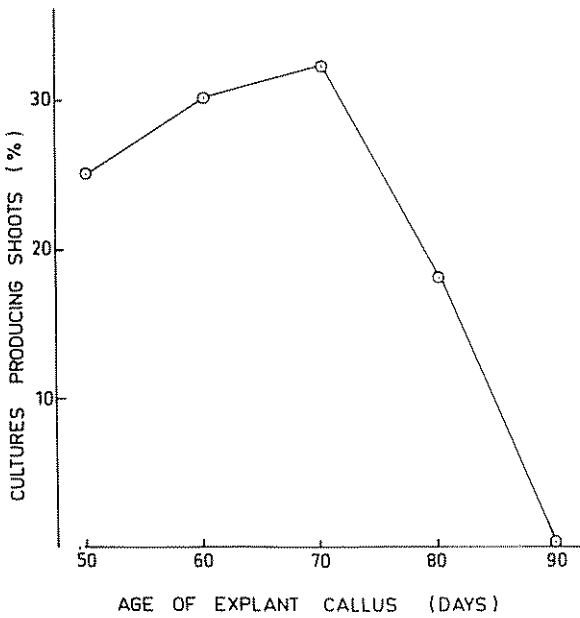


Fig 2. Effect of age of callus induced from explant on shoot bud regnerability

Discussion

In this investigation, callus has been induced from one, two or three locations on the embryo axis

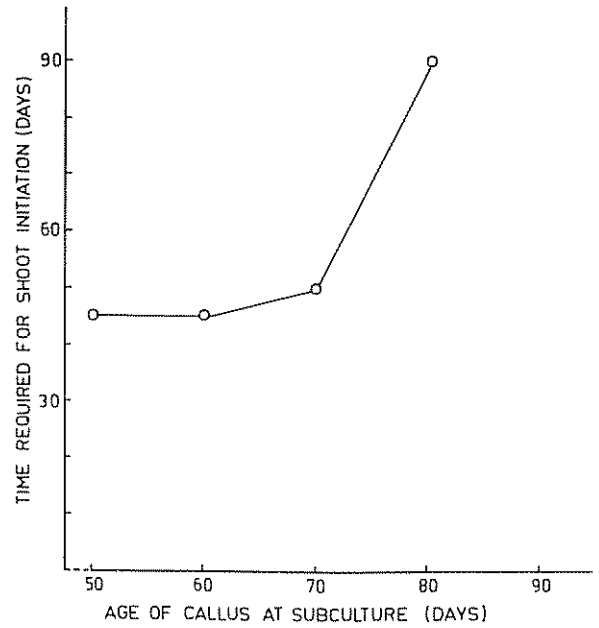


Fig 3. Effect of age of callus induced from explant on the period of adventitious shoot initiation.

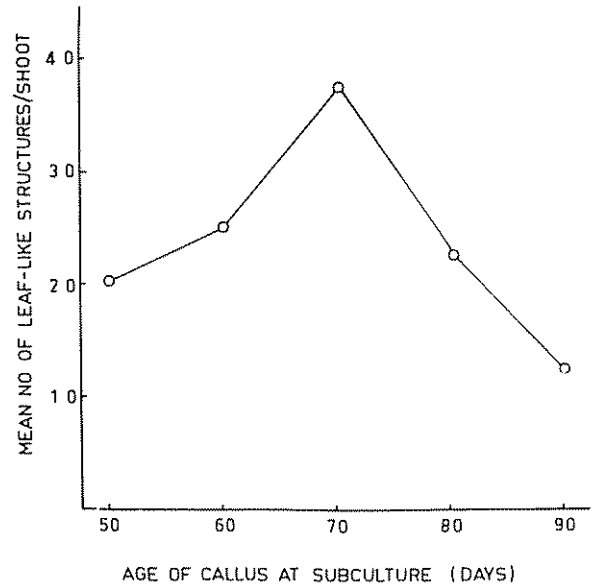
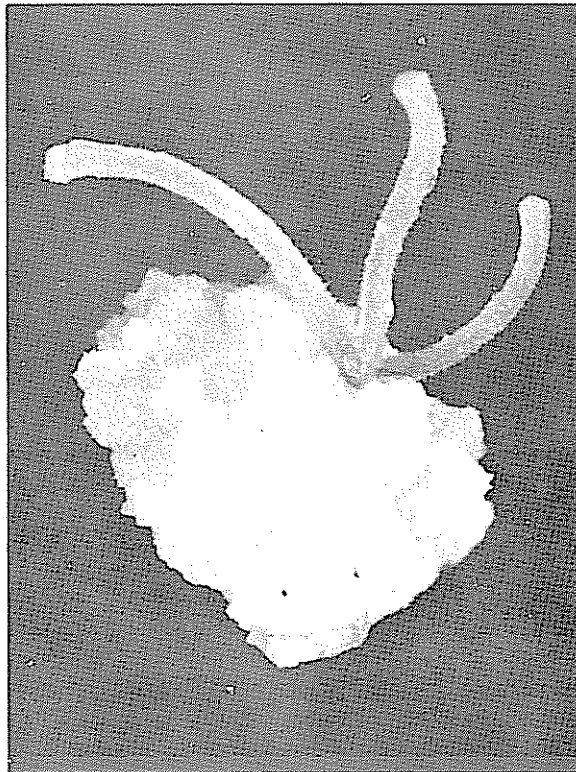
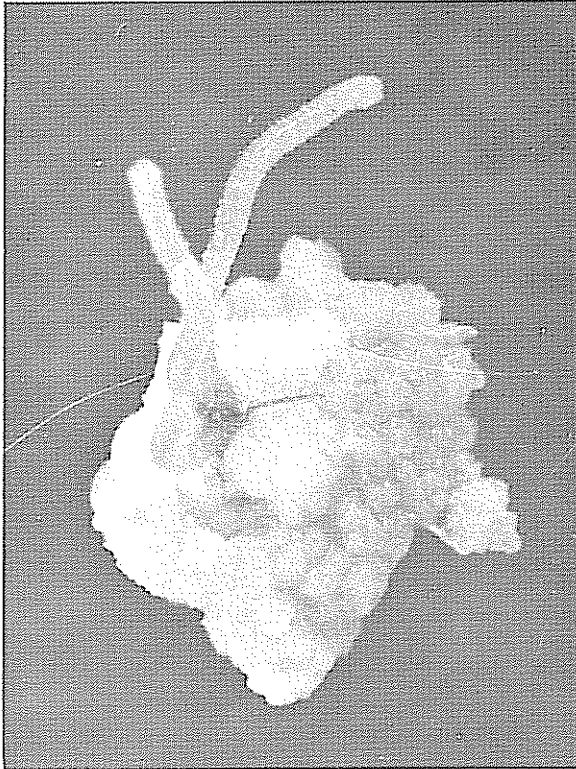


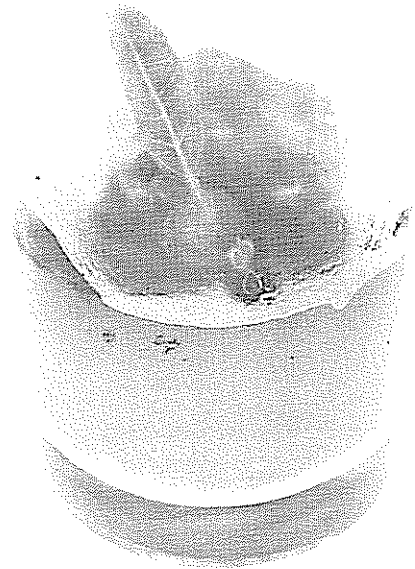
Fig 4. Effect of callus age on the number of adventitious leaves produced per shoot.

explant depending on the auxin concentrations employed and the genotype of the seed. Adventitious buds and shoots of cacao were induced in callus initiated from mature cacao embryo axis explants

Adventitious shoot regeneration from callus cultures has not been reported previously for *Theobroma cacao* L. or any other member of the family



Figs 5(a and b). Vestigial shoots from *Theobroma cacao* friable highly nodulated callus.



Figs 6(a and b). A culture vessel containing callus from which a shoot has been produced. In (b) the morphogenetic callus and shoot are removed from their containers so as to show details.

sterculiaceae. However a few reports do exist on successful propagation of a few other non-forest, commercial fruit trees through the application of tissue culture methods. The first regularly sub-cultured callus cultures were reported for *Salix caperea* (8) and *Gastanea vesca* (9), while several other tropical fruit tree tissues of economic importance have been used to demonstrate inherent capability for organogenesis and/or embryogenesis among tree species. These species include *Citrus* and



Figs. 7(a and b) Culture bottles containing morphogenetic calli from which etiolated shoots are emerging. In both cultures, the callus has necrosed at the surface (turned brown) However adventitious budding is occurring from the fresh whitish callus at the base nearest the medium. Note also a ball of callus being developed from the tip of the shoot in (b)

Citrus relatives (6), *Carica papaya* L. (4), *Coffea* species (16, 18). Nevertheless commercial feasibilities of these successes have not yet been attained. Furthermore, other fruit tree tissue cultures such as apples (11), avocado (1, 15), cacao (7) have even failed to respond organogenetically beyond rooting. Callus induction has been found to be easiest with embryos or sections from very young seedlings of tree species, and the most morphogenic callus have been produced from seed or seedling hypocotyl explants (13). This justifies the choice of the explant used in this investigation.

Observations in this investigation suggest that tissues in different parts, even when as small as the cacao bean embryo axis, tend to have different minimum auxin threshold for producing callus. This seems obvious in that while callus was readily induced in the radicle region, good shoot growth was still being enhanced at the plumule region even though both regions were in direct contact with the medium. A comparable observation, which was partly explained by polarity, has been described by Esan (6), when excised whole citrus nucellus was used as explants. The micropylar half remained embryogenic and produced nucellar embryos while on the other hand the chalazal region grew in a disorganised fashion and produced callus which also remained undifferentiated even after many subcultures and recultures.

Mehra and Mehra (12) had pointed out that even though equally fast growing calli are often obtained from different parts of the tree, the morphogenetic potentials of those calli often vary with the actual site of origin on the explant.

It is generally known that rhizogenesis *in vitro* occurs more readily than other forms of regenerative growths. Nevertheless shoot formation followed by rhizogenesis (rooting) has been reported to be characteristic of cultures that are freshly isolated from hypocotyls, stem meristems and young leaf segments (14). Accordingly, observations made in this investigation confirm this general claim.

This report also suggests that the age of callus being subcultured has direct effects on inherent morphogenetic potentials. Similarly, several workers have observed similar phenomena in callus cultures that have either aged or that had gone through several passages (17).

The adventitious shoots, which were differentiated in this investigation, have also been maintained for as long as 9 months without root development. Similar rootless shoots have been produced in callus cultures of some other plants such as *Pergularia* (3, 14, 19).

In conclusion, even though shoots have been regenerated from cacao callus cultures its practical importance and application for attaining the goal being aimed at is still far from being satisfactory. Nevertheless, this achievement will serve as a foundation for future work.

Abstract

Callus was induced from the plumule regions of mature cacao seeds when the embryo axis was cultured on a basal medium composed of Murashige and Skoog Salts, calcium nitrate supplement, sucrose, bacteriological peptone, naphthalene acetic acid, kinetin and inositol. When this callus was subcultured after 50, 60, 70, 80 and 90 days respectively on the basal medium modified to contain white's vitamins, adventitious buds were differentiated *in vitro*.

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ESTUDO COMPARATIVO DE VARIEDADES DE BATATA DOCE (*Ipomoea batatas*),
VISANDO APROVEITAMENTO EM INDUSTRIAS DE ALIMENTOS¹ /

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Summary

A comparative study of sweet potatoe varieties was conducted to estimate their utilization in food industry. Eighteen varieties were utilized in this study including varieties with pulp of different color (cream, orange and purple). The orange varieties were the most important to the food industries.

After harvesting, the material was analyzed for color of the pulp, dry weight, starch, α and β amylases, enzymatic browning, Brix of the crude and boiled broth, pH of the crude broth, viscosity of the boiled paste and general aspects.

It is concluded that some varieties have better chance for processing than others in the food industry.

Introdução

A batata doce tem sido pouco utilizada industrialmente no Brasil, embora possa ser economicamente empregada na indústria, competindo, até certo limite com a mandioca no fabrico de raspa, da fécula, do álcool, da glucose, etc. (4). No Brasil a batata doce ainda é pouco utilizada industrialmente. Algumas indústrias alimentícias brasileiras a utilizam na fabricação de doces em calda e em massa, embora em outros países existam produtos alimentícios bastante diversificados como enlatados (8); farinhas (5) e flocos (7). Nosso objetivo, neste trabalho é obter informações a respeito de características físico-químicas de variedades de batata doce, com vistas a um futuro aproveitamento industrial.

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Material e métodos

Material

Amostras de batata-doce

As amostras de batata doce foram obtidas de ensaio de campo conduzido na Fazenda Experimental São Manuel, município de São Manuel (S. P.). Após a colheita as raízes sofreram um processo de cura e foram encaminhadas ao laboratório para análise.

Preparo das amostras

As raízes sofreram os seguintes preparos:

- a) **Esfatiamento:** As batatas foram cortadas em rodela com faca de aço inoxidável. As fatias foram utilizadas na determinação da cor, matéria seca (umidade), extração do caldo cru e, após secagem, para determinação do amido.
- b) **Extração do Caldo Cru:** As fatias obtidas foram submetidas a pressão de 3.00 kg/cm² em prensa hidráulica, dentro de recipiente plástico dotado de perfurações por onde escorreu o caldo cru, que

foi recolhido em bequer de vidro para análise (6). As análises realizadas foram: pH, brix, α e β - amilase.

- c) **Cozimento:** As raízes foram envoltas em alumínio e cozidas em forno a 190°C por 70 minutos (10). Após o cozimento, as amostras foram utilizadas para determinação de brix, viscosidade, e aspecto geral.

Métodos

Umidade - matéria seca

Foi determinada pelo método Oficial da A.O.A.C. (2) por perda de massa em estufa a 100°C, até massa constante. O peso seco foi determinado por cálculo.

Amido

O teor de amido foi determinado por hidrólise ácida, seguida pela determinação de redutores por Eynon-Lane, segundo recomendação da A.O.A.C. (2).

Brix

A determinação do Brix foi feita após clarificação do caldo, por filtração, usando refratômetro marca Toko, de 0.1 sensibilidade de leitura.

pH

Foi determinado no caldo, utilizando pH-meter marca Corning Scientific Instruments Model 7, de sensibilidade de 0.1 de leitura.

α -amilase

Foi determinada pelo método padronizado pela OKA S/A Indústria e Comércio de Diástase Ltda, baseado no método Oficial da A.O.A.C. (2).

β -amilase

Foi determinada pelo método descrito por Walter Jr. *et al* (12) e os açúcares redutores resultantes da ação enzimática determinados pelo método de Somogy-Nelson.

Fenoloxidase

O escurecimento enzimático, decorrente da ação das enzimas do grupo das fenoloxidases foi avaliado por método descrito por Cereda *et al* (3)

Viscosidade

A viscosidade da pasta cozida foi avaliada pelo seguinte método: 50 g de batata doce cozida, foram homogeneizadas com 150 ml de água destilada. A pasta assim obtida foi submetida a ensaio em viscosímetro Saybolt, em orifício de 2 mm e expressos os resultados em S. U. Saybolt. As determinações foram feitas a 25°C (10-12).

Aspecto geral

O aspecto geral foi determinado em batatas cozidas e submetidas a juizes que deram notas que variaram de 0 a 12, levando em conta, individualmente, o sabor, aroma e a cor. O aspecto geral foi avaliado pela média das três notas.

Análise estatística

Os valores obtidos nas análises foram calculados em média, coeficiente de variação, desvio padrão e intervalo de confiança segundo Pimentel Gomes (9).

Resultados e discussão

Os resultados das análises realizadas estão relacionados no Quadro 1. Os valores que constam no quadro são médias de repetições. A reação é medida em minutos a maior aproximação possível.

Os resultados de cada análise, contida no Quadro 1, em separado, foram calculados para: média e intervalo de confiança, cujos valores estão representados no Quadro 2. Quanto a análise de regressão, foi feita, mas o trabalho ficaria longo demais pois algumas correlações obtidas não são simples. Optamos por colocá-las em outro trabalho, onde propomos as soluções para os resultados obtidos.

A interdependência das análises torna difícil a discussão em separado, por esta razão procuraremos, em função dos Quadros 1 e 2, e em função da importância para a indústria alimentícia, discuti-los em conjunto. Em relação à cor, as indústrias brasileiras tem demonstrado maior interesse para com as variedades de polpa laranja, devido à coloração que conferem ao doce em massa.

Do ponto de vista industrial, há interesse em variedades que apresentam menor teor de umidade, já que resulta em um maior teor de matéria seca e, portanto, menor quantidade de água a ser evaporada. Neste caso, embora não haja por parte da indústria um limite estabelecido para umidade, as variedades que se destacaram por um teor de matéria seca acima da média (64.67%) foram; por ordem decrescente:

Quadro 1. Resultados das análises de laboratório em variedades de batata doce.

Variedades	Materia seca g/100 g	Umidade g/100 g	β -amilase mg glucose/ml/ 5 min./100	pH	Brix		Amido g/100 g M.S.
					Caldo Cru	Caldo Cozido	
IAC 02/19	27.68	72.32	905.0	6.3	7.5	25.2	52.82
IAC 3/4	36.61	63.39	810.0	6.4	10.0	29.4	67.73
IAC 45/71	33.69	66.31	735.0	5.8	10.0	25.2	57.46
IAC 58/71	22.88	77.12	785.0	6.1	8.0	21.0	65.53
IAC 66/118	32.41	67.59	660.0	5.8	10.0	24.0	62.05
IAC 138-Z	41.63	58.37	870.0	6.4	13.0	29.4	70.08
SRI 066	40.09	59.91	704.5	6.0	14.0	30.0	65.18
SRI 072	45.88	54.12	856.5	5.8	12.0	28.8	78.72
SRT 129	31.78	68.22	890.0	5.7	13.0	19.2	42.61
SRT 225	33.58	66.42	735.0	6.1	14.0	30.0	54.29
SRT 230	38.14	61.86	535.0	6.5	11.0	27.0	73.47
SRT 250	24.05	75.95	887.5	5.4	13.0	21.6	51.70
SRI 252	37.47	62.53	777.5	6.3	10.0	36.0	72.38
SRI 253	37.01	62.99	772.5	6.5	12.0	22.8	69.87
SRI 257	40.47	59.53	725.0	6.6	16.0	31.8	59.14
SRT 263	48.21	51.79	762.5	6.5	15.0	36.0	69.47
SRI 269	37.92	62.08	762.5	6.2	12.0	26.4	69.47
SRT 272	26.47	73.53	887.5	6.1	13.0	21.0	56.25

Variedades	-amilase unidades/ g M.S.	Viscosidade pasta cozida S.U.**	Escurecimento D.O./g	Aspecto geral*				Cor da polpa
				Cor	Sabor	Aroma	Media	
IAC 02/19	1 142.86	29	0.00	11.0	10.0	10.0	10.3	Laranja
IAC 3/4	1 777.78	15	8.75	8.0	10.0	8.0	8.7	Creme
IAC 45/71	1 230.77	21	0.00	8.0	7.0	7.0	7.3	Creme
IAC 58/71	410.26	20	0.00	7.0	5.0	8.0	6.7	Creme
IAC 66/118	1 230.77	16	7.46	8.0	9.0	8.0	8.3	Creme
IAC 138-Z	2 285.71	20	12.36	9.0	9.0	10.0	9.3	Laranja
SRI 066	3 200.00	31	35.89	5.0	6.0	4.0	5.0	Creme
SRI 072	2 285.71	13	11.53	9.0	4.0	6.0	6.3	Creme
SRT 129	842.10	18	0.00	9.0	8.0	7.0	8.0	Laranja
SRT 225	2 285.71	38	2.96	10.0	10.0	10.0	10.0	Roxa
SRI 230	1 142.86	12	0.00	9.0	6.0	8.0	7.7	Creme
SRT 250	1 777.78	14	10.00	6.0	8.0	8.0	7.3	Laranja
SRT 252	516.13	48	0.00	9.0	6.0	8.0	7.7	Creme
SRI 253	516.13	16	4.76	4.0	8.0	6.0	6.0	Creme
SRT 257	1 777.78	15	10.55	2.0	4.0	5.0	3.7	Creme
SRT 263	1 777.78	18	10.28	2.0	2.0	2.0	2.0	Creme
SRT 269	1 000.00	23	10.49	11.0	11.0	10.0	10.7	Roxa
SRT 272	3 200.00	40	2.58	9.0	9.0	9.0	9.0	Laranja

* Média de Notas.

** Segundos Saybolt Universal.

SRT-263, SRT-072, IAC-138-Z, SRT-257 e SRT-066. Destas, a IAC-138-Z é de polpa laranja.

O teor de amido em batata doce é um fator de grande interesse para a indústria. É fato conhecido que a batata doce contém grandes quantidades de

amido, o qual é convertido, em parte, em maltose e dextrinas durante a cocção, devido à presença de diástases ativas (12). O teor de amido convertido por enzimas durante a cocção varia em torno de 53.8 a 95.4%, de acordo com a variedade (12). Os autores são unânimes em afirmar que o teor de amido

Quadro 2. Valores médios e intervalo de confiança calculados para os resultados das análises de batata doce.

Análise	Média	Intervalo de confiança
Matéria Seca	3.03 – 38.64 g/100 g	32.026 – 38.637
Umidade	61.36 – 67.97 g/100 g	61.363 – 67.974
β -amilase	736.36 – 825.97 mg glucose/ml/min./100	736.362 – 825.971
Brix do Caldo Cru	10.78 – 12.94 %	10.776 – 12.944
pH do Caldo Cru	5.98 – 6.30	5.985 – 6.298
Brix do Caldo Cozido	24.02 – 28.84 %	24.020 – 28.840
Amido	58.85 – 67.62 g/100 g	58.846 – 67.623
α -amilase	1 181.09 – 1 974.48 unidades/ml	1 181.092 – 1 974.478
Viscosidade	17.71 – 27.51 S.U. Saybolt	17.712 – 27.510
Escurecimento Enzimático	5.67 – 10.63	5.667 – 10.634
Aspecto Geral	6.51 – 8.69	6.506 – 8.688

tem influência direta sobre a viscosidade da pasta cozida e, indiretamente, através da ação das enzimas amilolíticas, sobre o estoque de açúcares produzidos durante a cura ou processamento sob calor. Destas enzimas, são encontradas na batata doce a α e a β -amilase.

A β -amilase parece ser a mais ativa das duas (12), dando origem a maltose, mesmo a altas temperaturas.

A ação da α -amilase sobre o amido dá formação à dextrinas e estas têm o seu ótimo de atividade a 70-75°C, a pH 6.0 (12). Esta atividade, à temperaturas elevadas é confirmada por Deobald (6).

No presente trabalho, as variedades apresentaram teor médio de amido de 63.23 g e as variedades que apresentaram teores acima desta média foram, em ordem decrescente: SRT-072, SRT-230, SRT-252, SRT-138-Z, SRT-253, SRT-263, SRT-269 e IAC-3/4. Destas, a SRT-138-Z, é de polpa laranja e a SRT-269 é de polpa roxa.

O pH médio foi de 6.14, portanto, de uma maneira geral as variedades apresentaram um pH próximo à faixa de atividade ótima das enzimas amilolíticas.

Quanto às enzimas amilolíticas, foram detectadas atividade tanto da α como da β -amilase.

A atividade média da α -amilase foi de 1577.78 unidades/ml, e apenas mostraram-se pouco ativas as variedades: IAC-58/71, SRT-129 (laranja), SRT-252 e SRT-253. As variedades SRT-272 (laranja) e SRT-066 apresentaram uma atividade de 3 200 unidades/ml.

A atividade da β -amilase foi avaliada pela produção de açúcares redutores em condições de ensaio e as variedades, de uma maneira geral apresentaram

altas atividades. A média calculada foi de 781.17 mg glucose/ml/minuto x 100 e as variedades mostraram atividade próxima desta média. Como consequência da atividade destas enzimas, pudemos notar, e era esperado, um aumento do Brix do caldo cru após a cocção.

A média calculada para o Brix do caldo cru foi de 11.9%, sendo que as variedades que apresentaram Brix mais elevado do que a média foram, por ordem decrescente: SRT-257, SRT-263, SRT-225 (roxa), SRT-066, IAC-138-Z (laranja), SRT-129 (laranja) e SRT-272 (laranja).

Quanto ao Brix do caldo cozido, a média calculada foi de 24.4%. Algumas variedades mostraram valores bem elevados; com a variedade SRT-263, mas partiram de um Brix inicial elevado, já a variedade SRT-252 havia apresentado um Brix de 10.0%, sendo o aumento devido a alta atividade enzimática. Se calcularmos o aumento de grau Brix após o cozimento, em termos de porcentagem, vamos verificar que algumas variedades, como a SRT-252, apresentaram um aumento de Brix da ordem de 260%, em relação ao Brix do caldo cru, o que mostra muito bem a importância das enzimas amilolíticas (Quadro 3).

Quanto à viscosidade, parece ser de grande importância quanto a qualidade da batata doce para indústria. Segundo Rao e Humphries (10) a viscosidade aparente é uma maneira definitiva de classificar os cultivares de batata doce quanto às qualidades organolépticas, principalmente quanto ao sabor. Assim, as variedades que apresentam viscosidade aparente elevada (> 2 000 c.p.) tendem a apresentar uma consistência "seca" e as abaixo de 1 000 c.p. são consideradas "úmidas". Como os dados do presente trabalho foram obtidos por outros métodos, torna-se difícil a comparação. Apesar disto, podemos constatar que para os valores obtidos isto parece não ocorrer, já

Quadro 3. Aumento, expresso em porcentagem, do Brix de batata doce submetida a cozimento.

Variedade	Brix do caldo cru %	Brix do caldo cozido %	Aumento do grau Brix %
IAC-02/19	7.5	25.2	223.33
IAC-3/4	10.0	29.4	194.00
IAC-45/71	10.0	25.2	152.00
IAC-58/71	8.0	21.0	162.50
IAC-66/118	10.0	24.0	140.00
IAC-138-Z	13.0	29.4	126.15
SRT-066	14.0	30.0	114.29
SRT-072	12.0	28.8	140.00
SRT-129	13.0	19.2	47.69
SRT-225	14.0	30.0	114.29
SRT-230	11.0	27.0	145.46
SRT-250	13.0	21.6	66.15
SRT-252	10.0	36.0	260.00
SRT-253	12.0	22.8	90.00
SRT-257	16.0	31.8	98.75
SRT-263	15.0	36.0	140.00
SRT-269	12.0	26.4	120.00
SRT-272	13.0	21.0	61.54

que variedades de alta viscosidade, SRT-272, por exemplo, tiveram uma média elevada para o aspecto geral enquanto outras que tiveram valores bem baixos de viscosidade, tiveram notas mais baixas em aspecto geral. Esta característica merece, ao nosso ver, estudos mais aprofundados. A viscosidade média encontrada foi de 22.61 S.U. Saybolt. Preferimos destacar a maior viscosidade, por acreditar que estas darão produtos processados mais consistentes. As variedades que se destacaram por uma viscosidade mais elevada foram: SRT-252, SRT-272, SRT-225, SRT-066, IAC-02/19, por ordem decrescente.

O escurecimento enzimático é fator importante para a industrialização da batata doce já que quando não são inativadas estas enzimas, ocorrem severas corrosões em latas (11). O autor observou que as variedades que causam mais corrosões têm também alta atividade de fenoloxidasas. Além disso, as batatas com alta atividade de fenoloxidasas escurecem rapidamente quanto cortadas, proporcionando, ao produto de sua industrialização, uma coloração escura que o deprecia. Como se pode observar pelo Quadro 1, obtivemos variedades que não apresentaram escurecimento (0.0 D.O./g) e outras com 35.89 de D.O./g, nas condições de ensaio. A média encontrada foi de 10.63, sendo que as variedades que não apresentaram escurecimento apreciável em condições de ensaio, foram: IAC-02/19, IAC-45/71, IAC-58/71, SRT-129, SRT-230 e SRT-252.

Quanto às qualidades organolépticas ensaiadas, a nota média obtida foi de 7.60 e as variedades que se

destacaram da média foram, pela ordem: SRT-269, IAC-02/19, SRT-225, IAC-138-Z e SRT-272.

Conclusões

Considerando os valores de maior importância para a indústria alimentícia como pontos ganhos, podemos concluir que se destacaram das outras variedades, pelo total de pontos e pela ordem, as seguintes: IAC-138-Z, SRT-263, SRT-225, SRT-272, SRT-257, SRT-066 e SRT-072. Destas, a IAC-138-Z e SRT-272 são de polpa laranja e a SRT-225 de polpa roxa. As demais são creme.

Resumo

O estudo comparativo das variedades de batata doce visou sua utilização em indústrias de alimentos. Dezoito variedades foram selecionadas, entre as quais haviam as de polpa creme, roxa e laranja, sendo a última a preferida pelas indústrias. Após a colheita as batatas foram remetidas ao laboratório onde foram realizadas as seguintes análises: cor, matéria seca, amido, α e β amilase, escurecimento enzimático, brix do caldo cru e cozido, pH do caldo cru, viscosidade da pasta cozida e aspectos gerais. Da análise dos resultados concluímos que algumas das variedades apresentaram características promissoras do ponto de vista do aproveitamento em indústrias de alimentos.

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Resumen

El estudio de las relaciones entre fuentes de sustratos y del desarrollo de botones florales de café después de la latencia, ha demostrado que se requiere un área foliar de aproximadamente 4 70 cm² para que una flor normal se abra. La fotosíntesis concurrente, dependiendo del área de la hoja, fue esencial para el proceso y excedió la contribución de las reservas almacenadas, ambas en las hojas y en la madera de las ramas. Los botones florales fueron capaces de utilizar sustrato procedente de hojas alejadas del botón, ya sea en dirección ascendente o descendente.

Introduction

During the formation and initial growth of coffee flower buds, a steady decline in the starch reserves in the leaves and branch wood is observed, indicating a mobilization of carbohydrates (Janardhan *et al.* 12). Following a short period of initial growth, the flower buds pass through a variable period of dormancy (Mes 15, Frederico and Maestri 7), when the metabolic activities are minimal (Gopal and Vasudeva 9). With the onset of the first rains, after a dry period or a sudden drop in the temperature, or both (Browning 2), the flower whorls expand rapidly until anthesis, with a marked rise in

the metabolism of carbohydrates (Croope *et al.* 6, Gopal *et al.* 10, Janardhan *et al.* 13) and with an increase in dry matter of over 500% in relation to the dormant buds (Mes 15, Croope *et al.* 6). Thus, a fast transport of organic substances to the flower buds is necessary at this time.

This paper reports on the contribution of different sources of assimilates for the growth of flower buds, from the break of dormancy to anthesis.

Material and methods

This study was carried out in a greenhouse in Viçosa, Minas Gerais from August through October 1979, using a local selection of the hybrid "Catimor" (*Coffea arabica* L. x *C. canephora* Pierre). The plants, which were approximately three years old and blossoming for the first time, were grown in soil in 20 kg cans. Flower bud dormancy was broken by plentiful re-watering of the soil, following an eight-day period of suspended irrigation. This procedure is considered to mimic the natural conditions (Cramer 5, Alvin 1).

The treatments, which followed a completely randomized design with four replications, were set in place before the re-watering. Basically, these consisted of the isolation of a node or a branch segment of one or a few internodes of a first order

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lateral branch by making one bark girdle above and one below the node or the branch segment under study.

The contribution of the concurrent photosynthesis was estimated by leaving six flower buds and leaves trimmed to areas of approximately 30.00, 22.50, 15.00, 7.50 and 2.75 cm² on each axil of girdle-isolated nodes. Parallel treatments, but with leaves covered with aluminum foil, were made to assess the contribution of assimilate reserves of the leaves (previous photosynthesis). Leafless segments of one, two and four internodes in length, with six flower buds in each axil of a single node, served to estimate the contribution of assimilate reserves in the wood. As controls, ungirdled nodes were left with six flower buds and 30.00 cm² of leaf area per axil, either covered or uncovered.

The attracting ability of floral buds for assimilates from sources located at various distances was measured by taking branch segments having six flower buds per axil of a node and a leaf area of 40.00 cm² per axil either on the node under study, or on the first, second or fourth node, either above or below the flower buds.

A possible lateral transport was investigated by leaving six flower buds on one axil and only a 40.00 cm² leaf on the opposite axil, either of the same node or of the node immediately above. The leaf area needed for the normal development of one bud was determined by leaving a leaf area of approximately 12.50 cm² and two, four and six flower buds on each axil of girdle-isolated nodes.

The competition ability for assimilates by the flower buds and by the apical vegetative bud was studied by taking girdle-isolated segments of first order branches, as comprised of the most mature distal node with six flower buds and the terminal bud, and with one 20.00 cm² leaf per axil, at an intermediate position, left between those two structures. Identical segments, either without flower buds or detipped, completed the treatment series.

Every other day, starting from the re-watering of the plants, the lengths of the flower buds were taken and their dry weight was estimated by the following relation previously established from direct measurements:

$$Y = 1.8541 + 0.3008X + 0.0096X^2$$

where Y is the dry weight, and X is the length.

Results and discussion

Mass transfer to the flower buds after the break of dormancy seemed to be a function of the available leaf area which reached a plateau at a leaf area of 28.80 cm² for six buds. This represented a requirement of an area of 4.70 cm² for the normal expansion of one bud (Figure 1). This should explain why the flower buds did not go on to anthesis when Magalhães and Angelocci (14) removed the leaves of a girdled flowering node. It also explains why Robusta coffee trees bear a larger number of flower per axil than do those of Arabica. In Figure 1, it can be observed that the ability of the flower buds to mobilize reserves from the leaves appeared to saturate from a rather low leaf area of about 15.00 cm². Beyond this value, the mass transfer seemed to be due to the concurrent photosynthesis.

Despite the rather low starch content in the branches of coffee trees (Cooil 4), reserves were readily available to the growth of buds, as shown in Figure 2. The slope of the curve suggests that mobilization ability of the flower buds might go beyond the fourth internode, which could explain Browning's (3) observation that some flower buds open even on defoliated branches.

Four sources of organic nutrients can participate in the final dry weight of flower buds after dormancy break: 1) concurrent photosynthesis in the leaves; 2) branches and 4) original matter of the dormant bud proper. In this study, the average dry weight of the dormant buds was 3.90 ± 0.80 mg and only the

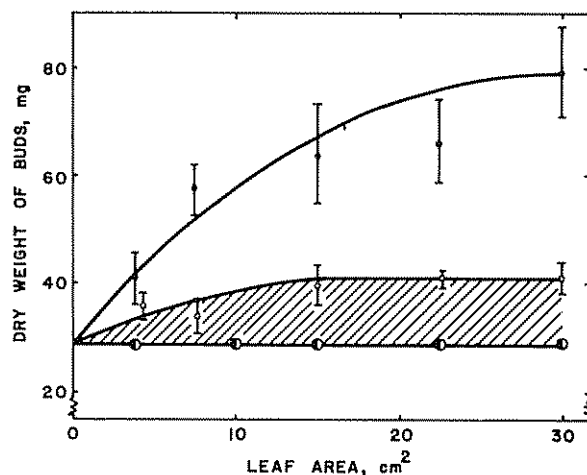


Fig. 1. Dry matter accumulation in six flower buds as a function of the leaf area. Leaves uncovered (●) or covered with aluminum foil (○). (■) dry matter contribution from the dormant bud. The vertical line represents the standard mean deviation in this and in the following figures.

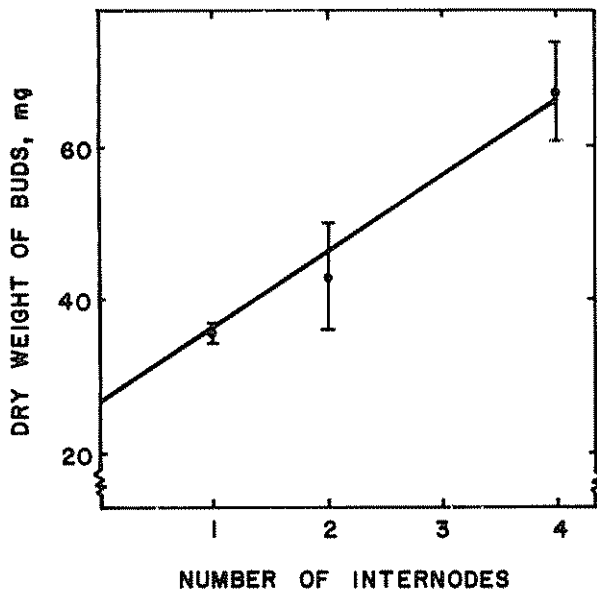


Fig 2. Dry matter transfer from internodes to six flower buds per axil. Dry matter accumulation is taken from the dry weight of the dormant buds as the start.

weight in excess of 4.70 mg, the upper limit of the range (shown in Figures 1-5 and 7 as 28.20 mg for six buds per axil), was considered as mass transfer. Although the chloroplasts of dormant flower buds are able to carry on the Hill reaction, they occur in such small numbers that their contribution is negligible (Janardhan and Gopal 11). Furthermore, after the second day following re-watering, when dry matter accumulation began to accelerate, the flower buds were already of a whitish colour. The contribution of the original matter of the dormant buds to the final weight of the open flowers is shown in Figure 3. This ranged from 100% in the dormant bud to 35% in the open flower. These values are likely affected by varietal and environmental differences as dry weight of the dormant buds can change from approximately 3.00 mg in Minas Gerais, Brazil (Frederico and Maestri 7), to 5.50 and 6.70 mg in different seasons in Southern India (Gopal and Vasudeva 9).

By assuming that the expanding flower buds can drain reserve assimilates from along the length of one internode (half internode lengths above and below the node under study), one internode could supply only 7.00 mg of dry matter for the flowers on one axil of a node, on the twelfth day after the start of re-watering (Figure 3). This accounted for 8.75% of the total dry weight and 13.5% of the mass transfer to six flowers. Considering that the average dry weight of a flower ranges from 13.00 to 15.00 mg, the contribution of one internode seemed to be

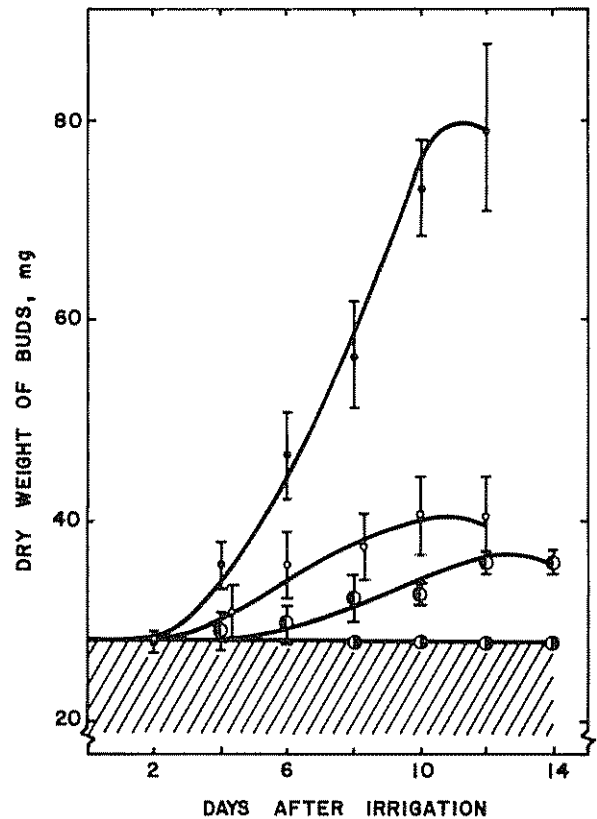


Fig 3. Expansion of six flower buds as a function of the source of assimilates ● - uncovered leaves, ○ - covered leaves, ◐ - internode, ◑ - original organic matter from the dormant bud.

insufficient for the expression of a single open flower (4.70 mg from the dormant bud plus 7.00 mg from the internode). Concerning this, Mes (15) had already observed that the food material from an explant of one node plus one internode was sufficient only for a limited growth of the buds, and these did not reach full anthesis. However, the mass transfer from several internodes to just one internode (Figure 2) could bring about some flower opening, as observed in this study and in the previously mentioned example of Browning (3). Therefore, although the flower buds easily were able to mobilize the reserves from the branches (as shown in Figure 2), the contribution of one internode for the expansion of the flower buds (Figure 3) seemed to be limited by its low storage content (Cooil 4). This is in clear contrast to many temperate woody plants whose flower buds open at the beginning of spring when the plants bear no leaves.

In spite of the high starch content in coffee leaves which can account for as much as 20% of their dry weight (Cooil 4), it seemed that the flower buds attracted only the food material stored in the

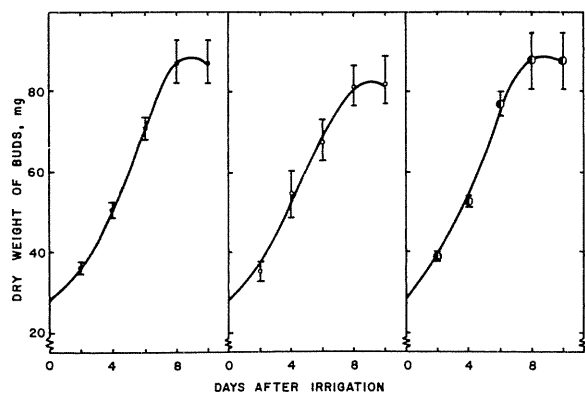


Fig. 4. Growth of six flower buds as a function of a 40.00 cm^2 leaf area left at each axil of the subtending node (\bullet), two nodes above (O), or two nodes below (O), the buds under study.

proximal parts of leaves, as indicated by the data in Figure 1. This seems to suggest that leaf reserves are not so readily available to flower bud growth as are the branch storages. With saturating leaf area, the contribution of leaf storage for the flowers did not exceed 13.00 mg (Figure 1), which represented 25.0% of the mass transfer and 10.5% of the total dry weight of the six flowers (Figure 3).

On the other hand, concurrent photosynthesis could supply 40.0% of the total dry weight and 61.5% of the mass transfer to the six flowers (Figure 3), and so was the most important source for flower bud growth. The other three sources previously discussed were naturally limited in supplying carbohydrates for flower bud growth. However, as shown in Figure 1, assimilates from concurrent photosynthesis could be transferred to the threshold of the sink capacity of the flower buds (see Figure 6, as well). Since the contributions of the leaf reserves saturated at a low leaf area (Figure 1), the formation of flowers with over 15.00 mg in dry weight, or the maintenance of a greater number of buds per axil, depend basically on an increase in the photosynthesis as obtained through the larger leaf area required for the flower expansion (Figure 1).

No difference was observed in the growth of flower buds on ungirdled axils, whether the subtending leaves were covered or not. Taking into account that the reserves in the leaves (Figure 1) and in one internode (Figures 2 and 3) are well below the threshold value for the opening of six flowers, a transport of assimilates must have occurred from more distant leaves to the flower buds on axils with covered leaves. This hypothesis was tested by taking a 40.00 cm^2 leaf per axil which either subtended six buds or was located one, two or four internodes

below or above the buds under study. Since there were no differences among the treatments, Figure 4 shows only the results from the control (flower buds on the same node as leaves) and from the flower buds two internodes apart, both above or below the source leaves. It can be seen that the buds attracted assimilates from sources located at some distance, irrespective of whether the flow direction was acropetal or basipetal. Even two days after rewatering, no differences in the growth of the buds were observed. In the case of distant sources, initial growth must have been due to storage reserves in the branches (Figure 2), until a direct connection of the sinks (buds) with the main sources (leaves) was established. Very likely, this occurs in the first growth stages of the buds. These facts would explain why, under natural conditions, open flowers can be seen all along a branch which retains only the most apical two or three leaf pairs. Growth of flower buds also did not differ even when the leaves were located on the opposite side of the same node or on the node immediately above (curves in Figure 5 compared to the curve on the far left in Figure 4). Thus apparently the transport system must be so interconnected, either at the node level or at the internode length, so that a radial flow of assimilates obtains.

Some difference is noted between the growth curves as given in Figure 3 and in Figures 4 and 5. While in the former, growth on the second day was slow, with anthesis occurring on the 10th to 12th day after irrigation, growth in the first two day, as shown

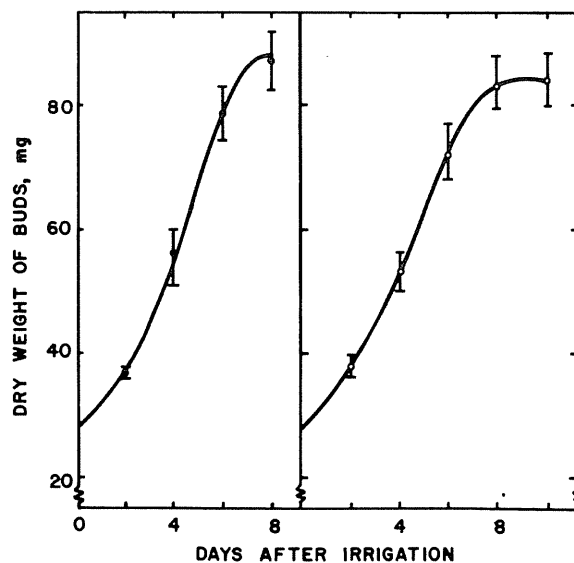


Fig. 5. Growth of six flower buds as a function of a 40.00 cm^2 leaf area left on the opposite side of the same node (\bullet) or of the node immediately above (O).

in Figures 4 and 5, was already very fast and the flowers opened on the 7th to 8th day after irrigation. Since the growth rate of coffee flower buds is directly related to temperature (Mes 15), it can be assumed that the above differences were due to lower temperatures which prevailed in Viçosa in August, when the data of Figure 3 were collected, than in October, at which time the data for curves of Figures 4 and 5 were taken.

The study on the change in the number of buds with relation to a fixed leaf area of approximately 12.50 cm² has shown that, despite a rise in net assimilation rate with an increase in the quantity of buds, the maximum number of normal open flowers remained at about 2.63 (Figure 6). That is, a leaf area of approximately 4.75 cm² is necessary for the expansion of a normal flower, quite in agreement with the data of Figure 1. Excessive numbers of flower buds per axil resulted in abortion, abscission, or abnormal flowers.

When flower buds and the terminal vegetative bud competed for the assimilates from a common source of 20.00 cm² leaf, which is insufficient to support

the expansion of six flower buds (Figure 1), it was observed that the flower buds grew as well as those on a branch without the terminal vegetative bud (Figure 7). On the other hand, the expansion of the terminal leaves was always much greater in the treatments in which the flower buds were removed than in those which had the competing flower buds. In the latter, the terminal leaves started expanding only after the 10th day, when flower buds had abscised or stopped growing. This indicates a larger sink strength for the flower buds than for the vegetative buds. This also happens in *Monodora tenuifolia* Benth, a tropical tree which bears vegetative and floral buds spatially conjugated and not so distant as the buds under the artificial conditions of the present work. The expansion of flower buds precedes the sprouting of new leaves which, too, points to a higher competitive ability of the flower buds (Njoku 16).

Conclusions

Since the reserves in the internode that were available for the expansion of the flower buds were

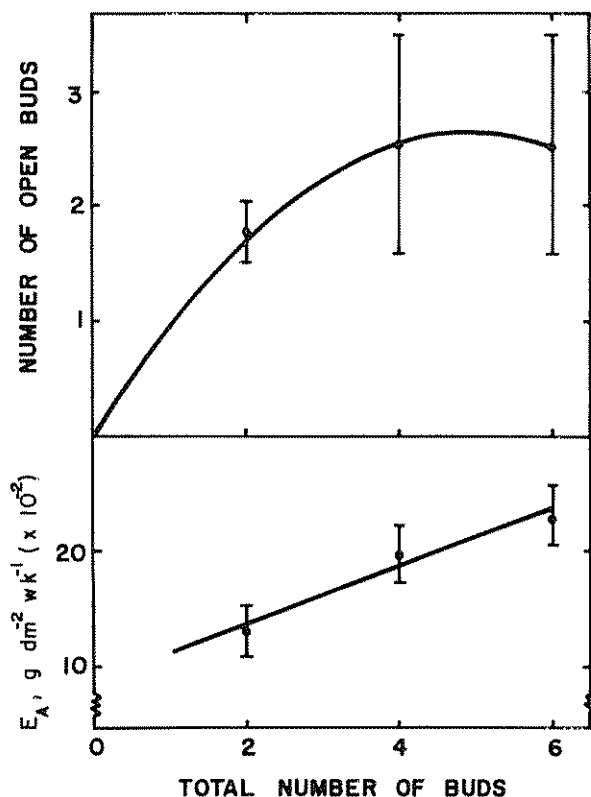


Fig. 6. Number of open flowers and net assimilation rate (E_A) as a function of the number of flowers per axil, with a constant leaf area of approximately 12.50 cm².

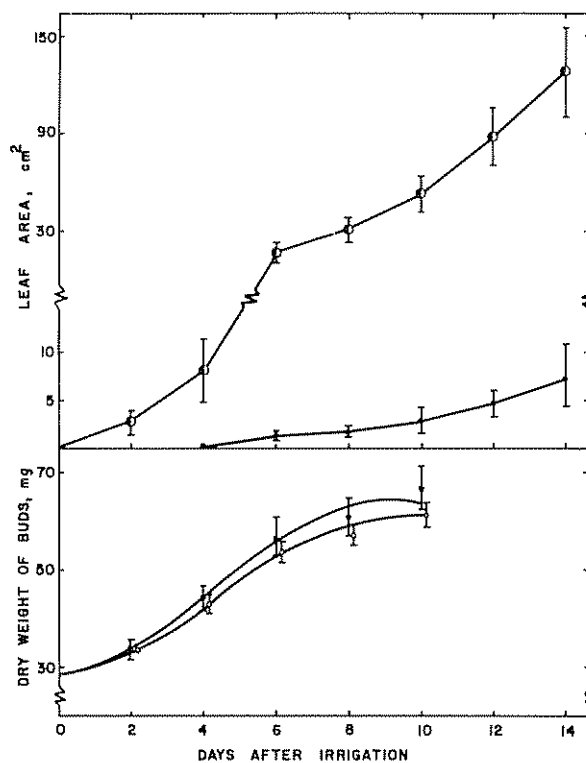


Fig. 7. Mass transfer from a 20.00 cm² leaf area left in an intermediate position between six flower buds and the terminal vegetative bud (O), or under otherwise similar conditions but with either the flower buds (O) or the terminal vegetative bud (O) removed

limited to 700 mg per axil (Figure 3), and the supply of reserves from the leaves saturated at a low leaf area (Figure 1) and, in consideration that under natural conditions the number of flower buds per leaf axil is well above six, the number tested in this study, the relative contributions of those sources of assimilates should be lower. Moreover, as a consequence of a higher leaf area, the participation of the concurrent photosynthesis should be higher than that observed in the present work. The information gathered here supports the contention that for the expansion of the flower buds, the contribution of the various sources followed the order: concurrent photosynthesis \gg storage reserves in the leaves $>$ storage reserves in the branch wood, not taking into consideration the dry matter of the dormant bud itself. In addition, concurrent photosynthesis may be the sole source that could be, in part, affected by the sink strength (Figure 6). These facts may explain the higher correlations of the number of normal opened flowers with the number of leaves rather than with branch reserves, as reported by Gopal and Raju (8).

The initial enlargement of the buds, after re-watering, did not differ statistically, whatever the sources of assimilates to which they were connected (Figure 3). If in this stage, the organic nutrients come preferentially from wood reserves in the branches, from reserves in the leaves or from concurrent photosynthesis, or from the two or three sources simultaneously, only further studies on assimilate mobilization can demonstrate this. One needs to explain why, in absolute quantities, the contribution of leaf reserves for the total final dry weight of the bud saturates at so low a leaf area (Figure 1), even though the leaf starch content could be high (Cooil 4).

Detailed anatomic studies of the transport system for assimilates are also needed to explain the reason for the existent up and down flow (Figure 4) and radial flow, as well (Figure 5).

Although the floral buds have potentially a higher competitive ability than the terminal vegetative bud, this may not be, in fact, a true competition, since the leaves subtending the floral buds were removed (Figure 7). Although some competition may be initiated, the processes of enlargement and anthesis in plants with gregarious flowering, such as coffee, occur over such a short time span that the vegetative growth would hardly be impaired; hence, the phenologic pattern of plant growth may not be affected.

Summary

A study of the relationship between sources of assimilates and the growth of flower buds of coffee, after dormancy break, has shown that a leaf area of approximately 4.70 cm² is required for the opening of one normal flower. Concurrent photosynthesis, as dependent on leaf area, was essential for the process and far exceeded the contribution of storage reserves, both in the leaves and in the branch wood. Flower buds were able to draw assimilates from distant source leaves, irrespective of the up or down flow direction. Lateral transport was also observed. Flower buds had a greater sink strength than the terminal vegetative buds of the branch.

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Reseña de libros

IOSHIDA, S. *Fundamentals of rice crop science* 1981. International Rice Research Institute. Los Baños, Philippines. 1981.

Este libro trata sobre aspectos fisiológicos del cultivo del arroz.

La obra consta de 269 páginas e incluye 479 referencias generadas en las últimas 3 décadas sobre la ciencia en el cultivo del arroz, destacándose las investigaciones generadas por el IRRI en estos últimos 20 años.

El libro cubre la mayoría de los aspectos fisiológicos del cultivo del arroz y su relación con otras disciplinas y enfatiza el hecho de que el arroz sigue siendo un alimento vital para más de la mitad de la población del mundo.

El primer capítulo pone en conocimiento del lector, estudiante o productor, todos los aspectos del crecimiento y desarrollo del cultivo. En el capítulo dos el autor muestra en forma práctica como los factores climáticos afectan el crecimiento y desarrollo del cultivo. Los capítulos tres y cuatro cubren todos los aspectos nutricionales, tanto los positivos como los desórdenes de tipo negativo.

Los fundamentos fisiológicos del cultivo adquieren su relevancia cuando se discuten en el capítulo cinco los aspectos de fotosíntesis y respiración siempre relacionados con el crecimiento del arroz. Los índices óptimos de área foliar se discuten en este capítulo y los aspectos bioenergéticos de producción del cultivo.

En el capítulo seis se tratan las características de la planta de arroz y su habilidad productiva; se destacan las nuevas variedades de alta producción adaptada a las condiciones del trópico y su relación con las características de plantas asociadas con el nuevo y viejo concepto del tipo de planta ideal.

El autor termina el libro con los aspectos fisiológicos y su relación con el análisis de rendimiento. Se encuentran y discuten dentro del texto el por qué de las diferencias de rendimiento con diferentes grados de tecnología y el máximo potencial productivo y los factores que actualmente limitan ese máximo productivo. En otras palabras, los capítulos seis y siete el autor discute los componentes y factores que influyen en el rendimiento.

En términos generales el libro está escrito en una forma muy comprensible. En sus 269 páginas incluye un total de 92 cuadros y 123 figuras, más 39 láminas a colores sobre deficiencias y desórdenes nutricionales del arroz. Los cuadros, láminas y figuras hacen muy amena la lectura y facilitan la comprensión de los aspectos fisiológicos del cultivo del arroz.

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INFLUÊNCIA DAS BACTÉRIAS LÁTICAS MESÓFILAS: *Streptococcus cremoris*,
Streptococcus lactis, *Streptococcus diacetylactis* e *Leuconostoc citrovorum* NAS
CARACTERÍSTICAS DO QUEIJO TIPO MINAS. NITROGÊNIO SOLÚVEL, NITROGÊNIO
NÃO PROTEICO E ÍNDICE DE MATURAÇÃO¹

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Summary

The objective of the present work was to compare the influence of some mesophilic lactic acid bacteria: Streptococcus cremoris, S. lactis, S. diacetylactis and Leuconostoc citrovorum, as they affect soluble nitrogen, non proteic nitrogen and ripening degree of Minas cheese

In cheeses prepared with different associations of bacteria species soluble nitrogen, non proteic and ripening degree were determined at 10, 20 and 30 days after elaboration.

The data verified that there was no definite relation among the obtained values and the different ratios of the utilized bacteria. However a gradual increase of these values during ripening was verified

Introdução

A utilização de microrganismos na elaboração de queijos, praticamente existe desde os primórdios de sua fabricação. Antigamente, quando o queijo era fabricado em condições empíricas com a utilização de leite cru, podia-se considerar que o mesmo tinha uma flora diversa. Com a melhoria da qualidade do leite, e com a utilização da pasteurização como etapa anterior à fabricação tornou-se imprescindível a adição de cultura láctica selecionada para fornecer os microrganismos necessários à obtenção de um produto adequado (9).

As bactérias lácticas mesófilas homofermentativas: *Streptococcus cremoris* e *Streptococcus lactis* são comumente usadas isoladamente ou em combinação com

as heterofermentativas *Streptococcus diacetylactis* e *Leuconostoc citrovorum* na fabricação de diversas variedades de queijos (6, 12). No Brasil estas espécies são utilizadas principalmente na elaboração de queijos de massa crua e semi cozida.

A presente pesquisa foi desenvolvida tendo por objetivo fazer um estudo comparativo sobre a influência destas espécies em relação ao nitrogênio solúvel, nitrogênio não proteico e índice de maturação do queijo tipo Minas. O trabalho foi estabelecido para estudar o comportamento isolado ou em combinação e também variações nas concentrações das diferentes espécies de bactérias lácticas. Simultaneamente, procurou-se verificar a evolução de nitrogênio solúvel, nitrogênio não proteico e índice de maturação no decorrer da cura do queijo Minas.

Materiais e métodos

Utilizou-se na presente pesquisa leite pasteurizado tipo C. No laboratório procedeu-se às análises do

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leite, conforme descrito em Bonassi *et al* (4). O leite utilizado apresentou valores de acidez expressa em ácido láctico de 0.17% a 0.19% e para a matéria graxa valores de 3.1 a 3.5%.

Estabelecidos os tratamentos a serem efetuados, foi feita a distribuição ao acaso em 4 ensaios. Para confirmar a casualização e para maior segurança nas determinações, foi constituído posteriormente o ensaio de número V. Esse, também foi formado ao acaso, englobando um tratamento de cada ensaio anterior.

O delineamento adotado foi o de blocos ao acaso, com esquema fatorial. A comparação das médias dos tratamentos foi feita pelo teste de Tuckey (8). Todos os ensaios foram realizados com queijos distribuídos em 3 blocos de 5 tratamentos. Cada bloco constituiu-se de peças de queijos obtidas de um mesmo leite de fabricação, repartidos em 5 tanques de aço inoxidável, para serem efetuados os tratamentos. Cada unidade experimental foi constituída por um queijo de 16 cm de diâmetro e 5 cm de altura. Os tratamentos foram:

ENSAIO I

1. Testemunha (T) — Sem adição de bactérias lácticas
2. 95% de *S. lactis* e 5% de *S. diacetilactis*
3. 95% de *S. lactis* e 5% de *L. citrovorum*
4. *S. cremoris*
5. 50% de *S. lactis* e 50% de *L. citrovorum*

ENSAIO II

1. Testemunha (T) — Sem adição de bactérias lácticas
6. 75% de *S. cremoris*, 5% de *S. lactis* e 20% de *S. diacetilactis*
7. *S. lactis*
8. 50% de *S. lactis* e 50% de *S. diacetilactis*
9. 95% de *S. cremoris* e 5% de *S. lactis*

ENSAIO III

1. Testemunha (T) — Sem adição de bactérias lácticas
10. 70% de *S. cremoris*, 5% de *S. lactis*, 20% de *S. diacetilactis*, 5% de *L. citrovorum*. Neste tratamento utilizou-se CH Normal 01 (10).
11. 50% de *S. cremoris* e 50% de *L. citrovorum*
12. 90% de *S. cremoris*, 5% de *S. lactis* e 5% de *L. citrovorum*
13. 95% de *S. cremoris* e 5% de *S. diacetilactis*

ENSAIO IV

1. Testemunha (T) — Sem adição de bactérias lácticas
14. 50% de *S. cremoris* e 50% de *S. lactis*
15. 50% de *S. cremoris* e 50% de *S. diacetilactis*
16. 25% de *S. cremoris*, 25% de *S. diacetilactis*, 25% de *S. lactis* e 25% de *L. citrovorum*
17. 95% de *S. cremoris* e 5% de *L. citrovorum*.

ENSAIO V

1. Testemunha (T) — Sem adição de bactérias lácticas
3. 95% de *S. lactis* e 5% de *L. citrovorum*
7. *S. lactis*
10. 70% de *S. cremoris*, 5% de *S. lactis*, 20% de *S. diacetilactis* e 5% de *L. citrovorum*. Neste tratamento utilizou-se a cultura CH normal 01 (10).
11. 50% de *S. cremoris* e 50% de *S. diacetilactis*

A fabricação foi efetuada segundo Bonassi *et al* (4). A cultura láctica, variou conforme os tratamentos, sendo utilizada na proporção de 1%, em relação ao leite de fabricação. O tempo de coagulação para todos os tratamentos foi 45 minutos (4).

Decorridos 10, 20 ou 30 dias após o início da fabricação, retirou-se da câmara de maturação um queijo de cada tratamento, eliminou-se uma porção de 2 cm correspondente à casca. Após serem raladas, as amostras devidamente acondicionadas em frasco de vidro foram armazenadas em congelador a $-18^{\circ}\text{C} \pm 1^{\circ}\text{C}$, sendo posteriormente efetuadas as análises químicas, num período máximo de 1 mes.

As determinações feitas foram: a) nitrogênio na forma solúvel em água (18), utilizando-se o método Kjeldahlmicro (2); b) nitrogênio não proteico (13), utilizando-se o método Kjeldahl-micro (2); c) o índice de maturação (% de nitrogênio solúvel em relação ao nitrogênio total) foi calculado pela fórmula: $\text{N solúvel} \times 100/\text{N total}$ (18)

Resultados e discussão

Os valores médios, obtidos para todos os tratamentos e a média dos resultados aos 10, 20 e 30 dias, para nitrogênio solúvel em água, nitrogênio não proteico (expresso em gramas/100 g de queijo) e índice de maturação (nitrogênio solúvel \times 100/nitrogênio total) são apresentados nos Quadros 1 a 5.

Pela observação aos Quadros 1 a 5, verifica-se que o teor médio de nitrogênio solúvel, para todos os tra-

Quadro 1. Teores médios de nitrogênio solúvel, nitrogênio não proteico e também do índice de maturação, com os respectivos níveis de significância, obtidos para o queijo integral e em relação aos valores calculados na matéria seca, no ensaio I.

Tratamentos	N SOLÚVEL		N NÃO PROTEICO		ÍNDICE DE MATURAÇÃO ($\frac{N \text{ sol} \times 100}{N \text{ total}}$)
	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	
1	0.332 a*	0.570 a*	0.130 a*	0.222 a*	9.29 a*
2	0.318 a	0.522 a	0.138 a	0.228 a	8.33 a
3	0.360 a	0.590 a	0.137 a	0.223 a	9.63 a
4	0.342 a	0.551 a	0.150 a	0.240 a	8.96 a
5	0.356 a	0.587 a	0.144 a	0.240 a	9.49 a
d. m. s.	0.043	0.086	0.022	0.035	1.55
10 dias	0.294 a*	0.533 a*	0.115 a*	0.181 a*	8.52 a*
20 dias	0.334 b	0.558 ab	0.142 b	0.236 b	9.08 ab
30 dias	0.384 c	0.591 b	0.159 c	0.245 b	9.59 b
d. m. s.	0.027	0.055	0.014	0.022	0.99
CV %	4.49	5.32	5.94	5.72	5.95

* Para cada determinação química em cada conjunto, letras iguais indicam não haver diferença ao nível de 5% de probabilidade pelo teste de Tukey

Quadro 2. Teores médios de nitrogênio solúvel, nitrogênio não proteico e também do índice de maturação, com os respectivos níveis de significância, obtidos para o queijo integral e em relação aos valores calculados na matéria seca, no ensaio II.

Tratamentos	N SOLÚVEL		N NÃO PROTEICO		ÍNDICE DE MATURAÇÃO ($\frac{N \text{ sol} \times 100}{N \text{ total}}$)
	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	
1	0.341 a*	0.547 a*	0.150 a*	0.238 a*	9.10 a*
6	0.358 a	0.561 a	0.163 a	0.256 a	8.68 a
7	0.359 a	0.572 a	0.154 a	0.243 a	9.18 a
8	0.370 a	0.573 a	0.156 a	0.244 a	9.11 a
9	0.373 a	0.560 a	0.174 a	0.274 a	9.61 a
d. m. s.	0.049	0.079	0.025	0.039	1.53
10 dias	0.315 a*	0.552 a*	0.127 a*	0.221 a*	8.70 a*
20 dias	0.347 b	0.549 a	0.167 b	0.263 b	8.88 ab
30 dias	0.418 c	0.618 b	0.185 c	0.269 b	9.83 b
d. m. s.	0.031	0.050	0.016	0.025	0.98
CV %	5.09	4.99	5.75	5.57	6.00

* Para cada determinação química, em cada conjunto, letras iguais indicam não haver diferença ao nível de 5% de probabilidade, pelo teste de Tukey

tamentos dos cinco experimentos realizados, variou de 0.318 a 0.419 g/100 g no queijo integral e de 0.503 a 0.636 g/100 g na matéria seca. O nitrogênio não proteico variou de 0.130 a 0.196 g/100 g no queijo integral e de 0.208 a 0.292 g/100 g na matéria seca. O índice de maturação variou de 8.07 a 10.61

Com relação aos resultados encontrados neste trabalho, pode ser verificado através dos Quadros 1 a 5 que não houve diferença significativa nos ensaios I, II, III e IV, para os diversos tratamentos, em todas as determinações efetuadas. O tratamento 15 do ensaio V, constituído de 50% de *S. cremoris* e 50% de

Quadro 3. Teores médios de nitrogênio solúvel, nitrogênio não proteico e também do índice de maturação, com os respectivos níveis de significância, obtidos para o queijo integral e em relação aos valores calculados na amostra seca, no ensaio III.

Tratamentos	N SOLÚVEL		N NÃO PROTEICO		ÍNDICE DE MATURACÃO ($\frac{N \text{ sol} \times 100}{N \text{ total}}$)
	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	
1	0.348 a*	0.579 a*	0.156 a*	0.260 a*	9.63 a*
10	0.360 a	0.552 a	0.169 a	0.258 a	9.15 a
11	0.358 a	0.542 a	0.167 a	0.253 a	8.81 a
12	0.358 a	0.557 a	0.157 a	0.248 a	8.93 a
13	0.357 a	0.560 a	0.158 a	0.249 a	8.91 a
d.m.s.	0.039	0.064	0.019	0.032	1.02
10 dias	0.311 a*	0.517 a*	0.125 a*	0.211 a*	8.39 a*
20 dias	0.342 b	0.534 a	0.159 b	0.250 b	8.73 a
30 dias	0.415 c	0.623 b	0.199 c	0.299 c	10.13 b
d.m.s.	0.025	0.041	0.012	0.021	0.65
CV %	3.97	4.18	4.36	4.62	4.07

* Para cada determinação química, em cada conjunto, letras iguais indicam não haver diferença ao nível de 5% probabilidade, pelo teste de Tukey.

Quadro 4. Teores médios de nitrogênio solúvel, nitrogênio não proteico e também do índice de maturação, com os respectivos níveis de significância, obtidos para o queijo integral e em relação aos valores calculados na amostra seca, no ensaio IV.

Tratamentos	N SOLÚVEL		N NÃO PROTEICO		ÍNDICE DE MATURACÃO ($\frac{N \text{ sol} \times 100}{N \text{ total}}$)
	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	
1	0.317 a*	0.521 a*	0.130 a*	0.211 a*	8.77 a*
14	0.332 a	0.503 a	0.151 a	0.232 a	8.07 a
15	0.357 a	0.544 a	0.136 a	0.208 a	8.78 a
16	0.353 a	0.540 a	0.140 a	0.214 a	8.63 a
17	0.336 a	0.527 a	0.137 a	0.213 a	8.32 a
d.m.s.	0.067	0.114	0.077	0.119	1.88
10 dias	0.280 a*	0.469 a*	0.121 a*	0.201 a*	7.66 a*
20 dias	0.318 a	0.502 a	0.125 ab	0.199 a	8.13 a
30 dias	0.418 b	0.610 b	0.170 b	0.247 a	9.76 b
d.m.s.	0.043	0.073	0.048	0.076	1.20
CV %	7.44	8.23	24.79	24.38	8.32

* Para cada determinação química, em cada conjunto, letras iguais indicam não haver diferença ao nível de 5% de probabilidade, pelo teste de Tukey.

S. diacetylactis mostrou os maiores teores de nitrogênio solúvel e de nitrogênio não proteico. Embora de acordo com Fryer (7), *S. diacetylactis* possua maior efeito proteolítico que outros estreptococcus lácticos, deve-se considerar que seu teor neste ensaio não foi estatisticamente diferente da testemunha

e no ensaio IV seu teor praticamente não diferiu das demais, fato esse que possibilita não levar em consideração esta diferença. Schrijver *et al.* (19), também não observaram diferença na degradação proteica e no nitrogênio solúvel, nos queijos fabricados unicamente com uma espécie homofermen-

Quadro 5. Teores médios de nitrogênio solúvel, nitrogênio não proteico e também do índice de maturação, com os respectivos níveis de significância, obtidos para o queijo integral e em relação aos valores calculados na amostra seca, no ensaio V.

Tratamentos	N SOLÚVEL		N NÃO PROTEICO		ÍNDICE DE MATURAÇÃO ($\frac{N \text{ sol} \times 100}{N \text{ total}}$)
	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	Queijo Integral (g/100 g)	Amostra Seca (g/100 g)	
1	0.389 ab*	0.624 b*	0.183 ab*	0.290 ab*	10.61 b*
3	0.377 ab	0.570 ab	0.170 a	0.257 a	9.46 ab
7	0.354 a	0.532 a	0.169 a	0.257 a	8.72 a
10	0.370 ab	0.560 ab	0.196 b	0.292 b	9.21 ab
15	0.419 b	0.636 b	0.196 b	0.292 b	10.32 b
d. m. s.	0.051	0.084	0.021	0.034	1.40
10 dias	0.326 a*	0.535 a*	0.139 a*	0.229 a*	8.89 a*
20 dias	0.390 b	0.595 b	0.183 b	0.279 b	9.78 ab
30 dias	0.429 c	0.623 b	0.226 c	0.237 c	10.31 b
d. m. s.	0.032	0.053	0.013	0.022	0.90
CV %	4.94	5.25	4.24	4.65	5.28

* Para cada determinação química, em cada conjunto, letras iguais indicam não haver diferença ao nível de 5% de probabilidade, pelo teste de Tukey.

tativa de estreptococo láctico ou em combinação com heterofermentativas, como a cultura do tipo BD (10).

Em todos os ensaios, a testemunha apresentou valores elevados de nitrogênio solúvel, nitrogênio não proteico e também de índice de maturação. Em alguns ensaios estes valores foram superiores a algumas combinações de bactérias lácticas; observados para nitrogênio solúvel na matéria seca e índice de maturação nos ensaios I, III, IV e V e para o nitrogênio não proteico na matéria seca nos ensaios III, IV e V. Resultados concordantes foram obtidos por Kiruchi *et al.* (11), que observaram fraca atividade proteolítica dos estreptococos lácticos comparativamente à outras bactérias e também com Thomas e Lowrie (20) que constataram deficiência em proteinase nas culturas lácticas da Nova Zelândia. Outros autores (1, 3, 14, 15, 16, 17, 21) além do mais, consideram que o coalho e as enzimas do leite ou da flora microbiana contaminante, tem maior efeito proteolítico que as bactérias lácticas.

Em relação às análises efetuadas aos 10, 20 e 30 dias, pode-se observar nos Quadros 1 a 5, que o nitrogênio solúvel, nitrogênio não proteico e índice de maturação. Em alguns ensaios estes valores foram superiores a algumas combinações de bactérias lácticas; observados para nitrogênio solúvel na matéria seca e índice de maturação nos ensaios I, III, IV e V e para

o nitrogênio não proteico na matéria seca nos ensaios III, IV e V. Resultados concordantes foram obtidos por Kiruchi *et al.* (11), que observaram fraca atividade proteolítica dos estreptococos lácticos comparativamente à outras bactérias e também com Thomas e Lowrie (20) que constataram deficiência em proteinase nas culturas lácticas de Nova Zelândia. Outros autores (1, 3, 14, 15, 16, 17, 21) além do mais, consideram que o coalho e as enzimas do leite ou da flora microbiana contaminante, tem maior efeito proteolítico que as bactérias lácticas.

Em relação às análises efetuadas aos 10, 20 e 30 dias, pode-se observar nos Quadros 1 a 5, que o nitrogênio solúvel, nitrogênio não proteico e índice de maturação, aumentaram com o desenvolvimento da cura. Estes resultados estão de acordo aos obtidos por Dahberg e Kosikowsky (5) em queijos tipo Cheddar.

Conclusão

Os resultados obtidos nas condições do presente trabalho possibilitaram concluir que, as diversas combinações de bactérias lácticas não alteraram o teor de nitrogênio solúvel em água, nitrogênio não proteico e índice de maturação do queijo tipo Minas. Foi constatado aumento gradual dos teores de nitrogênio solúvel, nitrogênio não proteico e índice de maturação durante a cura do queijo tipo Minas.

Resumo

O objetivo deste trabalho foi fazer um estudo comparativo da influência de algumas espécies de bactérias lácticas mesófilas: *Streptococcus cremoris*, *Streptococcus lactis*, *Streptococcus diacetylactis* e *Leuconostoc citrovorum* em relação ao nitrogênio solúvel, nitrogênio não proteico e índice de maturação do queijo tipo Minas

Nos queijos preparados com diferentes combinações dessas espécies bacterianas, foram feitas aos 10, 20 e 30 dias determinações de nitrogênio solúvel, nitrogênio não proteico e índice de maturação

Pela análise dos dados obtidos constatou-se que, não houve uma relação definida entre os valores encontrados e as diferentes combinações de bactérias utilizadas. Verificou-se porém, um aumento gradual desses valores durante a cura.

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INSTITUTO INTERAMERICANO DE COOPERACION PARA LA AGRICULTURA

Febrero de 1981

En virtud de que el IICA ha adoptado el Sistema Internacional de Unidades, nos permitimos anotar a continuación para los autores y colaboradores de las Revistas Turrialba y DRELA, así como para otras series de publicaciones del Instituto, las siguientes reglas principales.

En 1960, la Conferencia General de Pesas y Medidas (CGPM) y la Oficina Internacional de Pesas y Medidas (BIPM) decidieron por unanimidad en París, sede del BIPM, crear un sistema internacional de unidades de pesas y medidas (SIU). En 1975 había ya 44 países miembros del BIPM cuya tarea principal es asegurar la unificación mundial en torno del SIU. Hoy día los Estados Unidos de América e Inglaterra han adoptado también el uso del SIU.

Por ejemplo, el kilogramo es unidad de masa, y ya no de peso; el recurso al concepto de peso queda abolido, pues corresponde en realidad a la fuerza de atracción debida a la gravedad, y, por lo tanto, los cuerpos en el espacio interplanetario no tienen peso, pero sí conservan su masa. La unidad de fuerza es el newton (N), que corresponde a la necesaria para producir una aceleración de un metro por segundo sobre una masa de un kilogramo. La unidad de presión o esfuerzo es el pascal (Pa) y equivale a la noción abolida de kilogramos (fuerza) por centímetro cuadrado: $9\ 806\ 650\ \text{kg (fuerza)/m}^2 = 1\ \text{Pa}$.

Reglas principales para la consignación de las unidades SI

1. No se usan las mayúsculas en los nombres de unidades. Única excepción: grados Celsius.
2. Los símbolos no se escriben con mayúsculas. Excepciones: los derivados de nombres de personas.
3. Los prefijos métricos no se escriben con mayúsculas. Excepciones: tera T, giga G, mega M.
4. Los símbolos se escriben siempre igual, sean singular o plural, ej.: 5 mm, no 5 mms.
5. Cuando se escriben los nombres de unidades completos, se pluralizan normalmente, ej.: 10 kilogramos, 55 hectáreas.
6. No se usan los prefijos solos, sino acompañados de la unidad, ej.: 15 megawatts, no 15 megas.
7. No se usa el punto después del símbolo (24 m, no 24 m.), excepto al final de un párrafo.
8. Siempre se deja un espacio entre el número y el símbolo o unidad, ej.: 10 cm, no 10cm.
9. No se usan comas ni puntos para separar números largos; se deben separar de tres en tres. El punto marca el principio de la fracción decimal, ej.: 1 000 005.34, 30 000 y no 1,000,005.34 ó 30,000.
10. Siempre se coloca un cero a la izquierda del punto decimal, ej.: 0.77 y no .77.
11. Cuando se expresan unidades compuestas como kilómetros por hora, se usa la diagonal, ej.: 78 km/h, 50 m/s. Si se trata de newton metros se usa el punto, ej.: 5 N.m.

Continúa en la página 440

Summary

This is a preliminary assessment of the provenance test of Pinus caribaea var. hondurensis Barret and Golfari established by the Tropical Agriculture Research Training Center (CATIE) in Costa Rica

The analysis of the growth at two years of age revealed significant differences among the nine provenances tested. However the genetic variance was never higher than 6.4 percent for the traits assessed.

In the provenances Melinda, Guanaja, and Limones, slower growth was observed. This observation was supported by the principal component and cluster analysis, which grouped these three populations separately from the rest.

It was found that most of the variables studied showed highly significant differences among the three sites tested.

In this juvenile stage, most of the variation was detected between trees within provenances. It will be interesting to follow up these observations to determine if this pattern is maintained in coming years.

Introducción

Después de que *P. caribaea* var. *hondurensis* fue identificado, ha venido siendo extensivamente plantado en algunos países tropicales y subtropicales (7, 28). Sin embargo se ha dado poca atención al origen de las semillas, por lo que en ocasiones resulta difícil obtener conclusiones correctas de algunas plantaciones.

Los estudios preliminares muestran que los bosques naturales de esta variedad presentan gran variación en sistemas de ramificación, forma del fuste, altura, características del cono y semillas (1, 22, 25, 26, 27).

A la fecha, más de 40 países del trópico y subtrópico colaboran con el "Proyecto de investigación de procedencias de pino de América Central", promovido por el Commonwealth Forestry Institute (CFI), (5).

Resultados preliminares confirman que existe considerable variación entre procedencias y que es posible obtener una ganancia apreciable a través de la simple selección. En general estos estudios muestran a Mountain Pine Ridge, Poptún, Alamicamba y Brus Lagoon con un comportamiento sobresaliente en términos de producción, pero con un fuste y sistema de ramificación variables; Melinda y Culmi presentan un comportamiento inferior (2, 6, 17, 18, 29, 30, 34, 37).

Los resultados de las pruebas recientes establecidas bajo el control CFI, evidencian la existencia de variación genética entre procedencias en términos de crecimiento inicial así como la presencia de interacción genotipo-ambiente. Consistentemente Mountain Pine Ridge, Alamicamba, Brus Lagoon, Santa Cla-

1 Recibido para publicación el 14 de julio de 1982. Esta publicación está basada en la tesis de Ph D., presentada por el autor al Departamento Forestal de la Universidad de Oxford, Inglaterra, en junio de 1981.

* Gerente de Celulosa de Turrialba, S. A. Turrialba, Costa Rica

ra, y Culmi aparecen como las más promisorias en crecimiento diamétrico, altura y forma del fuste; aunque en algunos sitios se observa una alta incidencia de cola de zorro y fuerte ramificación (10, 12, 13, 16, 19, 31, 32, 36). Burley y Nikles (9) indican que la variedad *hondurensis* es superior en vigor a las otras dos variedades, pero esta importante característica normalmente está asociada con fuste y ramificación poco deseables, así como alta incidencia de cola de zorro. Huges (21) encontró que Mountain Pine Ridge tiene una alta variación entre árboles con respecto a propiedades de la madera.

Recientemente Barnes *et al.* (5) en un análisis de nueve procedencias en cinco países, encontraron que las características cualitativas tienden a estar bajo menor control genético que las características cuantitativas; en este caso, ninguna procedencia fue consistente en mostrarse superior en los cinco sitios en términos de productividad. En general un gran proporción de la variación fue detectada entre árboles; Santa Clara presentó árboles superiores en algunas localidades; Guanaja presentó alto volumen y alta densidad de madera; Alamicamba generalmente presentó los fustes más rectos y menor bifurcación aunque con baja densidad de la madera y sistema de ramificación poco deseable.

En general, y aunque las pruebas de procedencia son relativamente jóvenes, éstas indican que existe considerable variación genética en algunas características de producción.

El presente trabajo pretende, a través de técnicas biométricas, evaluar el grado de variabilidad entre y dentro de nueve procedencias de *P. caribaea* var. *hondurensis*, así como medir el grado de interacción

genotipo-ambiente de algunas características de producción, a los dos años de edad.

Materiales y métodos

Los estudios de laboratorio, así como los análisis estadísticos, se realizaron en el Departamento Forestal de la Universidad de Oxford, Inglaterra.

El "Proyecto de investigación de procedencias de pino de América Central" promovido por CFI dio inicio en 1979 con la recolección de semillas en toda el área de distribución natural; Kemp (24, 25) describe en forma detallada la técnica de muestreo. Como parte de este proyecto, el Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), estableció en setiembre de 1977 el experimento 77-1(112). Dyson (11) describe el procedimiento de vivero para la preparación de las plantas.

El diseño experimental fue suplido por el CFI; básicamente es un diseño de bloques completos al azar repetido en cinco sitios, cinco repeticiones por sitio, 12 procedencias por repetición y siete árboles en líneas por parcela.

Las procedencias son nueve de *P. caribaea* var. *hondurensis*, una de *P. caribaea* var. *caribaea* y dos de *P. oocarpa* Schiede. El presente estudio consideró sólo el *P. caribaea* var. *hondurensis*; el Cuadro 1 resume la información geoclimática de las nueve procedencias.

La Figura 1 muestra los cinco sitios de plantación. Este análisis no toma en consideración los sitios 4 y

Cuadro 1. Información geoclimática de las nueve procedencias.

Origen		Latitud ° N	Longitud ° O	Altitud (msnm)	Precipitación anual (mm)	Meses secos	Temperatura media (°C)
País	Localidad						
Nicaragua	Alamicamba (ALA)	13° 34'	84° 17'	25	2 610	3	20.7
Nicaragua	Río Coco (RIO)	14° 45'	83° 55'	75	2 863	2	25.4
Honduras	Guanaja (GUA)	16° 27'	85° 54'	75	2 308	3	27.1
Guatemala	Poptún (POP)	16° 21'	89° 25'	500	1 688	4	24.2
Honduras	Culmi (CUL)	15° 06'	85° 37'	550	1 325	6	24.3
Honduras	Brus Lagoon (BRU)	15° 45'	84° 40'	10	2 840	2	26.5
Honduras	Los Limones (LIM)	14° 03'	86° 42'	700	663	7	22.2
Belice	Mountain Pine Ridge (MPR)	16° 58'	89° 00'	487	1 558	3	23.9
Belice	Melinda (MEL)	17° 01'	88° 20'	12	2 137	2	26.9

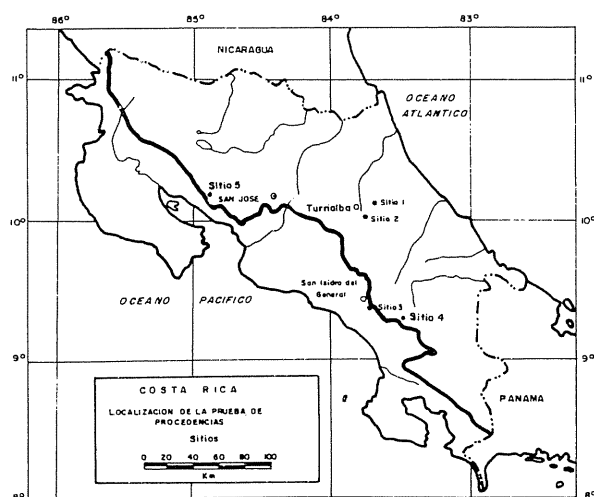


Fig. 1. Mapa de Costa Rica mostrando los sitios de plantación.

5; el primero porque mostró una alta mortalidad en los primeros meses por mal drenaje, y el segundo porque fue establecido seis meses después. El Cuadro 2 es el resumen de la información geoclimática de los tres sitios en estudio.

Sitio 1 (Celulosa de Turrialba): localizado a 15 kilómetros por carretera a Limón al noreste de Turrialba; ecológicamente es un bosque muy húmedo premontano (20) con topografía accidentada. El suelo es profundo y bien drenado. Antes de la plantación fue potrero dominado por regeneración natural.

Sitio 2 (Florencia Norte, CATIE, Turrialba): ecológicamente es un bosque premontano muy húmedo (20), con topografía ondulada. El suelo es profundo y bien drenado. Antes del experimento el sitio fue ocupado con pruebas de especies forestales.

Sitio 3 (Universidad Nacional, San Isidro de El General): sobre la carretera a Panamá; ecológicamente

es un bosque húmedo tropical, en la faja de transición premontano muy húmedo con topografía plana. El suelo es profundo y bien drenado. Antes del experimento estaba cubierto por gramíneas y especies arbóreas de regeneración secundaria.

La densidad de siembra fue de 2.5 x 2.5 metros utilizando arbolitos de 20 y 25 centímetros de altura.

La plantación se limpió a mano cinco veces el primer año y cuatro el segundo. La sobrevivencia en el Sitio 2 fue afectada adversamente por ganado y el Sitio 3 fue parcialmente destruido por el fuego en 1979.

La morfología de los árboles juveniles se evaluó a través del análisis de las siguientes variables: altura total en cm a 6, 12, 18 y 24 meses, diámetro en milímetros (mm) a 1.3 m de altura a 12, 18 y 24 meses; número de internudos y números de ramas a los 6 meses.

Se utilizó el siguiente modelo completamente al azar para evaluar la variación entre sitios, procedencia y la interacción sitio-procedencia de cada variable:

$$Y_{ijkl} = \mu + S_i + P_j + (SP)_{ij} + R_{k/j} + (PR)_{jk/i} + T_{ijkl}$$

Donde:

- Y_{ijkl} = valor promedio de la variable Y en 1th árbol en k^{th} repetición de j^{th} procedencia de i^{th} sitio;
- μ = efecto real de la media;
- S_i = efecto del i^{th} sitio; $i = 1, 2, 3$.
- P_j = efecto de la j^{th} procedencia; $j = 1, 2, 9$.

Cuadro 2. Información geoclimática de los sitios de plantación.

Sitio	Latitud °N	Longitud °O	Altitud (msnm)	Precipitación (mm)	Meses secos	Humedad relativa %	Temperatura °C		
							Med.	Máx.	Mín.
Celulosa de Turrialba	9°55'	83°37'	750	2 673*	1	88	22.2	26.9	17.6
CATIE	9°52'	83°40'	650	2 673	1	87	22.2	26.9	17.6
U. Nacional San Isidro de El General	9°22'	83°33'	670	3 030	3	87	24.3	31.0	17.5

* No hay registro. Considerado similar a CATIE del que dista de aproximadamente 5 km en línea recta.

(PS)_{ij} = efecto de la interacción entre el i^{th} sitio con la j^{th} procedencia;
 $R_{k/i}$ = efecto de k^{th} repetición en el i^{th} sitio;
 $k = 1, 2, 5$;

(PR)_{jk/i} = efecto de la interacción de la j^{th} procedencia en la k^{th} repetición en el i^{th} sitio.
 T_{ijkl} = efecto del i^{th} árbol en la k^{th} repetición de la j^{th} procedencia en el i^{th} sitio; $l = 1, 2, 7$.

En el Cuadro 3 se presenta la forma del análisis de variancia junto con los cuadrados medios esperados para cada fuente de variación, de acuerdo al modelo descrito. El programa de computación utilizado (ANOVAR 2) incluye la prueba de rango múltiple y la transformación de raíz cuadrada para variables discontinuas.

Dado que el análisis de variancia propuesto no presenta una fuente de error simple para probar el efecto de sitio, este fue probado por la combinación de los respectivos cuadrados medios $(3 + 4) - 5$. Para la corrección de los grados de libertad se utilizó la aproximación de Satterthwaite (3).

$$df = \frac{\sum_{i=1}^n \omega_i CM_i^2}{\sum_{i=1}^n \frac{\omega_i^2 CM_i^2}{f_i}}$$

df = grados de libertad
 ω_i = coeficiente usado por i^{th} cuadrado medio
 CM = el i^{th} cuadrado medio del total n cuadrados usados en la construcción del término de error

El análisis de componentes principales y análisis de agrupamiento fueron utilizados sobre la base de promedio por procedencia, para detectar la tendencia de las procedencias a agruparse en función de las variables analizadas. Estos análisis multivariados han probado ser eficientes en estudios taxonómicos, básicamente cuando se analizan muchas variables (4, 8, 14, 15, 35).

Resultados y discusión

En el Cuadro 4 se observa que el efecto de sitio fue significativamente diferente para ocho de las nueve variables evaluadas; no obstante, sólo el diámetro a 18 y 24 meses presenta 25.25 y 21.59% como la variación más alta detectada entre sitios. Esto indica que la uniformidad de los sitios es tal, que durante esta etapa juvenil las diferencias observables son mínimas. El diámetro aparenta ser el más susceptible al efecto de sitio principalmente de los 18 meses en adelante.

La diferencia entre procedencias aunque fue significativa y altamente significativa (Cuadro 4) para siete de las nueve variables, en ningún caso fue superior a 6.4%, que en este caso corresponde a número de ramas; Poptún y Cumi presentan 4.7 y 4.9 (Cuadro 5) ramas a los seis meses de edad, siendo el promedio 3.4. Esto indica que durante los dos primeros años, la morfología que exhiben las procedencias es bastante

Cuadro 3. Análisis de variancia y cuadros medios esperados.

No.	Fuente Variación	Grados de latitud (d.f.)	Prueba de ¹ significancia	Cuadros medios esperados
1	Sitios	(S-1)	(3 + 4) - 5	$\sigma^2 + t \sigma_{PR}^2 + tp \sigma_{R/S}^2 + tr \sigma_{SP}^2 + trp \sigma_S^2$
2	Procedencias (P)	(P-1)	3	$\sigma^2 + t \sigma_{PR/S}^2 + tr \sigma_{SP}^2 + trs \sigma_P^2$
3	S x P	(S-1) (P-1)	5	$\sigma^2 + t \sigma_{PR/S}^2 + tr \sigma_{SP}^2$
4	Repeticiones (R) en S	S(R-1)	5	$\sigma^2 + t \sigma_{PR/S}^2 + tp \sigma_{R/S}^2$
5	P x R en S	(P-1) S(R-1)	6	$\sigma^2 + t \sigma_{PR/S}^2$
6	Arboles (I) en RPS	SPR (I-1)		σ^2

1 Los números en esta columna se relacionaron con la de la primera columna

Cuadro 4. Resumen del análisis de variancia y componentes de la variancia.

No. Fuentes de variación	Grados de libertad	Prueba de significancia	6 meses			12 meses		18 meses		24 meses	
			ht	in	ra	ht	di	ht	di	ht	di
CUADRADOS MEDIOS											
1 Sitios (S)	2 (3+4)-5		1 130.0	6.84	9.57	93 660	24.28	185 000	167.9	634 200	298.1
2 Procedencias (P)	8	3	1 280.0	2.01	6.01	7 216	1.73	13 650	6.5	39 630	20.7
3 S x P	16	5	283.3	0.20	0.61	1 889	0.52	9 423	2.9	10 050	2.7
4 Repeticiones (R) en S	12	5	789.6	0.37	1.23	6 595	2.49	23 610	8.2	77 060	34.7
5 P x R en S	96	6	361.1	0.40	1.13	2 548	0.72	11 340	3.2	12 390	4.2
6 Arboles (t)	810		223.6	0.27	0.65	1 171	0.33	2 717	1.1	6 434	2.1
PRUEBA DE F											
S			NS	**	**	**	**	**	***	**	**
P			**	***	***	**	**	NS	NS	**	***
S x P			NS	NS	NS	NS	NS	NS	NS	NS	NS
R en S			NS	NS	*	***	***	***	***	***	***
P x R en S			***	***	***	***	***	***	***	***	***
COMPONENTES DE LA VARIANCIA %											
S			0.51	6.42	3.50	16.47	14.14	11.02	25.25	17.13	21.59
P			3.64	5.20	6.38	3.00	2.22	0.86	1.48	2.72	4.37
S x P			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R en s			2.60	0.00	0.25	3.80	5.66	4.14	3.96	9.90	12.34
P x R en s			7.32	5.81	8.52	11.63	11.31	26.20	14.85	8.20	7.72
T en R en P en S			85.73	82.57	81.35	69.24	66.67	57.78	54.46	62.05	53.98

* Significativo a $P < 0.05$ de probabilidad.

** Significativo a $P < 0.01$ de probabilidad.

*** Significativo a $P < 0.001$ de probabilidad.

similar. Aunque persistentemente Guanaja y Melinda presentan los más bajos crecimientos en diámetro y altura con respecto a las restantes siete procedencias (Cuadro 5). Este comportamiento puede considerarse como genético, ya que la matriz de correlación lineal simple (Cuadro 6) indica que las variables evaluadas, no están significativamente relacionadas con las variables climáticas en esta etapa juvenil.

Aunque el análisis de variancia (Cuadro 4) no mostró diferencias significativas para la interacción sitio-procedencia, en el Cuadro 7 se muestra que en el Sitio 2 (CATIE) la mayoría de las variables presentaron los valores más bajos. Además, la Figura 2 muestra la presencia de la interacción en el caso de altura y DAP, donde la mayoría de las procedencias mostraron una reducción a los 12, 18 y 24 meses principalmente en Sitio 2. La reducción no se presentó a los seis meses, posiblemente porque a esta edad aún perdurará el efecto de vivero. La mayor interacción en el Sitio 2 en cuanto a altura la presentaron Limones, Poptún, Alamicamba y Guanaja aunque

ésta no fue constante en todas las edades. La procedencia Melinda fue constante en presentar los más bajos crecimientos en los tres Sitios a las diferentes edades. En DAP, Poptún, Melinda, Alamicamba, Guanaja y Limones, también presentaron una fuerte interacción; en el Sitio 2 exhiben los mejores diámetros, aunque a los 24 meses la interacción fue mínima.

Resulta interesante observar (Figura 2) como los genotipos que presentan, los desarrollos más pobres en los Sitios 1 y 3, se comportan mejor en el Sitio 2. Sería interesante demostrar cuáles son los factores de sitio que provocan este tipo de interacción durante esta etapa juvenil.

En esta variedad ya se ha mencionado que las diferencias entre procedencias están influenciadas por factores de sitio Barnes *et al.* (5); Greaves, (18). Will y Hodgkiss (1977) informaron que el *P. radiata* D. Don en Nueva Zelanda, cuando crece en suelos con baja concentración de nitrógeno y

Cuadro 5. Promedios y parámetros de dispersión.

Procedencias	6 meses			12 meses		18 meses		24 meses	
	th	in	ra	th	di	th	di	th	di
A la micamba	65.49	1.17	2.20	150.90	0.88	221.93	2.19	347.87	4.63
Río Coco	63.38	1.69	3.82	148.46	0.81	214.70	2.12	352.19	4.97
Guanaja	63.97	1.41	2.93	131.72	0.54	198.04	1.74	302.64	3.90
Poptún	64.68	2.22	4.72	147.41	0.78	223.55	2.50	340.14	5.35
Culmi	66.02	2.17	4.87	155.20	0.87	214.81	2.18	352.34	5.24
Brus Lagoon	63.96	1.30	2.58	151.57	0.89	224.65	2.20	361.08	4.88
Los Limones	59.86	1.65	3.36	138.95	0.66	200.20	1.87	323.48	4.70
Mountain Pine	65.54	1.75	3.60	148.91	0.84	214.57	2.25	347.85	4.99
Ridge Melinda	55.18	1.77	3.40	133.95	0.61	195.20	1.77	317.48	4.40
\bar{X}	63.12	1.66	3.45	145.23	0.76	212.01	2.09	338.34	4.79
S \bar{x}	1.83	0.07	0.10	4.83	0.08	10.27	0.17	10.72	0.19
S \bar{e}	2.58	0.09	0.14	3.33	0.11	9.15	0.11	9.11	0.12
CV (%)	2.89	5.11	5.41	6.84	10.64	10.74	0.23	18.31	0.42

ht-Altura (cm)

in-Número de internudos a 6 meses

di-DAP (cm)

ra-Número de ramas a 6 meses

Cuadro 6. Matriz de correlación lineal simple (r).

No.	Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Latitud	1.00														
2	Longitud	0.67*	1.00													
3	Altitud	-0.05	0.32	1.00												
4	Precipitación	-0.03	-0.40	-0.93***	1.00											
5	Meses secos	-0.35	-0.03	0.86**	-0.88**	1.00										
6	Temperatura	-0.11	-0.48	-0.86**	0.90***	-0.78*	1.00									
7	th 6	-0.27	-0.32	0.17	0.11	0.08	-0.03	1.00								
8	in	0.37	0.37	0.64	-0.55	0.45	-0.41	-0.01	1.00							
9	ra	0.28	0.22	0.62	-0.49	0.42	-0.32	0.13	0.98***	1.00						
10	ht 12	-0.40	-0.37	0.18	0.10	0.07	-0.03	0.69*	0.18	0.24	1.00					
11	di 12	-0.39	-0.33	0.06	0.20	0.05	-0.02	0.63	0.02	0.08	0.98***	1.00				
12	ht 18	-0.29	-0.19	-0.01	0.31	-0.15	0.06	0.72*	0.02	0.08	0.83**	0.89**	1.00			
13	di 18	-0.10	0.11	0.27	0.04	-0.04	-0.15	-0.66	0.36	0.36	0.81**	0.80**	0.93***	1.00		
14	ht 24	-0.35	0.35	0.03	0.25	-0.11	0.09	0.54	0.06	0.13	0.96***	0.98***	0.80**	0.77*	1.00	
15	di 24	-0.13	0.06	0.52	-0.24	-0.26	-0.29	0.41	0.63	0.65	0.84**	0.75*	0.71*	0.86**	0.78*	1.00

* Significativo al P < 0.01 de probabilidad.

** Significativo al P < 0.01 de probabilidad.

*** Significativo al P < 0.01 de probabilidad.

ht 6, ht 18, ht 24, = Altura a 6, 12, 18, y 24 meses.

di 12, di 18, di 24 = DAP a 12, 18 y 24 meses.

lp = número de internudos.

ra = número de ramas.

fósforo presenta una reducción en el diámetro y tamaño de ramas. Namkoong and Davey (1976) mostraron que familias de *P. taeda* L. responden diferente a distintos niveles de nitrógeno.

La variación total entre árboles, varió entre 53.9 y 85.7%; esta condición ofrece una buena alternativa para estudios de progenie, con el fin de identificar individuos altamente productivos.

La prueba de rango múltiple (Figura 3) soporta el análisis de variancia, en el cual aunque existen diferencias significativas entre procedencias, esas diferencias son mínimas. La prueba indica que con respecto a la altura total y el diámetro, las proce-

dencias Limones, Melinda y Guanaja pueden ser consideradas como las de más bajo crecimiento en los dos primeros años. Es posible que estos genotipos hayan sufrido modificaciones específicas para adaptarse a condiciones especiales de sitio, como en el caso de Melinda donde los suelos son áridos. Suelos áridos, baja precipitación y siete meses de verano en el caso de Limones, y población aislada sobre suelos de baja fertilidad en el caso de Guanaja.

A través del análisis de componentes principales se encontró que el 65.4% de la variación total observada en las nueve variables estudiadas, depende del primer componente, el cual está formado básicamente por altura y diámetro a 12, 18 y 24 meses. El segundo

Cuadro 7. Promedios y prueba de rango múltiple para sitios.

Sitios	ht 6	in	ra	ht 12	di 12	ht 18	di 18	ht 24	di 24
1-Celulosa	61.83	1.84	3.72	146.12	0.70	222.03	2.35	364.36	5.25
2-CATIE	65.30	1.26	2.75	127.56	0.52	184.37	1.27	286.53	3.64
3-U									
Nacional	62.18	1.93	3.92	162.01	1.07	229.63	2.63	364.14	5.44
\bar{X}	63.12	1.66	3.45	145.23	0.76	212.01	2.09	338.34	4.78
$S\bar{x}$	1.58	0.03	0.06	4.58	0.09	8.66	0.16	15.64	0.33
$S\bar{e}$	2.24	0.05	0.09	6.47	0.13	12.24	0.23	22.12	0.47
CV (%)	2.51	2.66	3.73	3.15	11.66	4.08	7.71	4.62	6.93
Prueba de rango múltiple									
P<0.05)	3	3	3	3	3	3	3	1	3
		2	1	1	1	1	2	3	1
		1	2	2	2	2	1	2	2

ht = Altura (cm)

in = Número internudos a 6 meses.

ra = Número de ramas a 6 meses

di = Diámetro (cm)

Cuadro 8. Raíces latentes y porcentaje de variación por componente.

Componentes	Raíces	Variación en porcentaje	
		Simple	Acumulado
I	5.88	65.37	65.37
II	2.17	24.06	89.43
III	0.59	6.60	96.03
IV	0.28	3.07	99.11
V	0.03	0.38	99.49

componente formado por número de internudos y ramas contiene el 24.1% de la variación. El tercer componente formado por altura a seis meses contiene solamente 6.6% de la variación (Cuadro 8). El Cuadro 9 presenta el valor proporcional que cada componente extraído da a las variables.

El 89.4% de la variación total está representada por los componentes I y II; la representación gráfica de estos dos componentes (Figura 4) confirma los análisis anteriores. También en este caso, las poblaciones Melinda, Limones y Guanaja se agrupan en forma separada del resto de las poblaciones por su crecimiento menor. Las poblaciones Culmi y Poptún de Honduras y Guatemala respectivamente forman otro grupo, básicamente por presentar a los seis meses mayor número de internudos y ramas.

Los anteriores resultados son similares a los obtenidos en el análisis de agrupamiento (Figura 5), que muestra el grado de similitud entre poblaciones y permite extraer dos grupos principales de procedencias: Guanaja, Melinda y Limones por un lado y Brus Lagoon, Mountain Pine Ridge, Poptún, Alamicamba, Río Coco y Culmi por el otro.

Conclusiones

Los resultados de los diferentes análisis realizados son consistentes en cuanto que muestran las procedencias Melinda, Limones, y Guanaja con crecimientos iniciales comparativamente bajos. En este caso las variables altura total y diámetro resultaron ser buenos discriminantes. El bajo rendimiento de estas tres procedencias ya ha sido reportado.

Se considera riesgoso descartar cualquiera de estas procedencias por el comportamiento que presentan en la etapa inicial de desarrollo, máxime si existe interacción genotipo-ambiente que también varía con la edad. La mayoría de los genotipos estudiados presentan un crecimiento constante en cada sitio, aunque algunos muestran reducciones o incrementos bruscos en esta etapa juvenil.

El crecimiento inicial que muestran las nueve poblaciones tiene una tendencia ecotípica, posible-

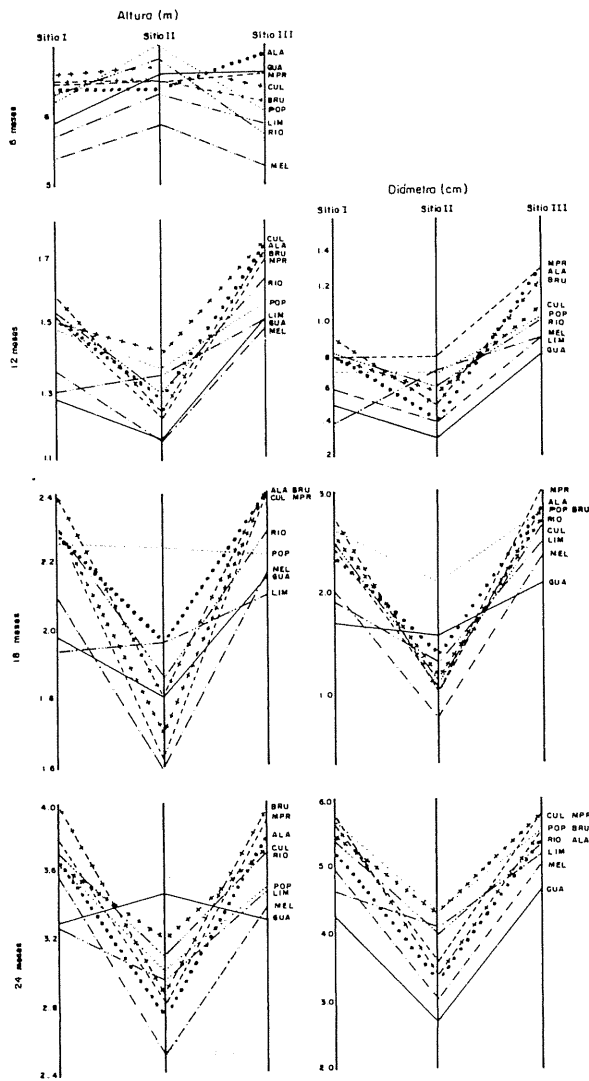


Fig. 2. Interacción sitio procedencia.

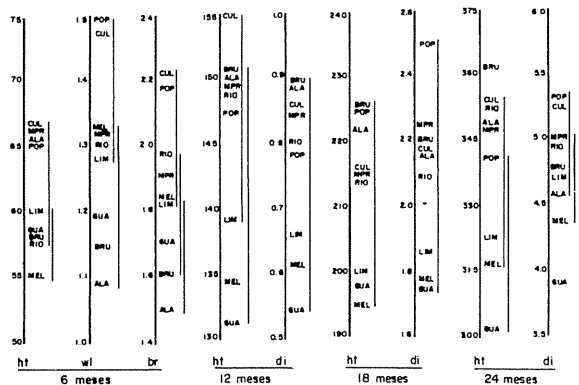


Fig. 3. Prueba de rango múltiple ($P < 0.05$).

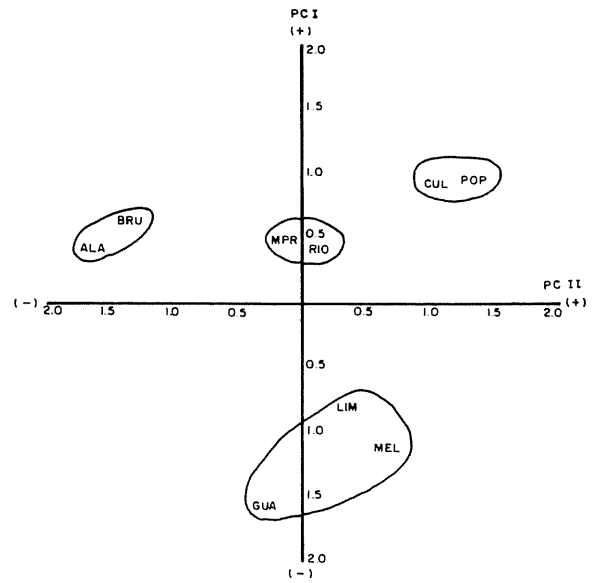


Fig. 4. Distribución de las procedencias dado por la combinación de los componentes I y II.

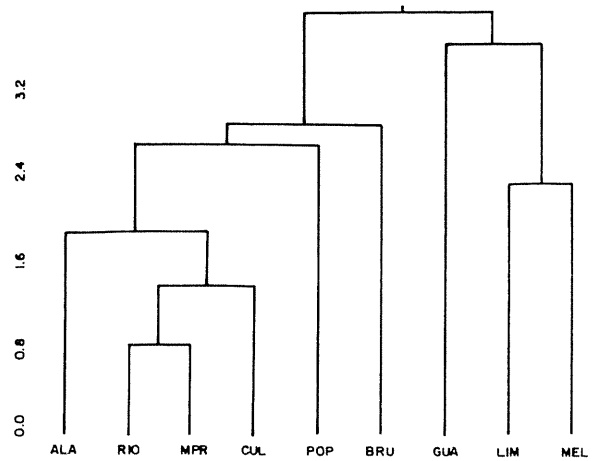


Fig. 5. Dendrograma presentando la agrupación de las procedencias como resultado del análisis de agrupamiento.

mente por tratarse de poblaciones naturales relativamente discontinuas creciendo sobre una amplia variedad de suelos, en condiciones climáticas variadas y en algunos casos con presencia de fuertes vientos. Estos factores pueden ser los responsables de provocar alteraciones genéticas en el proceso de adaptación, creando de esta manera la presencia de ecotipos.

Continuar observando el comportamiento de estas procedencias por un período más largo permitirá detectar si la tendencia inicial de crecimiento se mantiene, esto facilitaría la toma de decisiones desde los

Cuadro 9. Valor proporcional para cada variable por componente.

Variables	Vectores por componente				
	I	II	III	IV	V
ht 6	0.73	-0.26	1.00	0.46	-0.28
in	0.33	1.00	0.04	0.01	0.10
ra	0.39	0.96	0.16	0.34	0.62
ht 12	1.00	-0.16	-0.13	0.61	-0.01
di 12	0.97	-0.30	-0.26	0.37	-0.26
ht 18	0.96	-0.30	0.10	-0.73	1.00
di 18	0.97	0.07	0.16	-1.00	-0.38
ht 24	0.95	-0.24	-0.42	-0.39	0.32
di 24	0.93	0.40	-0.30	-0.16	-0.98

ht 6, ht 12, ht 18, ht 24 = Altura total a 6, 12, 18 y 24 meses.

di 12, di 18, di 24 = DAP a 12, 18 y 24 meses

in = Número de internudos ra = Número de ramas.

primeros años y acelerando de esta manera el proceso de mejoramiento.

Resumen

Se presenta un análisis preliminar de una prueba de procedencias de *Pinus caribaea* var. *hondurensis* Barret y Golfari, establecida por el Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) en tres sitios en Costa Rica. Las variables de crecimiento evaluadas mostraron diferencias significativas a los dos años de edad entre las nueve procedencias estudiadas. Las procedencias Melinda, Guanaja y Los Limones presentaron los crecimientos más bajos. Estos resultados fueron soportados por el análisis de componente principales y cluster análisis, los cuales consideran estas tres poblaciones como diferentes. No obstante, en ninguna de las variables analizadas la variación genética detectada fue superior al 6.4%.

Ocho de las nueve variables analizadas, mostraron diferencias altamente significativas entre los tres sitios en estudio, durante las cuatro edades juveniles estudiadas.

Se encontró que a este estado juvenil, la mayor parte de la variación se presentó entre árboles dentro de procedencias. Será de mucho interés comprobar si esta tendencia persistirá en los próximos años.

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Notas y comentarios

Academia de Ciencias de Cuba. Recientemente hemos recibido siete publicaciones de la A.C.C., cuatro Informes Científico-técnico (no. 21, 104, 124 y 175) y tres publicaciones no seriadas, todas relacionadas con la Ciencia del Suelo. Los trabajos recibidos cubren aspectos de génesis, mineralogía y materia orgánica en suelos cubanos y son distribuidas por la Editorial de la Academia de Ciencias de Cuba, La Habana 2, Cuba

Publicaciones

Esta publicación titulada *Pinus oocarpa* es la segunda de la serie de bibliografías que el autor A. Greaves ha preparado sobre especies forestales tropicales de reconocido valor comercial.

En esta oportunidad se presentan en forma resumida 310 revisiones bibliográficas sobre *Pinus oocarpa* Schiede; conífera de rápido crecimiento, nativa de zonas altas de México y América Central.

Esta especie ha demostrado tener amplias posibilidades para el establecimiento de plantaciones comerciales en las zonas tropicales y subtropicales, razón por la que en los últimos años esta conífera ha sido ampliamente investigada.

Las 310 citas resumen en forma bastante clara la mayoría de las investigaciones, que sobre esta especie han sido realizadas desde 1936 hasta 1980.

Para facilitar la utilización de la bibliografía, el autor clasifica las citas en los siguientes campos: Revisión de literatura, Poblaciones naturales, Silvicultura, Evaluación de la especie, Fisiología, Crecimiento y producción, Genética, Plagas y enfermedades, Propiedades de la madera, Utilización.

Este tipo de bibliografías son de gran valor, ya que le ofrecen al técnico información en forma concisa sobre una amplia variedad de temas; y además, porque ponen a su disposición una considerable cantidad de información, que de otra forma le sería de muy difícil adquisición.

Publicaciones

El grupo británico de Investigaciones Geomorfológicas ha publicado 30 boletines técnicos con el fin de reunir la metodología aplicada a problemas de geomorfología por temas específicos.

El presente boletín titulado "Soil aggregate stability tests for the geomorphologist", trata sobre métodos para estimar la estabilidad de agregados y cubre tanto aspectos teóricos de formación de agregados como los principales métodos para su cuantificación desde el punto de vista geomorfológico.

La publicación puede ser adquirida por medio de Geo Abstracts Ltd., Regency House 34 Duke Street, Norwick NR 3 3AP, England.

A COMPARISON OF WATER BALANCE COMPONENTS IN NATURAL AND PLANTATION
FORESTS IN EL SALVADOR, CENTRAL AMERICA¹ /

NORMAN W PRICE*

Resumen

La precipitación bajo el bosque, el flujo de precipitación por los tallos, la humedad de suelo, la evaporación y la transpiración fueron medidas en cuatro tipos de bosques en el área de Metapán-Montecristo, El Salvador. El tipo de bosque (latifoliadas vs. coníferas) tiene gran importancia sobre el balance hídrico de una región.

Los cuatro tipos de bosques incluyeron: una plantación de ciprés (Cupressus lusitanica Mill.), una plantación de pino (Pinus pseudostrobus Lindl.), un bosque mixto de pino-roble (P. oocarpa Schiede y Quercus peduncularis NEE), y un bosque de regeneración natural.

Según un análisis de los cinco factores estudiados, se llegó a la conclusión de que la plantación de ciprés tiene el mayor impacto sobre el balance hídrico del área, seguida en orden por el bosque mixto de pino-roble, la regeneración natural dominada por latifoliadas, y la plantación de pino.

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Introduction

Reforestation with rapid-growth tree species in tropical regions may lead to unwanted changes in local and/or regional water balances. An understanding of the impact of rapid-growth species, particularly the conifers, on the water resources is thus essential for evaluation of land-use and water management priorities.

Differences between the impact of conifers and natural hardwood stands on the water balance have been established for temperate and some tropical regions. Higher percentages of interception of rainfall by conifers have been established by several researchers (1, 9, 23), and Swank, *et al.* (30, 31, 32) showed that lower streamflow result from conversion of natural hardwood stands to pine due to evaporation of the greater amount of intercepted rainfall.

The objective of this research was to compare conifers and natural hardwoods, in relation to their respective impact on the water balance, in a tropical region. The field research was undertaken between July 1976 and March 1977.

Research Area

The field research was carried out in the Metapan-Montecristo region of northeastern El Salvador, an area rough and mountainous, underlain by faulted sedimentary strata and strongly dissected by rivers and ravines. Slopes are variable, but average about 60% and usually exceed 50 m in length. Drainage is typically dendritic.

The four sites used in this investigation were between 1600 and 1800 masl with a mean annual rainfall of 1940 mm and a temperature range of 8°C to 23°C. Thus, they fall within the sub-tropical wet forest zone. The area is covered by a mosaic of conifer plantations of various ages, various stages of succession, mixed stands of oak-pine, and pasture.

Generally, during the study, rainfall was well below the six year average though still within the limits of standard deviation. Air temperatures corresponded to the long-term average with a tendency towards warmer days and cooler nights.

The four sample areas consisted of a nine-year-old plantation of *Pinus pseudostrobus* LINDL. (pinabete), situated at 1800 m, a nine-year-old plantation of *Cupressus lusitanica* MILL. (ciprés) at 1600 m, a natural stand of *Quercus aff. sapotaeifolia* LIEBM. (encino), *Q. peduncularis* NEE. (roble) and *P. oocarpa* SCHIEDE (pino ocote) at 1760 m, and a stand of natural regeneration, dominated by *Leucaena* sp. (guaje), *Roupala montana* AUBL. (zorri-illo), *Winneria cyclocarpa* RADLK. (loroncito), *Cassia* sp. (vainillo), and *Sauravia pseudorubriformis* BUSC (siete pellejos) at 1800 m.

The plantations of pinabete and ciprés were both situated in an area slightly to moderately sloping. Spacing in both was 2.5 by 2.5 m. DBH and heights were 15 cm/7.6 m and 12 cm/10.5 m respectively for the pinabete and ciprés. Undergrowth was dense and controlled by slashing about once a year.

The mixed oak-pine was situated on relatively flat terrain. The dominants averaged 38 cm in DBH with heights ranging from 13-26 m. A moderately dense second storey canopy occurred at 9 m with stems ranging from 4 to 10 cm in diameter. The age of the stand was approximately 60 years.

A site approximately 35 years of age on a bench near a large ravine was the location of the natural regeneration treatment. DBH varied between 10 and 51 cm with an average of 18. Heights were on the average about 8 m.

Further detail with respect to the different forest stands can be found in Price (24), "Highland Deforestation and Approaches to Forest Recovery in the American Tropics: the Metapan-Montecristo example of El Salvador."

Throughfall and Stemflow

Materials and Methods: Rain gauges were constructed from 6.5 cm diameter plastic funnels attached to 5 litre plastic reservoirs. The capture surface was set 45 cm above the ground since this height had been shown by experience to be the best compromise to avoid additions from splash-back or interference from wind.

Stemflow channels were formed from plasticine. The channel was concave and extended about 2 cm from the trunk. Polyethylene tubing led from the end of the channel to a litre plastic jug which served as the reservoir.

Throughfall and stemflow were measured in the morning before the afternoon rains, though occasionally two or three days delay occurred.

The gauges were positioned in a regular pattern throughout the site, with one gauge being situated in the most open location and another situated at the most closed; six to eight gauges were used. Stemflow was measured at five locations at each site, based on DBH, with trees representing the average and a range of diameters being selected.

Results: It was assumed that the meteorological station at Los Planes, which is within 300 meters of the Natural Regeneration site, would provide gross precipitation data to represent the above canopy condition. However, variation between sites suggests that this assumption did not hold. The variation mentioned above is evident in the summary of mean throughfall by site as a percentage of gross precipitation (i.e. meteorological station) given in Table 1.

To avoid the assumption of uniform precipitation over the entire area, analysis of throughfall was restricted to the specific site by calculating mean throughfall as a percent of the gauge with the largest consistent catch. The assumption in this case is that rainfall over a particular site on any given occasion

Table 1. Mean throughfall by sites as a percentage of mean rainfall in open*.

	Date	Natural Regeneration	Mixed Oak-Pine	Pine	Cypress
WET SEASON	July 27				
	Aug. 13		$\frac{1.60^a + 100 = 34}{4.7^b}$	30	30
	Aug. 14		42	60	41
	Aug. 15		102	89	129
	Aug. 17	76	60	53	50
	Aug. 24	72	85	99	103
	Aug. 31	105	103	159	163
	Sept. 2	4	9	59	126
	Sept. 3	23	4 543	2 917	4 254
	Sept. 7	76	56	69	66
	Sept. 8	55	75	39	99
	Sept. 21	75	206	250	223
	Sept. 23	78	67	64	62
	Sept. 24	66	83	48	63
	Oct. 5	37	76	76	81
TRAN- SITION	Oct 29- Nov. 9	33	9	20	19
DRY SEASON	Nov. 17	578	55	58	69
	Nov. 18	124	21	114	25
	Nov. 20	75	80	69	57
	Nov. 25	98	0	1	0
	Dec. 1	134	29	93	78
	Dec. 6	54	0	0	0
	Dec. 7	42	0	0	0
	Dec. 9	82	0	0	0
	MEAN	94.5	286.8	209.8	286.9

* As recorded at the Los Planes de Montecristo Meteorological Station.

a Throughfall as recorded at site.

b Rainfall as recorded by Meteorological Station.

was evenly distributed. Considering the relatively small size of the sites, this is a reasonable assumption. Also assumed is that the gauge with the most consistently high catch gives a good estimate of the gross precipitation. Three means for throughfall were calculated for each site; one for the wet season, one for the dry and the third for the overall record (Table 2). During the rainy season, both the Natural Regeneration site and the Pine site appear to permit the greatest amount of throughfall, with mean throughfall percentages close to 85 percent. Also closely grouped together are the Mixed Oak-Pine and Cypress sites with a lower mean throughfall of 68 percent. This same pattern follows into the early dry season, though a change in the distribution of rainfall, favouring the Natural Regeneration site, is noted. In point of fact, the Mixed Oak-Pine, Pine, and Cypress received no further precipitation after December 6, while the Natural Regeneration

site was frequently under low, wind-blown cloud. A change is apparent at the Natural Regeneration site as well. This can largely be explained by much more open site conditions resulting from the combination of dry conditions and strong winds causing a degree of defoliation of the vegetation.

When considered in relation to the area of capture, stemflow does not appear as a significant quantity with means of 0.06 mm/m², 0.02 mm/m², 0.06 mm/m², and 0.04 mm/m² respectively, for Natural Regeneration, Mixed Oak-Pine, Pine, and Cypress. However, as a "per stem" measure (Table 3) the contribution of stemflow to the soil is more readily apparent. Stemflow at the Natural Regeneration and Pine sites is the greatest, being 5.1 and 4.4 times greater than the maximum mean gauge catch for their respective sites. The Mixed Oak-Pine and Cypress sites are grouped closely with stemflows

of 2.6 and 2.8 times their maximum mean gauge catches

Discussion: Precipitation variability, in mountainous terrain, is a complex function of large- and small-scale topographic parameters super-imposed upon weather conditions (5). As long as the measurements of rainfall are confined to a given watershed a small density network of gauges is adequate to provide a coefficient of variation of less than 10 percent (21). However, inter-gauge distance and elevational differences are highly correlated with variation in catch (5). This correlation with inter-gauge distance, and possibly elevational differences, is apparent in the mean throughfall data by site as a percentage of gross precipitation (i.e. meteorological station). In fact, even distances as short as 300 meters, such as that between the meteorological station and the Natural Regeneration site, are significant enough to cause extreme variation. The degree of variation between the meteorological station is more surprising than similar differences

between this station and the other three sites, all of which are on a different watershed (though at similar elevation and relatively close).

A trend towards a redistribution of rainfall is evident from the rainy season into the dry season. This has not been previously investigated in the area and could be an important consideration in the silvicultural treatment of present and future plantations, which deserves further attention.

The literature indicates differences in the results of researchers interested in forest-type effects on interception and throughfall, particularly in relation to hardwoods versus softwoods. Whereas some authors (9, 10, 29) have pointed to or found significant differences between hardwoods and softwoods, others (16, 25, 26) have not. The results of the present study seem to lie between these two groups. Throughfall, based on a comparison of mean gauge catch with the particular sites' gauge with the maximum mean catch, at the Natural Regeneration

Table 2. Mean throughfall as a percent of the gauge with largest receipt.

	Date	Natural Regeneration	Mixed Oak-Pine	Pine	Cypress
WET SEASON	Aug 13	—	74	79	33
	Aug 14	—	87	115	62
	Aug 15	—	81	62	84
	Aug 17	288	105	74	80
	Aug 24	65	88	72	74
	Aug 31	83	61	79	73
	Sept 2	75	48	88	86
	Sept 3	68	77	74	77
	Sept 7	95	56	71	66
	Sept 8	106	40	127	51
	Sept 21	—	63	69	59
	Sept 23	88	69	77	59
	Sept 24	79	48	98	56
Oct 5	102	66	67	82	
TRANSITION	Oct. 29-Nov. 9	30	6	58	—
DRY SEASON	Nov 17	98	59	131	25
	Nov 18	101	71	—	25
	Nov 20	98	68	97	52
	Nov 25	179	0	0	0
	Dec. 1	101	56	123	30
	Dec. 6	250	0	0	0
	Dec 7	—	0	0	0
	Dec. 9	128	0	0	0
	Overall Mean	101.89	53.17	70.95	60.59
	Wet Season Mean	84.9	68.79	82.29	67.29
Dry Season Mean	136.43	33.87	50.14	48.82	

Table 3. Summary of mean stemflow by site and 'event'.

Date	Natural Regeneration	Mixed Oak-Pine	Pine	Cypress
Aug 13	—	—	24.24	5.63
Aug 14	—	2.65	229.04	38.80
Aug 15	—	15.70	57.02	38.70
Aug 17	—	166.71	210.31	59.66
Aug 19	—	108.99	252.53	150.81
Aug 24	200.64 ^a	65.70	188.25	210.86
Aug 31	0	0	21.41	31.44
Sept 2	0	174.73	196.65	134.06
Sept 3	252.53	191.24	152.53	184.49
Sept 7	46.19	1.15	17.78	5.92
Sept 8	252.53	95.97	237.60	166.93
Sept 21	2.66	188.95	252.53	234.35
Sept 23	56.08	18.68	48.32	18.75
Sept 24	252.53	252.53	252.53	0.36
Nov 9	3.68	0.32	0.47	0
Nov 17	44.62	0	1.52	5.56
Nov 18	51.59	0	145.71	5.56
Nov 20	233.33	191.57	234.57	0
Nov 25	252.53	0	0.06	0
Dec 1	6.18	0	0.25	0
Dec 6	214.67	no precip.	no precip.	no precip.
Dec 7	26.32	—	—	—
Dec 9	253.53	—	—	—
Dec 15	8.99	—	—	—
Dec 18	54.20	—	—	—
Dec 22	3.54	—	—	—
MEAN	105.6	77.6	131.2	85.4
	mm/stem	mm/stem	mm/stem	mm/stem
As a % of Max.				
Mean Gauge Catch	511	259	437	278

a Each datum represents the average of 5 stemflow gauges.

and Pine sites was found to be the highest, at 85 and 82 percent respectively. In a similar grouping, the Mixed Oak-Pine and Cypress sites had the lowest throughfall (or conversely, the highest rate of interception), at 68 and 67 percent. These percentages apply during the rainy season; similar groupings arise from the overall (i.e. both rainy and dry seasons) means but are distorted as a result of changes in precipitation patterns during the transition and early dry season. The close similarity between the Mixed Oak-Pine and Cypress sites is interesting considering the difference in development of the two stands; the Mixed Oak-Pine being a mature stand, some 60 + years old, whereas, the Cypress plantation is only nine years old. Depending upon silvicultural treatment, the amount of interception can be expected to increase at the Cypress site as the trees continue to grow and expand their leaf surface area.

Extreme variation is the rule rather than the exception in the pattern of interception, and, consequently, for its complement, throughfall (7). Though not discussed in any detail in the literature, differences in the amount of variation between forest types appears to be significant. This is important in determining patterns of soil moisture and is a factor to be considered in inter-forest-type sampling of throughfall, if comparable data are to be produced.

Stemflow is frequently calculated in precipitation studies using the area of capture of the trees to which gauges are attached. Determined in this manner, stemflow is often concluded to be unimportant. Some authors, however, prefer to consider stemflow as a "concentrated application of water to the soil where conditions are ideal for entry" (13). Calculated on a millimeter per square meter basis stemflow, in the present study, was found to

be minimal. However, the mean "per stem" quantities for stemflow, as measures of a "concentrated application of water to the soil" are significant. Consequently, the greater amount of stemflow, such as indicated between the Pine plantation and the other three sites, particularly the Mixed Oak-Pine and the Cypress, is certain to favour better soil moisture conditions. This could well be important in an area that has already been shown to receive less rainfall than other areas such as the Natural Regeneration site. Similarly, the lower amount of stemflow at the Cypress site, situated in the same area as the Pine, could in time limit productivity by limiting soil moisture. As to differences between hardwoods and conifers, data reviewed by Geiger (8) indicated that hardwoods tend to have more stemflow than conifers. In the present study, there is a split between the two hardwood stands and the two conifer stands, with the Natural Regeneration stand aligned beside the Pine as the greatest conductors of stemflow and the Mixed Oak-Pine and Cypress closely grouped together with an average stemflow of about two-thirds that of the former two sites.

Soil Moisture

Materials and Methods: Percent Available Soil Moisture, at the different sites, was monitored with a Bouyoucos Moisture Meter (Beckman Instruments, Inc.) and a dozen CEL-WFD Gypsum Moisture blocks. Three gypsum blocks were placed at each site in sequence at 10.2, 30.5 and 61 cm depths. The gypsum blocks were fitted into an undisturbed column of soil as per instructions as supplied by the manufacturer.

Readings were taken from the blocks during the regular routine of visiting the site. Readings were discontinued during the dry season after all sites at all

three levels had reached zero percent available soil moisture.

Soil moisture, field capacity and wilting point measurements were carried out as a part of soil sampling done early in the study and again at the end by the laboratories at the Centro Nacional de Tecnología Agropecuaria (CENTA) and the Dirección-General de Riego y Drenaje (i.e. Drainage and Irrigation). The final sampling consisted of soil moisture samples taken at one-foot (30.5 cm) intervals to a five-foot (152.5 cm) depth.

Results: Differences with respect to field capacity, wilting point, and storage capacity are evident between sites, and within sites, between organic and mineral soil horizons (Table 4). Considering storage capacity as the most relevant comparative measure, since this factor represents the particular soils ability to hold a given quantity of water, the data shows the Mixed Oak-Pine site to have the greatest storage capacity, followed closely (18% difference) by the Pine site. The Cypress and Natural Regeneration have the lowest values.

The month of September is noted as the time of the year, from a meteorological point of view, that the Intertropical Zone of Convergence is the closest to El Salvador. Characteristically, this is a month of high rainfall, much of it resulting from "temporales," interspersed with prolonged periods of low, dark clouds. The record of percent available soil moisture (Figures 1a and b) for the Natural Regeneration site appears to reflect this, with soil moisture at all depths at about 90 percent availability broken by a period of 12 days (i.e. from September 9 to 20) of no rain in which soil moisture availability markedly decreased. Available soil moisture returned to higher levels with the advent of new storms and remained high until into mid-October, after which a continual decline

Table 4. Summary of field capacity, wilting point and storage capacity for the organic and mineral soils for each site.

	Natural Regeneration		Mixed Oak-Pine		Pine		Cypress	
	0-16 cm	16 + cm	0-12 cm	12 + cm	0-12 cm	12 + cm	0-7 cm	7 + cm
Field capacity (%) (0.33 atm)	41.25	39.74	47.28	42.90	33.65	49.47	56.96	59.26
Wilting point (%) (15 atm)	31.65	29.29	23.65	20.40	18.22	31.90	44.54	39.06
Storage capacity (mm/cm)	0.93	0.98	2.35	2.33	1.89	1.93	0.88	1.59

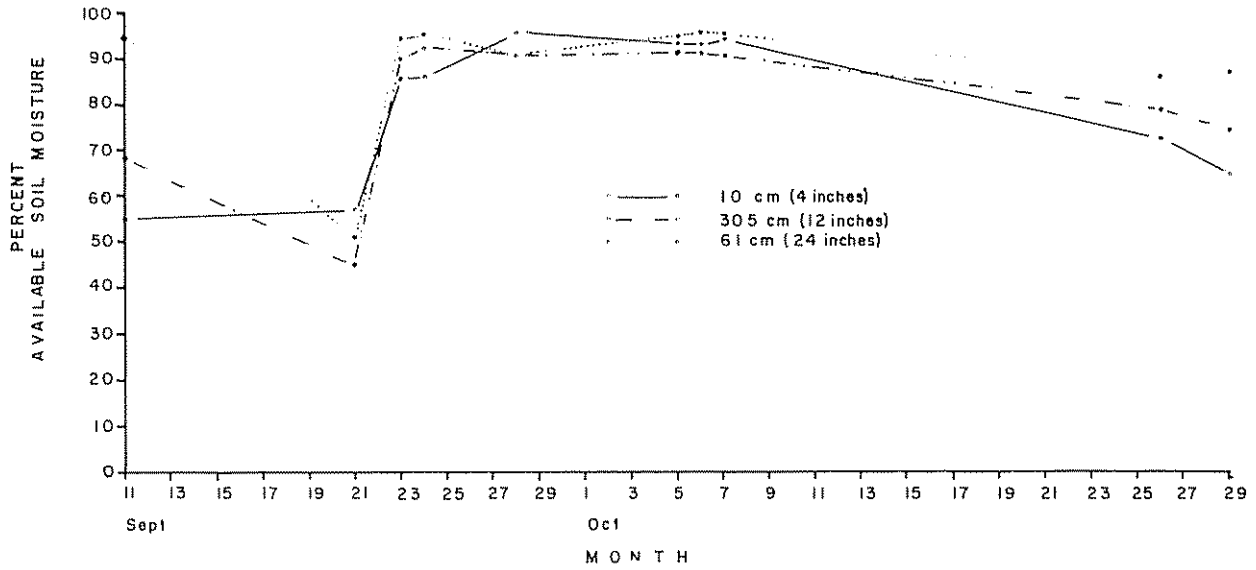


Fig 1a. Recorded percent available soil moisture between sept. 11 and oct. 20 at natural regeneration site.

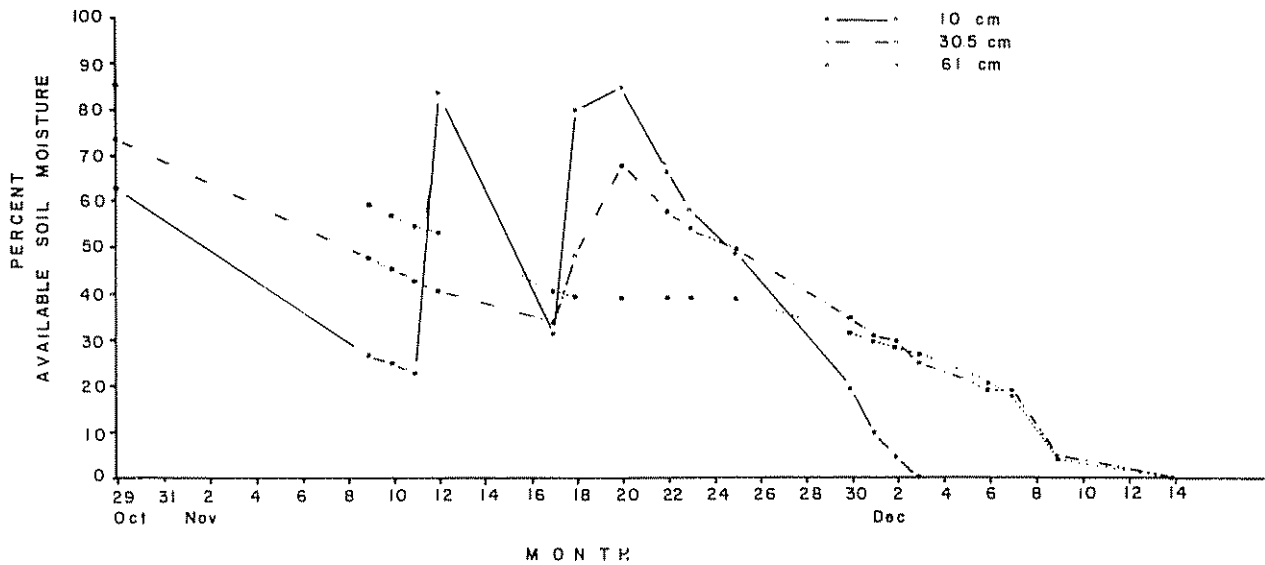


Fig 1b Percent available soil moisture between oct 29 and dec. 14 at natural regeneration site.

began, broken only by a couple of heavy storms in mid-November, with all depths recording zero percent available soil moisture by December 14th.

The curve shows a deficit from mid-December to late-April (transition from dry to wet season) and then fluctuates around 90 percent available soil moisture during the rainy season, except for July. In July

there is a retreat of the Intertropical Zone of Convergence and the occurrence of "caniculas," periods of sunny rainless days, resulting in a low rainfall for the month. Consequently, soil moisture depletion would occur at this time (possibly causing a deficit at certain sites). Soil moisture depletion would again occur after late September (i.e. the end of the rainy season) leading to a deficit by mid-December.

Inter-site comparisons (Figures 2, 3 and 4) are limited to the period between November 9 and December 15, when data for all four forest-types is available. The dominant characteristics of the soil moisture curves are as follows. The trend is towards depletion with the Cypress site having the most rapid rate at all three levels sampled. At the 10 cm level, Cypress is closely mimicked, with the exception of the early part of the curve, by the Natural Regeneration site. The Natural Regeneration and Mixed Oak-Pine sites have very similar curves at both the 30.5 and 61 cm levels. Except for the 10 cm level the curves for the Mixed Oak-Pine site are consistently, at all levels, those with the lowest rates of moisture depletion.

Discussion: Field capacity, wilting point and storage capacity is determined for the most part by the nature of the soil (2). In the particular situations investigated in this study the data obtained reflects the historical development of each site, including the evolution of the present forest types now present. Though it is accepted that individual species do affect the physical and chemical properties of the soil (6), the available data in this instance are not adequate to definitely indicate possible contributions

by the forest species found at the different sites towards the variation in these three factors, between sites. Nonetheless, certain inferences seem reasonable, although they are made guardedly. For instance, Hoover (13) has indicated that forest soils under old-growth tend to have an almost unlimited infiltration capacity; undoubtedly as well, these same soils also have high storage capacities. The high storage capacity at the Mixed Oak-Pine site does appear to suggest this. The lowest storage capacities at the Pine and Cypress sites, consequently, may reflect their youth and, as well, their recent disturbance (i.e. 9 years past) during planting. The slower rates of decomposition of conifer leaves (8) may also be a factor too, with attendant retardation in organic soil buildup. In the case of the Natural Regeneration site, with its very low storage capacity, greater age does not seem to have resulted in greater storage capacity.

Some of the differences seen between the sites at the 10 and 30.5 cm depths are explainable on the basis of previously presented data for throughfall. A radical variation in soil moisture at the Natural Regeneration site, relative to the other sites, is apparent between November 11 and 12. At this time, while

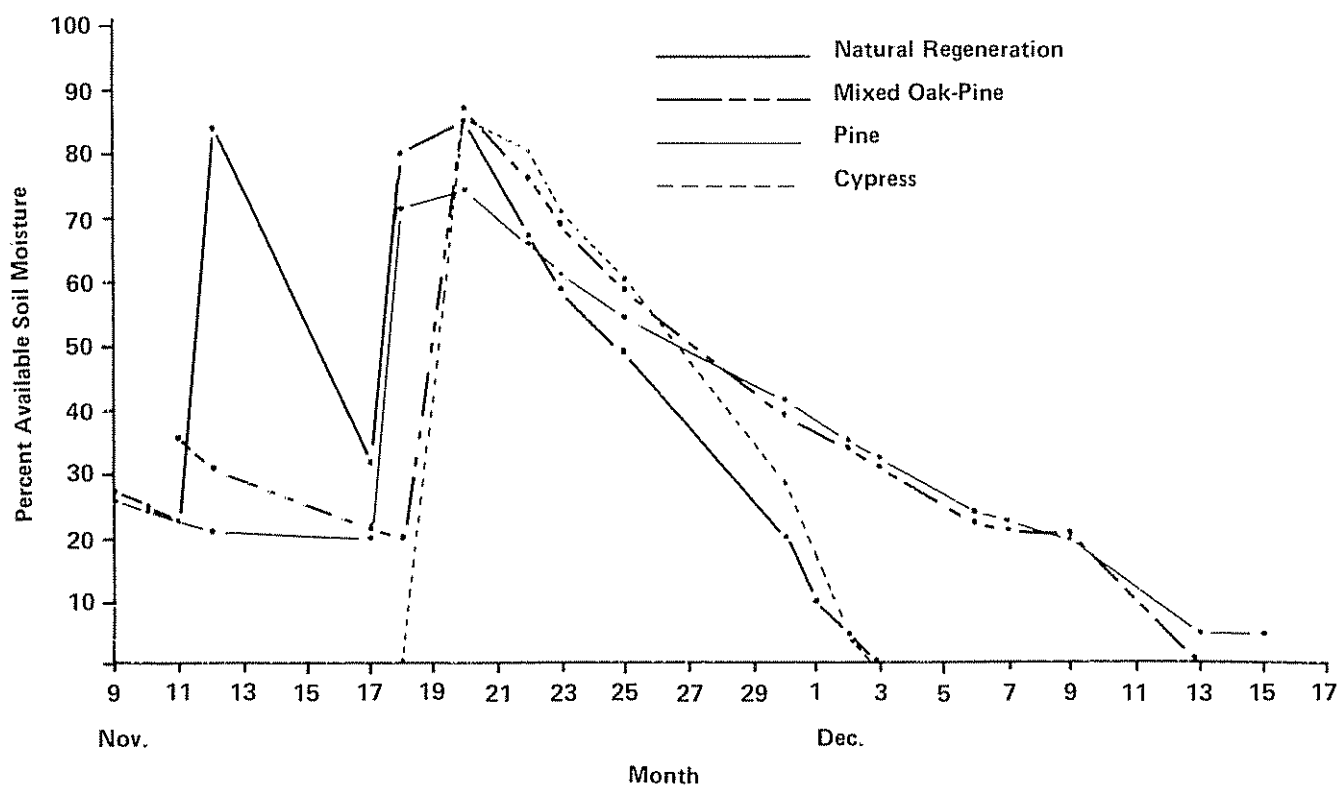


Fig. 2. Soil moisture trends by site at the 10 cm depth.

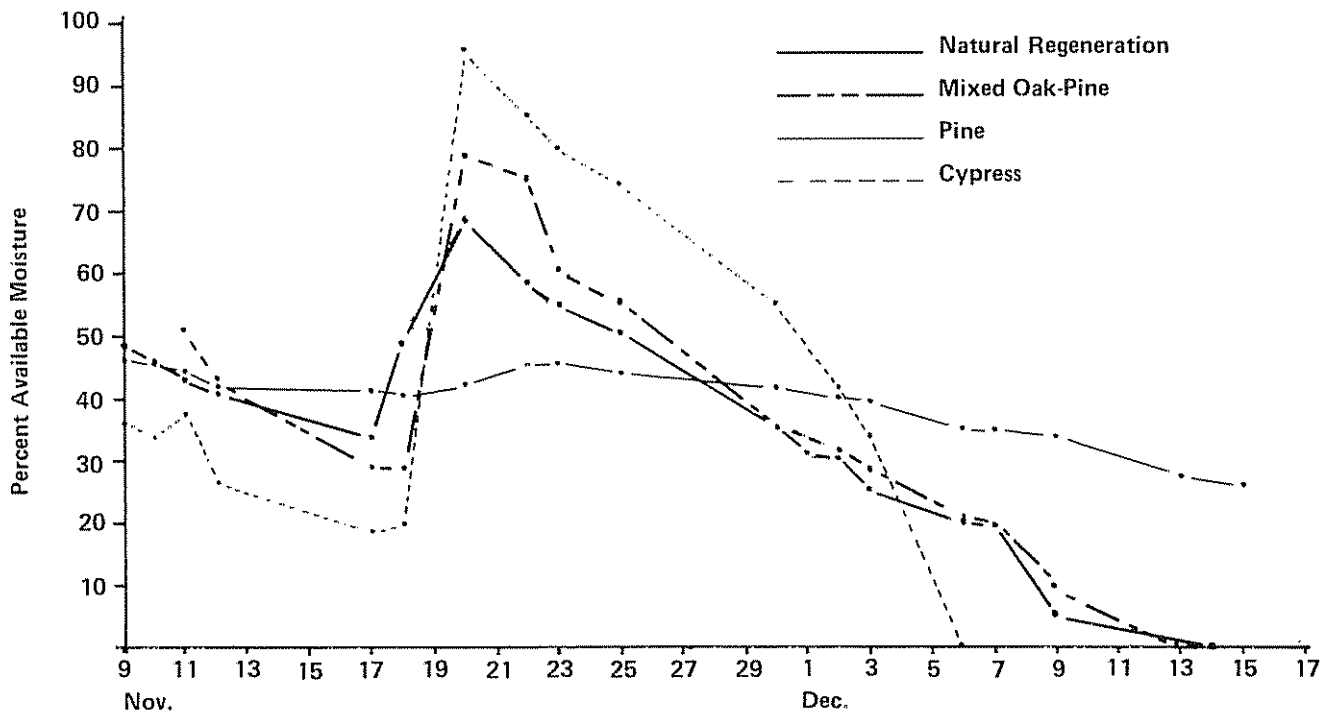


Fig. 3. Soil moisture by site at 30.5 cm depth.

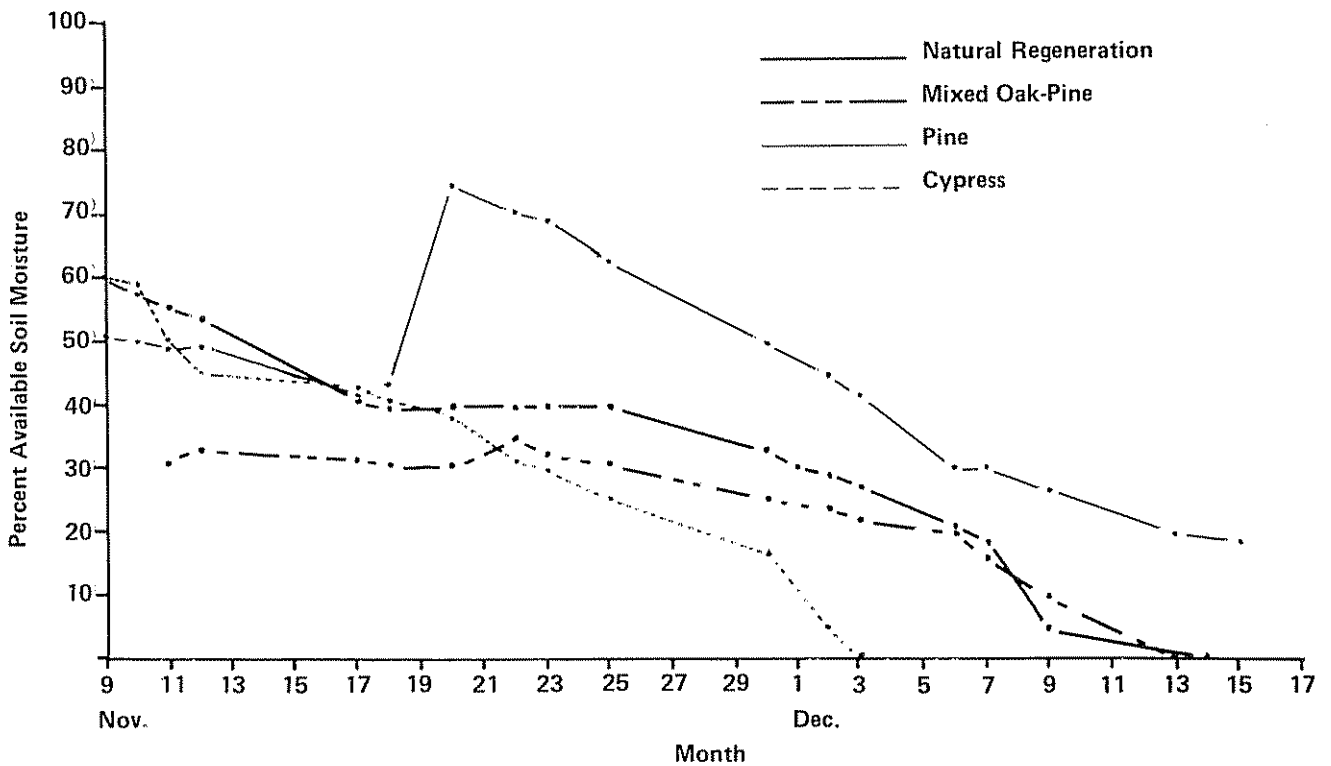


Fig. 4. Soil moisture trends by site at 61 cm depth.

other sites experienced a decline in soil moisture, the Natural Regeneration jumps from 26 percent available soil moisture to 84 percent. Though no field data is available on throughfall at the sites, 8.8 mm of precipitation was recorded at the meteorological station at Los Planes. Throughfall was checked on the 17th at which time it was noted that the Natural Regeneration site had received some six times as much rain as the other sites. Consequently, this departure from the dominant trend appears to reflect a situation of unequal distribution of precipitation over the area.

Evaporation

Materials and Methods: The objective of the evaporation measurements was to obtain a comparative measure of "evaporation potential" or "evaporativity" near the ground in the different forest types. This was carried out using Livingston Spherical Atmometers (white) as part of an evaporimeter. Construction and operation of the evaporimeters were adapted from Livingston (17) and Read (25, 26).

The atmometer bulbs were pre-calibrated at the factory before shipping and each carried a correction coefficient which standardized readings for all the atmometers. Directions for maintenance, as given in Livingston (17) were followed as closely as possible. Atmometers were allowed to operate for three months before being replaced with new unused atmometer bulbs.

Readings consisted of recording the amount of distilled water needed to bring the reservoir back to a mark. The date and hour were also recorded for later use in calculating the rate of evaporation. There were three evaporimeters placed at each site, except for Natural Regeneration, the third instrument from which was placed at the meteorological station at Los Planes. Readings were taken every day or every other day, though longer periods between readings occasionally occurred.

One evaporimeter was suspended in the canopy at each site in order to observe the difference in evaporativity with height. These evaporimeters were suspended on the same platforms used for transpiration trials.

Results: A comparison of the mean values (Table 5) for evaporativity for the sites indicate a close similarity between the Mixed Oak-Pine, Natural Regeneration and Pine. The Cypress site, with a mean of 0.79, has an evaporativity 1.58 times that of the Pine whose mean of 0.50 is the greatest of the three remaining sites.

One-way analysis of variance (Table 6) for the combined data for evaporativity, including data from the meteorological station at Los Planes, indicates the presence of highly significant variation at the 0.001 level of probability.

The intersite comparison showed no significant difference among the Mixed Oak-Pine, Natural Regeneration and Pine, as would be expected from the previous comparison of the means. All sites, except the Cypress, had highly significant differences when compared to the Meteorological Station; the Cypress had a significant difference with the Meteorological Station at the 0.05 level of probability. Differences between the Cypress and the other three sites were all highly significant.

Differences between the Cypress and Pine sites could be expected to approach each other more closely as the Pine site was possibly affected by a line of giant cypress which formed a windbreak up-wind of the site.

Three things are indicated by Figures 5 and 6; these are intersite variation, the fluctuating nature of evaporation and the marked difference in evaporativity between the Rainy/Transition Season and the Dry Season. The difference between the Cypress site and the other three sites is highlighted, particularly during the period of measurement in the Dry Season.

Table 5. Summary statistics of evaporativity (ml/hr) by site.

	Number of Cases	Mean	Standard Deviation	Minimum	Maximum	Coefficient of Variation (%)
Mixed Oak-Pine	51	0.47	0.22	0.06	1.16	47.0
Natural Regeneration	49	0.43	0.23	0.05	0.91	53.0
Pine	51	0.50	0.23	0.06	0.94	45.3
Cypress	54	0.79	0.30	0.14	1.50	38.4

Table 6. Summary of intersite T-test results.

Meteorological Station	Mixed Oak-Pine	Natural Regeneration	Pine	Cypress
Meteorological Station	8.28 ^(a) *** ^(b) 10.4 ^(c)	8.68***	7.81***	3.35*
Mixed Oak-Pine		0.90	-0.67	-6.16***
Natural Regeneration			-1.54	-6.77***
Pine				-5.55
				103

(a) = T-value; (b) = Significance; (c) = Degrees of Freedom.
 * = Probably Significant at 0.05; ** = Significant at 0.01; *** = Highly Significant at 0.001.

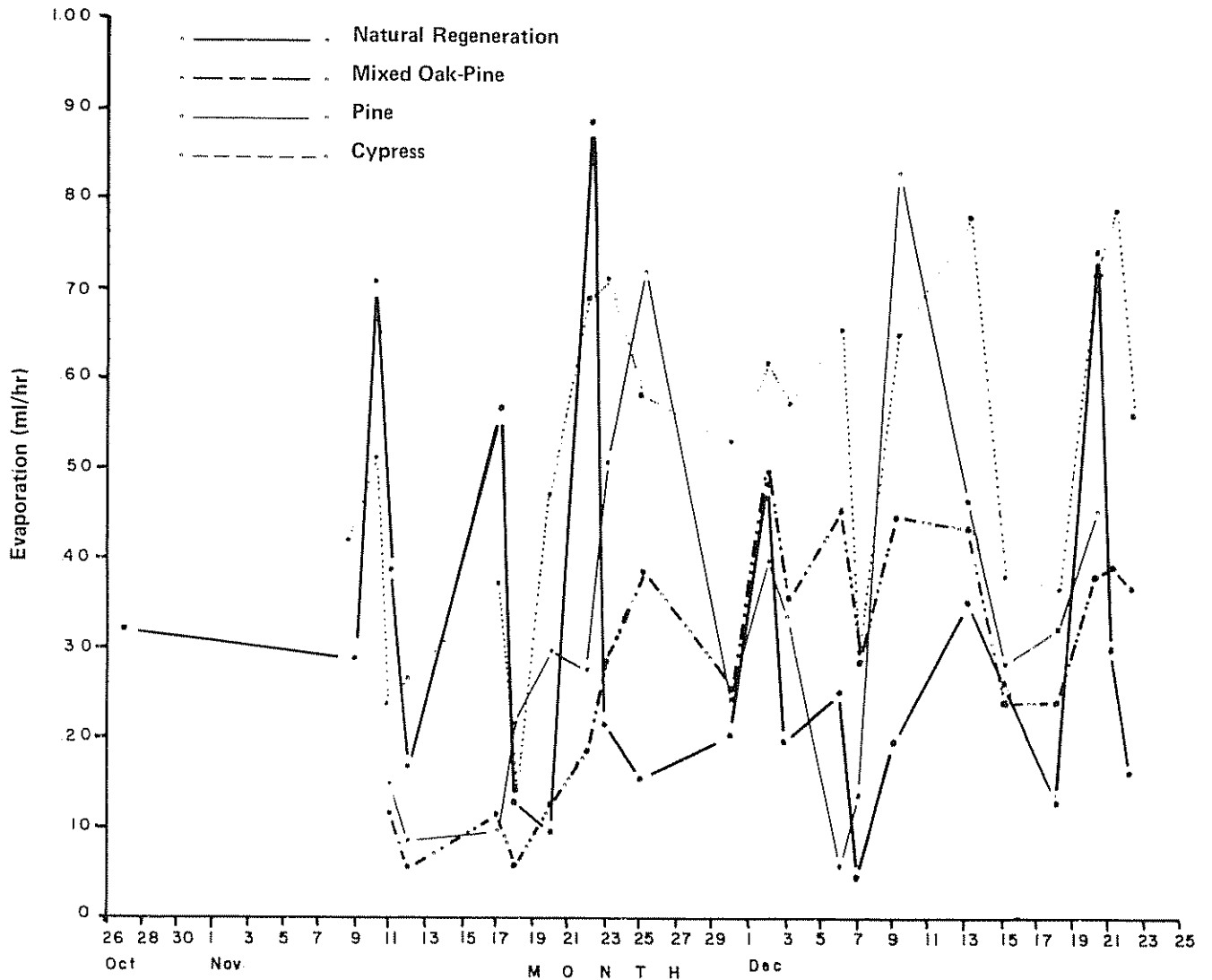


Fig. 5. Summary of evaporativity data for the four forest-types.

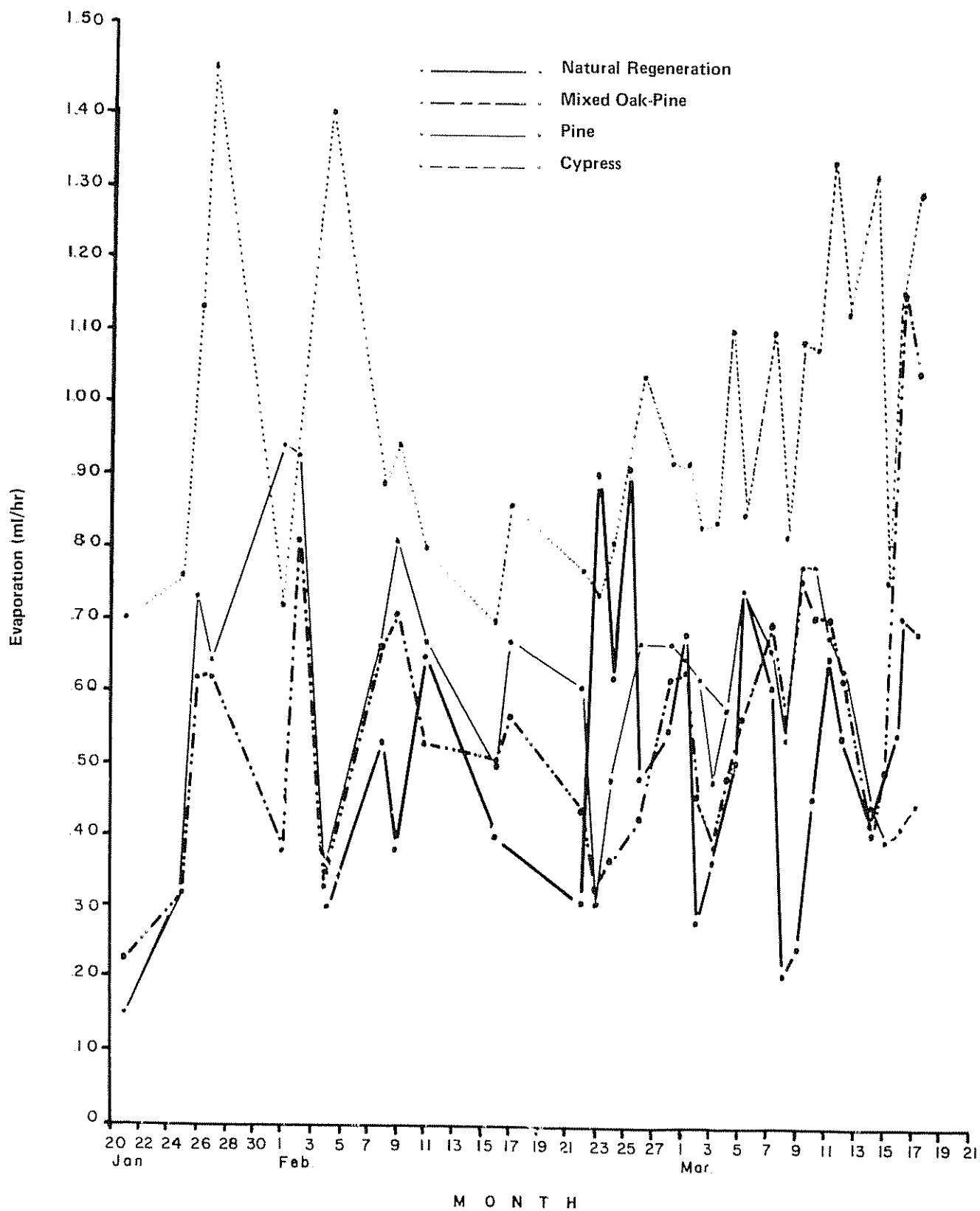


Fig. 6. Summary of evaporativity data for the four forest-types

(i.e. Jan. 25 – Mar. 17). Variation between the sites is mainly reflected in different rates of evaporation; however, though all the sites tend to follow the same trend in fluctuations, there occurred a number of occasions when a particular site was not consistent with the others in relation to this trend. Differences in trend are probably a reflection of a particular topographic location. This explanation would account for the greater number of occasions on which the Natural Regeneration site was not consistent with the others, situated as it was the head of a small valley down or up which were channeled the prevailing winds, in contrast to more dispersed movements over the other three sites. The contrast between mean evaporativity for the Late Rainy/Transition Season and the Dry Season (Table 7) is marked by rates of evaporation during the Dry Season of approximately twice those during the former season. These higher rates are a result of the generally dryer conditions and the influence of the cool, dry northerly winds which prevail during this

period. The Average Percent Relative Humidity during January, February and March (March value for 1976) was 72, 75 and 70% compared to values of 85, 83 and 80% for the months of October, November and December. Average wind speeds were about 19 km/hr, with maximums in the 80's or near 80's. Mean Monthly Maximum and Minimum temperatures showed a trend towards increasing through August to March, particularly during March and the preceding month of February. The monthly means presented in Table 8 are more indicative than absolute, based as they are on an incomplete monthly record; however, the trend towards increasing temperatures and the differences between sites are clear and consistent. A comparison of the mean rates of evaporation at the sites during the Rainy/Transition Season and Dry Season, as indicated in Table 7 correspond well with the mean maximum temperatures for the corresponding periods, with the exception of the Mixed Oak-Pine site during the wet period. The Mixed Oak-Pine site, though experiencing

Table 7. Comparison of mean near-ground evaporativity (ml/hr) for Late Rainy Season/Transition and Dry Season.

	Oct. 26 – Jan. 21	Jan. 25 – Mar. 17	Ratio
Mixed Oak-Pine	0.286	0.580	0.286/0.580 = 0.49
Natural Regeneration	0.320	0.526	0.320/0.526 = 0.61
Pine	0.332	0.606	0.332/0.606 = 0.55
Cypress	0.530	0.983	0.530/0.983 = 0.54

Table 8. Mean maximum and minimum temperatures for August through March at sites.^a (°C).

	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Mixed	21 ^b	20	23.3	22.2	21	22.2	25.5	26.6
Oak-Pine	12.2 ^c	13.3	12.7	13.3	13.3	8.8	12.7	12.7
Natural	18.8	18.3	18.8	17.7	17.7	19.4	21	21.6
Regeneration	12.2	12.2	12.2	11.6	11.6	7.7	10.5	11.6
Pine	18.8	18.8	21	20.5	21	21.6	23.8	26.1
	11.6	11.6	12.2	13.3	12.7	8.8	12.2	12.2
Cypress	21.6	21.6	22.2	22.7	23.8	21.6	25	26.1
	12.7	12.7	12.7	12.7	12.7	8.8	11.6	12.7

a = Average number of records equals 8

b = Maximum

c = Minimum

temperatures similar to the Cypress site, nonetheless, had the lowest mean evaporation rate for this latter period. This may reflect local site conditions such as higher relative humidities and less sub-canopy turbulence which would tend to suppress vapour pressure deficits and in turn evaporativity.

Evaporativity at mid-canopy was one third greater than the corresponding level near the ground (Table 9), with the exception of the Cypress site. Excessive shading may be the cause of differences found between the Cypress canopy evaporimeter results and the other sites.

Discussion: The higher evaporativities at the Pine and Cypress sites agree with the results of Swank *et al.* (30, 31, 32) which indicated that coniferous stands lose more water to evaporation than similar deciduous stands. In part this is due to the large leaf area of conifers (average of 59.5 m² and 65.9 m² respectively, for *P. pseudostrobus* and *C. lusitanica* found in plantations studied) which provides a large interceptive, and consequently evaporative, surface. The open and uniform nature of plantations permits the development of organized mass air flows which also favors greater loss of moisture to evaporation both from the soil and the vegetation.

The evaporativity at canopy level and at the Meteorological Station was much higher than the near-ground forest evaporativity as was expected from previous studies (26, 33).

Transpiration

Materials and Methods: The objective behind the measurement of transpiration was to obtain a comparative measure of transpirational water loss for the different forest types. The "cut-stem potometer" technique used for measuring transpiration in this study was adapted from Weaver *et al.* (34).

In choosing stems, attention was paid to maintaining consistency within and between trials with respect to size, position in canopy (e.g. all stems were taken from mid-canopy), and exposure in order to reduce variation within species.

Stems were cut using garden shears attached to a long pole and were placed into water contained in a 250 ml graduated cylinder within seconds of being cut. A one-holed cork stopper held the stem in place and partially sealed the cylinder. The cylinders were further sealed using plasticine around the rim and around the stem and opening of the stopper. Additional sealing was accomplished using a fast drying cement (Seal-All, Allen, Stevenson Products).

At each of the plantation sites, four replicates were prepared during each trial, since only one species was involved. For the natural regeneration site, five different species were tested and during a given trial, four species with two replicates each were involved. Three species were tested at the Mixed Oak-Pine site, one Pine (Pino ocote), one Oak (Encino) and one sub-canopy species (Trompillon).

Once the potometers for a particular site were all prepared, they were then all raised into mid-canopy on a small platform where they were left for a period of 48 hours. Measurement involved determining water loss from the graduated cylinder and leaf area of sample.

Though the method does not give quantitatively reliable data in an absolute sense, it has been shown to be an economical and relatively simple method to obtain data sufficient to establish transpirational differences among forest types and species (15, 34).

Results: A comparison of the mean transpiration rates (Table 10) for the different sites indicates that the Pine Plantation with a mean of 2.37 the lowest rates. The Mixed Oak-Pine, with a mean of 3.98 is

Table 9. Summary of canopy evaporativity (ml/hr).

	Mixed Oak-Pine	Natural Regeneration	Cypress	Pine
Number of Readings	9*	22	9*	23
Mean	0.89	0.86	1.00	0.95
Standard Deviation	0.35	0.31	0.28	0.25
Ratio of Near Ground** to Canopy Evaporativity	0.64	0.64	0.98	0.65

* Instruments were damaged during high winds, which prevented further measurements.

** Readings for Near Ground Evaporativity were recorded together with those for the Canopy at the same time.

Table 10. Summary statistics of transpiration data ml/m²/hr by species and site.

Species	Sites	Mixed Oak-Pine	Natural Regeneration	Cypress	Pine
		<i>Quercus</i> aff. <i>Sapotaefolia</i> Liebm.	<i>Leucaena</i> sp.	<i>Cupressus lusitanica</i>	<i>Pinus</i> <i>pseudostrobus</i>
Mean		3.35	2.89	4.03	2.27
S.D. ¹		2.19	2.34	1.25	1.09
		<i>Cleyeratheaeoides</i> (sw) Choicy	<i>Roupala montana</i> Audi		
Mean		5.48	6.26		
S.D.		1.53	1.86		
		<i>Pinus oocarpa</i> Schiede	<i>Sauravia pseudo-</i> <i>rubrififormis</i> Buse		
Mean		3.11	6.75		
S.D.		0.77	3.64		
			<i>Cassia</i> sp.		
Mean			8.78		
S.D.			1.43		
			<i>Winmeria</i> <i>cyclocarpa</i> Radlk		
Mean			9.12		
S.D.			5.34		

1 Standard Deviation.

1.76 times greater than the Pine and is followed closely by the Cypress Plantation at 4.03 (i.e. 1.77 times Pine). The Natural Regeneration site, with a mean of 6.76 experiences transpiration rates some 2.98 times greater than that of the Pine Plantation.

Statistical variation among the cross-species comparisons ranged from not significant to highly significant. Differences in mean transpiration rate between Pinabete and Ciprés, four of the Natural Regeneration species (the exception being Guaje), and Trompillon from the Mixed Oak-Pine site were highly significant. A significant difference was also found between Pinabete and Ocote pine. Besides the highly significant difference found with Pinabete, Ciprés also showed highly significant differences with all species from the Natural Regeneration site except Guaje. No significant differences were uncovered between Encino and Trompillon or Ocote pine of the Mixed Oak-Pine though a possible significant difference was indicated between Trompillon and Ocote pine. None of the three species from the Mixed Oak-Pine site has a statistically important variation when compared with Guaje of Natural Regeneration site. However, both Encino and Ocote pine had highly significant differences with Zorrillo, Vainillo and Lloroncito of the Natural

Regeneration species and a significant difference with Siete Pellejos. Amongst the Natural Regeneration species, a highly significant difference was found between Guaje and Zorrillo, Vainillo and Lloroncito. Significant differences were found between Guaje and Siete Pellejos, and Zorrillo and Vainillo.

The results of the one-way ANOVA (Table 11) comparing the grouped data for the broad-leaved species to the grouped data for the conifers indicated a highly significant difference between the two groups. The mean transpiration rates for the broad-leaved and coniferous groups were, respectively, 6.62 and 3.08. The broadleaved group has, overall, a mean transpiration rate some 2.14 times greater than that of the conifers.

Discussion: Published transpiration rates obtained by Weaver *et al.* (34), using similar techniques to those used in the present study are comparable in range to that found in the broadleaves species. Data from McLean (18) is similar in its lower range as is data from Odum *et al.* (22). In cross-species comparisons, data from Henrici (11) indicates lower transpiration rates for both Cypress (*C. lusitanica*) and Pine (*P. radiata*) when compared with *Acacia* and *Eucalyptus*. This relationship held true for five of the

Table 11. Analysis of variance (grouped broadleaved versus grouped conifers).

Source	D.F.	Sum of Squares	Mean Squares	F. Ratio	F. Prob.
Between Groups	9	349.2043	38.8005	4.149	0.000
Within Groups	102	953.9250	9.3522		
Total	111	1303.1294			

seven broadleaved species in the present study. However, no significant difference was found between any of the conifers in the study and Encino. Oaks are the principal dominants in mature natural stands in the study area. Transpiration rates for Cypress and *Pinus radiata* were almost the same in Henrici's study, while in the present report Cypress has a rate of 1.30 times greater than *P. oocarpa* and 1.78 times greater than *P. pseudostrabus*. Kittredge (14) found average daily transpiration for pines greater than various species of Oaks, whereas, in this case no significant statistical difference was found between Encino and either pine. Transpiration rates for pines in the present study are also comparable to those found by Minkler (19).

Consideration of the exact meaning of transpiration rates, in terms of water loss, must include comparative measure of the leaf surface area of the species involved. The two plantation species, *Pinus pseudostrabus* and *Cupressus lusitanica*, had mean leaf surface areas of 59.5 m² and 65.9 m², respectively. Data for broadleaf species was not obtained nor found in the literature, but are likely to be less based on subjective impressions from field observations. Some of the broadleaved species, notably *Leucaena* sp. and *Cassia* sp., experience partial defoliation during the dry season, which would lessen their impact on available soil moisture.

Conclusions

It is clear that the Cypress site is the driest of the four sites. Low throughfall, high evaporation and moderately high transpiration (together with a large leaf surface area) combined to give the most rapid rate of soil moisture depletion and produce very dry site conditions. Following in order of dryness were the Natural Regeneration and Mixed Oak-Pine sites, both of which had similar site conditions when all factors were considered. The Pine site, with greater throughfall, moderate evaporation rates and low transpiration rates, was the least dry of the four sites.

These conclusions have important implications for the area. In terms of the water balance the implications are: 1) run-off, and, consequently, streamflow, is reduced under plantations of conifers, particularly cypress and 2) higher evapotranspirational losses will lead to increased rate of regression of streamflow as present plantations mature and new ones are planted.

The principal management implications are: 1) reduced streamflow will reduce the danger of flooding during the rainy season of the San Jose River; 2) vegetative cover, in the form of a canopy and litter, as provided by the conifer plantations, provide protection for the soil and reduce the potential for soil erosion; 3) rapid rates of soil moisture depletion, as found under cypress, along with a deep layer of litter and abundant brush lead to dry conditions which increases fire hazard, and 4) reduced streamflow, arising from greater evapo-transpiration by the forest, leads to reduced availability of irrigation water and thus will prolong the effective drought season.

These implications apply to the immediate area in which the study was undertaken and may extend to other areas in El Salvador where reforestation is occurring as well.

Management priorities will determine the importance of reduced streamflow from the area. A number of factors argue strongly for placing the management for water yield as the first priority. The most compelling being that El Salvador, as Moore (20) has stressed, is first and foremost an agricultural country dependent upon export for foreign exchange earnings. Moreover, the country experiences an annual drought period of approximately five months and, consequently, is a country where water is an extremely valuable resource sufficiently scarce already that rationing occurs throughout a portion of the year in many parts of the country.

Reforestation is an urgent necessity in order to preserve soil resources and, as well, to provide jobs and revenues in many areas of the world. Undoubtedly,

various species of conifers such as *Pinus* sp. and *Cupressus* sp., have an important role to play; it is not the intent of this research to discredit their utility. However, the choice of reforestation with one species versus another can have important implications, as this study indicates. Yet, huge gaps in our present knowledge concerning the characteristics of forest species in relation to factors such as evapotranspiration exists, which prevent proper evaluations of such choices and their long-term significance. It is towards the objective of reducing this gap that this research has been directed and the discussion of the advantages and disadvantages of conifers versus natural broadleaved species addressed.

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Summary

To study the effects of foliar fertilization with nitrogen in different stages of plant growth, two field experiments were set out in a sandy loam soil. The first experiment was conducted during the wet season and the second during the dry season.

Nitrogen (32-0-0) was applied on the leaves at the level of 20 l/ha on three different dates divided twice: 15 and 30, 30 and 45, 45 and 60 days from seedling emergence. All of the plots received a basic soil fertilization with 80 kg/ha of P₂O₅, 30 kg/ha of K₂O applied in the row plus 30 kg/ha of N topdressed 15 days after seedling emergence.

Foliar nitrogen fertilization at 30 and 45 days after seedling emergence increased bean production by 18% in the wet season and 39% in the dry season. It was noticed that late season application of nitrogen on the leaves caused an increasing in the thousand-grain weight.

Introdução

A prática da adubação foliar vem aumentando nos últimos anos devido às respostas rápidas e satisfatórias que vêm sendo obtidas em algumas culturas, e ainda ao baixo custo da aplicação, principalmente se associada à aplicação de defensivos. Contudo, em nosso meio, são escassas as pesquisas sobre essa prática na cultura do feijão, e vários aspectos carecem ainda de estudos ou confirmações.

Alguns autores (3, 4) justificam o estudo sobre o assunto no feijoeiro devido a facilidade de aplicação e custo relativamente baixo quando em associação ao controle fitossanitário.

Um dos aspectos ainda não definidos do problema, é a época de aplicação que poderia promover os melhores resultados.

Com relação a este aspecto, tem sido demonstrado que, para a cultura da soja, aplicações de adubos foliares no estágio reprodutivo da planta tem dado bons resultados (5, 10), embora existam trabalhos que mostrem resultados negativos para essa prática (1, 8).

Na cultura do feijoeiro, foi demonstrado (7) que aplicações tardias podem dar bons resultados, em determinadas condições. Por outro lado, encontram-se citações (5) em que os adubos foliares nitrogenados proporcionaram melhores resultados quando aplicados no estágio vegetativo das plantas anuais.

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Com a finalidade de contribuir para o estudo de épocas de aplicação de adubo nitrogenado foliar em feijoeiro foi conduzido o presente trabalho.

Material e métodos

O trabalho constou de 2 ensaios conduzidos em condições de campo na Estação Experimental de São Manuel, Município de São Manuel, Estado de São Paulo, sendo um na safra das águas e o outro na safra da seca do ano agrícola de 1979/80.

O solo utilizado para ambos os ensaios foi classificado como Latossol Vermelho Escuro-fase arenosa, que revelou as seguintes características: pH = 5.5; M. O. = 0.67%; K^+ = 0.17 emg; PO_4^{3-} = 0.05 emg; $Ca^{+2} + Mg^{+2}$ = 1.53 emg e Al^{+3} = 0.4 emg por 100 g de TFSA, em amostragem realizada antes do 1.º ensaio.

A cultivar utilizada foi a carioca.

O delineamento experimental utilizado foi em blocos casualizados, com 4 tratamentos e 5 repetições.

Os tratamentos constaram de:

- 1) Testemunha
- 2) Adubação foliar aos 15 e 30 dias após a emergência das plantas.
- 3) Adubação foliar aos 30 e 45 dias após a emergência das plantas.
- 4) Adubação foliar aos 45 e 60 dias após a emergência das plantas.

A adubação foliar constou da aplicação de 20 l. por hectare da fórmula 32-0-0, parcelas em duas vezes, sendo, o produto utilizado, conhecido comercialmente como URAN.

Em cada uma das aplicações foram utilizados 300 l/ha de água mais 1,0 ml de espalhante adesivo por litro de água.

Todos os tratamentos receberam adubação normal no solo na base de 30-80-30 kg/ha de N, P_2O_5 e K_2O , respectivamente nas formas de sulfato de amônio, superfosfato simples e cloreto de potássio. Os adubos fosfatados e potássico foram aplicados por ocasião da semeadura, em sulcos situados 5 cm ao lado e abaixo daqueles destinados às sementes.

A adubação nitrogenada foi efetuada na sua totalidade, em cobertura, aos 15 dias após a emergência das plantas.

Cada parcela experimental constou de cinco linhas de cinco metros de comprimento, espaçadas entre si de 0.40 m.

Considerou-se como área útil da parcela as 3 linhas centrais, eliminando-se 0.50 m de cada extremidade dessas linhas, que foram consideradas como bordaduras.

As semeaduras do feijão foram efetuadas a 5 de outubro de 1979 e 14 de fevereiro de 1980 respectivamente para a época das águas e da seca. O final de emergência das plantas foi observado aos 15/10/79 e 21/02/80, respectivamente para o ensaio da safra das águas e da seca e na mesma ordem a colheita foi realizada em 10 de janeiro e 13 de maio de 1980. Após a colheita de ambos os ensaios foi procedida a secagem, a limpeza e a pesagem do produto. A partir dos grãos colhidos na safra da seca, foi determinado o peso de 100 grãos conforme prescreve as regras para Análise de Sementes (2).

Na ocasião da colheita do ensaio da safra da seca foram coletados ao acaso 10 plantas por parcela e determinado o número de vagens, o número total de grãos, o número de grãos desenvolvidos e o número de grãos não desenvolvidos. A média dos dados obtidos com as 10 plantas foi utilizada para cálculos dos parâmetros dos ensaios.

Resultados e discussão

O desenvolvimento vegetativo das plantas foi normal durante todo o ciclo, ocorrendo leve incidência de bacteriose que não chegou a prejudicar os ensaios. Contudo a aplicação do adubo foliar aos 15 dias após a emergência das plantas ocasionou uma leve queimadura na parte marginal do limbo dos folíolos. A medida em que foram retardadas as aplicações, os sintomas diminuíram de intensidade e frequência. O desenvolvimento das plantas injuriadas foi normal o que leva a concluir que as injúrias não chegaram a afetar o crescimento das mesmas.

Os dados médios da produção de feijão das safras das águas e da seca, encontram-se na Figura 1.

A análise estatística dos dados de produção não revelou diferença significativa entre os tratamentos para época das águas, entretanto notou-se uma tendência de se obter maior produção (18%) quando se faz aplicação do adubo foliar aos 30 e 45 dias após a emergência das plantas (Figura 1).

Na safra da seca a produção verificada para o citado tratamento foi significativamente superior (39%) a

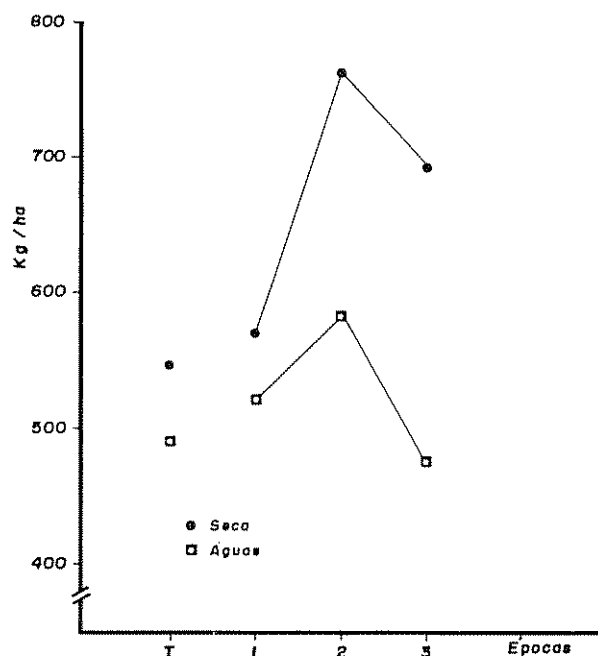


Fig. 1 Efeito de épocas de aplicação foliar de nitrogênio (T... testemunha; 1... 15 e 30; 2... 30 e 45; 3... 45 e 60 dias da emergência) na produção do feijoeiro, cultura das águas e da seca

produção da testemunha e também em relação a produção obtida para a aplicação do adubo aos 15-30 dias após a emergência das plantas.

A média de produção das duas safras vem realçar os dados obtidos na época das secas, onde verifica-se que o tratamento em que foi efetuada a aplicação do adubo foliar aos 30 e 45 dias após a emergência das plantas produziu 29% mais que a testemunha.

Os dados médios do número total de grãos, número de grãos desenvolvidos e o número de grãos não desenvolvidos por planta acham-se na Figura 2.

A análise estatística dos parâmetros mencionados, não revelou diferenças significativas entre os tratamentos, contudo pode-se verificar que a aplicação de adubo foliar aos 30-45 dias após a emergência, revelou uma tendência, não significativa, de apresentar maior número total de grãos por planta e maior número de grãos desenvolvidos por planta e menor número de grãos não desenvolvidos por planta. Tal fato vem explicar em parte a maior produção obtida para este tratamento, anteriormente vista. Esses resultados levam a presumir que a aplicação do adubo foliar na citada época contribuiu para o fornecimento de níveis maiores de nitrogênio, permitindo o desenvolvimento de maior número de grãos que os demais tratamentos. Essa premissa poderia ser feita com maior segurança caso os resultados obtidos fossem estatisticamente diferentes.

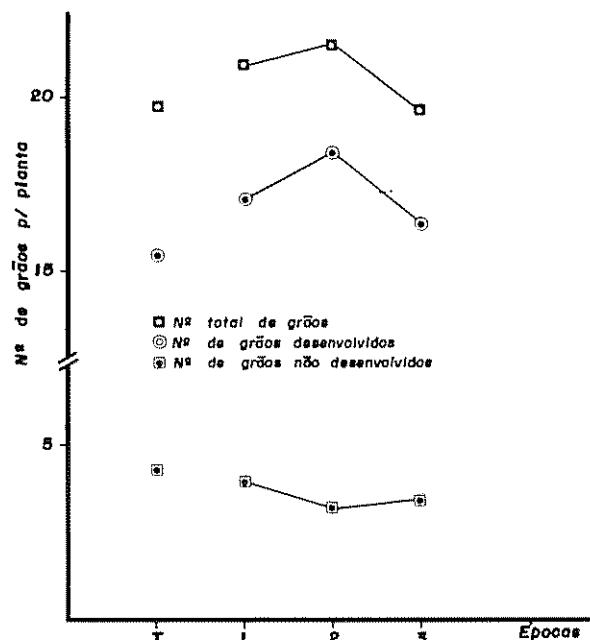


Fig. 2 Efeito de épocas de aplicação foliar de nitrogênio (T... testemunha; 1... 15 e 30; 2... 30 e 45; 3... 45 e 60 dias após emergência) no número de grãos por planta de feijão, cultura da seca.

Os dados médios do número de vagens por planta e do peso de 100 grãos encontram-se na Figura 3. A análise estatística desses dados não revelou diferença significativa para o número de vagens por planta, entretando em todos os tratamentos que se utilizou o adubo foliar resultou em maior número de vagens que da testemunha. Tal fato leva a formular a hipótese de que a aplicação de adubo nitrogenado por via foliar influenciou na retenção e formação das vagens. Contudo, deve-se ressaltar que tais resultados não foram estatisticamente diferentes.

Ainda pela Figura 3, com referência ao peso de 100 grãos pode-se observar que a aplicação de adubo nitrogenado foliar aos 45 e 60 dias após a emergência das plantas resultou em grãos significativamente mais pesados que os da testemunha e da aplicação de foliar aos 15 e 30 dias após a emergência das plantas. Contudo o citado tratamento não diferiu da aplicação aos 30 e 45 dias após a emergência da planta. Pode-se dizer que a medida que se retardou a aplicação dos adubos foliares obteve-se sementes mais pesadas.

Alguns autores têm observado que a manutenção de níveis adequados de nitrogênio nos estágios tardios da planta pode manter por mais tempo a taxa fotossintética das folhas (1, 7, 9). Isso parece ser acontecido na safra da seca do presente trabalho uma vez que ocorreu um aumento no peso de 100 sementes, que pode ter sido uma função da manutenção da síntese de carboidratos por um tempo maior, ou em maior intensidade, nos estágios finais da cultura.

Quadro 1. Médias dos dados de produção, obtidas para tratamentos dos ensaios conduzidos na safra das águas e na safra da seca.

Tratamento	Safra das águas produção: kg/ha	Safra da seca produção: kg/ha	Média das 2 safras	%
TEST.	494 a	546 b	520	100
15-30	527 a	570 b	549	106
30-45	581 a	758 a	669	129
45-60	479 a	680 ab	579	111
D.M.S (5%) (Tukey)	152	144		
C.V. (%)	15.54	12.02		

Nota: Médias seguidas da mesma letra não diferem entre si significativamente.

Quadro 2. Médias dos dados de número de vagens por planta, número de grãos por planta e número de grãos por vagem, obtida no ensaio conduzido na safra da seca.

	Número de vagens por planta	Número de grãos por planta			Peso de 100 grãos	Número de grãos por vagem		
		total	desen- volvidos	não desen- volvidos		total	desen- volvidos	não desen- volvidos
Testemunha	4.08 a	19.66 a	15.44 a	4.22 a	22.89 a	4.90	3.79	1.10
15 e 30	4.44 a	20.92 a	17.02 a	3.90 a	23.08 a	4.75	3.86	0.89
30 e 45	4.42 a	21.48 a	18.30 a	3.18 a	23.45 ab	4.81	4.14	0.68
45 e 60	4.52 a	19.62 a	16.30 a	3.32 a	24.14 b	4.33	3.59	0.80
D.M.S 5% (Tukey)	1.62	7.02	5.53	2.09	0.58			
C.V. (%)	19.78	18.31	17.55	30.02	2.28			

Nota: Médias seguidas da mesma letra não diferem entre si significativamente.

Entretanto, quando se analisa os dados de produção (Figura 1), observa-se que a contribuição do peso de 100 grãos isoladamente não foi suficiente para elevar substancialmente a produção, uma vez que a aplicação de adubo foliar aos 25 e 60 dias após a emergência das plantas que revelou maior peso de 100 grãos, não chegou a provocar aumentos significativos de produção em relação a testemunha e, resultou inclusive em uma produção inferior a obtida no tratamento aos 30 e 45 dias, embora não sendo estatisticamente significativa.

Provavelmente, a soma de benefícios revelados por cada uma dos componentes isoladamente é que foi a responsável pela maior produção verificada pela aplicação do adubo foliar aos 30 e 45 dias após a

emergência das plantas. Assim contribuíram para maior produção, o maior número de grãos desenvolvidos e o maior peso de 100 grãos, sendo que cada componente influiu com uma parcela que na soma resultou em maior produção.

Tais resultados contrariam a hipótese de autores (6), que trabalhando com soja, recomendaram a aplicação de adubos foliares em estágios tardios das plantas, visando o fornecimento de níveis adequados de nutrientes para que houvesse boa translocação dos mesmos para órgãos o que seria suficiente para aumento de produção. Deve-se levar em consideração que o hábito de desenvolvimento diferentes das duas espécies, venham justificar as diferenças com os resultados do citado autor.

Conclusões

- 1) A aplicação de adubo nitrogenado foliar parcelado aos 30 e 45 dias após a emergência das plantas resultou em significativo aumento de produção (39%) em relação a testemunha na safra da seca e em aumento razoável (18%) na safra das águas e em aumento médio de 29% nas duas safras.
- 2) A aplicação do adubo nitrogenado foliar parcelado aos 45 e 60 dias resultou em sementes mais pesadas que a não realização da pulverização foliar.
- 3) Em face dos resultados obtidos no presente trabalho, novos estudos são necessários, principalmente visando verificar o efeito em épocas isoladas, bem como do fornecimento de outras doses mais elevadas.
- 4) A aplicação de adubo nitrogenado foliar não ocasionou efeitos significativos no número de vagens por planta, número total de grãos, número de grãos desenvolvidos e número de grãos não desenvolvidos.

Resumo

Com a finalidade de estudar o efeito da aplicação de adubo nitrogenado via foliar em diferentes estágios de desenvolvimento do feijoeiro, foram conduzidos dois ensaios em condição de campo na Estação Experimental São Manuel, Município de São Manuel, Estado de São Paulo, em um solo classificado como Latossolo Vermelho Escuro-fase arenosa, sendo um na safra das águas e outro na safra da seca, no ano agrícola de 1979/80.

Os 4 tratamentos constaram de uma testemunha e da aplicação por via foliar de 20 l/ha da fórmula 32-0-0 divididos em 2 aplicações: aos 15 e 30; 30 e 45 e 60 dias após a emergência das plantas. Foram utilizados 300 l/ha de água mais espalhante adesivo. Todos os tratamentos receberam adubação fundamental de 80 kg/ha de P_2O_5 e 30 kg/ha de K_2O , mais 30 kg/ha de N, aplicado em cobertura aos 15 dias após a emergência das plantas.

No ensaio conduzido na época das águas não foi verificada resposta significativa quanto à produção de grãos, notando-se apenas uma tendência de aumento da mesma (18%) quando o adubo foi aplicado aos 30 e 45 dias da emergência. Os resultados obtidos na época da seca evidenciaram que a aplicação de adubo foliar no citado estágio resultou em aumento significativo da produção. Verificou-se ainda que

à medida que se retardou a aplicação do adubo foliar, houve aumento no peso de 100 sementes de feijão.

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Reseña de libros

W. G. ROCKWOOD (ed.) Soil related constraints to food production in the Tropics. International Rice Research Institute, Los Baños, Filipinas, 1980. p. 468.

Se reconoce en general que los factores edáficos forman parte de las principales limitantes de la producción agrícola en los trópicos. Existe bastante experiencia a nivel local sobre estos problemas, pero no se ha resumido la información individual con el fin de obtener una visión global de los problemas y las soluciones propuestas.

Este volumen incluye los 21 trabajos y los resúmenes de las discusiones presentadas de un simposio realizado en el Instituto Internacional para Investigación en Arroz (IRRI).

Los primeros trabajos se refieren a aspectos generales del problema; el primero analiza la posible cooperación en suelos y el segundo identifica las limitantes de suelos que afectan el desarrollo agrícola de los trópicos. Este segundo capítulo resalta algunas limitantes pocas veces reconocidas como por ejemplo la limitación a la producción por excesiva temperatura del suelo. También se reconoce claramente la necesidad de establecer las relaciones entre las propiedades de suelos y de los climas en relación a los requerimientos de cultivos específicos.

La segunda subdivisión del simposio se dedicó a la identificación de las limitantes de suelo en relación a su clasificación en el primer trabajo y en diferentes partes del mundo en los cuatro restantes. Las cuatro regiones consideradas son América Tropical, la parte húmeda de África Tropical, la parte sureste de Asia y

los trópicos semiáridos con referencia especial a India. Esta parte, que consiste en casi la tercera parte del volumen, incluye un capítulo muy general y en otros cuatro discute los problemas de las regiones mencionadas en términos de sistemas de agricultura. Estos capítulos tienen bibliografías bastante amplias reflejando la investigación de los grandes centros internacionales en estas regiones. Se da mucha relevancia en estos trabajos a las necesidades de investigación en las diferentes regiones. Considerando el alto nivel de los autores de los trabajos, se debe considerar estas recomendaciones, ya que vienen de personas con la visión general de las necesidades.

La tercera subdivisión estudia individualmente las áreas donde falta información sobre limitantes de suelos. En 14 trabajos, que incluyen más de la mitad del volumen, se estudian problemas como el de la acidez de los suelos bien drenados. Como el tópico es muy amplio es difícil evitar que se omitan apreciables áreas; se descuida la cuantiosa experiencia con boro en Latinoamérica y se exagera los problemas de los propios países en el caso de capítulos como el de elementos menores.

A juicio de este revisor el problema de deficiencia de P pudo tratarse en un poco más que el 3% del volumen. Se estima también que faltan muchas citas importantes que podrían haber contribuido a este capítulo. En forma similar el capítulo sobre K cubre solamente parte de la situación con énfasis en la India. Así se cita solamente un trabajo no publicado sobre Latinoamérica, en comparación con más de una docena de trabajos de la India.

Los capítulos tienen resúmenes útiles y en la bibliografía se cita muchos de los trabajos fundamentales en el campo. Por desgracia el volumen no tiene índice, lo que le hace mucha falta. El inglés de la obra es claro y comprensible. El volumen representa otra adición útil a la literatura agrícola en los trópicos, proveniente del IRRI.

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ADUBAÇÃO FOLIAR DO FEIJOEIRO (*Phaseolus vulgaris* L.): II. EFEITOS
DO NITROGÊNIO COM E SEM COBERTURA NITROGENADA¹ /

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Summary

In two field experiments, the effects of nitrogen fertilizers applied in the soil and/or on the leaves on bean production were studied. The first experiment was conducted during the wet season and the second during the dry season.

Nitrogen (32-0-0) was applied on the leaves on 5 levels (0, 12, 24, 36 and 48 l/ha divided into a applications (15, 30, 45 and 60 days from seedling emergence), in the presence or absence of 30 kg/ha of topdressed nitrogen.

The topdressed nitrogen always increased bean production. In the wet season there was a small production increase due to foliar fertilization in the absence of topdressing, but in the dry season a tendency to quadratic response to foliar fertilization was noticed irrespective of topdressing. Nitrogen application on the soil (20 days from seedling emergence) caused an increase in number of pods per plant whilst the foliar fertilization with nitrogen in the absence of topdressing increased the thousand-grain weight.

Introdução

Trabalhos conduzidos por Bulisani e outros (2, 3) demonstraram que a adubação foliar do feijoeiro pode ser uma prática viável, uma vez que existe a possibilidade da mesma ser efetuada juntamente com os tratamentos fitossanitários. Os autores relatam que ocorreram aumentos de produção de 18 a 35% com a adubação foliar, dependendo do produto utilizado, da dosagem e da presença ou ausência de adubação mineral no solo. Os tratamentos

que receberam adubação no solo produziram mais do que aqueles que receberam adubos apenas pelas folhas, e a resposta ao adubo foliar foi menor quando em presença de adubação no solo.

Em Israel, foram estudados alguns aspectos da adubação foliar do feijoeiro (6), que concluem ter ocorrido aumentos de 10 a 40% na produção quando foram feitas 1 ou 2 aplicações foliares de nutrientes (NPKS), em consequência principalmente do aumento no número de sementes por planta e do peso de 100 sementes. Os autores explicam que estes efeitos foram devidos não propriamente ao fornecimento de nutrientes, mas sim a um retardamento da queda da taxa fotossintética das plantas.

Apesar disso, alguns resultados de pesquisas conduzidas no Brasil (8) tem demonstrado que a adubação foliar do feijoeiro chegou a deprimir a produção.

Em todos os casos relatados, foram utilizadas fórmulas NKP em adubação foliar, o que não permite

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atribuir o efeito, benéfico ou maléfico, a um determinado nutriente. Desta maneira, entre outros aspectos, é importante o estudo dos efeitos de determinados nutrientes, quando aplicados via foliar, na produção do feijoeiro e em seus componentes, para que se possa chegar a uma recomendação segura desta prática.

Dentre os macronutrientes primários, tem-se obtido respostas do feijoeiro à adubação nitrogenada com relativa frequência, o que torna fundamental o estudo do emprego de fertilizantes nitrogenados à cultura em questão (5), principalmente se for levado em consideração que a translocação do nitrogênio das folhas para os grãos apressa a senescência das folhas, como foi observado na cultura da soja (1, 10).

Material e métodos

O trabalho constou de 2 ensaios conduzidos em condições de campo, na Estação Experimental de São Manuel, Município de São Manuel, Estado de São Paulo, sendo uma na safra das águas e o outro na safra das secas do ano agrícola de 1979/80.

O solo utilizado para ambos ensaios foi classificado como Latossol Vermelho Escuro-fase arenosa que revelou as seguintes características químicas $\text{pH} = 5.5$; $\text{M. O.} = 0.67\%$; $\text{PO}_4^{3-} = 0.05$ emg; $\text{K}^+ = 0.17$ emg; $\text{Ca}^{2+} = 1.19$ emg; $\text{MG}^{2+} = 0.34$ emg e $\text{Al}^{+3} = 0.4$ emg por 100 g de TFSA, em amostragem feita antes do 1.º ensaio. Foi efetuada calagem na dose de 2 t/ha.

A cultivar utilizada em ambos ensaios foi a carioca.

O delineamento experimental utilizado para cada ensaio foi um fatorial 2×5 , com quatro repetições, em blocos casualizados. Os tratamentos constaram das aplicações de 0, 12, 24, 35 e 48 litros por hectare de adubo nitrogenado (32-0-0) via foliar, na presença ou não de adubação nitrogenada no solo. As doses foram divididas em quatro aplicações feitas aos 15, 30, 45 e 60 dias após a emergência das plantas. Em cada uma das aplicações foram utilizados 300 litros/ha de água e 1 ml de espalhante adesivo por litro de água. A adubação básica constou da aplicação de 80 e 30 kg/ha de P_2O_5 e K_2O , respectivamente, nas fórmulas de superfosfato simples e cloreto de potássio, aplicadas por ocasião da semeadura, em sulcos situados 5 cm ao lado e abaixo daqueles destinados às sementes.

Nos tratamentos destinados a receber adubo nitrogenado no solo foi aplicado 30 kg/ha de N como sulfato de Amônio, em cobertura, aos 15 dias após a emergência das plantas.

Cada parcela experimental constou de cinco linhas de cinco metros de comprimento, espaçadas entre si de 0.40 m. Foi considerado como área útil da parcela as 3 linhas centrais eliminando-se 0.50 m de cada extremidade.

A semeadura foi efetuada a 5 de outubro de 1979 e 14 de fevereiro de 1980 respectivamente para a época das águas e da seca. Considerou-se final de emergência das plantas 15/10/79 e 21/02/80 respectivamente para o ensaio da safra das águas e o da seca e na mesma ordem a colheita foi efetuada em 10 de janeiro e 13 de maio de 1980. Por ocasião da colheita, na época da seca, foram amostradas 10 plantas por parcela, onde foram determinados: número de vagens, número de grãos, tamanho das vagens e a produção por planta. Foi efetuada análise de variância dos dados obtidos e tentou-se correlacionar entre si os resultados.

Resultados e discussão

Desenvolvimento das plantas:

O desenvolvimento vegetativo das plantas foi normal durante todo o ciclo, ocorrendo leve incidência de bacteriose que não chegou a prejudicar os ensaios. Entretanto após a aplicação do adubo foliar nitrogenado houve uma queimadura que afetou a parte marginal do limbo dos folíolos. Essa ocorrência foi verificada para a aplicação do foliar na dose de 48 l/ha, e em menor intensidade e frequência para a dose de 36 l/ha. Os demais tratamentos não chegaram a mostrar sintomas de fitotoxidez. A injúria ocorreu com menor intensidade e frequência nas aplicações mais tardias, estando presente em maior intensidade e frequência quando o adubo foi aplicado aos 15 dias após a emergência ou seja quando as plantas apresentavam a 3ª folha trifoliada, sendo extremamente leve quando se aplicou o foliar aos 60 dias após a emergência.

Tanto as plantas injuriadas como as não injuriadas desenvolveram-se normalmente, o que leva a concluir que aparentemente as queimaduras verificadas não chegaram a afetar o desenvolvimento das mesmas. Alguns autores tem atribuído diminuições na produção com aplicação de adubos foliares à queima das folhas pelos produtos (6, 7), o que não ocorreu no presente caso.

Produção:

As produções obtidas nos ensaios da época das águas e da época da seca encontram-se na Figura 1

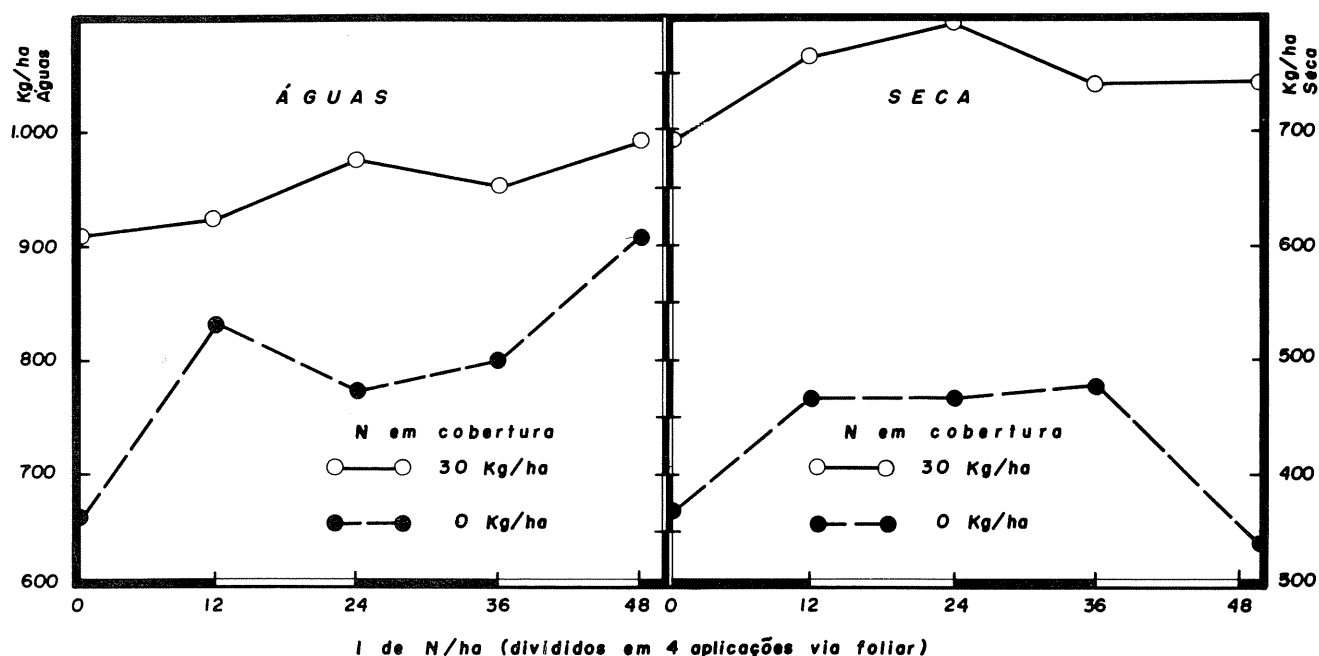


Fig. 1. Produções de feijão na época das águas e da seca, em função da aplicação de N via foliar, na presença e ausência de adubação em cobertura.

Pode-se notar pela referida figura que ocorreram algumas tendências de resposta à adubação foliar, embora não se tenha obtido diferença estatisticamente significativa para os tratamentos foliares. Houve resposta significativa apenas para a aplicação do nitrogênio em cobertura no solo.

É interessante ressaltar na Figura 1 a tendência quadrática dos resultados obtidos na época da seca, demonstrando que a partir de certo ponto apareceu um novo agente limitante da produtividade, provavelmente a água através de sua interação com a nutrição da planta, como pode ser visto na Figura 2, onde estão as precipitações ocorridas no período. Reforçando essa hipótese existem trabalhos demonstrando que a adubação foliar apresenta melhor resultado sob condições hídricas satisfatórias (4).

Outro ponto a ser discutido é o fato de que a aplicação de aproximadamente 20 kg de N/ha via foliar (48 l/ha de 32-0-0), proporcionou uma produção de feijão muito semelhante àquela obtida com 30 kg N/ha em cobertura, no solo, na época das águas, mas não na época da seca (Figura 1). Novamente poderia ser levantado o problema das condições hídricas encontradas em cada caso.

Componentes da produção:

Os resultados obtidos para os diversos componentes da produção do feijoeiro na época da seca podem ser vistos em Quadros 1 e 2.

Na Tabela 1 pode-se notar que a aplicação de nitrogênio em cobertura sempre proporcionou resultados com valores maiores do que sem a aplicação de nitrogênio no solo, com exceção do tamanho das

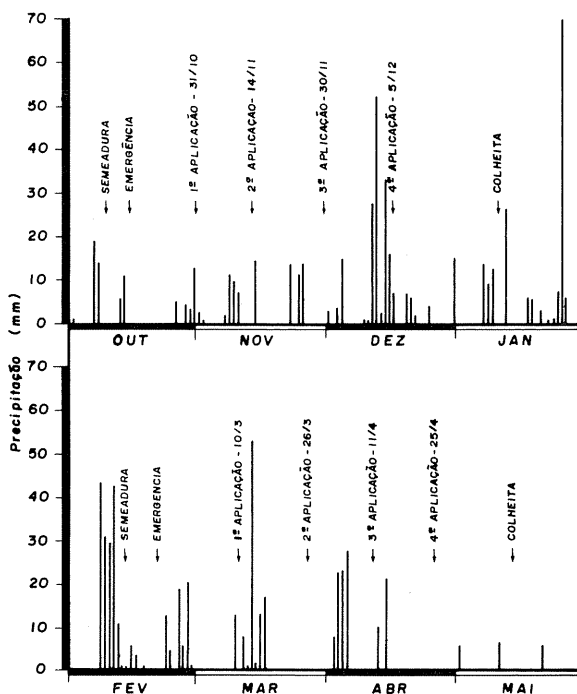


Fig. 2. Precipitações ocorridas durante a condução dos ensaios.

Quadro 1. Efeito da adubação foliar com nitrogênio (32-0-0) em presença e ausência de cobertura nitrogenada no peso de 100 sementes, produção por plantas, n^o de vagens por planta e tamanho das vagens do feijoeiro, safra da seca.

Cobertura	l/ha* 32.0.0	Produção g/planta	Peso de 100 sementes**	Número de vagens por planta	Tamanho das vagens (cm)
0 kg N/ha	0	21.2	21.3 x	2.6	8.9
	12	22.6	22.3 y	2.8	9.6
	24	22.7	22.2 y	2.6	8.4
	36	22.6	22.5 y	2.8	7.8
	48	17.6	21.0 x	2.5	7.8
	Média	21.3 b	21.9 b	2.6 b	8.5 b
30 kg N/ha	0	29.5	22.0	3.7	8.2
	12	30.6	22.5	3.7	8.6
	24	33.9	22.5	3.7	8.3
	36	29.6	22.4	3.5	8.2
	48	29.2	22.5	3.2	9.5
	Média	30.5 a	22.4 a	3.6 a	8.6 a
C.V. %		19.3	1.5	15.9	17.2
f. ad. foliar		n.s.	**	n.s.	n.s.
f. ad. cobertura		**	**	**	n.s.
f. interação		n.s.	**	n.s.	n.s.

* Doses divididas em 4 aplicações aos 15, 30, 45 e 60 dias da emergência das plantas

** a e b demonstram diferenças significativas ($P < 0.5$) para N em cobertura; x e y indicam diferenças entre tratamentos foliares.

Quadro 2. Efeito da adubação foliar com nitrogênio (32-0-0) em presença e ausência de cobertura nitrogenada no número de grãos por planta e número de grãos por vagem do feijoeiro, safra da seca.

Cobertura	l/ha* 32-0-0	n ^o de grãos/planta**			n ^o de grãos/ vagem
		desenvolvidas	não desenvolvidas	total	
0 kg N/ha	0	9.6	2.3	11.9	4.54 x
	12	10.8	3.8	14.6	5.28 y
	24	11.5	2.3	13.8	2.32 y
	36	11.6	2.4	14.0	5.19 y
	48	8.4	2.5	10.9	4.80 x
	Média	10.4 b	2.7 a	13.03 b	4.93 a
30 kg N/ha	0	12.9	2.6	15.6	4.67
	12	13.2	3.0	16.2	4.40
	24	13.9	3.7	17.6	4.57
	36	12.2	4.0	16.2	4.25
	48	12.1	3.1	15.2	4.75
	Média	12.9 a	3.3 a	16.6 a	4.53 a
C.V. %		16.5	32.3	17.8	15.00
f. ad. foliar		n.s.	n.s.	n.s.	n.s.
f. ad. cobertura		**	n.s.	**	n.s.
f. interação		n.s.	n.s.	n.s.	**

* Doses divididas em 4 aplicações aos 15, 30, 45 e 60 dias da emergência das plantas.

** a e b demonstram diferenças significativas ($P < 0.5$) para N em cobertura; x e y indicam diferenças entre tratamentos foliares.

vagens. Apesar da não significância estatística entre as diferenças observadas, na maioria dos casos, os resultados obtidos explicam os resultados obtidos para produção (Figura 1), sendo que no caso da ausência de N em cobertura foram obtidos coeficientes de correlação altamente significativos entre a produção por planta e peso de 100 sementes com a produção de grãos em kg/ha

Na Tabela 2 encontram-se o número de grãos desenvolvidos, não desenvolvidos e total por planta e o número de grãos por vagem. Novamente o teste *f* foi significativo apenas para adubação em cobertura. Cumpre ressaltar que, na ausência de nitrogênio no solo, obteve-se correlação altamente significativa e positiva entre o número de grãos desenvolvidos, número total de grãos por planta e número de grãos por vagem com a produção de feijão, ao passo que a correlação obtida para grãos não desenvolvidos foi negativa. Estes resultados permitem inferir que a tendência observada para a produção foi devida a uma melhor desenvolvimento dos grãos.

Os resultados obtidos no presente trabalho, na época da seca, permitem ainda inferir que a resposta fisiológica do feijoeiro parece ser diferente quando o nitrogênio é aplicado no solo ou via foliar, pois o aumento de produção devido à aplicação de nitrogênio no solo deveu-se principalmente ao aumento no número de vagens por planta, uma vez que não foram obtidas correlações significativas dos outros parâmetros estudados com a produção (Quadros 1 e 2). Por outro lado a adubação foliar, quando na ausência da cobertura, fez aumentar o peso de 100 sementes e número de grãos por vagem (Quadro 2), o que ficou claro pelo estudo das correlações que foram obtidas, sendo que a adubação em cobertura não modificou o número de grãos por vagem significativamente (Quadro 2).

Estes resultados estão de acordo com a teoria segundo a qual a adubação foliar, através da manutenção de um nível adequado de nitrogênio nas folhas, poderia aumentar, ou manter por mais tempo, a taxa fotossintética das mesmas (1, 6, 9). Por outro lado, as mesmas tendências podem não ter sido observadas na presença de nitrogênio aplicado no solo em razão de um nível mais elevado do nutriente, uma vez que uma rápida senescência e queda na taxa fotossintética de folhas foi associada com níveis supra ótimos de nutrientes nas mesmas (6).

Resumo

O presente trabalho foi realizado com a finalidade de estudar as respostas do feijoeiro ao nitrogênio aplicado em cobertura, no solo, e por via foliar. Para

tanto foram instalados dois ensaios em condições de campo, sendo um na época das águas e outro na época da seca, em um solo classificado como Latossol Vermelho Escuro-fase arenosa.

Foram empregadas as doses de 0, 12, 24, 36 e 48 l/ha da fórmula 32-0-0, divididas em quatro aplicações foliares aos 15, 30, 45 e 60 dias da emergência das plantas, na presença ou ausência de adubação nitrogenada em cobertura no solo, na dose de 30 kg/ha. O adubo foi diluído em 300 l/ha de água mais espalhante adesivo. Todas as parcelas, em ambos os ensaios, receberam adubação fundamental de 80 e 30 kg/ha de P_2O_5 e K_2O , respectivamente

Com relação à produção de grãos não foram verificadas diferenças significativas devidas à adubação foliar em ambas as épocas de cultivo. A adubação em cobertura sempre proporcionou maiores produções em relação às parcelas

Com relação à produção de grãos não foram verificadas diferenças significativas devidas à adubação foliar em ambas as épocas de cultivo. A adubação em cobertura sempre proporcionou maiores produções em relação às parcelas que não receberam nitrogênio no solo. Na época das águas a adubação foliar proporcionou uma tendência de aumento nas produções apenas das parcelas sem adubação em cobertura, ao passo que na época da seca foi observada uma tendência quadrática de resposta à adubação foliar, independentemente de adubação em cobertura. Na ausência de nitrogênio em cobertura, a adubação foliar proporcionou maior peso de 100 sementes, maior produção por planta e maior número de vagens por planta, ao passo que a adubação em cobertura fez com que aumentasse o número de vagens por planta.

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PROTEIN CONTENTS OF CASSAVA CULTIVARS AND ITS HYBRID WITH WILD *MANIHOT* SPECIES¹

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Resumen

Se calculó el contenido proteínico de los tubérculos de dieciséis cultivares de yuca y un híbrido de yuca con la variedad silvestre Manihot oligantha. También se analizó la semilla de yuca y de ocho variedades silvestres de Manihot. El porcentaje de proteína en el tubérculo pelado de cultivares de yuca variaba entre el 0.9% y el 1.4%, y era de 4.5% en el tubérculo pelado del híbrido.

La proteína en las semillas de las variedades silvestres de Manihot variaba entre el 27.9% y el 37.3%, comparado con el 26.8% en la semilla de yuca.

Introduction

Cassava, the sixth major staple food in the world, has the poorest protein content in comparison to the five leading food crops: wheat, rice, maize, sorghum and barley. Cassava is the major source of food energy in several tropical countries. As 50-80% of calories consumed by people in these countries are from cassava, their diets are often protein deficient (12).

Recently attention was given to correcting this protein deficiency either by selecting clones with high protein content or by studying the proper utilization of other parts of cassava such as the seeds or leaves. Some attempts were made to select clones with protein rich tubers but no outstanding success was achieved (13).

This paper reports protein content in sixteen cassava clones maintained in our germplasm collection and a hybrid of cassava with the wild species *M. oligantha*.

Materials and methods

Tubers of sixteen cassava clones, 8 months old maintained in the germplasm collection at the Experimental Station, University of Brasilia were analyzed chemically for protein content. Total nitrogen and dry matter basis was determined by the A.O.A.C. procedures (2). Percent protein was obtained by multiplying percent N by the factor 6.25. For every clone two samples were analysed, one from small tuber (50 g) and the second from older tubers (200 g). Tubers were peeled, and protein was estimated in both peel and pulp. Tubers of hybrid between cassava clone Catelo and *M. oligantha* were analysed in the same way. Seed of cassava and wild *Manihot* species maintained in the living collection at University of Brasilia were analysed for protein content by the same procedures.

Results and discussion

The concentration of protein in tubers of cassava clones and tubers of the hybrid is presented in Table

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1. It is seen that protein percent ($N\% \times 6.25 =$ protein percent) ranged from 0.7% to 1.2% in larger tubers (bigger than 200 g) while the range was 0.9% to 1.4% in tubers less than 50 g. In the same clone, protein content of the pulp was higher in small tubers than bigger ones. These numbers agree with those found by Akinrele (1) who reported 0.7% for protein content in peeled tuber on dry matter basis and Chada (3) who found 1.2%. However, these researchers did not pay attention to the effect of tuber size on protein content. Jennings (4) stated that protein in cassava root

tends to be concentrated in the outer zone of the root. This may explain why the small tubers have higher protein content since they have a larger proportion of the outer zone than older and bigger ones. The very little variation of protein content among the sixteen clones collected from all over Brazil shows that selection for this characteristic in cassava clones will not bring any notable improvement. From Table 1 it is seen also that protein is higher in peel than in pulp in all the examined samples and in all clones. This may be explained by the above mentioned statement of Jennings that

Table 1. Protein content of tubers of cassava clones.

Clone	Approximate size	Protein in peel %	Protein in pulp %
CBM 0206	200 g	2.13	0.90
	50 g	2.09	1.22
EAB 348	200 g	1.41	0.85
	50 g	1.69	1.04
BGM 188	200 g	—	—
	50 g	1.68	1.45
CPM 0231	200 g	—	—
	50 g	1.56	1.26
CPM 2002	200 g	—	—
	50 g	2.08	0.99
CPM 0232	200 g	2.00	1.02
	50 g	1.82	1.15
BGM 808	200 g	1.63	0.93
	50 g	—	—
CPM 0225	200 g	1.38	0.89
	50 g	1.25	0.95
BGM 204	200 g	1.24	1.06
	50 g	—	—
CPM 1805	200 g	1.14	0.72
	50 g	1.37	1.00
EAB 1156	200 g	1.58	0.84
	50 g	1.28	1.16
EAB 484	200 g	1.96	1.07
	50 g	—	—
BGM 048	200 g	1.41	0.82
	50 g	1.11	1.17
BGM 020	200 g	1.80	0.98
	50 g	1.53	1.23
CPM 1060	200 g	—	—
	50 g	1.58	1.19
EAB 675	200 g	1.36	0.70
	50 g	1.51	0.93
Hybrid	200 g	6.63	4.56
	50 g	8.06	4.56

protein in cassava roots tends to be concentrated in the outer layers.

The analysis of hybrid tubers showed a notable increase in the hybrid of cassava with *M. oligantha* as it is 4.6%. In an earlier paper, the senior author reported the high protein content in *M. oligantha* (9) as it was 7.1% on dry matter basis. Moreover, crosses of this species with cassava were highly fertile (6, 8). This author also showed low HCN content in the wild species *M. oligantha* (10). This may exclude any possibility that high protein content in the wild species is due to HCN nitrogen (11).

Table 2 shows the results of seed analysis of some wild *Manihot* species maintained in our living collection. The highest protein content is that of *M. brachyandra* followed by *M. alutacea*. *M. brachyandra* is native to Western Pernambuco and Northern Bahia, one of the driest areas in Brazil. The senior author had reported that the seed of wild cassava is eaten by the population of these regions particularly in famine times. Jones (5) reported that cassava seed is eaten in several parts of West and Central Africa. Thus, this discovery of the high protein content in native cassava hybrids may open a new door to better protein balanced food for people of the tropical world.

Table 2. Protein content in wild *Manihot* species seed on dry matter basis.

Species	Protein %
<i>M. glaziovii</i>	30.09
<i>M. caerulescens</i>	27.91
<i>M. brachyandra</i>	35.35
<i>M. pseudoglaziovii</i>	31.15
<i>M. alutacea</i>	37.33
<i>M. zehntneri</i>	28.99
<i>M. dichotoma</i>	29.24
<i>M. reptans</i>	33.25
<i>M. esculenta</i>	26.81

Summary

Protein contents of tubers of sixteen cassava cultivars and a hybrid of cassava with the wild species *Manihot oligantha* were estimated. Seed of cassava and eight wild *Manihot* species were analyzed also. Protein percent in peeled tuber of the cassava cultivars ranged from 0.9% to 1.4% while it was 4.5% in the hybrid peeled tuber. Protein in wild *Manihot* species seed ranged from 27.9% to 37.3% compared with 26.8% in cassava seed.

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EVALUACION DE LOS DAÑOS CAUSADOS EN FRIJOL POR LARVAS Y ADULTOS DE LOS
CRISOMELIDOS *Diabrotica balteata* LECONTE Y *Cerotoma facialis* ERICKSON¹ /

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Summary

Damage to common beans (Phaseolus vulgaris) by feeding of larvae and adults of Diabrotica balteata LeConte and Cerotoma facialis Erickson was evaluated under greenhouse and field conditions. Second and third instar larvae were more damaging than first instars. Stand losses due to larval attack were in some cases as high as 100%. Both species caused significant reductions in leafarea in plants infested when 1, 4 and 7 days old. No significant reduction in leafarea was found when 14-day-old or older plants were infested with 10 larvae/plant.

*Mixed and pure adult populations of these species at the rate of 2 and 4 adults/plant caused yield losses of up to 60% during the initial period of plant growth (8-15 days) and to a lesser extent during flowering. No significant effect was detected by such infestation levels at other stages of the growth cycle. Adult damage to flowers and pods was also considered, however, no yield reduction was detected presumably because the adults prefer feeding on the foliage. When maize and beans were planted in association, adults of *C. facialis* caused more damage to the beans than those of *D. balteata*. The results are discussed in relation to the management of chrysomelid infestations in common beans.*

Introducción

Existen varios géneros de crisomélidos que atacan al frijol común (*Phaseolus vulgaris*) en América Latina. Entre ellos *Diabrotica*, *Systema*, *Epitrix*, *Maecolaspis*, *Colaspis* y *Disonycha* son los más prevalentes (10). Las especies *Diabrotica balteata* LeConte y *Cerotoma facialis* Erickson son importantes en diversas áreas de Centro y Sur América y aunque son polífagas muestran cierta preferencia por los cultivos de frijol (4, 11).

Los crisomélidos *D. balteata* y *C. facialis* pueden causar daño al frijol en tres formas: por el ataque de las larvas a las semillas en germinación, a las plántulas recién germinadas y a las raíces de plantas en

desarrollo (2, 7); por los adultos (3, 10) y como agentes transmisores de enfermedades virales de importancia económica (6)

En estudios preliminares, tanto Gent (7) como Boonekamp (2) encontraron que en condiciones de laboratorio las larvas de *D. balteata* pueden criarse en maíz pero no en raíces de frijol, mientras que las de *C. facialis* pueden criarse en frijol pero no en maíz. Sin embargo, en ocasiones se ha detectado ataques de larvas de *D. balteata* en campos comerciales de frijol, lo cual sugiere que en condiciones naturales puede haber alguna supervivencia de larvas de esta especie cuando se alimentan de plántulas en este cultivo

Los crisomélidos han sido ampliamente estudiados como vectores de enfermedades virales. Gámez (6) publicó una revisión sobre su importancia como transmisores de por lo menos seis enfermedades del frijol en América Latina. Por el contrario, prácticamente no hay literatura sobre el efecto del daño

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mecánico de las larvas y de los adultos cuando se alimentan de la planta de frijol.

En este artículo se resumen los resultados de cinco ensayos de invernadero y ocho ensayos de campo dirigidos a evaluar el efecto del daño causado por las larvas y por los adultos de *D. balteata* y *C. facialis* en el desarrollo y rendimiento de plantas de frijol. Como los trabajos se desarrollaron en una zona en la cual el ataque por virus es muy bajo, no se estudió el efecto de los adultos actuando como vectores de virus.

Materiales y métodos

Los trabajos se desarrollaron en invernaderos y campos experimentales de la estación principal del CIAT localizada en Palmira, Colombia a 1024 metros sobre el nivel del mar. El daño por larvas fue estudiado en condiciones de invernadero (25.4°C; 87% H. R.). Las larvas utilizadas para infestar plantas fueron obtenidas en el laboratorio a 27°C y 80% H. R. La evaluación del efecto del daño mecánico por adultos se realizó en el campo a 24°C y 80% H. R.

La cría y manipulación de larvas de *D. balteata* se hizo siguiendo los métodos descritos por Pitre y Kantack (9) y Chalfant y Mitchell (4), con algunas modificaciones. Las de *C. facialis* fueron criadas de acuerdo con la metodología desarrollada por González y Cardona (8). Para obtener huevos se colectaron adultos de ambas especies en cultivos de frijol mediante el uso de una aspiradora de motor tipo D-Vac. Los adultos se introdujeron en jaulas y allí se separaron las hembras de cada especie, las cuales se introdujeron en grupos de a 10 en cajas Petri de 25 mm de alto por 140 mm de diámetro, provistas de una tapa con abertura de ventilación. Como alimento para los adultos se utilizaron hojas, flores y vainas tiernas de frijol colocadas sobre una capa de gasa húmeda que sirvió como sustrato de oviposición. Los huevos obtenidos por este procedimiento se colocaron en cajas de Petri (200/caja) en un incubador a 27°C y 80% H. R. Se revisaron diariamente y a la eclosión las larvas fueron transferidas con un pincel a semillas de maíz en germinación en el caso de *D. balteata* y de frijol en el caso de *C. facialis*. El alimento fue renovado periódicamente y la cría se programó de tal manera que siempre se tuvieran a disposición todos los instares de ambas especies.

Efecto del daño causado por larvas

En el invernadero se sembraron semillas de la variedad Diacol-Calima en potes plásticos de 10 cm de

altura y 10 cm de diámetro (1 semilla por pote). Se infestó con 10 larvas/planta y las siembras se hicieron en fechas distintas, de tal manera que se lograra infestar simultáneamente plántulas de 0, 1, 4, 7, 14 y 21 días de edad. Cada edad se infestó con cada uno de los tres instares larvales y todos los tratamientos (combinación edad de la planta — instar larval) así como el testigo absoluto (no infestado) se replicaron 10 veces en un diseño de bloques completos al azar.

Para medir los efectos de la infestación con los diversos instares a diferentes edades de las plántulas se estimó el porcentaje de germinación, el porcentaje de plantas supervivientes y el porcentaje de área foliar a los 10, 15 y 20 días después de la infestación. El área foliar se midió por el método de Grid (planímetro) o en ocasiones con un medidor electrónico. Veinte días después de la infestación, las plantas se cortaron a ras del suelo y los potes se cubrieron con una malla para detectar la emergencia de adultos.

Efecto del daño causado por adultos

Para medir el efecto de la infestación con adultos también se usó la variedad Diacol-Calima. Se sembraron parcelas de 4 surcos de 4 m de longitud con distancias de 0.60 m entre surcos y 0.10 m entre plantas. Se utilizaron niveles de infestación de 0, 2, 4 y 6 adultos/planta. Para confinar los adultos se usaron jaulas de malla de 2.5 m de longitud por 1 m de ancho y 0.80 m de altura, las cuales fueron colocadas sobre los dos surcos centrales de cada parcela. Las parcelas testigo también fueron enjauladas con el fin de medir el efecto de jaula en los rendimientos. Las infestaciones se hicieron por períodos de 1 ó 2 semanas comenzando a los 8, 15, 22, 29, 36, 43, 50 ó 57 días después de la siembra del frijol. Al final de cada período de infestación los adultos se colectaron y contaron y las jaulas fueron trasladadas al tratamiento siguiente. En algunos experimentos se infestó con una combinación de *D. balteata* y *C. facialis* en proporción 1:1; en otros se infestó con las especies separadas. Todos los tratamientos se replicaron 3 veces en un diseño de bloques completos al azar.

Para prevenir la infestación de insectos, las plantas no cubiertas con jaulas fueron protegidas con aplicaciones semanales de Malathion a la dosis de 0.9 kg ia/ha. A los 70 días de edad se determinó la altura promedio y el número de vainas/planta en una muestra de 10 plantas tomadas al azar por parcela. A la cosecha se midieron los rendimientos (14% de humedad).

En otro de los experimentos se evaluó el efecto de una infestación con 4 adultos/planta de ambas es-

pecies en la primera etapa de desarrollo (8 a 15 días después de la siembra) sobre los rendimientos de frijol sembrado con maíz. El frijol se sembró a 0.60 m entre surcos y 0.10 m entre plantas; el maíz a 0.50 m entre plantas, 2 plantas/sitio, en el mismo surco del frijol y simultáneamente con éste. El diseño experimental y la metodología general fueron iguales a los de otros experimentos.

Para determinar el daño causado por *C. facialis* a las estructuras reproductivas de la planta (botones, flores y vainas), se diseñó un experimento en el cual se infestó con 4 adultos/planta por un período de una semana empezando a los 29, 36, 43, 50 y 57 días después de la siembra usando la misma metodología de los otros ensayos.

Se utilizaron 6 repeticiones, en tres de las cuales se evaluó el daño a las estructuras reproductivas tomando 5 plantas al azar por parcela a los 2, 4 y 7 días después de haber infestado. Las plantas fueron llevadas al laboratorio para hacer los recuentos detallados del número de estructuras sanas y dañadas. Para mantener el nivel de infestación de 4 adultos/planta, en cada evaluación se retiraron los adultos correspondientes a las 5 plantas muestreadas, es decir 20 adultos/parcela. Las otras 3 repeticiones se dejaron sin disturbar y en ellas se tomaron los datos de rendimiento.

Resultados y discusión

Efecto del daño causado por larvas

Cuando las larvas de *D. balteata* y *C. facialis* se alimentaron de los embriones de las semillas, causaron pérdidas en germinación que variaron entre 36 y 70%. Si la semilla logra germinar, entonces puede ocurrir que las plántulas mueran antes de emerger

pero el efecto depende del instar que esté atacando y de la edad de la planta afectada. Así, las larvas de primer instar no influyeron en la germinación, independientemente de la edad de la plántula o de la especie de insecto y la mayoría de las plantas sobrevivieron al ataque (Cuadro 1). Las larvas de segundo instar sí se alimentaron de las semillas en germinación y causaron mortalidad significativa de plántulas infestadas entre 0 y 4 días después de la siembra. Las de tercer instar de ambas especies causaron la muerte de la mayoría de las plantas, aún de aquellas infestadas 7 días después de la siembra. Todas las plantas infestadas a los 14 y 21 días de edad sobrevivieron.

Cuando la semilla o la plántula logran sobrevivir al ataque, las plantas que emergen muestran daño en las hojas primarias y trifolio parecido al causado por adultos (perforaciones). El follaje puede aparecer deformado y muchas plantas muestran retardo en el crecimiento y una reducción del área foliar que depende del instar que ataca. Las larvas de primer instar de ambas especies causaron menor reducción del área foliar que las de segundo y tercer instar (Cuadro 2). El segundo instar de *D. balteata* sólo causó reducción significativa del área foliar en plántulas de 0 y 1 día de edad mientras que éste instar de *C. facialis* causó el mayor daño en plántulas infestadas a los 0, 1 ó 4 días.

Las larvas de tercer instar fueron las más dañinas, en especial las de *C. facialis* en plantas de hasta de 7 días de edad. Ninguna de las especies afectó las plantas infestadas 14 y 21 días después de la siembra (Cuadro 2).

Al estudiar en mayor detalle el daño por larvas, se confirmó que las de segundo y tercer instar de ambas especies fueron más dañinas que las de primero y que *C. facialis* fue más dañino que *D. balteata* (Cuadro 3). Las observaciones detalladas permitieron de-

Cuadro 1. Porcentaje de supervivencia de plantas de frijol (variedad Diacol-Calima) infestadas a diferentes edades con 10 larvas/planta de *Diabrotica balteata* o *Cerotoma facialis* en condiciones de invernadero.

Instar	Especies	Edad de la planta al infestar (Días después de la siembra)						Promedio
		0	1	4	7	14	21	
Primero	<i>Diabrotica</i>	100	100	100	100	100	100	100.0
	<i>Cerotoma</i>	100	100	90	100	100	100	98.3
Segundo	<i>Diabrotica</i>	10	60	100	100	100	100	78.3
	<i>Cerotoma</i>	0	0	30	100	100	100	55.0
Tercero	<i>Diabrotica</i>	20	10	0	30	100	100	43.3
	<i>Cerotoma</i>	10	10	10	40	100	100	45.0
TESTIGO		100	100	100	100	100	100	100.0

Cuadro 2. Porcentaje de área foliar con respecto al testigo de plantas de frijol (Variedad Diacol-Calima) 10 días después de haber sido infestadas a diferentes edades con 10 larvas/planta de *Diabrotica balteata* o *Cerotoma facialis* en condiciones de invernadero.

Instar	Especie	Edad de las plantas al infestar (Días después de la siembra)						Promedio
		0	1	4	7	14	21	
Primero	<i>D. balteata</i>	73.2	54.8	91.8	95.5	85.3	106.7	84.5
	<i>C. facialis</i>	105.8	81.2	87.0	88.4	87.6	83.5	88.9
Segundo	<i>D. balteata</i>	72.5	21.1	84.4	72.6	94.9	94.0	73.3
	<i>C. facialis</i>	73.9	2.8	26.1	76.1	95.9	87.6	60.4
Tercero	<i>D. balteata</i>	74.3	20.6	33.0	50.1	112.9	82.6	62.2
	<i>C. facialis</i>	23.3	8.4	10.3	32.3	107.8	92.3	45.7

Cuadro 3. Porcentaje de área foliar con respecto al testigo de plantas de la variedad Diacol-Calima 10 días después de haber sido infestadas a diferentes edades con 10 larvas/planta de *Diabrotica balteata* o *Cerotoma facialis* en condiciones de invernadero.

Instar	Especie	Edad de las plantas al infestar (Días después de la siembra)			
		0	1	4	7
Primero	<i>Diabrotica</i>	91.3 a*	70.5 a	111.4 a	98.2 a
	<i>Cerotoma</i>	74.9 a	68.3 a	89.3 b	99.6 a
Segundo	<i>Diabrotica</i>	3.2 c	5.9 b	79.4 b	96.3 a
	<i>Cerotoma</i>	0 d	0 c	24.2 c	68.8 ab
Tercero	<i>Diabrotica</i>	8.4 b	3.8 b	0 e	18.8 c
	<i>Cerotoma</i>	12.5 b	4.7 b	3.3 d	19.4 c

* En sentido vertical, las cifras seguidas por la misma letra no son significativamente diferentes al nivel de 5% (Duncan).

notar daño no sólo en las hojas primarias sino también a los cotiledones, el hipocótilo y los puntos de crecimiento de las plantas en desarrollo. En algunos casos las larvas abren galerías en los tallos desde el sistema radicular hasta el primer nudo. En este caso las plantas mueren.

La supervivencia de larvas en estos ensayos fue relativamente baja. La emergencia de adultos de *C. facialis* (promedio: 19.9%) fue mayor que la de *D. balteata* (promedio 9.1%). Esto confirmó los resultados de Boonekamp (2) quien concluyó que *C. facialis* es una especie mejor adaptada al frijol que *D. balteata*.

Efecto del daño causado por adultos

La magnitud del daño mecánico causado por adultos de *D. balteata* y *C. facialis* dependió del número de adultos por planta y de la edad del cultivo; los resultados fueron consistentes a través de diferentes experimentos. En general, la infestación no afectó la altura de las plantas pero sí tuvo un efecto significativo en los rendimientos. En el Cuadro 4 se ilustra una respuesta típica de plantas de la variedad Diacol-

Calima al ataque de crisomélidos. Las parcelas infestadas con 2 y 4 adultos/planta durante el periodo inicial de crecimiento (8-15 días de edad) rindieron 54 y 60% menos que el testigo, respectivamente. También se encontró un efecto significativo cuando se infestó con 4 adultos/planta durante la época de floración (29-36 días). En este caso la reducción en rendimiento fue del 37.8%. No hubo efecto de las infestaciones en las otras épocas del cultivo.

Se obtuvieron resultados similares cuando se infestó con *D. balteata* o *C. facialis* por separado (Cuadro 5); de nuevo, las épocas críticas fueron la primera semana de edad del cultivo y la floración. Cuando se probó con *C. facialis* en forma individual, se encontró que esta especie fue más dañina que *D. balteata* (Cuadro 5). Con ambas especies, el daño fue más severo cuando se infestó con 4 adultos/planta por un periodo de dos semanas.

En una asociación maíz-frijol infestada con 4 adultos/planta en la primera semana del cultivo, también *C. facialis* fue más dañino que *D. balteata* (Figura 1). Los porcentajes de reducción en rendimiento causados por *C. facialis* en el monocultivo de frijol y en la

Cuadro 4. Efecto de la infestación en diferentes épocas del cultivo con 4 niveles de población de adultos de crisomélidos¹ en los rendimientos (gramos/planta) de la variedad Diacol-Calima.

Nivel de infestación (adultos/planta)	Períodos de infestación (días después de la siembra)							
	8-15	15-22	22-29	29-36	36-43	43-50	50-57	57-64
0	6.1 a*	6.6 a	7.1 a	5.1 ab	5.2 a	3.7 a	3.9 a	4.7 a
1	4.0 ab	5.3 a	6.7 a	5.7 a	4.4 a	4.4 a	4.5 a	5.0 a
2	2.8 b	5.6 a	4.9 a	4.3 ab	3.6 a	4.0 a	3.3 a	5.1 a
4	2.4 b	5.3 a	5.9 a	3.7 b	3.0 a	4.1 a	4.3 a	5.3 a

1 *D. balteata* y *C. facialis* en proporción 1:1.

* En sentido vertical, las cifras seguidas por la misma letra no son significativamente diferentes al nivel del 5% (Duncan).

Cuadro 5. Efecto de la infestación en diferentes épocas del cultivo con 2 niveles de población de adultos de crisomélidos en los rendimientos (gramos/planta) de la variedad Diacol-Calima.

Especie	Nivel de infestación (adultos/planta)	Períodos de infestación (días después de la siembra)							
		Infestación por 7 días					Infestación por 14 días		
		8-15	15-22	22-29	29-36	36-43	8-22	22-36	36-50
<i>D. balteata</i>	0	9.5 a*	8.9 a	9.6 a	8.3 a	8.4 a	9.4 a	10.2 a	7.1 a
	4	5.2 b	7.5 a	8.3 a	8.9 a	6.4 b	5.0 b	7.7 b	5.7 b
<i>C. facialis</i>	0	6.0 x*	9.2 x	5.8 x	4.8 x	4.8 x	7.5 x	2.1 x	2.4 x
	4	1.1 y	0.1 y	0.8 y	1.9 y	5.9 y	0 y	0.1 y	3.6 y

* En sentido vertical, las cifras seguidas por la misma letra no son significativamente diferentes al nivel del 5% (Duncan). Cada especie probada y analizada por separado.

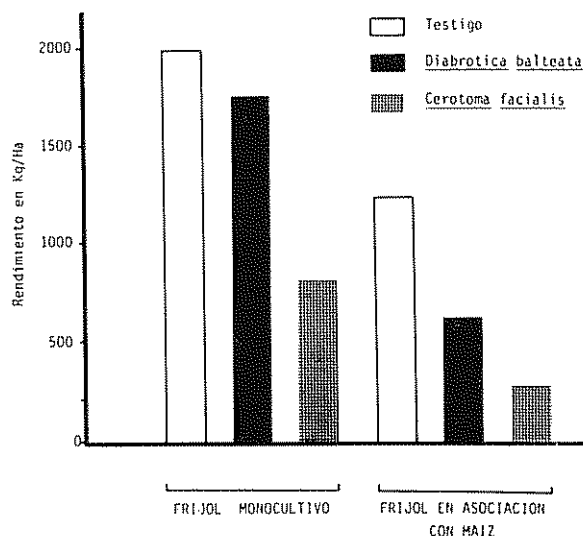


Fig 1. Rendimientos de frijol Diacol-Calima sembrado en monocultivo y asociación e infestado con 0 (testigo) y 4 adultos/planta de *Diabrotica balteata* y *Cerotoma facialis* entre los 8 y los 15 días de edad del cultivo.

asociación con maíz fueron de 59.3 y 76.6%, respectivamente. *D. balteata* dio lugar a pérdidas del 13.1% en monocultivo y del 48.0% en la asociación. Al nivel de infestación utilizado en estos experimentos, ambas especies fueron más importantes en la asociación; sin embargo, es de anotar que en esta modalidad de siembra no se han observado poblaciones tan altas de crisomélidos y por el contrario, las poblaciones tienden a ser menores que en monocultivos de frijol (3).

Cuando se estudió en detalle el daño a las estructuras reproductivas (Cuadro 6), se encontró que los adultos de *C. facialis*, la especie mejor adaptada al frijol, atacan mayor proporción de vainas formadas (períodos 43-50 y 50-57 días después de siembra) que flores (períodos 29-36 y 36-43 días). El daño en vainas no es muy importante porque los adultos de limitan en general a raspar superficialmente, por lo cual las vainas se recuperan, las lesiones cicatrizan y los granos no se ven afectados. Esto podría explicar por qué a pesar de haberse encontrado hasta 20% de vainas con daño superficial, sin embargo

Cuadro 6. Porcentajes de estructuras con daño y rendimientos (gramos/planta) de la variedad Diacol-Calima después de la infestación con 4 adultos/planta de *Cerotoma facialis* en diferentes épocas del cultivo.

Variable	Período de infestación (días después de siembra)			
	29-36	36-43	43-50	50-57
Flores con daño (%)	4.8	4.9	20.6	—
Vainas con daño (%)	—	1.2	18.2	20.7
Rendimiento con 4 adultos por planta	5.3 b*	6.1 a	6.0 a	6.4 a
Rendimiento del testigo sin infestar	6.4 a	5.2 a	6.0 a	5.1 a

* En sentido vertical, los promedios de rendimiento seguidos por la misma letra no son significativamente diferentes al nivel del 5% (Duncan).

los rendimientos en los periodos 36-43, 43-50 y 50-57 días después de la siembra no fueron significativamente diferentes a los del testigo. Por el contrario, al infestar entre los 29 y los 36 días se detectó efecto significativo en el rendimiento aunque el daño en flores fue inferior al 5%. Esto no sólo confirma resultados anteriores sobre la importancia del daño en floración, sino que sugiere que el efecto puede deberse más a la defoliación que al consumo de estructuras reproductivas. La importancia de la defoliación en frijol fue estudiada por Appadmai y Rajakarma (1) y por Gálvez *et al* (5) quienes concluyeron que las mayores pérdidas ocurren cuando la defoliación coincide con la floración y formación de las primeras vainas (30-43 días después de siembra).

Bajo las condiciones de invernadero y los altos niveles de infestación utilizados en estos experimentos, las larvas de crisomélidos causaron daño significativo a las semillas y plántulas de frijol. Sin embargo, sólo en raras ocasiones se han detectado ataques de importancia económica en algunas zonas de América Latina y la mayoría de los agricultores nunca usan insecticidas del suelo para prevenir el daño por larvas. Esto podría deberse a que las poblaciones generalmente no llegan a los niveles críticos, no son detectadas, o los daños son confundidos con problemas relacionados con mala calidad de la semilla. Usualmente las infestaciones son menores de una larva/planta. Por otra parte, la mayoría de los agricultores se preocupan por el daño visual causado por los adultos y aplican insecticidas, una práctica que de acuerdo con nuestros resultados rara vez se justifica. Las poblaciones promedio en cultivos comerciales (0.6-1.0 adultos/planta) están por debajo de los niveles críticos de 2 a 4 adultos/planta aquí establecidos. Además los muestreos de campo han indicado que las mayores poblaciones ocurren hacia el final del periodo vegetativo, una época en la cual se ha demostrado que no se causan pérdidas económicas.

Por estas razones, en áreas en las cuales los crisomélidos no están transmitiendo virus, el control químico de estas plagas debería ser recomendado sólo en casos especiales y limitado a la primera semana del cultivo o a la época de floración.

Resumen

Se evaluó bajo condiciones de invernadero y campo el daño causado al frijol común (*Phaseolus vulgaris*) por larvas y adultos de *Diabrotica balteata* LeConte y *Cerotoma facialis* Erickson. Los instares larvales segundo y tercero causaron más daño que los primeros instares. La pérdida de poblaciones de plántulas por larvas fue de 100%. Ambas especies causaron significantes reducciones en el área foliar en plántulas de 1.4 y 7 días de edad. No hubo reducción significativa en el área foliar cuando se infestaron plantas de 10 días o más con 10 larvas por planta. Poblaciones de adultos puros y mezclados de estas especies en una proporción de 2 a 4 adultos por planta causaron pérdidas en rendimiento hasta un 60% durante el periodo inicial de crecimiento (8-15 días) y en una menor proporción durante la floración. No se registraron efectos significativos a estos niveles de infestación ni en otros periodos de crecimiento de la planta de frijol. El daño de los adultos a flores y vainas también fue considerado; sin embargo, no se registró reducción en rendimiento probablemente porque los adultos prefirieron alimentarse del frijol. Cuando frijoles y maíz se sembraron en asociación, los adultos de *C. facialis* causaron más daño a los frijoles que los de *D. balteata*. Los resultados se discutieron en relación al manejo de infestaciones de crisomélidos en frijol común.

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Unidades básicas del SI*

Especie	Unidad	Símbolo	Especie	Unidad	Símbolo
Longitud	metro	m			
Masa	kilogramo	kg	Temperatura	kelvin	K
Tiempo	segundo	s	Intensidad luminosa	candela	cd
Corriente eléctrica	ampere	A	Cantidad de sustancia	mole	mol

Unidades suplementarias

Angulo plano	radián	rad	Angulo sólido	steradián	sr
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Unidades derivadas que tienen nombres y símbolos aprobados por el SI:

Especie	Unidad	Símbolo	Fórmula	Especie	Unidad	Símbolo	Fórmula
Frecuencia	hertz	Hz	1/S	Conductancia eléctrica	siemens	S	A/V
Fuerza	newton	N	Kg m/s ²	Flujo magnético	weber	Wb	V s
Presión	pascal	Pa	N/m ²	Densidad de flujo	tesla	T	Wb/m ²
Trabajo	joule	J	N m	Inductancia	henri	H	Wb/A
Potencia	watt	W	J/s	Flujo luminoso	lumen	lm	cd/sr
Cantidad electricidad	coulomb	C	A s	Iluminación	lux	lx	lm/m ²
Potencial eléctrico	volt	V	W/A	Radiactividad	bequerel	Bq	1/s
Capacidad eléctrica	farad	F	C/V	Dosis absorbida	gray	Gy	J/kg
Resistencia eléctrica	ohm	Ω	V/A				

Definiciones de las unidades básicas del SI*

El metro. Es la longitud equivalente a 1 650 763 73 longitudes de onda en el vacío de la radiación electromagnética emitida por el átomo de criptón 86, correspondiente a la transición entre $2p_{10}$ y $5d_5$ (su símbolo es m).

El kilogramo. Corresponde a la masa del kilogramo prototipo adoptado internacionalmente (su símbolo es kg).

El segundo. Es la duración de 9 192 631 770 períodos de la radiación electromagnética correspondiente a la transición entre dos niveles hiperfinos del estado base en el átomo de cesio 133 (su símbolo es s).

El ampere. Es la corriente eléctrica constante en dos conductores paralelos de longitud infinita y de sección transversal insignificante que, colocados a un metro de distancia entre sí en el vacío, se atraen con fuerza igual a 2×10^{-7} newton por metro de longitud (su símbolo es A).

Continúa en la página 446

INFLUÊNCIA DE NUTRIMENTOS ORGÂNICOS NA PERSISTÊNCIA DO CARBARIL EM SOLOS¹

RODOBIKO HIRATA*, LUIZ CARLOS LUCHINI**, TEREZINHA BONANHO MESQUITA**, ELZA FLORES RÜEGG***

Resumen

La degradación del carbaril, evaluada por la técnica de centelleo líquido fue estudiada en dos tipos de suelos tratados con diferentes fuentes de nutrientes orgánicos.

Los resultados obtenidos muestran que después de 2 semanas de tratamiento, la degradación del carbaril bajo la influencia de las fuentes de carbono, a excepción de celulosa pura y papel de filtro fue mayor en el Latosol Rojo-Amarillo. En el Latosol todo el insecticida se degradó, no siendo este el caso del suelo Gley Húmico.

La incubación previa de los suelos con hojas de vegetales y papel de filtro durante 2 semanas, alteró de manera similar la persistencia de carbaril en los dos suelos.

Introdução

Matéria orgânica tem grande influência na fertilidade dos solos, pois além de melhorar suas propriedades físicas e químicas, mantém ativa a comunidade microbiológica. A incorporação de resíduos de plantas ao solo é um dos recursos para repor o conteúdo orgânico consumido com o crescente uso agrícola (6). Essa modificação em nutrientes orgânicos influencia a flora microbiana e conseqüentemente, o destino dos resíduos dos pesticidas no solo.

Recentemente, investigamos a influência da adição de fertilizantes (NPK) e de uma fonte de carbono (sacarose) na persistência do carbaril em dois solos com diferentes conteúdos de matéria orgânica (4). No presente trabalho, os mesmos solos foram tratados com outras fontes de carbono, algumas menos acessíveis à metabolização do que a sacarose, com a finalidade de verificar se a diminuição na persistência do carbaril com a adição de nutrientes orgânicos aos solos, já observada, é um fenômeno geral.

Material

Solos

Dois tipos de solos (4) coletados nos arredores do Instituto Biológico foram utilizados nas experiências. Um deles, o solo Gleí Húmico, é caracterizado pelo maior teor de matéria orgânica (4.33%) e o outro, o Latossolo Vermelho-Amarelo, pela pequena quantidade de matéria orgânica (0.36%). Antes dos experimentos, os dois solos, secos ao ar, foram passados numa peneira com malha de 2 mm.

Pesticida

¹⁴C-carbaril (1-Naftil N-metilcarbamato), marcado no grupo carbonílico, foi adquirido no Centro de

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Radioquímica, Amersham, Inglaterra, em solução benzênica, com pureza radioquímica de 99% e atividade específica de 57 mCi/mmol. Para os ensaios, preparou-se uma solução aquosa de carbaril grau técnico (obtido no Instituto Biológico) contendo 2 µg/ml de carbaril "frio" à qual se adicionou carbaril radioativo resultando solução com cerca de 70.000 dpm/ml.

Fontes de carbono

Foram utilizadas as seguintes fontes de carbono:

- a) sacarose pura (Hoechst)
- b) leite (fonte protéica com 1 e 14% de teor em gordura)
- c) óleo vegetal (de soja)
- d) celulose microcristalina pura (Merck)
- e) papel de filtro (Klabin)
- f) folha vegetal (de soja)

Métodos

Tratamento dos solos

Amostras de 10 g de solo foram pesadas em frascos esmerilhados de 250 ml. A massa (0.1 g) de cada fonte de carbono foi adicionada aos solos de acordo com os procedimentos abaixo:

- a) **sacarose:** 2.7 ml de uma solução aquosa de sacarose de concentração 37 mg/ml.
- b) **leite com 1 e 14% de teor de gordura:** 2.7 ml de uma solução de leite de concentração 37 mg/ml.
- c) **óleo de soja:** 0.1 g de óleo mais 2.7 ml de água.
- d) **celulose pura:** 0.1 g de celulose mais 2.7 ml de água.
- e) **papel de filtro:** foram feitos dois tratamentos. Em um deles 0.1 g de papel foi coberto com solo, adicionou-se em seguida 2.7 ml de água e deixou-se a mistura incubar por duas semanas. No outro, à 0.1 g de papel coberto com solo foi adicionado 2.7 ml de água.
- f) **folha de soja "seca":** 0.1 g de folha, desidratada por um período de 24 horas em dessecador, mais 2.7 ml de água.
- g) **folha de soja "verde":** foram feitos dois tratamentos como os descritos para o papel de filtro. Tomou-se 0.4 g de folhas "verde", recentemente colhidas, correspondentes a 0.1 g de folha "seca"

e mais 2.7 ml de água que foram adicionados às amostras dos solos. Um dos grupos de amostras foi incubado durante duas semanas.

Após esses tratamentos adicionou-se 1.0 ml de solução de carbaril radioativo. Este procedimento elevou a umidade nas amostras de solo Glei Húmico para 2/3 de sua capacidade de campo, mas foi necessário acrescentar 0.8 ml de água ao Latossolo Vermelho-Amarelo para atingir aquele conteúdo de umidade. Amostras em duplicata de cada tratamento foram analisadas em diversos intervalos.

Procedimento analítico

Extração

Cada 10 g de solo foi extraído por agitação com 20 ml de diclorometano durante 2 horas. Deixou-se a mistura em repouso e separou-se o solvente por decantação. O solo remanescente foi extraído mais duas vezes com porções de 20 ml de diclorometano, os extratos combinados e o volume ajustado para 50 ml em frasco volumétrico. Um volume de 5.0 ml de extrato foi evaporado até a secura em frasco de cintilação, adicionando-se em seguida 10 ml de solução cintiladora composta de 200 mg POPOP, 4 g PPO, 500 ml Triton-X e 500 ml de xileno ou tolueno por litro de solução.

Cromatografia em camada delgada

Secou-se uma alíquota de 5.0 ml de extrato de solo com sulfato de sódio anidro antes de concentrar a 1.0 ml para análise por cromatografia em camada delgada de sílica gel com indicador fluorescente, usando-se hexano-acetona 4:1 como solvente. As placas foram divididas em seções, e a sílica raspada para frascos de contagem de cintilação líquida. A maior parte da radioatividade apresentava o mesmo R_f (0.38) das amostras de referência do carbaril "frio", localizadas pelo "quenching" de fluorescência sob luz ultravioleta.

Combustão úmida do solo

Após a extração, o radiocarbono remanescente no solo foi determinado por combustão úmida a $^{14}\text{CO}_2$ usando-se o procedimento de Smith *et al.* (5). O $^{14}\text{CO}_2$ resultante de amostras de 2.0 g de Latossolo Vermelho-Amarelo e 1.0 g de solo Glei Húmico, foi absorvido em 2.0 ml de monoetanolamina dissolvido em 20 ml de coquetel de cintilação contendo 5.5 g/l PPO em tolueno (2 partes por volume) e éter etilenoglicol monometílico (1 parte).

Determinação da radioatividade

As medidas radiométricas foram realizadas em espectrômetro de cintilação líquida da Nuclear Chicago, modelo Mark 1. As amostras foram contadas durante 10 minutos, e os resultados corrigidos em função da radiação de fundo e do "quenched", que foi estimado usando-se o método de razão de canal com fonte externa.

Resultados e discussão

As taxas de degradação do carbaril em dois solos modificados pela adição de várias fontes de nutrientes orgânicos, são mostradas nas Figuras 1 e 2 para os solos Glei Húmico (GH) e Latossolo Vermelho-Amarelo (LVA), respectivamente. Encontram-se no Quadro 1 as porcentagens do ^{14}C recuperado dos solos, duas semanas após a aplicação do ^{14}C -carbaril; ensaios por cromatografia em camada delgada do material marcado com ^{14}C , extraído com diclorometano, mostrou que pelo menos 95% da radioatividade presente nestes extratos tinha o mesmo R_f que o carbaril padrão.

A degradação do carbaril foi relativamente menor no solo LVA não modificado, isto é, 53% comparado aos 46% remanescente no solo GH após sete semanas. Esses valores são semelhantes àqueles observados por Carazo *et al.* (2) trabalhando com os mesmos solos.

Adição de fontes de nutrientes, leite, sacarose e óleo de soja, induziu um aumento moderado na velocidade de degradação do carbaril no solo GH, sendo este mais evidente com leite contendo 14% de gordura, observando-se 25% de carbaril remanescente após sete semanas (Figura 1B). Entretanto, dos tratamentos com celulose pura, papel de filtro e folhas de soja, somente aquele com folhas de soja "verde" propiciou um pequeno aumento na taxa de degradação, os outros tratamentos não tendo praticamente nenhum efeito (Figura 1A).

Todas as fontes de nutrientes adicionadas aumentaram acentuadamente a velocidade de degradação do carbaril no solo LVA, as menos acessíveis sendo celulose pura e o papel de filtro (com ou sem incubação prévia). Nos outros tratamentos onde os nutrientes orgânicos foram mais efetivos, menos que 10% de carbaril restou após quatro semanas (Figuras 2A e 2B).

A velocidade de degradação do carbaril aumentou no solo LVA muito mais que no solo GH pela adição de substâncias nutritivas, a despeito da degradação ser levemente menor no solo LVA não modificado que

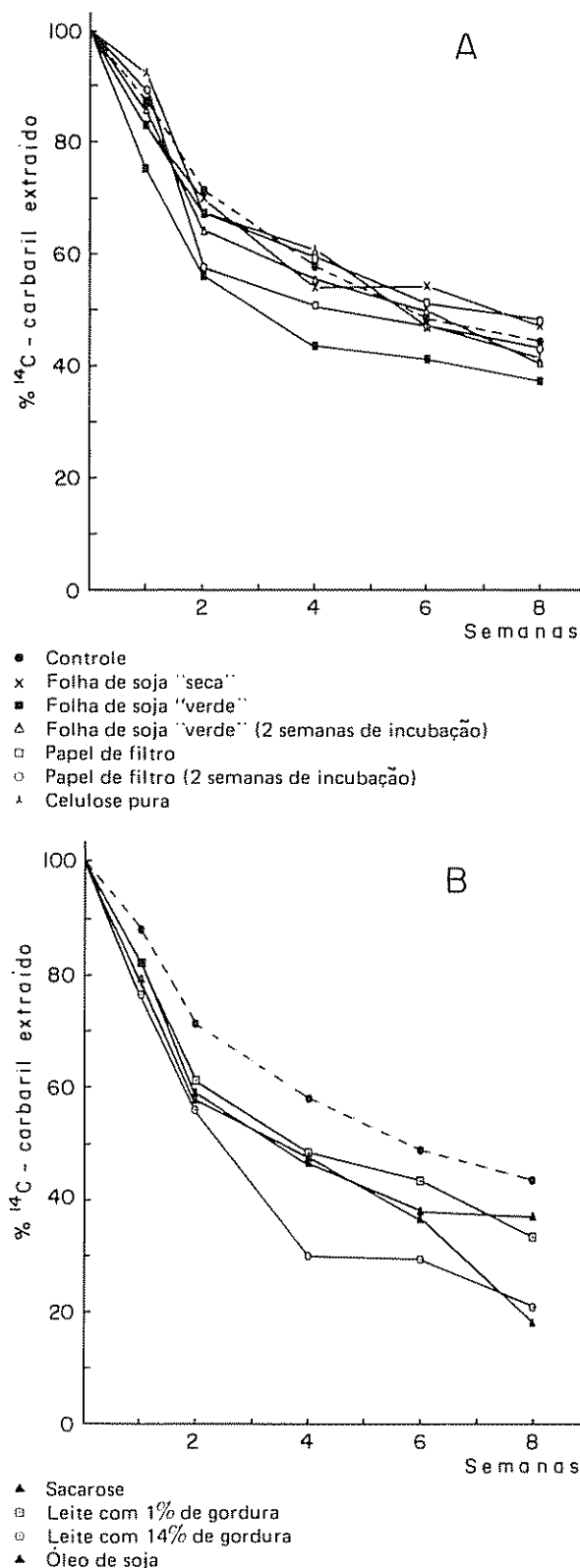


Fig 1. Porcentagem de carbaril extraído do solo Glei Húmico após adição de diferentes fontes de carbono (A = fontes com celulose; B = fontes sem celulose)

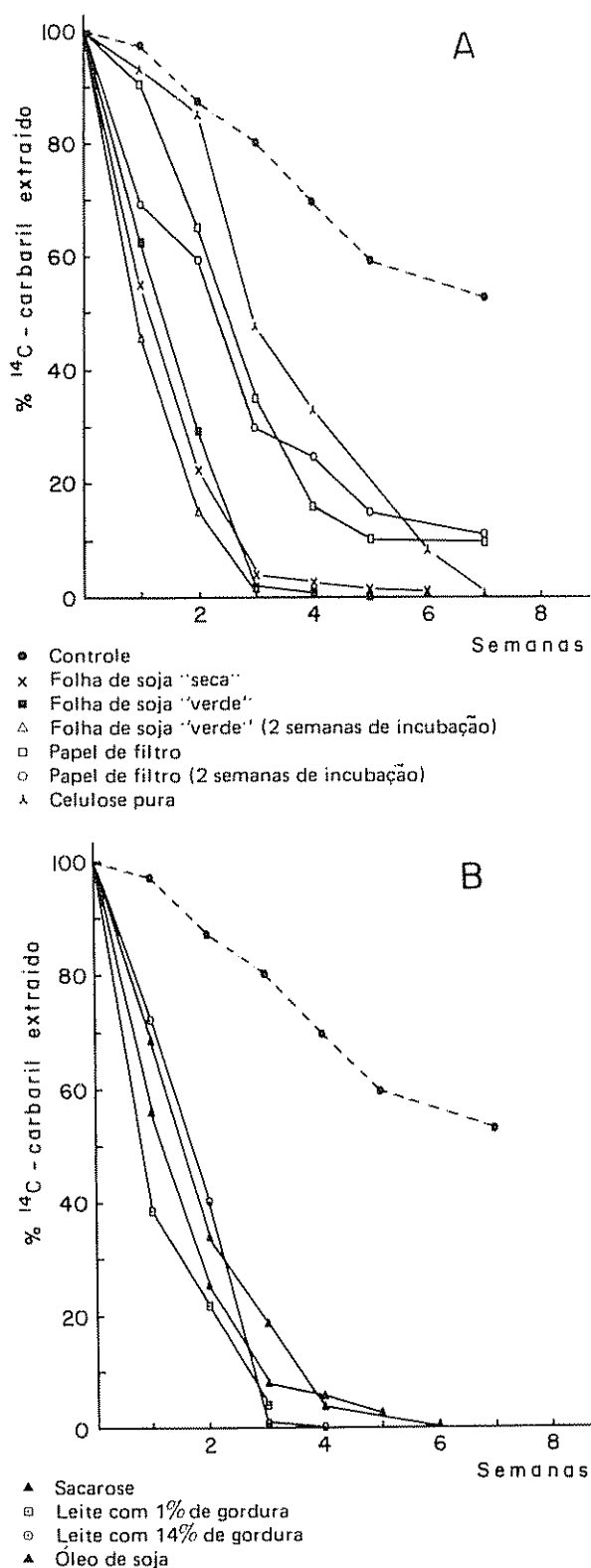


Fig. 2 Porcentagem de carbaril extraído do Latossolo Vermelho-Amarelo após adição de diferentes fontes de carbono (A = fontes com celulose, B = fontes sem celulose).

no solo GH. Hirata *et al.* (4) também obtiveram efeitos semelhantes no comportamento do carbaril nesses mesmos solos tratados com sacarose.

Como no trabalho citado (4), não há razão para supor que microorganismos naqueles dois solos responderiam diferentemente às fontes de nutrientes adicionadas: a provável razão para a falta de resposta na degradação do carbaril no solo GH aos nutrientes adicionados é o maior conteúdo de matéria orgânica deste solo que, causando adsorção mais forte do carbaril (coeficiente de absorção 128 comparado a 0.65 do LVA (3), reduziria a disponibilidade do inseticida para a degradação.

Das fontes de nutrientes que aumentam a degradação do carbaril as mais eficientes são aquelas mais facilmente disponíveis para os microorganismos. Então, por exemplo, enquanto celulose pura e papel de filtro são metabolizados muito lentamente, folhas de soja contêm compostos como a hemicelulose e amido que são mais facilmente atacadas por microorganismos do que a própria celulose.

Adicionalmente, as folhas, o leite e óleo de soja contêm alguns compostos nitrogenados; podemos então assumir que aquelas fontes de nutrientes mais facilmente disponíveis sustentam uma população de microorganismos mais elevada nos solos, resultando numa degradação mais rápida do carbaril.

Quantidades de ^{14}C não extraível, presente nos solos após duas semanas da adição do ^{14}C -carbaril, medidas após combustão a $^{14}\text{CO}_2$ foram, geralmente, maiores no solo GH que no LVA, a média total de todos tratamentos sendo 15.4 e 6.7% do aplicado, respectivamente (Quadro 1).

Esta diferença pode ser devida ao nível mais alto da matéria orgânica no solo GH, os produtos de degradação do carbaril reagindo com a matéria orgânica para formar resíduos não extraíveis.

Assumiu-se que o radiocarbono não encontrado por extração ou combustão tenha evoluído como $^{14}\text{CO}_2$, isto ocorrendo especialmente com o solo LVA modificado.

Neste estudo verificamos que diferentes fontes de carbono, mesmo aquelas menos acessíveis à metabolização como a celulose, podem influir na fertilidade do solo e na persistência do carbaril, modificando a flora e a fauna em solos com baixos teores de matéria orgânica.

Quadro 1. Efeitos da adição de diferentes fontes de carbono na distribuição do radiocarbono em dois solos, duas semanas após aplicação de ^{14}C -carbaril (70.000 dpm/ml).

Adição ao solo	^{14}C recuperado com % do aplicado					
	Glei Húmico			Latossolo Vermelho-Amarelo		
	Extraído ^a	Combustão	Total	Extraído ^a	Combustão	Total
Nenhuma	71	28	99	87	11	98
Sacarose	59	16	75	26	17	43
Leite (1% gordura)	62	12	74	22	5	27
Leite (14% gordura)	56	12	68	40	4	44
Óleo vegetal (soja)	58	14	72	34	17	51
Celulose pura	67	13	80	85	3	88
Papel de filtro	68	14	82	65	6	71
Papel de filtro incubado 2 semanas	57	20	77	60	5	65
Folha de soja "seca"	70	18	88	23	5	28
Folha de soja "verde"	57	22	79	29	4	33
Folha de soja "verde" incubada 2 semanas	64	13	77	15	1	16

^a Análise dos extratos por cromatografia em camada delgada mostrou que pelo menos 95% do ^{14}C extraído era carbaril.

Resumo

Investigou-se a degradação do inseticida carbaril em dois solos tratados com diferentes fontes de nutrientes orgânicos, utilizando-se técnicas radioisotópicas de cintilometria em líquido.

Resultados obtidos mostram que após seis semanas de tratamento a degradação do carbaril, sob a influência das fontes de carbono exceto celulose pura e papel de filtro, aumentou no Latossolo Vermelho-Amarelo onde todo inseticida foi degradado, comparado ao solo Gleí Húmico.

O tratamento com folhas de vegetais, com ou sem incubação prévia por duas semanas, modificou de modo semelhante a persistência do carbaril em ambos os solos. Comportamento análogo foi observado usando-se papel de filtro, que é fonte de carbono de metabolização mais lenta.

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El kelvin. Corresponde a $1/273.16$ de la temperatura termodinámica del punto triple del agua (su símbolo es K).

La candela. Es la intensidad luminosa --en dirección perpendicular y con una superficie igual a $1/600\,000$ de metro cuadrado-- de un cuerpo negro, a la temperatura de solidificación del platino a una presión de $101\,325$ newton por metro cuadrado.

La mole. Es la cantidad de sustancia de un sistema que contiene tantas entidades elementales como átomos hay en 0.012 kilogramos de carbono 12 .

El radián. Es la medida de un plano cuyo vértice coincide con el centro de un círculo y cuya abertura es igual a la longitud de su radio subtendido como arco.

El steradián. Es la medida de un ángulo sólido con su vértice al centro de una esfera y que abarca sobre su superficie el área de un cuadrado cuyos lados tienen la longitud del radio.

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CHARACTERIZATION OF *Thanatephorus cucumeris* ISOLATES CAUSING WEB BLIGHT OF BEANS
IN COSTA RICA¹ /

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Resumen

Setenta y un aislamientos de *Thanatephorus cucumeris* fueron obtenidos del tejido de hojas de frijol infectadas en el campo y recolectadas en diferentes áreas de cultivo de Costa Rica, localizadas entre 50 y 1 200 m sobre el nivel del mar. Otros dos aislamientos fueron obtenidos a partir de lesiones sobre las hojas de las malezas *Sida acutifolia* y *Rhizoctonia exaltata* en un campo de frijol cerca de Esparza. Con base en las características del micelio, a la condición multinuclear y a la estructura del septo (tipo doliporo), todos los aislamientos correspondieron típicamente a *Rhizoctonia solani*, el estado imperfecto de *T. cucumeris*. La tasa de crecimiento lineal, determinado sobre agar papa - dextrosa (PDA) a 25°C varió mucho entre los aislamientos, siendo dicha variación entre 10 y 29 mm en 24 horas. Todos los aislamientos produjeron esclerocios de color pardo a pardo oscuro, variando en diámetro desde 0.5 a 9.0 mm.

Todos los aislamientos fueron patógenos hacia el tejido de hojas e hipocotilos de frijol (cultivar Mexico 27), siendo sus virulencias significativamente variables. Hubo una correlación positiva entre la tasa de crecimiento de un aislamiento y su virulencia a hojas e hipocotilos de frijol. De los 73 aislamientos probados, 26 y 38 pertenecen a los grupos de anastomosis (AG) 1 y 2, respectivamente. Los nueve aislamientos restantes no hicieron anastomosis con ninguno de los cuatro AG de ensayos usados.

Introduction

In the humid lowlands of Latin America and the Caribbean, web blight (WB) is one of the most destructive diseases of beans (*Phaseolus vulgaris* L.) (3, 5, 6, 8, 10, 11, 19, 25). It causes a very rapid defoliation and sometimes even complete crop failure during the rainy season in the lowland tropics

(8, 10, 25). Weber (24) was the first to demonstrate that WB of beans in Florida is caused by *Rhizoctonia solani* Kühn and its perfect state, *Thanatephorus cucumeris* (Frank) Donk. Both the perfect and imperfect states of this fungus often have been observed and reported occurring on beans in Costa Rica (5, 6, 7, 8, 11) and other bean growing areas in the tropics (3, 10, 19, 24, 25).

In nature, *T. cucumeris* exists in the form of many strains that differ in cultural appearance and pathogenicity. Such variability has been demonstrated to occur among isolates obtained from within one field or from different bean fields (9). It has been shown that *T. cucumeris* generally consists of 4 anastomosis groups (AG) that are characterized by distinctive morphological, physiological, and pathogenic traits, although overlapping may occur among the groups (1, 16, 20).

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The WB isolates of *T. cucumeris* have a faster growth rate, produce larger number of sclerotia, and are more sensitive to CO₂ than the subterranean isolates of this fungus (4). Nevertheless, the aerial isolates were found capable of causing damping-off and both hypocotyl and root rot of beans. In contrast, the subterranean bean isolates of *T. cucumeris* with a fast growth rate were able to cause aerial infection under conditions of high humidity and temperature (9). Similar results have been reported with *T. cucumeris* isolates from cowpea (17) and soybean (23).

The objectives of this study were to characterize and to determine the range of variability among isolates of *T. cucumeris* causing WB of beans in Costa Rica.

Materials and methods

Isolations and identification of *T. cucumeris* from naturally infected leaf tissues. Leaf samples were collected from bean fields in the Central Plateau and the eastern and western regions of Costa Rica during the rainy season (August-December) of 1979. Small leaf pieces were obtained from the margin of expanding lesions, surface sterilized for 1-2 min in 0.05% NaOCl solution, and then plated on potato-dextrose agar (PDA) or the selective medium of Ko and Hora (14). After 24-48 hr of incubation at 24°C, hyphal tip transfers were made to 0.5% yeast-PDA (Y-PDA) slants from colonies showing the mycelial characteristics of the imperfect state, *R. solani* (2). Stock cultures were maintained by periodic transfer to Y-PDA slants at 20°C. All isolates were examined for the presence of the characteristic dolipore septal apparatus and the multinucleate condition of the hyphal tip cells (12, 13). The isolates were grown on 2% water agar plates at 25°C until the colony had almost covered the surface of the agar plates. Several drops of a wetting solution (1 ml each of Tween 20 and 85% lactic acid/L distilled water) were placed about 2-cm proximal to the advancing hyphal tips and spread thoroughly with a bent spatula. The wetted areas were then stained with a drop of 0.05% trypan blue in lactophenol. Pieces of agar (1 x 1 cm) containing the stained hyphae were thinly cut, placed on a slide, covered with a slip, and examined microscopically after 5-10 min.

Cultural variability and pathogenicity of WB isolates of *T. cucumeris*. Growth rate, sclerotial formation, zonation, and color of the isolates were determined as described previously (9). Four to 6 plates were used for each isolate per determination and were incubated at 25°C. Sclerotial formation

and color of the colonies were identified according to Butler and Bracker (2) and Koppers (15), respectively.

The bean cultivar Mexico 27 was used in the pathogenicity tests. Nontreated seeds were surface disinfested for 5 min in 0.25% NaOCl and rinsed several times in sterile distilled water. Pasteurized greenhouse-prepared soil mix (PS) (equal parts of top soil and sand) was used in this study. Test plants were watered daily and maintained in a greenhouse at 20°-24°C and relative humidity that varied between 60% -90%. Inoculum preparation, methods of inoculation, and incubation periods were essentially as reported earlier (9).

To test the pathogenicity of the isolates on hypocotyl tissues, 5 seeds were planted 2-cm deep in 20-cm clay pots, three-fifths filled with PS. Six to 8 days after planting, the plants were thinned to 3/pot and a layer about 4-cm thick of *T. cucumeris*-infested soil (14) (10%, v/v of inoculum source soil/PS) was added to the pots around the bean hypocotyls. Check plants were covered with PS that was treated identically as the *T. cucumeris*-infested soil. All treatments were replicated 5 times. After 14 days, plants were removed from the soil and their roots were washed and rated for disease severity. A rating of 0 to 5 was used with 0 indicating no apparent disease, whereas 5 referred to most severe disease (dead plants).

The pathogenicity of isolates of *T. cucumeris* was compared using the detached leaf method (9). Detached leaves of 20-day-old plants were placed on a wire mesh about 3 cm above the bottom of plastic moist-chamber boxes (10x20x30 cm). High humidity was maintained by addition of water to the bottom of the boxes and by lining the sides with filter paper that extended to the water. The leaves were also sprayed thoroughly with sterile distilled water using an atomizer. The check treatments received noninfested PDA discs. Six leaves were used per box per isolate. Disease severity was recorded after 5 days' incubation at 25°C using a scale of 0 (no apparent lesion development) to 5 (100% of leaf surface is affected).

Anastomosis grouping. The 4 AG testers used in this study were obtained from Dr. E. E. Butler, Department of Plant Pathology, University of California, Davis, CA 95616. Stock cultures of these isolates were maintained by periodical transfers to Y-PDA slants. Anastomosis between isolates was determined by using the method of Parmeter *et al.* (18). The anastomosis reactions recognized in this study (perfect fusion, imperfect fusion, and contact

fusion) were based on the definitions and descriptions previously reported in the literature (18). Isolates which did not anastomose with any set of the testers were paired in all possible combinations with other isolates. Pairings among isolates were made at least 2 different times with each of the testers.

Results

Identity of the WB isolates. The majority of the 81 isolates examined were recovered from lesions that appeared to be initiated either by sclerotial or mycelial form of inoculum (24). However, 3 isolates (R-70, R-72, and R-76) were obtained from lesions characteristic of basidiospore infection (7). All of the isolates possessed the mycelial characteristics of *R. solani* (imperfect state of *T. cucumeris*) as described in the literature (2). Eight of the isolates were discarded as they failed to grow after several transfers to either PDA or Y-PDA slants. The remaining 73 isolates exhibited the presence of a

dolipore septal apparatus and the cells of their young hyphal tips were multinucleate (12).

Cultural variability among the collected isolates of *T. cucumeris*. **Growth Rate.** Significant differences in growth rate were found among the 73 WB isolates of *T. cucumeris* obtained from several bean growing regions of Costa Rica (Table 1). Average colony linear growth of these isolates ranged from 10 to 29 mm per 24-hr incubation on PDA at 25°C. Generally, isolates of the fungus collected from the bean growing regions at elevations between 200 and 750 m (Esparza, 200 m; San Isidro, 650 m; and Turrúcares, 700 m) appear to have the highest growth rate. In contrast, isolates collected from bean fields at elevations of 50 m (Upala) and above 750 m (Central Plateau region) exhibited a relatively slower growth rate on PDA.

Zonation. Zonate formation is due to the periodical changes in mycelial growth and density

Table 1. Anastomosis groups, growth rate, and virulence to beans of web blight isolates of *Thanatephorus cucumeris* in Costa Rica.

Isolate no. ^s	Locality and altitude (m)	Growth rate (cm/24 hr) ^t	Virulence (0-5) ^{uv}		Anastomosis group ^y
			Hypocotyl ^w	Leaf ^x	
R-37	Turrúcares; 700	5.76	4.14	5.00	1-P
R-66	San Isidro; 650	5.72	4.14	5.00	1-P
R-49	Pasoagres; 200	5.70	3.60	5.00	1-P
R-53	Turrubares; 500	5.38	3.40	5.00	1-P
R-67	San Isidro; 650	5.36	4.14	4.83	1-P
R-25	Turrúcares; 700	5.20	3.67	5.00	1-I
R-80	Esparza; 200	5.18	4.07	5.00	1-I
R-55	San Mateo; 400	5.16	3.87	5.00	1-P
R-68	San Isidro; 650	4.98	4.27	4.83	1-P
R-75	Esparza; 200	4.94	4.07	5.00	1-P
R-26	Turrúcares; 700	4.86	4.07	5.00	1-P
R-14	Turrúcares; 750	4.74	3.80	5.00	1-I
R-21	Turrúcares; 700	4.64	2.95	4.00	1-I
R-44	Turrúcares; 750	4.36	3.80	5.00	1-I
R-73	Esparza; 200	4.36	3.87	4.83	1-P
R-76	Esparza; 200	4.24	3.40	5.00	1-P
R-69	Esparza; 200	4.22	3.47	5.00	1-P
R-74	Esparza; 200	4.22	3.54	5.00	1-P
R-72	Esparza; 200	4.18	3.80	4.83	1-P
R-24	Turrúcares; 700	4.08	3.14	5.00	1-P
R-65	San Isidro; 650	3.78	3.87	4.33	1-I
R-45	Turrúcares; 700	3.52	3.07	4.00	1-P
R-70	Esparza; 200	3.38	3.47	4.67	1-P
R-52	Orotina; 500	3.28	2.67	3.67	2-P
R-78	Esparza; 200	3.28	2.73	3.67	1-P
R-63	Fabio Baudrit; 850	3.02	3.87	3.83	2-P
R-71	Esparza; 200	3.02	2.47	3.33	1-P
R-60	San José; 1 000	2.90	2.67	3.50	1-C
R-4	Palmares; 900	2.76	1.27	3.66	2-P

Table 1 (Cont)

R-23	Dulce nombre; 850	2.74	2.67	3.83	— ^z
R-51	Riogrande; 800	2.72	2.07	3.50	2-I
R-56	Dulcenombre; 850	2.70	3.20	4.00	1-P
R-11	Palmares; 1 100	2.64	1.20	3.33	2-I
R-2	Palmares; 950	2.58	2.14	3.33	2-I
R-34	Upala; 50	2.58	2.20	3.33	2-I
R-62	Fabio Baudrit; 850	2.58	2.07	3.33	2-P
R-7	Naranjo; 1 000	2.56	1.47	3.00	2-C
R-64	Fabio Baudrit; 850	2.56	2.40	3.33	2-C
R-29	Upala; 50	2.52	1.73	3.00	2-C
R-53	Villa Bonita; 900	2.52	1.20	3.00	—
R-61	Fabio Baudrit; 850	2.52	2.47	3.33	2-C
R-22	Dulcenombre; 850	2.48	1.00	3.00	2-I
R-59	San José; 1 000	2.48	2.07	2.83	—
R-20	Santa Eulalia; 900	2.44	2.20	2.50	2-C
R-8	Palmares; 900	2.42	1.47	3.00	—
R-19	Santa Eulalia; 900	2.42	2.14	2.50	—
R-10	Palmares; 900	2.40	1.00	3.00	2-C
R-28	Upala; 50	2.34	1.00	2.33	2-P
R-57	San José; 1 000	2.34	2.60	3.50	2-P
R-58	San José; 1 000	2.34	1.74	2.67	2-C
R-17	Santa Eulalia; 900	2.32	1.00	1.83	2-C
R-27	Dulcenombre; 850	2.32	1.20	2.50	2-C
R-46	Monserrat; 900	2.32	1.20	2.83	2-P
R-54	Santa Eulalia; 900	2.30	2.60	2.33	2-C
R-32	Upala; 50	2.30	2.00	2.16	2-P
R-3	Palmares; 1 000	2.22	0.74	2.33	—
R-6	Palmares; 1 100	2.18	0.74	1.67	2-C
R-15	Palmares; 900	2.16	1.20	1.83	—
R-30	Upala; 50	2.16	1.00	2.00	—
R-9	Palmares; 900	2.12	0.54	2.00	2-C
R-12	Palmares; 900	2.12	1.00	1.33	2-C
R-40	Ciruelas; 800	2.12	0.60	1.83	—
R-41	Ciruelas; 800	2.08	0.60	2.00	2-I
R-33	Upala; 50	2.08	1.47	1.16	2-I
R-36	Upala; 50	2.08	1.26	1.00	2-P
R-42	Villa Bonita; 900	2.08	0.54	1.83	2-C
R-31	Upala; 50	2.06	1.26	2.33	2-C
R-47	Dulcenombre; 850	2.06	1.00	1.83	2-C
R-50	Estanquillo; 1 200	2.06	0.60	1.00	2-I
R-1	Palmares; 900	2.04	1.60	1.66	2-P
R-16	San Ramón; 1 100	2.04	1.20	1.50	2-C
R-39	Ciruelas; 800	2.04	0.47	1.33	2-P
R-48	Dulcenombre; 850	2.04	1.00	1.33	2-I
	LSD: 0.05	0.019	0.030	0.021	

^s Refers to locality or regions in Costa Rica where the isolates were collected.

^{t-u} Each number is an average of 6 and 5 replicates, respectively.

^v Disease severity rating was recorded using a scale of 0 to 5, with 0 referring to no apparent disease symptoms and 5 indicating 100% of inoculated tissues were infected.

^{w-x} Disease rating was determined 14 days and 5 days after inoculation, respectively.

^y Anastomosis grouping was according to the designation given by Parmeter *et al.* (18). P, I, and C refer to perfect, imperfect, and contact fusion, respectively.

^z These isolates failed to anastomose with any of the tester isolates or any of the other isolates used in this study.

resulting in the production of a sparse and a dense aerial mycelial mat regions (21). All the isolates examined in this study failed to exhibit zonation. However, concentric rings composed of the aggregation of sclerotia were observed in cultures of isolates R-6, R-7, R-9, R-31, R-32, R-33, R-41, R-57, R-58, R-63, and R-69 that were incubated in the dark, but none was found under artificial illumination.

Sclerotial Formation. All the isolates of *T. cucumeris* included in this study produced dark brown sclerotia. However, these sclerotia varied in size and distribution on the surface of the agar plates. Small sclerotia (2-4 mm diam) that were uniformly distributed on the surface of the colony were produced by 47 isolates which exhibited low to intermediate growth rate (Figure 1c). Within this group, 2 isolates (R-11 and R-15) showed aggregates of sclerotia in the center of the plate, whereas 10 others produced aggregates of sclerotia in the form of concentric rings. The latter were produced when the isolates were incubated in darkness but not under continuous artificial illumination. Isolates of *T. cucumeris* with fast growth rates produced sclerotia that were evenly distributed on the agar plates and were either few and large (7-9 mm diam; isolates R-24, R-26, R-37, R-66, R-67, R-68, and R-69) (Figure 1a) or numerous and very small (0.5-1.0 mm diam; R-49, R-71, R-72, R-73, R-74, R-75, and R-76) (Figure 1b).

Color of Vegetative Hyphae. The color of the mycelium of all isolates studied was typically brown. The brown color of most of these isolates is characterized as N90 A60 M60 and N99 A50 M50 for the mycelium and sclerotia, respectively (15). Few isolates had a mycelial and sclerotial color of dark brown which was designated as N99 A50 M50. The latter isolates had a fast growth rate and produced small sclerotia (0.5-1.0 mm diam).

Virulence on hypocotyls and leaves. Significant differences were found in the relative virulence of the 73 isolates of *T. cucumeris* to bean hypocotyls and leaves (Table 1). Virulence of the isolates was found to be related to their relative growth rates. The isolates with the highest growth rate were the most virulent, whereas those with the slowest growth rate exhibited the least virulence.

The highly virulent isolates of *T. cucumeris* caused a rapid and severe necrosis on leaves and branches of bean seedlings. Lesions first became visible after 36 hr of incubation and the foliage of inoculated plants was completely affected within 5 days. Small sclerotia (0.5-1.0 mm diam) were produced on infected tissues 5 days after inoculations. The least

virulent isolates incited restricted necrotic lesions that did not coalesce nor result in infection of

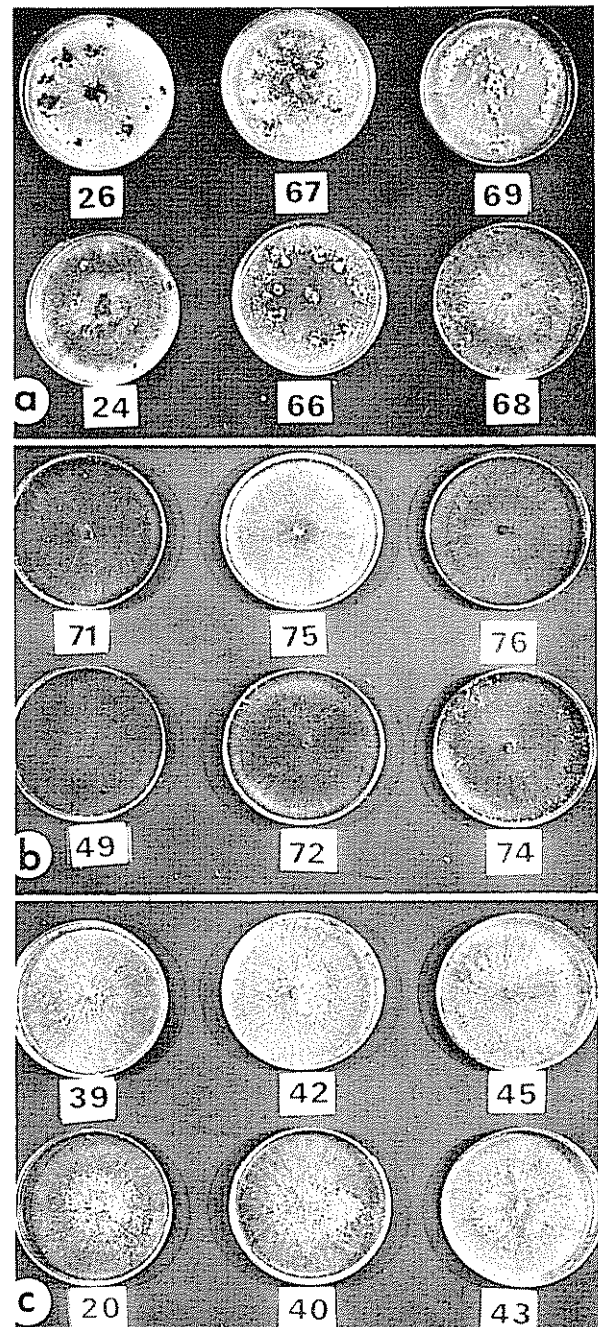


Fig. 1. Representative cultures of *Thanatephorus cucumeris* isolates causing web blight of beans in Costa Rica after 14 days' growth on potato-dextrose agar at 25°C with continuous light. a-b) Large (7-9 mm diam) and small (0.5-1.0 mm diam) sclerotial isolates of Anastomosis Group (AG) 1, respectively. c) Isolates of AG-2 showing sclerotia (2.0-4.0 mm diam) that often aggregate toward the center of the culture.

branches. Sclerotia were not visible around such restricted lesions over the duration of the test.

Sunken lesions were produced by the highly virulent isolates on hypocotyl tissues. Lesions were dark brown and sometimes coalesced resulting in girdling of the stem tissues at the soil line or slightly above. The soil surface of the pots infested with isolates of *T. cucumeris* often were covered by numerous small sclerotia (0.5-1.0 mm diam) which first became evident 3 days after inoculations. Brick-red sunken lesions were produced on hypocotyl tissues by the least virulent isolates. The most virulent isolates generally were collected from the bean growing regions of eastern, central, or western parts of Costa Rica with an altitude between 200 and 700 m (San Isidro de El General, Turrúcares, and Esparza).

Anastomosis grouping. Sixty-four out of the 73 WB isolates of *T. cucumeris* examined were assignable to the anastomosis groups designated by Parmeter *et al.* (18). Of these, 26 and 38 were found to belong to AG-1 and AG-2, respectively. None of the isolates were members of AG-3 or AG-4 (Table 1). Nine isolates failed to anastomose with the tester strains, among themselves, or with any of the web blight isolates belonging to AG-1 or AG-2. The 3 types of the anastomosis reaction observed in this study were similar to those reported in the literature (18). The "contact fusion" reaction was observed with 18 of the isolates in AG-2, the "imperfect reaction," with 7 isolates of AG-1 and 9 in AG-2, whereas the "perfect fusion" was observed with 19 isolates of AG-1 and 11 isolates in AG-2.

Isolates within AG-1 varied in color of mycelium and in size and number of sclerotia. Several isolates including R-66 exhibited a light brown and sparse mycelium with few large (6-9 mm diam) sclerotia that were dark brown in color. Other isolates including R-74 (Figure 1b) had an appressed and coarse mycelium with numerous small (0.5-1.0 mm diam) sclerotia. Sclerotia were imbedded in the agar, uniformly distributed on agar surface, and along with the mycelium were dark brown in color. Isolates of both groups had a fast growth rate and were highly virulent on hypocotyls and leaves.

Isolates within AG-2 showed only minor variation in color of mycelium and in size and number of sclerotia as compared to the isolates in AG-1 (Figure 1c). The mycelium was appressed to moderately aerial and brown in color. Sclerotia were 2-4 mm diam, often aggregated into compound sclerotia, and had a darker brown color than the mycelium. Isolates in AG-2 were characterized by slow to intermediate

growth rates as well as low to moderate in virulence to bean hypocotyls and leaves.

Discussion

Only *T. cucumeris* was isolated from naturally infected bean leaves in Costa Rica exhibiting typical symptoms of WB. Identification of the collected isolates was based on the mycelial characteristics of the imperfect state, *R. solani* (2), possession of a prominent dolipore septal apparatus, and the multinucleate condition of the young hyphal tip cells. The last 2 features provide reliable diagnostic criteria in distinguishing *T. cucumeris* and its imperfect state from many Rhizoctonia-like fungi, which are similar in cultural appearance and mycelial morphology (12, 13). The binucleate Rhizoctonia-like fungi have been reported as causal agents of foliar blights on grasses and sugarbeets (13).

Echandi (5, 6, 7, 8) and González (11) previously had reported on the occurrence of *T. cucumeris* and the severity of WB of beans in Costa Rica. Parmeter *et al.* (18) were successful in producing the perfect state under laboratory conditions using isolates of the fungus previously recovered from naturally infected bean leaves collected in Costa Rica. The basidial state of the fungus also has been reported occurring on beans (7) and tobacco (22) under field conditions in Costa Rica. The basidial state was also observed during the present study on bean tissues in fields in Esparza, San Isidro, and several areas in the Central Plateau. In this study, considerable cultural variability was demonstrated to exist among the WB isolates of *T. cucumeris* in Costa Rica. Cultural and morphological variability has been reported in the literature among isolates of *R. solani* that are associated with WB diseases on different crops and from different geographical regions (5, 23). Variability in cultural appearance also has been reported in the literature among the subterranean isolates of *R. solani* (9).

Rapid growing mycelium is characteristic of *T. cucumeris* isolates. Among the different habitats in which the fungus is found in nature (aerial, surface or subterranean), the aerial forms have been reported as having the fastest growth rates (4). However, several isolates of this fungus obtained from infected bean hypocotyls and roots previously were shown to have a comparable growth rate to the aerial WB isolates (9). The WB isolates examined in this study varied significantly in their growth rates which were also closely associated with their virulence to beans. The fastest growing isolates were distinguishable from the others by cultural appearance, higher virulence on

bean hypocotyls and leaves, and they were all in group AG-1.

All isolates of *T. cucumeris* obtained from WB lesions on beans were pathogenic to both hypocotyl and leaf tissues, although they varied considerably in their virulence (Table 1). Pathogenicity of the aerial isolates of *R. solani* to roots and hypocotyl tissues also have been reported on cowpeas (17), soybeans (23), and beans (9). In addition, *R. solani* isolates recovered from infected roots and hypocotyls have been shown to be pathogenic to the foliage of beans (9) and cowpeas (17). These results suggest that both foliar and root isolates of *T. cucumeris* have the capacity to infect the above-ground or subterranean bean tissues depending on the conditions prevailing in their habitats.

The sclerotia produced by the isolates of *T. cucumeris* in culture differed markedly from those formed on infected bean tissues. Sclerotia in culture were variable in shape and size (3-7 mm diam) and sometimes formed aggregates (1-2 cm diam), whereas those produced on infected tissue appeared round and very small (0.5-1.0 mm diam). This behavior also has been observed with *R. solani* isolates obtained from WB lesions on beans in Florida (24), but not from soybeans (23).

The majority of the WB isolates of *T. cucumeris* studied in this investigation were assignable to anastomosis groups AG-1 or AG-2. The characteristics of these isolates were in agreement with those given by Sherwood (20). Isolates of *T. cucumeris* in AG-1 and AG-2 have been previously isolated from naturally infected bean leaves in Costa Rica (18). Nine isolates obtained in this study failed to anastomose with any other isolates tested. The latter suggests that either anastomosis is rare among these isolates or that additional AG exist in nature as suggested by Parmeter *et al.* (18). Recently, field isolates from Japan have been placed in a new AG (1, 16).

The *T. cucumeris* isolates collected from the diverse geographical regions where beans are grown in Costa Rica varied greatly in cultural characteristics and virulence to bean tissues. Generally, the isolates obtained from Esparza, San Isidro de El General, and several from Turrúcares were highly virulent to beans. In contrast, isolates from Upala, San Ramón, Palmares, and some from Turrúcares were only weak to moderately virulent. Esparza, San Isidro, and Turrúcares are at altitudes of approximately 208, 650, and 700 m, respectively. WB is endemic in these areas and often occurs in epidemic proportion as a result of favorable environmental conditions for

disease incidence and development during the rainy season. In addition, the basidial state often has been observed occurring on beans and other crops in these areas. Thus, there are more opportunities for continued variability of the fungus and the prevalence of more variants than in areas where disease incidence and severity are lower, such as Upala (elevation 50 m) and Estanquillo (elevation 1200 m).

Summary

Seventy-one isolates of *Thanatephorus cucumeris* were obtained from naturally infected bean leaf tissues collected from several bean growing areas of Costa Rica that ranged in elevation from 50 to 1200 m. Two other isolates were obtained from lesions on leaves of the weed species *Sida acutifolia* and *Rhynchospora exaltata* in a bean field near Esparza. Based on mycelial characteristics, multinucleate condition, and presence of the dolipore-type septal structures, all isolates were typically *R. solani* which is the imperfect state of *T. cucumeris*. Linear growth rate, as determined on PDA at 25°C, varied greatly among these isolates and ranged from 10 to 29 mm in 24 hr. All isolates produced brown to dark brown sclerotia which varied in size from 0.5 to 9.0 mm diam. All isolates were pathogenic to bean leaf and hypocotyl tissues (cultivar Mexico 27), but their virulence varied significantly. There was a positive correlation between growth rate of an isolate and its virulence to bean leaves and hypocotyls. Of the 73 isolates tested, 26 and 38 isolates belonged to anastomosis groups (AG) 1 and 2, respectively. The remaining 9 isolates failed to anastomose with any of the 4 AG testers used.

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gánicos resistentes en suelos. Se discute en un segundo subcapítulo la formación y química de estos compuestos y se termina con conclusiones y una bibliografía de casi 300 referencias.

En el segundo capítulo se estudia el análisis "in situ" de los componentes de origen biológico del suelo. Hace tiempo se estima deseable realizar estudios con materiales no extraídos, sin que hasta el presente haya sido posible este tipo de estudios. El reciente desarrollo en microscopía electrónica y otras técnicas instrumentales hacen ahora posible estudios sin extracción, indicando el lugar preciso de la materia orgánica en la estructura del suelo. Se discute en el capítulo la localización e identificación, además de la cuantificación de estos derivados.

La combinación oxidativa de compuestos aromáticos por enzimas de microorganismos de suelos es el tópico del tercer capítulo. Se discute el tópico en cuatro subdivisiones dedicadas a las enzimas que promueven la combinación oxidativa que producidas por microorganismos del suelo y aparte del suelo, la diferenciación de las enzimas de combinación oxidativa producto de las reacciones de combinación oxidativa y finalmente los significativos biológicos y ecológicos de las reacciones de combinación oxidativa.

En el cuarto capítulo se estudia el control de las transformaciones de urea en suelos. Se discute aquí las propiedades de la ureasa en suelos, los efectos de los inhibidores de ureasa y los efectos de los inhibidores de la nitrificación sobre las transformaciones de urea en el suelo. Por ser la urea una forma de abono nitrogenado muy importante, es crítico el conocer sobre sus transformaciones en suelos; aquí se presenta un resumen tanto sobre los aspectos teóricos como los prácticos de sus cambios de forma.

En el quinto capítulo se presenta la información sobre la química y la distribución de los amino azúcares en suelos y organismos de suelos; se resume la información sobre la química de los amino azúcares, su distribución en organismos vivos, su determinación y las cantidades y formas de amino azúcares, su distribución en organismos vivos, su determinación y las cantidades y formas de amino azúcares en suelos. Se presenta el conocimiento actual sobre la transformación de estos compuestos en suelos.

El sexto capítulo se dedica al tópico de los derivados de petróleo en suelos. Se analiza su bioquímica, ecología y microbiología. Este capítulo, el segundo más largo del volumen, reúne información de una serie de ciencias. Se discuten las sustancias de importancia en este campo, su comportamiento en suelos y sus efectos sobre el mismo. Se incluye también informa-

Reseña de libros

PAUL, E.A. y J.N. LADD (eds.). *Soil biochemistry*. Vol. 5. M. Dekker Inc., New York 1981, 480 p.

Este es el primer volumen donde el Prof. McLaren, iniciador de la serie no figura entre los editores. Su muerte dejó un apreciable vacío entre los que practican la bioquímica de suelos. El volumen consiste de diez capítulos independientes que reflejan áreas con investigación activa en los últimos años y no tratados en volúmenes anteriores.

El primer capítulo se refiere a la química y las transformaciones de los compuestos naturales resistentes a una descomposición rápida. Este capítulo discute las evidencias de la existencia de compuestos or-

ción sobre la dinámica de dos reacciones y el comportamiento en sistemas acuáticos.

La fijación biológica del nitrógeno es el tópico del séptimo capítulo. Este tema, que ha recibido mucha atención recientemente, es cubierto con base a la literatura de la última década. Los principales aspectos discutidos son la fijación de N_2 , los organismos involucrados, la bioquímica del proceso y sus relaciones con O_2 . Se discuten también sistemas donde asociaciones entre microorganismos, raíces y plantas, especialmente tropicales, resultan en fijación de N_2 .

La desnitrificación es el tópico del octavo capítulo. Se estudian aquí los organismos responsables y la fisiología y la bioquímica del proceso. Se le da consideración a los métodos de medición del proceso y el sistema del suelo donde ocurre. Se considera la producción no biológica de formas volátiles de N_2 .

El capítulo noveno se dedica a los metales pesados en la biología y bioquímica de suelos. Se analizan las formas principales de interacción entre estos elementos, esenciales o no, y los procesos biológicos o bio-

químicos en suelos. Se considera más que todo los aspectos microbiológicos del problema, tratados con menos profundidad en otros trabajos de revisión.

En el décimo capítulo se estudia la biomasa microbiana en suelos, su medición y transformaciones. Se presenta su medición por microscopía directa, por medición de componentes específicos de la biomasa, la velocidad de respiración y otras técnicas. Se analiza la concordancia entre las diferentes técnicas.

Un breve índice final ayuda al lector a encontrar el material deseado en este volumen de alto nivel que presenta y resume mucho material de difícil acceso y de gran actualidad. El libro requiere conocimientos básicos en microbiología de suelos y buenas bases en bioquímica para su aprovechamiento y se le recomienda para los especialistas y estudiantes de posgrado en el campo.

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Summary

During a year the longevity of buried and imbibited seeds was studied, also the effect of the spectral composition of the light on seed germination was investigated, on Verbesina greenmanii, a secondary growth plant from the tropical rain forest. The seed were placed in plant growth chambers with modified light composition, and under natural light conditions in the forest. Other experiments were carried out on the effect of the red (660 nm) and far red light (730 nm). The results show that the seeds may remain dormant in the soil and in imbibed condition. The spectral composition of the light plays an important role on the regulation of seed germination. The seeds are stimulated to germinate by the red light and inhibited by the far red light.

Introducción

El estudio de la ecofisiología de la germinación de las semillas permite comprender en forma más precisa los mecanismos que regulan la longevidad de las semillas en el suelo, el rompimiento de la latencia, la germinación y el establecimiento de las plantas en condiciones naturales. Este aspecto de la biología de las plantas es de mucho interés en el estudio de aquellas especies que se establecen solamente en áreas alteradas o destruidas de la vegetación madura, ya que en la vegetación no alterada dichas plantas persisten solo en forma de semilla.

Con respecto a las semillas de algunas de estas especies, hay evidencias de que la alteración de la cubierta vegetal natural es la fuente de estímulos ambientales que desencadenan la germinación en el suelo (18).

Las poblaciones de algunas plantas pioneras heliofilas de corta vida dependen para sobrevivir de poseer mecanismos eficientes de conservación de semillas viables en el suelo, que germinen sólo cuando las condiciones ambientales externas sean propicias para el desarrollo de los individuos; o sea, cuando la cubierta vegetal natural ha sido alterada y la luz solar incide directamente sobre el suelo (13, 19, 20).

V. greenmanii es una compuesta arbustiva muy abundante en la vegetación secundaria temprana de terrenos en barbecho y claros amplios de la selva, en regiones cálidas y semicálidas de México. Las plantas de esta especie producen aquenios pequeños, alados que se diseminan por el viento en la época seca del año. Se han reportado semillas en el suelo de la selva madura (6). Esta especie es abundante en la Estación de Biología Tropical de Los Tuxtlas de la Universidad Nacional Autónoma de México en el Estado de Veracruz. De ese lugar proceden las semillas empleadas en la investigación y en él se efectuaron los experimentos y observaciones de campo.

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Este trabajo fue parcialmente financiado por el Consejo Nacional de Ciencia y Tecnología de México (Proyecto PCECNAL 790222). Los experimentos en cámaras y cajones de composición espectral de la luz controlada fueron efectuados en el Departamento de Botánica de la Universidad de Leicester en Gran Bretaña, contando con el asesoramiento y apoyo del Dr. Harry Smith.

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Las características de la Estación han sido descritas en detalle por Lot (10).

En un trabajo previo (18) se describieron los efectos de la temperatura y la germinación en luz y obscuridad en *V. greenmanii* y otras compuestas pioneras: *Bidens pilosa* L., *Eupatorium macrophyllum* L., *Epittierii* Klatt y *Vernonia deppeana* Less. Todas ellas presentaron semillas fotoblásticas, al igual que *V. caracasana* Rob. & Greenm. de Sudamérica (14).

Con el antecedente anterior se procedió a realizar experimentos de longevidad de semillas en el suelo de la selva y en condiciones de imbibición y se estudió el efecto de la calidad de la luz sobre la germinación, tanto en condiciones artificiales como en el campo, para profundizar en el entendimiento de los factores que regulan la conservación de semillas viables en el suelo y el disparo de la germinación cuando ocurre una perturbación del dosel vegetal, los experimentos de viabilidad complementan al estudio de la latencia, ya que permiten integrar un panorama del más probable comportamiento de las semillas en condiciones naturales, al obtenerse simultáneamente información acerca de las posibilidades que las semillas tienen de sobrevivir en el suelo y de los factores externos que impiden o desencadenan su germinación.

Es sabido que la luz solar sufre un notable cambio en composición espectral al ser transmitida o reflejada por el follaje verde de la vegetación (8, 12). Este cambio produce una modificación en la distribución fotónica del espectro luminoso, se reduce la energía en la porción correspondiente al color rojo (660 nm), sufriendo un incremento relativo en la porción correspondiente al infrarrojo cercano y en particular en el color rojo lejano (730 nm). Las mediciones efectuadas en la selva madura con un espectrofotómetro portátil (2) indicaron valores de ς (sigma) 0.01 a 0.3 en la luz difusa, a nivel del suelo, en diferentes puntos dentro de la selva. A valores similares han llegado otros autores que han medido la composición de la luz en la vegetación tanto tropical (8) como bajo otros tipos de doseles (15).

Según Monteith (12), ς expresa el valor de la relación Rojo/Rojo lejano que resulta de dividir la energía del espectro luminoso concentrada en 660 nm entre la que se encuentra en la franja de 730 nm (1).

Cuando las semillas se encuentran en medios iluminados cuya luz presenta un valor de ς muy bajo, su fitocromo se mantiene en su forma inactiva y las semillas permanecen latentes. La germinación se inicia al producirse un incremento energético en la porción roja del espectro que tiene lugar al aumentar

la intensidad de la luz que llega al suelo sin incidir sobre follaje verde (5, 9, 14, 17, 20). El papel del pigmento fitocromo como sensor ambiental en las semillas es bien conocido (3, 4, 11).

Materiales y métodos

Las semillas fueron colectadas a partir de varios individuos diferentes en el momento que estaban siendo diseminadas. Un ejemplar de herbario que respalda la identificación se encuentra depositado en el Herbario MEXU con el número Vázquez—Orozco 54.

Todos los experimentos salvo el de semillas enterradas, fueron efectuados en cajas de Petri de 10 cm de diámetro sobre agar puro al 1% en agua destilada, empleando 100 semillas por caja.

Los experimentos realizados fueron: 1) longevidad durante un año en semillas enterradas, 2) longevidad de semillas imbibidas, almacenadas en obscuridad, 3) efecto de la composición espectral de la luz en cámaras de crecimiento, 4) efecto de la luz difusa de la selva en condiciones naturales y 5) efecto de exposiciones breves y alternantes a luz de color rojo y rojo lejano.

- 1) El experimento de longevidad en el suelo consistió en mezclar 200 semillas con 10 g de suelo de la selva esterilizado, para eliminar semillas previamente presentes en él. La mezcla fue introducida en bolsas de malla de nylon que se cerraron y enterraron en el suelo de la selva madura a 5 cm de profundidad, cada mes se extrajo una bolsa y las semillas se pusieron a germinar en un cristizador dentro de una cámara de crecimiento a 25°C y 12 h de fotoperiodo.
- 2) El experimento de longevidad por un año en imbibición consistió en sembrar semillas en cajas de Petri que fueron cubiertas con pliegos de papel de aluminio y polietileno y después almacenadas en la obscuridad a 25°C. Cada mes una caja sembrada era desenvuelta y expuesta a la luz en la cámara Conviron después de verificar la germinación en oscuridad.
- 3) El experimento de germinación en cámara de crecimiento, en condiciones de calidad de luz controlada, fue efectuado en las cuatro cámaras construidas por Heathcote *et al.* (7). Las cuatro cámaras funcionaron a 25°C con un fotoperiodo de 12 horas. El valor de la relación R/RL para cada cámara fue: la cámara uno $\varsigma = 2.3$ representando una área descubierta, la cámara dos $\varsigma = 0.58$ represen-

tando una sombra vegetal producida por un dosel delgado; la cámara tres, $\zeta = 0.23$ representando sombra de un dosel relativamente denso y la cámara cuatro, $\zeta = 0.20$ representando la sombra de un dosel muy denso. La energía luminosa total de las cámaras es de 10 a 15 veces mayor que la que existe en el suelo de la selva a mediodía.

- 4) Los experimentos de germinación en el suelo de la selva se efectuaron con cajas de Petri sembradas que se colocaron en cuatro lugares de selva madura, dos lugares de vegetación secundaria de aproximadamente 14 años de edad y bajo una sombra no vegetal en descubierto que produce una luz difusa de composición espectral muy diferente a la de una sombra vegetal. La germinación fue verificada después de un mes.
- 5) Los tratamientos de exposición a luz de color rojo y rojo lejano fueron efectuados en dos cajones contruidos exprefeso. El cajón de luz roja estaba provisto de un plafón luminoso formado por tubos fluorescentes "daylight" (60 w) cuya luz atraviesa una capa de perpej rojo del número 400 (de ICI) y un vidrio transparente incoloro. El cajón de luz color rojo lejano tiene un plafón luminoso formado por bombillas de tunsteno-halógeno de 60 w parcialmente sumergidas en una cámara de agua corriente para enfriar y eliminar el infrarrojo. La luz producida atravesó una capa de perpej rojo y otra de perpej verde. Ambos tipos de lámparas producen luz de diferente composición espectral que al pasar por los filtros perpej adquiere las propiedades requeridas (14). Las semillas fueron sembradas en la oscuridad y después de dos días se expusieron durante períodos de 10 a 30 minutos al efecto de la luz de una u otra cámara o de ambas consecutivamente, para verificar si el fitocromo de las semillas es activado y desactivado por la luz de color rojo y rojo lejano respectivamente. Después del tratamiento las cajas de Petri fueron almacenadas en la oscuridad a 25°C por un mes antes de verificar la germinación.

Resultados

Los resultados de los experimentos de viabilidad en el suelo y en condiciones de imbibición se presentan en la Figura 1. Se observa que en ambos casos a) viabilidad en el suelo; b) condiciones de imbibición, hubo semillas que permanecieron viables pero el porcentaje final de germinación fue bajo, ya que disminuyó de más de 50% a menos de 10% en un año. Los resultados obtenidos en las cámaras de calidad de luz controlada se presentan en la Figura 2. Se observa que la disminución del valor de ζ disminuye la germina-

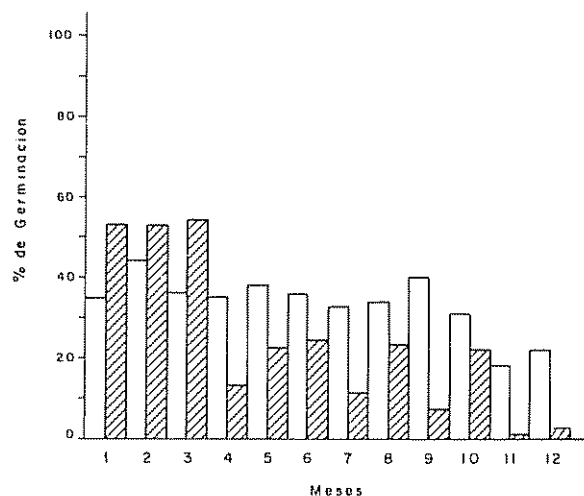


Fig. 1. Se muestra el porcentaje de germinación obtenido después de cada mes de almacenamiento. Las columnas rayadas corresponden a la germinación obtenida después de desterrar las semillas y las columnas blancas corresponden a la germinación obtenida después de exponer a la luz las semillas almacenadas imbibidas en la oscuridad.

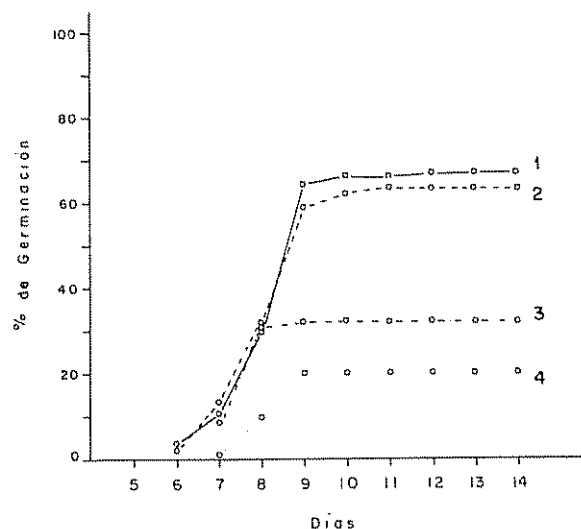


Fig. 2. Desarrollo de la germinación en las cuatro cámaras de calidad de luz controlada que simulan: 1- terreno descubierto, 2- dosel delgado, 3- dosel intermedio, 4- dosel denso.

ción y la velocidad con la que ésta se presenta. Los resultados obtenidos del experimento de germinación en el campo se presentan en el Cuadro 1, se observa menos germinación bajo la sombra vegetal, que la que se obtiene bajo una sombra artificial. Los resultados de exponer las semillas a luz de color rojo y rojo lejano se presentan en el Cuadro 2, se observa que el efecto estimulante de la luz roja es totalmente revertido por el rojo lejano cuando este tratamiento

Cuadro 1. Germinación de semillas de *Verbesina greenmanii* en diferentes ecosistemas.

Localidad	Germinación (%)	\bar{X}
Bosque maduro		6.25
1	7	
2	7	
3	7	
4	4	
Bosque secundario		10.50
5	17	
6	7	
Sombra artificial al descubierto		53.00
7	55	
8	51	

Cuadro 2. Efecto de tratamientos de luz de color rojo (R) y rojo lejano (RL) sobre la germinación de *Verbesina greenmanii*.

Tratamiento ¹	Germinación (%)
R ₁₀	11
R ₂₀	16
R ₃₀	17
RL ₁₀ R ₁₀	14
RL ₂₀ R ₂₀	20
RL ₃₀ R ₃₀	28
R ₁₀ RL ₁₀ R ₁₀	16
R ₂₀ RL ₂₀ R ₂₀	19
R ₃₀ RL ₃₀ R ₃₀	31
RL ₁₀	0
RL ₂₀	0
RL ₃₀	0
R ₁₀ RL ₁₀	0
R ₂₀ RL ₂₀	0
R ₃₀ RL ₃₀	1
RL ₁₀ R ₁₀ RL ₁₀	0
RL ₂₀ R ₂₀ RL ₂₀	0
RL ₃₀ R ₃₀ RL ₃₀	0

¹ La cifra indica el tiempo (minutos) de irradiación aplicado en cada tipo de luz.

es aplicado en último término, lo cual corresponde con lo observado en varios experimentos de diversos autores en especies de semillas fotoblásticas (4, 9, 14, 17).

Discusión

El éxito de las plantas pioneras, al igual que el de las hierbas anuales (13) depende de la potencialidad de sus semillas para sobrevivir latentes en el suelo y de germinar solo cuando las condiciones ambientales externas representan una razonable probabilidad de sobrevivencia de las plántulas y de alcanzar la edad reproductiva; por esta razón, es de esperar que este tipo de especies presenten una latencia impuesta por factores externos que prevenga la germinación cuando las condiciones para el establecimiento sean desfavorables, pero que no la impida cuando estas se tornen favorables.

Las semillas de *V. greenmanii* presentan una viabilidad relativamente baja al ser liberadas, pero la porción de semillas viables es capaz de sobrevivir en el suelo por varios meses y en condiciones de imbibición. La viabilidad decae más rápidamente en el suelo que en cajas de Petri, pero al año de enterradas las muestras aún presentan semillas viables, lo cual puede permitir una mayor acumulación de semillas viables en el suelo cuando se presenta una nueva estación de fructificación; o sea, una parcial superposición de generaciones de semillas.

Al igual que en otras compuestas tropicales las semillas son fotoblásticas y permanecen latentes en bajos valores de ζ (14, 18). Este fenómeno puede ser de particular importancia en tanto las semillas permanecen en la superficie del suelo después de ser diseminadas; ya que, cuando las semillas quedan enterradas o alojadas en cavidades oscuras del suelo, la total oscuridad de esos medios impide la germinación.

Cuando el dosel vegetal es destruido por una perturbación, el aumento de valor de ζ puede desencadenar la germinación abrupta de aquellas semillas que se encuentran al alcance de la luz.

Las semillas de *V. greenmanii* en condiciones naturales pueden formar parte del banco de semillas del suelo y que probablemente ese banco tenga características similares a las descritas por Thompson y Grime (16), como del tipo tres; o sea, una parte de las semillas producidas en una estación de fructificación germina al caer en terrenos descubiertos. De aquella porción de semillas que cae en terrenos cubiertos de vegetación, una parte importante se integra al banco de semillas latentes del suelo, del cual las pérdidas principales son por pérdidas de viabilidad, parasitismo y probablemente también por predación, hasta que ocurre una perturbación del dosel vegetal que desencadena la germinación.

Esta predicción surge del análisis conjunto de los resultados de campo y de laboratorio que se plantearon en esta investigación.

Resumen

Durante un año se estudió la longevidad en el suelo y en condiciones de imbibición de las semillas de *Verbesina greenmanii* Urban, que crece en una región de selva tropical húmeda, formando parte de la vegetación secundaria. Se investigó también el efecto de la composición espectral de la luz sobre la germinación en cámaras de composición espectral controlada, en el medio ambiente y bajo el efecto de luz de color rojo (660 nm) y rojo lejano (730 nm). Los resultados indican que las semillas pueden permanecer viables y latentes en el suelo y que la composición espectral de la luz juega un papel importante en el control de la germinación. La germinación es estimulada por la luz roja e inhibida por la de color lejano.

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Reseña de libros

OLSON, G.W. Soils and the environment. A guide to soil surveys and their applications. Chapman and Hall, New York, N.Y. 1981. 178 p.

Este texto fue preparado para proveer información generada por la descripción de perfiles y mapas de suelos, indicando los principios de agrupamiento de

suelos, y para discutir algunos aspectos específicos de su aplicación. El lector a quien se dirige este libro es la persona común, o sea cualquiera interesado en el uso del suelo y de la tierra.

Los primeros capítulos introducen al lector en la terminología de aspectos morfológicos del suelo, análisis de laboratorio y nomenclatura del sistema americano de clasificación de suelos. Hasta el capítulo 4 se trata de una discusión edafológica simplificada.

A partir del capítulo 5 se describe un sistema de computarizar información y su posterior interpretación al clasificar tierras, controlar la erosión, establecer correlaciones con rendimientos de cosecha, consideraciones y planeamiento para el futuro.

La forma en que se presenta la información está de acuerdo con la audiencia a la que está dirigida la obra. Quizá por esta razón no debe buscarse datos cuantitativos nuevos ni adelantos científicos sobresalientes del tema. El autor no incluye valiosa información generada sobre inventario de recursos naturales y publicada por el centro de enseñanza al cual pertenece.

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EFFECT OF DIFFERENT CENTRAL AMAZONIAN SOILS ON GROWTH, NODULATION, AND OCCURRENCE OF N₂-FIXING *Azospirillum* spp. IN ROOTS OF SOME CROP PLANTS¹ /

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R. SYLVESTER-BRADLEY**

Resumo

Foram plantados feijão de asa, soja, feijão caupi e milho em diferentes solos da Amazônia Central, numa área protegida de chuva. As taxas de crescimento das plantas, nodulação e ocorrência de Azospirillum em latosolo amarelo argiloso e solo podsólico arenoso não queimados foram muito baixas. Em latosolo argiloso queimado o crescimento das plantas e a ocorrência de Azospirillum foram maiores, mas ocorreu pouca nodulação. Soja var. Jupiter nodulou espontaneamente em solo aluvial ("várzea"). Com inoculação de Rhizobium a nodulação de soja aumentou em solos da várzea e terra preta dos índios. A nodulação de feijão de asa e feijão caupi não aumentou com inoculação sem adubação em latosolo ou solo podsólico, mas em solo de várzea o crescimento e a nodulação de feijão de asa inoculado aumentaram. O crescimento e nodulação de feijão de asa inoculado e adubado aumentaram, especialmente em solo podsólico arenoso, enquanto o crescimento de feijão caupi aumentou em todos os solos, e nodulação somente em solo podsólico queimado e solo da várzea. Os dados indicam que a ausência de nodulação em latosolo argiloso era parcialmente devida à presença de nitrogênio no solo.

Introduction

Yellow latosol occurs over a large proportion of the Central Amazonia (4, 5, 8, 10). Much of this soil is very heavy, acidic (pH 4.0 – 5.0), has a high aluminium saturation level (over 50% of total C. E. C.) and low available phosphorus (1-2 ppm). Areas of a more sandy soil (red-yellow podsol) occur on the valley sides. This sandy soil is

grey in colour at the surface but appears similar to yellow latosol when analysed by routine chemical tests. These two soils will be distinguished here by use of the terms latosol and podsol. Sandy podsol is often used in preference to clayey latosol for subsistence agriculture by the local inhabitants.

The natural vegetation on these two soils is forest which showed a living biomass of 473 t ha⁻¹ (dry weight), containing 2 983 kg N ha⁻¹ (6). However, when three parameters were used to estimate nitrogen-fixing activity (nodulation, acetylene-reducing activity and occurrence of N₂-fixing *Azospirillum* spp.), little activity was found in association with roots of trees growing in areas of undisturbed forest on clayey latosol (12). Plants growing in sandy podsol and more fertile soils showed higher levels of activity.

Areas of forest in this region are being cleared for agricultural development, the trees being burned after felling which releases some plant nutrients into the soil and atmosphere. Growth of *Pennisetum purpureum* planted in recently cleared latosol near

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Manaus was enhanced tenfold by fertilization with phosphorus but nitrogen gave little response. Maize and cowpea also showed little response to nitrogen in the first year of cultivation, but in succeeding years the response increased (1). This implies that nitrogen was not the limiting nutrient immediately after deforestation. However, continuous cultivation of the soil, especially if fertilized with phosphorus alone would rapidly cause depletion of the soil nitrogen and other nutrients. It is thus important that nitrogen-fixing plants should be incorporated into the agricultural systems being introduced in order to avoid the use of costly inorganic nitrogen fertilizers.

The objective of the investigations described here was to determine whether crop plants growing in representative Central Amazonian soils fix nitrogen and whether this activity can be enhanced by fertilization and inoculation of the appropriate bacteria.

Materials and Methods

Experiment I: Soil samples were collected from five sites considered to represent Central Amazonian soil types as follows: "alluvial" soil from the north bank of the Rio Solimões about 1 km above its confluence with the Rio Negro; "sandy podsol" at an experimental site at km 14, AM-010; "clayey latosol" at the Instituto Nacional de Pesquisas da Amazonia (INPA) Reserve (Estação Experimental de Silvicultura Tropical, EEST) at km 45 of the Manaus-Caracará road BV-8; "burned clayey latosol" in an area at EMBRAPA-UEPAE-Manaus (km 30, AM-010) which had recently been deforested and burned, and which therefore contained ash; "black earth" from the Estrada do Aleixo, Manaus, on the north bank of the Rio Negro opposite its confluence

with the Rio Solimoes. Soil analyses and descriptions of alluvium and black earth are shown in Table 1. Samples were collected at five points at each site from 0-20 cm depth and mixed. The soil was distributed in plastic bags of 1.6 kg capacity covered with aluminium foil to prevent excessive temperatures. Soybean cv. Jupiter, winged bean (*Psophocarpus tetragonolobus* cv. IRI 30-91 from the Legume Research Institute, Singapore) and maize seeds were surface-sterilized in 0.1% acidified HgCl₂ and planted in four replicate bags of each soil. The plants were irrigated with distilled water. Plant dry weight, dry weight of nodules on the legumes, *Azospirillum* enrichment root culture activity for the maize, acetylene-reducing activity of the intact system and total foliar nitrogen were determined after 42 days growth in a rain-proof area. *Azospirillum* enrichment root culture activity was determined as previously described (12). Acetylene reduction was determined by enclosing the entire plant and soil system in a polyethylene bag and incubating with acetylene for three hours, after which gas samples were analysed for ethylene by gas chromatography (2).

Experiment II: Samples of the same soils as those used in Experiment I were taken. Eight bags of each soil type were planted with surface-sterilized soybean cv. Jupiter seeds, four with rhizobium inoculum and four without. The rhizobium culture used for the inoculum had been isolated from nodules on soybeans grown in Experiment I in alluvial soil. Dry weight of nodules and plants were determined after 45 days growth.

Experiment III: Clayey and medium latosol and sandy podsol were collected from three sites close to the INPA EEST reserve at km 45 BV-8. Samples at each site were collected from primary forest and

Table 1. Analyses of soils used in Experiments I and II.*

Soil	Type	P ppm	K ppm	Ca + Mg	Al	pH	Organic matter (%)	(Total) N %	C/N
				meq./100 g					
Alluvium	(A)	>30	144	14.3	0.8	5.0	0.4	0.12	2
Podsol	(P)	15	28	0.7	0.5	4.7	0.8	0.07	7
Clayey latosol	(CL)	2	66	0.5	2.5	3.6	5.0	0.32	9
Burned clayey Latosol	(BCL)	1	56	1.7	0.5	4.6	2.2	0.18	7
Black earth	(BE)	3	44	6.3	0.1	5.2	3.1	0.17	11

* Analyses carried out at EMBRAPA-Km 47-RJ. Black earth ("terra preta dos índios") is a fertile soil containing many pieces of broken ceramics presumed to be left by the Indians who once inhabited these sites (9). Alluvial or "várzea" soil is the fertile alluvial soil deposited in annual floods on the banks of sediment-loaded rivers such as the Rio Amazon.

from adjacent areas which had recently been deforested and burned. These as well as alluvial soil from the banks of the Rio Solimões were obtained using the same methods as those described for Experiment I. Surface-sterilized and germinated winged bean cv. IRI-30-91 and cowpea [*Vigna unguiculata* cv. VITA 3 (TVU 1190)] seeds were planted in bags of each soil with three treatments: untreated, inoculated and inoculated and fertilized, with five replicates of each treatment. The rhizobium inoculants used were prepared from cultures isolated from nodules on the respective plants growing locally and pretested for efficient nitrogen fixation on plants in sterile sand and nutrient solution. Fertilization rates of triple super phosphate, KCl and lime were calculated from the soil analyses (Table 2) following the recommendations of Souza (11). Nodule and plant dry weight were determined after 75 days growth.

Results

Table 3 shows that in Experiment I plant dry weight, nodule weight, *Azospirillum* enrichment culture activity and total plant nitrogen were all relatively low in unburned clayey latosol and sandy podsol.

In the other three more fertile soils (burned clayey latosol, black earth and alluvial soil), soybeans and winged beans grew equally well, whereas maize grew better in burned latosol than in alluvial soil or black earth.

The pattern of occurrence of nodulation and *Azospirillum* enrichment culture activity in the three more fertile soils was different for each plant. Soybeans

showed some nodulation in alluvial soil, and negligible amounts in other soils. Winged beans showed good nodulation in both alluvium and black earth. They did not nodulate well in burned clayey latosol even though plant weight was relatively high in this soil. On the other hand activity of *Azospirillum* enrichment cultures from maize roots was higher in burned latosol and alluvium than black earth.

Total plant nitrogen was particularly high in burned clayey latosol for all three plants. The acetylene reduction assays carried out on the intact system at the end of the experiment showed acetylene reduction only by winged beans in alluvial soil and black earth.

Experiment II was set up in order to test further the nodulation of uninoculated soybeans in alluvial soil observed in Experiment I. Table 4 shows that inoculation of soybeans with *Rhizobium* isolated from the nodules formed on soybeans in alluvial soil in Experiment I caused better nodulation in alluvial soil and black earth. However, plant weight was only slightly higher in the inoculated treatment in alluvial soil.

The results of Experiment III are shown in Table 5. Although growth of the winged beans and cowpeas was better in burned than unburned latosol and podsol in no case was it as good as when the soil was fertilized. Even in alluvial soil growth of cowpeas was better with fertilization. Cowpeas in unburned unfertilized latosol and podsol either failed to germinate or died shortly after germination, despite various replantings.

Neither winged beans nor cowpeas nodulated better in burned than unburned latosol and podsol

Table 2. Analyses of soils* and fertilization rates used in Experiment III.

Soil Type**	P ppm	K ppm	Mg Ca Al			pH	Organic matter %	Lime t ha ⁻¹	Fertilizer applied	
			meq./100 g						TSP** kg ha ⁻¹	KCl kg ha ⁻¹
C	2	24	0.1	0.2	0.7	3.9	3.4	4.10	336	101
BC	4	102	0.6	1.3	0.1	5.0	5.0	0.65	285	60
M	2	28	0.2	0.2	0.7	4.0	3.4	3.98	336	101
BM	3	28	0.1	0.0	0.5	4.0	2.2	3.32	336	101
P	2	17	0.1	0.0	0.9	3.8	3.6	4.79	336	101
BP	2	21	0.1	0.0	1.0	3.6	4.2	4.95	336	101
A	52	106	2.0	9.9	0.1	5.0	1.9	0.00	143	101

* Carried out at IAC, CAMPINAS, SP.

** C = clayey latosol; BC = burned clayey latosol; M = medium latosol; BM = burned medium latosol; P = podsol; BP = burned podsol; A = alluvium; TSP = triple super phosphate.

Table 3. Levels of four plant parameters in different Central Amazonian soils (Experiment I). Values of the same parameter anotated with different letters are significantly different at the 50% level (Tukey's test).

Plant parameter	Soil type*	Soybean	Winged bean	Maize
Dry weight (g) plant. ⁻¹	A	1.39 ^a	1.20 ^{ab}	0.96 ^b
	P	0.24 ^b	0.75 ^b	0.20 ^b
	CL	0.24 ^b	0.52 ^b	0.56 ^b
	BCL	1.29 ^a	1.69 ^a	2.30 ^a
	BE	1.21 ^a	1.01 ^{ab}	0.90 ^b
Nodule weight (mg) plant. ⁻¹ (legumes) and root enrichment assay nmol C ₂ H ₄ culture. ⁻¹ h. ⁻¹ (maize)	A	20.70	62.70 ^a	205.08 ^a
	P	0.00	1.67 ^b	12.78 ^b
	CL	0.00	0.85 ^b	24.99 ^b
	BCL	0.00	0.47 ^b	198.29 ^a
	BE	0.10	41.37 ^{ab}	20.80 ^{ab}
Total N (mg) plant. ⁻¹	A	19.00 ^b	51.28 ^b	10.63 ^b
	P	6.88 ^c	26.38 ^e	3.64 ^b
	CL	8.51 ^c	22.66 ^e	9.48 ^b
	BCL	38.10 ^a	84.22 ^a	24.15 ^a
	BE	24.87 ^b	43.40 ^{cd}	9.93 ^b

* As in Table 1.

Table 4. Means and coefficients of variation (in brackets) of dry weights of nodules and whole soybean cv Jupiter plants in different inoculated and uninoculated Central Amazonian Soils (Experiment II).

Soil Type	mg nodules plant. ⁻¹		dry weight plant. ⁻¹ (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Alluvium	30.75 (187)	224.85 (33)	2.49 (30)	2.73 (10)
Podsol	0.00	22.75 (31)	0.81 (33)	0.65 (3)
Clayey latosol	0.00	0.50 (141)	0.53 (34)	0.41 (24)
Burned clayey latosol	0.00	63.85 (80)	2.27 (32)	1.67 (47)
Black earth	0.00	229.30 (27)	2.33 (13)	1.90 (44)

without fertilizer, even in the presence of inoculated *Rhizobium* spp. In alluvial soil inoculation alone improved both nodulation and growth of winged beans, but not of cowpeas.

In the fertilized and inoculated treatments winged beans nodulated well, particularly in the sandy podsol. Cowpeas on the other hand only showed good nodulation in fertilized and inoculated burned podsol and alluvial soil.

Discussion

The growth of winged beans, soybeans and maize in Experiment I varied markedly between the

different soil types. The good growth observed in burned clayey latosol was presumably due to the nutrients contained in the ash. The legumes did not however nodulate in this soil, which indicates that it contained sufficient nitrogen to meet the plants' needs. Indeed, the total nitrogen data show that the plants grown in burned clayey latosol contained relatively high nitrogen levels.

Azospirillum enrichment culture activity was high in burned clayey latosol, but this does not necessarily imply that nitrogen was being fixed in the roots. Acetylene reduction assays of the intact maize plants in this soil were negative, although this might have been due to poor diffusion of the gases in and out of the soil. The higher enrichment culture activity

observed may have been due to an increase in total numbers of bacteria including *Azospirillum* in the roots stimulated by more abundant energy-rich root exudates. The low *Azospirillum* enrichment culture activity observed in black earth was unexpected: previous observations showed abundant N₂-fixing *Azospirillum* in black earth samples (12). The difference may be due to the black earth having been collected from an uncultivated site, whereas previous investigations used samples from inhabited sites planted with fruit trees, cassava and other crops.

Soybeans are usually considered to require inoculation with *Rhizobium japonicum* for the establishment of effective nitrogen-fixing nodules, but rhizobia of the cowpea miscellany can form nodules on soybeans, although they are frequently ineffective (7, 13). Effective nodulation of low-yielding soybeans of Asian origin by naturally-occurring cowpea rhizobia has been observed in African soils (3). In Experiment I and II surface-sterilized seed and soil from areas not previously planted with soybeans were used. The occurrence of nodules in uninoculated alluvial soil thus implies that this soil naturally contains rhizobia able to promote nodulation in Jupiter.

In Experiment II inoculation greatly increased nodulation of Jupiter in both alluvial soil and black

earth, and to a certain extent in sandy podsol and burned clayey latosol. The lack of nodulation in the uninoculated black earth samples and its presence in the alluvial soil indicates that the rhizobia causing nodule formation were indeed of soil rather than seed origin because if they were of seed origin nodulation would have occurred in uninoculated black earth. However plant growth was no greater in inoculated than uninoculated treatments which implies that the strain of rhizobium used was inefficient on this variety of soybean. Further investigations would be needed to determine the specificity and efficiency of these naturally-occurring rhizobia.

The better growth of plants in fertilized than burned latosol and podsol observed in Experiment III indicates that the ash did not contain sufficient nutrients to support good growth. The quantity of nutrients contained in burned soil depends on the efficiency of the burning, the degree of leaching and run-off, and other factors. Results obtained with burned soil thus vary between experiments. It is possible that if crops were planted after a good burn before significant leaching had occurred they would not require additional fertilization.

In Experiment III inoculation alone did not overcome the lack of nodulation of winged beans or

Table 5. Means and coefficients of variation (in brackets) of dry weights of nodules and plants of winged bean and cowpea in different Central Amazonian soils (Experiment III).

Plant parameter	Soil type*	Winged Bean			Cowpea		
		Untreated	Inoculated	Inoculated and Fertilized	Untreated	Inoculated	Inoculated and Fertilized
Dry weight (g) plant ⁻¹	C	0.42 (62)	0.62 (27)	4.88 (29)	—	0.30 (—)	3.95 (18)
	BC	1.87 (24)	1.84 (28)	5.15 (11)	0.69 (45)	0.66 (26)	4.38 (25)
	M	0.29 (21)	0.31 (—)	3.55 (18)	—	—	3.66 (43)
	BM	2.13 (37)	1.69 (31)	3.38 (23)	1.77 (18)	1.70 (43)	3.18 (24)
	P	0.46 (37)	0.22 (55)	5.29 (14)	—	—	3.00 (24)
	BP	2.28 (15)	2.56 (36)	4.05 (17)	1.05 (38)	1.09 (34)	4.38 (33)
	A	2.78 (29)	4.04 (25)	3.26 (32)	2.72 (16)	2.22 (28)	3.24 (4)
Dry nodule weight (mg) plant ⁻¹	C	0.0	0.0	54.0 (103)	—	0.0	0.5 (200)
	BC	2.5 (200)	0.0	33.0 (86)	0.0	0.0	0.0
	M	0.0	0.0	46.0 (101)	—	—	1.3 (100)
	BM	0.0	0.0	64.0 (80)	0.0	0.0	1.4 (273)
	P	0.0	0.0	129.0 (72)	—	—	0.0
	BP	0.0	1.0	146.0 (23)	0.4 (225)	0.8 (225)	70.0 (137)
	A	59.0 (21)	183.0 (112)	121.0 (72)	13.2 (133)	12.2 (147)	45.0 (75)

* As in Table 2.

— Plants failed to germinate or died shortly after germination.

cowpeas growing in latosol or podsol even when it was burned. Once fertilized, however, the winged beans did nodulate. The more abundant nodulation of fertilized winged beans in sandy podsol than medium or clayey latosol, despite equally good growth in all three soil types indicates that there was more nitrogen available in medium and clayey latosol, thus partially inhibiting nodulation. A similar difference between clayey and sandy soil was observed when examining roots of vegetation (12) and is presumably due to more rapid leaching of nitrogen from sand than clay.

Cowpeas only nodulated in two of the inoculated and fertilized treatments: burned sandy podsol and alluvial soil. Plant growth was however more or less equal in all the treatments. This implies that cowpeas nodulate less readily than winged beans when nitrogen is present in the soil. Further experiments are needed to show the efficiency of nitrogen fixation by winged beans and cowpeas in different soils and whether it can be improved by inoculation of *Rhizobium*.

The failure of cowpeas to grow altogether or their death shortly after germination in unfertilized unburned latosol and sandy podsol was overcome both by burning and by fertilization. This may have been due to the greater vigour of the plants in more fertile soils enabling them to resist disease.

The data indicate that neither the winged bean nor the cowpea varieties used in these experiments would grow well in latosol or podsol without fertilization. Even the nutrients available after recent burning were not sufficient to support good growth and nodulation. It is also implied that the soils tested contained levels of nitrogen which were sufficient to inhibit nodulation in some cases. However, selection of the varieties used in the experiments described here was carried out without the determination of characteristics that would enable them to grow in soils of low natural fertility. The differences in nutrient uptake between varieties observed in these experiments imply that a wider range of varieties should be evaluated for ability to grow at low levels of nitrogen, phosphorus and lime, association with mycorrhizae and high rates of nitrogen fixation. It might then be possible to distribute these varieties to Amazonian farmers, thus avoiding as far as possible the application of expensive inorganic fertilizers.

Summary

Winged beans, soybeans, cowpeas and maize were planted in pots containing different Central

Amazonian soils in a rainproof area. Plant growth, nodulation, and occurrence of *Azospirillum* in unburned yellow clayey latosol and sandy podsol were very low. In burned clayey latosol plant growth and incidence of *Azospirillum* increased but nodulation was still low. Soybeans cv. Jupiter nodulated spontaneously in alluvial soil, and inoculation with *Rhizobium* isolated from these nodules increased nodulation but not growth in alluvial soil and black earth. Inoculation alone did not increase nodulation of winged bean or cowpea in latosol or podsol, but in alluvial soil growth and nodulation of inoculated winged beans increased. Inoculated and fertilized winged beans showed an increase in both plant growth and nodulation, especially in podsol, whereas cowpeas showed an increase in plant growth in all the soils, and an increase in nodulation in burned podsol and alluvial soil. The data indicated that the lack of nodulation in clayey latosol was at least partially due to the presence of nitrogen in the soil.

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Summary

The forms and availability of potassium for sorghum (Sorghum vulgare L.) were determined in 31 soil horizons from the orders Entisol, Inceptisol, Alfisol, Ultisol and Vertisol from the tropical humid region of Peru. Available K was also extracted with 2, 4, 6 and 8N sulfuric acid; 0.5 N hydrochloric acid; N sodium acetate, and N ammonium acetate.

Total K ranged from 1761 to 4548 ppm with the acid, the sandy soils having the lower amounts. No relationship was found between soluble K and the other forms. However, the ratio fixed/exchangeable K was a good index to group the soils. This ratio was higher in the more acid soils. Exchangeable K was directly related to clay content and inversely related to the degree of weathering of the soils. Sulfuric and hydrochloric acid extracted from 30% to 50% more K than ammonium acetate (exchangeable K), except in those soils with 2:1 expandible clays. Sodium acetate extracted only 20% to 65% of the exchangeable K.

The decrease in exchangeable K with cropping explains from 80% to 100% the uptake of K by plants in soils having more than 70% base saturation, while it only explains 50% of K uptake in soils having less than 50% base saturation. A tentative critical level for deficient soils is proposed at 0.15 meq K/100 g soil.

Introducción

La disponibilidad de nutrimentos para las raíces es un aspecto de la nutrición mineral de las plantas estudiado con cierto énfasis en los suelos de la Costa y la Sierra, pero no en los suelos de la Selva peruana. En vista de la alta meteorización que existe en esta última región, es de esperar que la lixiviación del potasio sea intensa y, consecuentemente, que muchos de los suelos sean deficientes en este nutrimento. La agricultura en la Selva empie-

za normalmente con la tumba y quema del bosque, lo cual eleva el contenido de K cambiante del suelo, para luego disminuir cuando éste último se cultiva en forma continua (19). Sin embargo, es probable que esta disminución en el contenido de K esté condicionado al tipo de suelo, clima y especie vegetal o cultivar. Para zonas con precipitaciones de alrededor de 2 000 mm/año, se ha encontrado que en los Ultisoles la deficiencia de K se presenta al segundo año de cultivo continuo después de la quema del bosque (19), mientras que un Inceptisol con dos años de cultivo no presenta la deficiencia aún al ser cultivado en macetas (20).

Los estudios realizados con suelos de Perú indican resultados contradictorios en cuanto a la evaluación del K disponible. Durand (6) encontró que el ácido nítrico N fue el extractante más adecuado para suelos de diferentes partes del Perú, incluyendo la Selva. Pero, los resultados encontrados por Oré (13)

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sugieren que la capacidad fijadora de K de los suelos de la Selva es menor que los de la Costa y Sierra y que por lo tanto, el ácido nítrico no es un buen extractante en estos suelos. Es probable que estos resultados estuvieran en función del grado de meteorización del suelo y del tipo de arcilla predominante. En los suelos donde predomina la caolinita y la montmoriolinita la fijación de K es baja, y es bastante probable que el K cambiabile sea la principal fuente de K disponible para las plantas (11).

El presente experimento se efectuó para determinar las formas del K en 31 muestras de suelos del valle de Chanchamayo y evaluar la forma más disponible para las plantas de sorgo (*Sorghum vulgare* L.).

Materiales y métodos

Suelos

Se estudiaron siete perfiles de suelos con un total de 31 horizontes del valle de Chanchamayo, Selva Alta del Perú. Estas muestras representan a seis grandes grupos de suelos extensamente distribuidos en la Selva peruana y que tienen un amplio rango de niveles de K y de mineralogía. Los perfiles están localizados en el trayecto de San Ramón hacia Pampa Silva, excepto uno que fue tomado camino a Villarica. El clima de la zona es subtropical húmedo (17), con una temperatura promedio mensual que oscila entre 21 a 25°C; la precipitación anual media es de 1900 mm. La altitud en la zona muestreada va desde 800 a 900 msnm. Algunas de las características generales de cada perfil y de los horizontes estudiados se presentan en el Cuadro 1.

Análisis químicos

Las muestras de suelos fueron secadas al aire, molidas con un rodillo de madera y pasadas por un tamiz de 2 mm. Los análisis de caracterización de los suelos se efectuaron utilizando los métodos normales del Laboratorio de Análisis de Suelos y Plantas del Departamento de Suelos de la Universidad Nacional Agraria-La Molina. Estos métodos fueron: composición mecánica por el método del hidrómetro usando calgon al 10% como dispersante; pH en una relación suelo: agua de 1:1; carbonatos por gasovolumetría; materia orgánica por el método de Walkley y Black; y nitrógeno total con el Microkjeldahl. El fósforo disponible fue extraído con bicarbonato de sodio 0.5 M a pH 8.5, empleando el ácido ascórbico como reductor. La capacidad del intercambio catiónico (CIC) se determinó con acetato de amonio N a pH 7.0 y en el extracto se midieron el calcio

(Versenato), magnesio (amarillo de tiazol), potasio y sodio (fotómetro de llama). La acidez cambiabile fue extraída con cloruro de potasio N y titulada con hidróxido de sodio, utilizando fenolstaleína como indicador. Debido a la limitación de no disponer de información mineralógica específica, la CIC de la arcilla fue calculada asumiendo una CIC de 1.34 meq/g de materia orgánica, de acuerdo con los resultados obtenidos en suelos de la zona (5).

El K del suelo fue fraccionado de la siguiente forma. El K soluble se extrajo de 10 g de suelo agitados en 50 ml de agua destilada. El K cambiabile fue extraído con acetato de amonio N a pH 7.0. Para la determinación del K fijado se utilizó la técnica de Wood y de Turk (21) en la cual se hierven por 10 minutos 10 g de suelo en ácido nítrico N, después de extraer el K cambiabile. El K total fue extraído con la mezcla de los ácidos nítrico y perclórico calentados a 220°C (4). El K residual es la diferencia entre el K total y la suma del K soluble, cambiabile y fijado.

El K disponible fue extraído con: 1) ácido sulfúrico en cuatro concentraciones diferentes: 2, 4, 6 y 8 N, en una relación suelo: ácido 1:5 (9); 2) dos extracciones sucesivas con ácido clorhídrico, 0.5 N en una relación suelo: ácido 1:10 consideradas por separado y en conjunto; 3) acetato de sodio N a pH 4.8 en una relación 1:7 (14) y 4) acetato de amonio N a pH 7.0 (14).

El K total de la planta fue determinado en una digestión con agua oxigenada y ácido sulfúrico (8). El K en los extractos de suelo fue determinado por fotometría de llama, mientras que en los extractos foliares fue determinado por espectrofotometría de absorción atómica.

Experimento de invernadero

Las muestras de los 31 horizontes fueron cultivadas en el invernadero en macetas con 300 g de suelo. Se tuvieron cinco plantas de sorgo por maceta y cuatro repeticiones por tratamiento. Todas las muestras recibieron una dosis constante de 200 ppm de N, 200 ppm P, 30 ppm Mg, 50 ppm S, 5 ppm Mn, 5 ppm Fe, 5 ppm Zn, 3 ppm B, 1 ppm Cu y 0.5 ppm Mo. Se realizaron dos cortes sucesivos de sorgo a los 25 y 50 días de la siembra. Después del primer corte se aplicaron 200 ppm adicionales de N y de P. No se aplicó K en ninguno de los dos cortes. Las muestras foliares fueron secadas a 79°C y analizadas para K, como se describió anteriormente.

El análisis estadístico se efectuó de acuerdo a lo propuesto por Calzada (2). Adicionalmente se reali-

Cuadro 1. Algunas características de los perfiles y horizontes de suelos estudiados.

Horizonte	Prof. cm	Arena %	Arcilla %	pH	M.O. %	Calcareao %	CIC	Cambiable			CIC* de la
								Ca meq /100 g	Mg	Al	Arcilla
PERFIL 1. Sipa Alto (San Ramón). Rhodudult.											
A1	0 - 20	59	21	4.0	4.9	0.0	7.8	2.9	0.3	2.1	12
A3	20 - 35	56	25	4.9	1.4	0.0	4.6	2.7	0.1	2.1	11
B1 1	35 - 55	39	27	5.1	0.9	0.0	4.3	1.5	0.1	2.2	11
B2 1t	55 - 80	36	47	5.4	0.3	0.0	5.9	1.7	0.1	2.9	12
B2 2 t	80 - 100+	34	47	5.0	0.1	0.0	5.6	2.3	0.1	3.8	12
PERFIL 2. Sipa Bajo (San Ramón) Troporthent											
Ap	0 - 17	54	18	5.0	3.7	0.0	9.7	5.6	0.6	0.8	26
AC	17 - 60	58	20	5.1	1.6	0.0	6.2	4.6	0.2	1.6	20
C	60 - 100+	57	22	5.2	1.9	0.0	5.8	3.8	0.2	2.3	15
PERFIL 3. La Breña (San Ramón) Dystrocept.											
Ap	0 - 18	65	5	6.0	2.7	0.0	6.2	5.3	0.5	0.2	52
AC	18 - 25	64	7	5.2	0.8	0.0	3.6	2.9	0.1	0.6	36
C1	25 - 40	57	18	5.0	0.3	0.0	4.2	1.7	0.1	1.2	21
C2g	40 - 55	57	17	5.3	1.5	0.0	3.6	2.2	0.1	1.0	9
C3g	55 - 80+	51	25	4.9	1.3	0.0	4.1	2.4	0.2	1.2	9
PERFIL 4. Cumbre Pampa Walley (Pampa Silva) Rhodudalf.											
A1	0 - 15	54	13	6.3	3.1	0.0	7.0	5.5	0.1	0.0	22
A3	15 - 37	52	17	6.2	1.2	0.0	5.4	4.9	0.1	0.0	22
B1 1	37 - 70	50	20	6.2	0.6	0.0	8.2	4.0	0.3	0.0	37
B1 2	70 - 90	52	23	6.2	0.5	0.0	3.8	2.6	0.7	0.0	14
B2	90 - 120+	43	37	6.4	2.0	0.0	5.6	3.9	1.2	0.0	8
PERFIL 5. Pampa Silva Cafetal (Pampa Silva). Eutrocept.											
Ap	0 - 20	52	13	6.5	3.0	0.9	9.6	7.9	1.1	0.0	43
Cca	20 - 40	56	14	7.6	1.6	0.9	12.4	11.1	0.7	0.0	73
C2	40 - 70	54	16	6.7	1.7	0.1	8.4	7.0	0.8	0.0	38
C3	70 - 100+	57	16	6.8	0.8	0.1	7.6	6.6	0.5	0.0	41
PERFIL 6. Entaz Bajo (Villarrica). Rhodudalf.											
Ap	0 - 18	20	37	6.6	1.7	0.0	0.6	9.3	0.9	0.0	22
B2 1t	18 - 48	17	49	5.2	0.9	0.0	18.2	15.5	0.9	1.3	35
B2 2t	48 - 64	21	45	5.8	0.2	0.0	22.3	20.2	0.9	0.7	49
B3	64 - 84	26	35	5.9	1.3	0.0	26.0	22.9	0.7	0.4	69
Cca	84 - 100+	34	25	7.1	1.3	2.2	17.4	16.7	0.5	0.0	63
PERFIL 7. Pampa Silva Vertisol (Pampa Silva). Chromudert											
A1	0 - 8	31	26	6.4	5.1	0.0	28.0	26.0	1.1	0.0	81
B2 1	8 - 60	30	31	6.6	2.9	0.0	30.1	28.4	1.2	0.0	85
B2 2	60 - 80	38	33	6.4	1.3	0.0	30.6	28.6	1.3	0.0	87
C	80 - 100+	60	18	6.0	0.2	0.0	28.7	26.8	1.3	0.0	158

* Calculada asumiendo una CIC de 1.34 meq/g de materia orgánica.

zaron correlaciones múltiples y ecuaciones de regresión lineal y cuadrática utilizando las computadoras del Centro de Cómputo de la Universidad Nacional Agraria - La Molina.

Resultados y discusión

Formas de K en el suelo

Los resultados de las determinaciones de las formas de K en los suelos antes del cultivo con sorgo se presentan en el Cuadro 2. El K total promedio por perfil estuvo en el rango de 1761 ppm (Perfil 2) a 4548 ppm (Perfil 7). Los bajos contenidos de K en el Entisol y en el Ultisol (Perfiles 1 y 2) se explican por la naturaleza común del material original consistente en granito con predominancia de feldespato sódico (C. A. Villachica, Comunicación Personal). La diferencia en posición fisiográfica, Perfil 1 en ladera alta del cerro y Perfil 2 en la base del mismo cerro, condiciona la diferencia en pedogénesis entre ambos suelos. En cambio, el contenido de K total varió notablemente entre los Inceptisoles, ya que en el Distropept (Perfil 3, material madre lutita) el K total disminuyó con la profundidad, mientras que en el Eutropept (Perfil 3, material madre calcáreo) el K total aumentó con la profundidad. Este resultado refleja la diferencia en meteorización y en material madre entre ambos suelos. Los resultados del contenido del calcáreo y de la CIC presentados en el Cuadro 1 apoyan esta hipótesis. Los mayores contenidos de K total encontrados en los Perfiles 6 y 7 (Alfisol y Vertisol, respectivamente) con respecto al Perfil 4 (Alfisol), pueden estar asociados en parte al mayor contenido de arcilla, probablemente del tipo 2:1 (Cuadro 1). De lo expuesto se deduce que los contenidos más bajos de K total están en los suelos más meteorizados y en los de textura más arenosa. Sin embargo, en los materiales recientemente depositados y que no han sido muy afectados por la pedogénesis, el contenido de K total estará determinado por el material original.

El K soluble representó en promedio de 0.11 al 0.43% del K total y del 4 al 32% del K cambiante (Cuadro 2). Sin embargo, no existió una relación definida entre el K soluble y las demás formas de K en el suelo, como lo sugirió la falta de correlación entre el K cambiante, total o residual (15).

El K cambiante promedio por perfil estuvo en el rango de 24 ppm (Perfil 2) a 136 ppm (Perfil 7, Cuadro 2), correspondiendo las mayores cantidades a aquellos suelos con mayor contenido de arcilla y con las arcillas de mayor CIC (Cuadro 1). El K fijado estuvo presente en menores cantidades

(88 ppm) en los Perfiles 2 y 3 y en cantidades más altas (166 ppm) en los Perfiles 5 y 6. Con base en la relación K fijado/K cambiante, promedio por perfil, los suelos podrían ser reunidos en tres grupos. El primer grupo estará conformado por el Vertisol, con una cantidad de K fijado equivalente a la cantidad de K cambiante (razón 1:1). El segundo grupo estará conformado por los Alfisoles, el Ultisol y el Inceptisol eutrófico (Eutropept), con 1.2 a 2.3 veces el K fijado en relación al K cambiante. El tercer grupo estará formado por el Inceptisol distrófico (Dystropept) y el Entisol (Troporthent) con más de tres veces K fijado con respecto al K cambiante. Es posible explicar estas diferencias en función del tipo de arcilla predominante en cada suelo. Por ejemplo, la montmorillonita, arcilla predominante en los Vertisoles, se caracteriza por presentar una baja fijación relativa del K. La alta fijación observada en el Entisol y en el Inceptisol distrófico, con respecto al K cambiante, así como aquella observada en el Ultisol, presumiblemente se debe a la presencia de illita y montmorillonita mal cristalizada en cantidades altas en estos suelos (11, 13). Es de esperar que la presencia de caolinita en el Ultisol (13) disminuya la fijación del K. Las cantidades medias de K fijado en los Alfisoles sugiere la presencia de caolinita y de arcillas fijadoras de K de tipo 2:1 (18).

Extracción química del K disponible

Al comparar las cantidades de K extraídas por los diferentes reactivos, se observa (Cuadro 3) que el ácido sulfúrico extrajo de 60 a 200% más K que el acetato de amonio en el Ultisol, el Entisol y en los Inceptisoles. En uno de los Alfisoles (Perfil 4) las extracciones con ácido sulfúrico fueron 30 a 50% mayores que aquellas del K cambiante. En el otro Alfisol (Perfil 6) y en el Vertisol no existió mayor diferencia entre las cantidades medidas con ambos extractantes, lo que probablemente se explique por la existencia de una mayor cantidad de minerales de arcilla expansible. La correlación entre el K extraído con ácido sulfúrico y el K cambiante fue significativa sólo para el ácido sulfúrico 8 N, con un coeficiente de determinación de 0.96 (15), lo cual sugiere que las diferentes ubicaciones del K absorbido en el complejo de cambio de estos suelos son igualmente accesibles para ambos reactivos.

En el Cuadro 3 también se observa que la primera extracción con ácido clorhídrico liberó cantidades de K similares a las del K cambiante, excepto en un Alfisol (Perfil 6) y en el Vertisol. La segunda extracción con ácido clorhídrico removió aproximadamente la mitad de lo extraído la primera vez. Este resultado indica que en los primeros cinco perfiles la primera

Cuadro 2. Formas del potasio en el suelo antes del cultivo.

Horizonte	Forma de Potasio			
	Total	Soluble	Cambiable	Fijado
ppm				
PERFIL 1 Sipa Alto (Rhodudult)				
A1	1 613	7	41	109
A3	1 950	3	16	103
B1 1	1 462	4	16	129
B2 1t	2 219	4	86	158
B2 2t	3 120	4	133	170
PERFIL 2. Sipa Bajo (Troporthent)				
Ap	1 657	6	47	103
AC	1 821	8	14	62
C	1 804	9	12	100
PERFIL 3. La Bretaña (Dystropept).				
Ap	4 664	10	65	98
AC	4 926	7	16	62
C1	2 072	4	23	84
C2g	2 365	2	16	94
C3g	1 487	4	20	97
PERFIL 4 Cumbre de Pampa Walley (Rhodudalf)				
A1	3 169	15	118	166
A3	2 291	8	59	184
B1 1	3 388	6	68	158
B1 2	4 097	5	74	166
B2	4 266	5	152	156
PERFIL 5. Pampa Silva Cafetal (Eutropept)				
Ap	2 486	7	82	179
Cca	3 754	6	51	191
C2	3 705	7	70	146
C3	5 094	9	74	133
PERFIL 6. Entaz Bajo (Rhodudalf)				
Ap	4 485	2	63	142
B2 1t	4 069	6	86	98
B2 2t	4 662	4	98	115
B3	4 248	12	90	99
Cca	4 440	8	76	196
PERFIL 7. Pampa Silva Vertisol (Chromudert).				
A1	4 973	6	177	123
B2 1	4 661	7	121	127
B2 2	4 217	3	117	103
C	4 339	3	129	121

Cuadro 3. Cantidad de potasio extraída del suelo por diferentes métodos y antes del cultivo (promedio por perfil).

Método de extracción	Perfil No.						
	1	2	3	4	5	6	7
	Rhodudult	Troporthent	Dystropept	Rhodudalf	Eutropept	Rhodudalf	Chromudert
	ppm K						
H ₂ SO ₄ 2N	92	72	66	124	107	74	127
H ₂ SO ₄ 4N	93	84	79	136	111	97	141
H ₂ SO ₄ 6N	100	71	68	142	120	87	160
H ₂ SO ₄ 8N	102	50	53	140	124	90	133
NH ₄ OAc pH 7.0	58	24	28	94	68	87	134
HCl 1a ext.	62	32	39	94	70	49	100
HCl 2a ext.	26	17	16	42	36	32	38
HCl Total	86	49	55	135	105	81	143
NaOAc pH 4.8	35	13	19	51	34	18	35

extracción con ácido clorhídrico desplazó mayormente el K cambiante, mientras que en la segunda oportunidad se desplazó otras formas de K. En el Alfisol de Entaz y en el Vertisol se necesitaron dos extracciones sucesivas con ácido clorhídrico para desplazar una cantidad de K similar al K cambiante. En este sentido, el alto coeficiente de determinación obtenido al correlacionar las cantidades de K cambiante y aquel medido en la primera extracción con ácido clorhídrico ($R^2 = 0.94^{**}$) apoya la hipótesis de que la primera extracción con ácido clorhídrico desplaza mayormente al K cambiante (Cuadro 3). El acetato de sodio extrajo entre 20 y 68% del K cambiante y tuvo una baja correlación con el K cambiante ($R^2 = 0.23^{**}$) y con el K fijado ($R^2 = 0.38^{**}$). Luego, se concluye que el ácido sulfúrico 8 N y el ácido clorhídrico en primera extracción son los que desplazan la mayor cantidad de K cambiante en los suelos estudiados, concordando con los resultados obtenidos por otros investigadores (3, 7, 10).

Absorción del K por las plantas

El K total absorbido por dos cortes de sorgo en cada muestra estuvo en el rango de 3 a 124 ppm (Cuadro 4). Las muestras de los Perfiles 1, 2 y 3, que tuvieron las menores cantidades de K cambiante, presentaron una absorción menor a 12 ppm de K, en promedio de cada perfil. En cambio, la absorción del K fue media (37 ppm) en los perfiles 5 y 6 correspondiendo con el nivel medio de K cambiante en estos suelos. Las mayores absorciones promedio de K se produjeron en los suelos de los perfiles 4 y 7

(54 y 78 ppm de K, respectivamente), lo que estuvo de acuerdo con su mayor contenido de K cambiante (Cuadro 2).

Los resultados presentados en los Cuadros 2 y 4 evidencian que el K limitó el desarrollo del sorgo al primer corte en las muestras del Ultisol y en las muestras subsuperficiales del Entisol y del Inceptisol distrófico. Para el segundo corte, los rendimientos obtenidos en las muestras de los perfiles 1 al 4 fueron notoriamente menores que aquellos obtenidos al primer corte, mientras que en las muestras de los perfiles 5 a 7 la disminución en el rendimiento no fue tan espectacular. Los menores rendimientos observados en el Ultisol probablemente también se explican parcialmente por una mayor saturación con Al en este suelo (Cuadro 1). En el Alfisol de Pampa Walley, el Inceptisol eutrófico y en el Vertisol, el rendimiento de materia seca al segundo corte fue del orden 60, 78 y 73%, respectivamente, de aquel obtenido al primer corte (Cuadro 4). En el Alfisol y en Inceptisol esto correspondió a una disminución en la absorción de K al segundo corte a niveles de 66 y 85% del K absorbido al primer corte (Cuadro 4). En el Vertisol (Perfil 7), la reducción en la absorción del K al nivel de 44% de lo absorbido al primer corte, pudo deberse a un consumo de lujo durante los primeros 25 días de crecimiento del sorgo, ya que el rendimiento de materia seca al segundo corte solo disminuyó al nivel de 74% con respecto al primer corte.

El K absorbido al primer corte correspondió aproximadamente al 30% del K cambiante inicial, excepto en los Perfiles 1 y 7 (Cuadro 2 y 4). En las muestras de los Perfiles 1 y 7, la relación fue del orden de

Cuadro 4. Rendimiento de materia seca (mg) por maceta y cantidad promedio de potasio acumulado en la parte aérea del sorgo (mg de de K por kg de suelo).

Horizonte	Materia seca/corte		K acumulado/corte		
	1°	2°	1°	2°	Total
PERFIL 1. Sipa Alto (Rhodudult)					
A1	503	76	9.3	2.0	11.3
A3	275	10	3.7	0.1	3.8
B1 1	213	10	2.7	0.1	2.8
B2 1t	208	10	5.7	0.1	5.8
B2 2t	241	10	6.7	0.1	6.8
PERFIL 2. Sipa Bajo (Troporthent)					
Ap	790	272	18.4	6.1	24.5
AC	438	10	7.4	0.1	7.5
C	273	10	3.4	0.1	3.5
PERFIL 3. La Bretaña (Dystropept)					
Ap	857	487	19.1	8.4	27.5
AC	499	172	4.5	2.6	7.1
C1	316	283	7.2	5.1	12.3
C2g	277	10	5.5	0.1	5.6
C3g	315	10	6.6	0.1	6.7
PERFIL 4. Cumbre de Pampa Walley (Rhodudalf)					
A1	995	479	48.7	25.4	74.1
A3	729	469	25.3	27.4	52.7
B1 1	861	485	36.4	16.3	52.7
B1 2	663	405	32.0	21.7	53.7
B2	458	408	20.3	16.2	36.5
PERFIL 5. Pampa Silva Cafetal (Eutropept)					
Ap	940	811	20.7	16.5	37.1
Cca	844	606	15.5	15.1	30.6
C2	676	492	19.4	16.1	35.5
C3	535	425	27.8	14.6	42.4
PERFIL 6. Entaz Bajo (Rhodudalf)					
Ap	806	746	22.3	14.9	37.2
B2 1t	603	596	40.0	15.5	55.5
B2 2t	477	619	25.9	17.5	43.4
B3	626	519	20.5	11.8	32.3
Cca	531	485	8.7	10.8	19.5
PERFIL 7. Pampa Silva Vertisol (Chromudert)					
A1	1.093	867	87.8	35.9	123.7
B2 1	967	682	43.5	20.8	64.3
B2 2	930	655	31.6	18.6	50.2
C	916	677	55.7	19.7	75.4

10% y de 45%, respectivamente, debido a los ya mencionados factores de la acidez cambiante y del consumo de lujo, respectivamente.

El K total absorbido por las plantas correlacionó muy bien con el K extraído con acetato de amonio (K cambiante). con ácido sulfúrico 8 N y con ácido

clorhídrico 0.5 N primera extracción (Cuadro 5). Las correlaciones fueron menores cuando se sumaron las cantidades obtenidas en las dos extracciones sucesivas con ácido clorhídrico, lo que indicó la eficiencia de la primera extracción con ácido clorhídrico en predecir la disponibilidad del K para el sorgo. También se observaron correlaciones menores cuando se usó ácido sulfúrico 6 N o acetato de sodio. No se obtuvo correlación significativa entre las cantidades de K determinadas en ácido sulfúrico 2 N y 4 N y aquellas absorbida por el sorgo. Los resultados evidencian que aquellos métodos que miden el K cambiante son los que proveen un mejor estimado del K del suelo absorbido por las plantas de sorgo. Por ejemplo, cerca del 92% de la variación en la absorción de K al primer corte y del 97% de la absorción total fue predecida por las variaciones en el K cambiante extraído con acetato de amonio. Una respuesta similar es obtenida con el ácido sulfúrico 8 N. Resultados concordantes fueron observados por Nelson (10) en diferentes suelos de Mississippi donde el K extraído con acetato de amonio también fue un buen índice del K absorbido por las plantas de girasol.

Fuentes de K disponible para la planta

Los análisis de las muestras de suelos tomadas antes y después del cultivo con sorgo indican que, en el Entisol, el Inceptisol eutrófico, los Alfisoles y el Vertisol, se puede atribuir la mayor parte del K cambiante (Cuadro 6) En el Inceptisol distrófico, el K absorbido pudo provenir de cantidades equivalentes de K cambiante y de K residual. Es probable que algún factor, presumiblemente el Al, limitó la absorción del K por las plantas en el Ultisol, lo que permitió la acumulación del K cambiante al

segundo corte. De los resultados presentados en los Cuadros 4 y 6 se puede deducir que el K absorbido por las plantas de sorgo provino en 80 a 100% del K cambiante en los suelos con más de 70% de saturación de bases y en 50% del K cambiante y 50% del K fijado en el Inceptisol de baja saturación de bases (Perfil 3).

Los resultados del Cuadro 6 indican que en todos los suelos, excepto el Inceptisol eutrófico (Perfil 5), los cambios en el K cambiante estuvieron acompañados de cambios en el K residual y muy poco en el K fijado. Este resultado no debe interpretarse como que el K fijado no varía, puesto que es bien conocido el equilibrio que existe entre las formas de K en el suelo. La explicación que se asume en este caso es de que probablemente el equilibrio estuvo dirigido a mantener los niveles de K cambiante y fijado en el suelo, a partir del K residual

La disminución en el K total en el suelo con el cultivo (Cuadro 6) explica en todas las muestras las cantidades de K absorbidas por las plantas de sorgo (Cuadro 4). Es interesante resaltar la baja cantidad del K absorbido por los dos cortes de sorgo en relación al K total del suelo (máximo 1.7% en el Vertisol, Cuadro 6). Los porcentajes de K absorbido por las plantas en relación al K total son menores que aquellos informados por otros investigadores (12), debido a que en el presente experimento sólo se pudo obtener dos cortes de sorgo en los suelos que tenían menos de 5.4 de pH (Perfiles 1, 2 y 3).

Luego, los resultados indican que el K estuvo presente en cantidades deficientes al primer corte en el Ultisol, el Entisol y el Inceptisol distrófico. En los otros suelos el K disponible estuvo en un nivel adecuado, pero empezó a limitar los rendi-

Cuadro 5. Coeficientes de correlación entre el potasio disponible por diferentes extractantes químicos y el absorbido por las plantas.

Extractante	K absorbido 1° corte	K absorbido 2° corte	K absorbido total
H ₂ SO ₄ 2N	0.146	0.007	0.327
H ₂ SO ₄ 4N	0.106	-0.329	0.097
H ₂ SO ₄ 6N	0.307	0.151	0.505**
H ₂ SO ₄ 8N	0.938**	0.957**	0.845**
NH ₃ OAc pH 7.0	0.953**	0.948**	0.879**
HCl 1°	0.951**	0.982**	0.826**
HCl 2°	0.651**	0.553**	0.781**
HCl Total	0.331	0.180	0.533**
NaOAc pH 4.8	0.378	0.283	0.481**

** Significativo al 1%.

Cuadro 6. Cambio de las formas de potasio en el suelo y potasio absorbido por el sorgo (promedio por perfil).

Perfil No.	Suelo	No. de horiz.	Forma de K ¹			K absorbido
			Cambiable ²	Fijado	Resid.	K total suelo
			ppm			%
1	Rhodudult	5	+ 18.8	-14.4	-32.1	0.3
2	Troporthent	3	- 9.7	+27.7	-104.7	0.7
3	Distropept	5	- 6.1	+ 2.2	- 6.2	0.4
4	Rhodudalf	5	- 59.8	-18.0	-84.2	1.6
5	Eutropept	4	- 37.8	-30.4	+18.7	1.0
6	Rhodudalf	6	- 29.6	+13.4	-78.9	0.9
7	Chromudert	4	-93.2	-30.2	-188.6	1.7

1 Los signos indican aumento (+) o disminución (-) con respecto al contenido inicial.

2 Incluye al K soluble

mientos al segundo corte, excepto en el Alfisol serie Entaz. Tentativamente se puede sugerir que el nivel crítico entre los suelos deficientes y adecuados en K disponible esta alrededor de 0.15 meq K/100 de suelo. A partir de 0.30 meq K/100 g de suelo se observa el consumo de lujo del K por las plantas. Resultados similares fueron encontrados por Sobulo (16) en diferentes suelos de Nigeria. En vista de que la agricultura de la Selva peruana esta orientada principalmente hacia el establecimiento de plantaciones de especies exigentes en K, tales como el café y los frutales, se hace evidente la importancia de la capacidad de suministro de K a largo plazo en estos suelos. Los resultados indican que en muchos de los suelos ácidos, la fertilización potásica será una práctica obligada para obtener buenos rendimientos en los cultivos.

Resumen

En 31 horizontes de suelos de los órdenes Entisol, Inceptisol, Alfisol, Ultisol y Vertisol del trópico húmedo peruano se caracterizaron las formas de K y su disponibilidad para las plantas de sorgo (*Sorghum vulgare* L.). El K disponible también se extrajo con ácido sulfúrico 2, 4, 6 y 8 N; ácido clorhídrico 0.5 N en dos extracciones sucesivas; acetato de sodio N, pH 4.8 y acetato de amonio N, pH 7.0.

El K total varió entre 1761 y 4548 ppm, encontrándose las menores cantidades en los suelos ácidos y en los arenosos. No se encontró relación entre el K soluble y las otras formas de K. En cambio, la relación K fijado/K cambiable fue un buen índice para agrupar los suelos. Esta relación fue mayor en los suelos más ácidos. Por el contrario, el K cambiable

estuvo presente en mayores cantidades en los suelos menos meteorizados y con mayor contenido de arcilla. El ácido sulfúrico y el ácido clorhídrico extrajeron de 30 a 50% más K que el acetato de amonio (K cambiable), excepto en aquellos suelos con arcillas 2:1 expandibles. El acetato de sodio extrajo sólo de 20 a 65% del K cambiable. La disminución en el K cambiable con el cultivo explica en 80 a 100% la absorción de este nutrimento por el sorgo en los suelos con más de 70% de saturación de bases, mientras que esta disminución sólo puede explicar el 50% del K absorbido por las plantas en los suelos con menos de 50% de saturación de bases. Tentativamente se propone que los suelos deficientes en K tuvieron menos de 0.15 meq de K/100 g de suelo, mientras que sobre 0.30 meq de K/100 g de suelo existió consumo de lujo.

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CERAMBYCIDAE ASSOCIATED WITH THE HOST GENUS *Nothofagus* IN CHILE AND ARGENTINA¹

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Resumen

Los árboles del género *Nothofagus* se consideran importantes para la producción comercial de madera en Chile. En la madera de algunas de estas especies se encuentra comúnmente graves defectos causados por insectos de la familia Cerambycidae. Este trabajo reúne la dispersa información publicada sobre los cerambycidos que se desarrollan en especies de *Nothofagus* en Chile y Argentina. Además, se presentan observaciones originales sobre nueve especies de cerambycidos con datos sobre huéspedes para *Acanthinodera cummingi* Hope, *Microplophorus castaneus* Blanch., *Callisphyris semicaligatus* Fairm. & Germain, *Lautarus concinnus* (Philippi), *Planopus laniniensis* Bosq., *Platynocera lepturoides* Blanch., *Calydon submetallicum* (Blanch.), *Chenoderus testaceus* (Blanch.), y *Holopterus chilensis* Blanch. Existe información sobre 27 especies de cerambycidos que se desarrollan en *Nothofagus*; entre ellos 22 se encuentran en *N. dombeyi* (Mirb.) Oerst., cinco en *N. pumilio* (Poepp. et Endl.) Oerst., 10 en *N. antarctica* (Forst.) Oerst., dos en *N. alpina* (Poepp. et Endl.) Oerst., y seis en *N. obliqua* (Mirb.) Oerst.

Introduction

Beech forests of the genus *Nothofagus* are restricted to the southern hemisphere in the South Pacific region. Similarities between indigenous forests of Argentina and Chile and the related forests in New Zealand are discussed by Clarke (14) and Schmithüsen (36). Unique ecological relationships have developed in these forests, particularly between *Nothofagus* spp. and the wood boring beetles of the family Cerambycidae that utilize these trees as hosts (10, 30).

There are ten species of beech trees in the genus *Nothofagus* native to Chile. The ranges of several of these species also extend into Argentina. Raulí, *N. alpina* (Poepp. et Endl.) Oerst.; coigüe, *N. dombeyi* (Mirb.) Oerst.; roble, *N. obliqua* (Mirb.) Oerst.; and lenga, *N. pumilio* (Poepp. et Endl.) Krasser are of major importance to the timber industry in Chile. Nirre *N. antarctica* (Forst.) Oerst., a widely distributed species often found in association with lenga, is a shrub or a relatively small tree of little commercial importance. Chilean foresters have noted that the timber of some *Nothofagus* species often has much defect due to cerambycid damage. Cerambycids not only cause defect and occasional mortality, but also open infection courts for wood destroying fungi.

The literature on cerambycids associated with *Nothofagus* spp. in Chile and Argentina consists primarily of taxonomic studies, with only scattered information on host preferences. Biological investigations are practically non-existent.

The following authors report numerous original records of cerambycid species which develop in

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Nothofagus spp.: Bosq (3, 6), Fairmaire and Germain (18), Havrylenko and Winterhalter (23), Monrós (28), and Peña (31, 32). In a monograph of the immature stages of the neotropical cerambycids, Duffy (16) has described the larval and pupal stages, and listed distributions and records of host plants for many Chilean and Argentine species. A biological sketch for *Cheloderus childreni* Gray in association with *N. dombeyi* in Chile has been described by Cameron and Real (8). No other detailed accounts of biologies of Chilean cerambycids are available in the literature.

The purpose of this paper is to review known associations of cerambycid larvae with *Nothofagus* spp., to report several new host records and to encourage further work in this area.

Methods

From 1971 to 1974 cerambycid larvae and adults were collected from *Nothofagus* species in the provinces of Cautín and Valdivia in southern Chile. Larval host relationships were established by rearing larvae and pupae from portions of branches and logs in screened laboratory rearing cages, or by physically extracting specimens from the inner bark and wood of trees showing external signs of beetle attack, such as entrance holes or frass. Adult specimens were identified by the authors and some identifications were confirmed by M. Cerda*. Numerous *Holopterus chilensis* Blanch. were captured by placing small wire cages (5x5x10 cm) over exit holes on the boles of host trees. The biology of this species was studied in detail and will be reported in a subsequent paper.

Results

The cerambycid species observed in this study and those reported in the literature to develop in *Nothofagus* in Chile and Argentina are listed in Table 1. Species are grouped by subfamily and listed in alphabetical order within each subfamily. Indicated for each cerambycid are: the countries in which it has been collected, the *Nothofagus* species in which it has been reported to develop, whether or not it has been reported to develop in host genera other than *Nothofagus*, and the condition of the host (live, dead or dying, not specified by the author). The authors of these citations are referred to by numbers which correspond with the numbered list of literature cited

In summary, twenty-seven species of cerambycid beetles are reported to develop in *Nothofagus* spp. Five additional species have been collected in the adult stage from — or the larvae are suspected to develop in — *Nothofagus* spp., but host relationships remain to be confirmed. Twenty-two species develop in coigüe, five in lenga, ten in ñirre, two in rauli and six in roble.

Seven species of cerambycids were observed to develop in *Nothofagus* species in this study, five of which are new host records. A brief summary for each of these observations follows.

Callisphyris semicaligatus Fairm. & Germain — One adult was extracted from a pupal chamber in a live 40-year old rauli (23.5 cm in diameter, 22 m in height and approximately 40 years old). This tree was located in a thinned stand of second growth rauli in the Villarrica Forest Reserve, northeast of Pucón. Approximately 40 percent of the trees in this stand had signs of cerambycid attacks. The larvae construct galleries in the center of the main stem, occasionally making holes to the exterior to extrude frass. The galleries reached a maximum of about 8 x 12 mm in cross section and a length of 1 to 2 m and contain a mixture of fibrous and granular frass.

Calydon submetallicum (Blanch.) — Numerous adults were reared from bolts of *N. obliqua* taken from fallen trees at Fundo Los Pinos, near Valdivia, and Fundo Quechuco, near San José de la Mariquina. The larvae feed in the phloem throughout their development and, upon maturity, bore into the wood to form the pupal chamber. The winding galleries are filled with tightly packed granular frass. Numerous parasitic wasps also emerged from the bolts containing larvae and pupae of *C. submetallicum*.

Chenoderus testaceus (Blanch.) — One adult emerged from dead wood of a *N. dombeyi* branchlet severed by a *Platynocera lepturoides* larva. The branch was collected near the city of Valdivia.

Holopterus chilensis Blanch. — Numerous adults were captured in wire cages as they emerged from pupal chambers within the boles of *N. obliqua* on the University Austral campus in Valdivia, on the Fundos Forestales of the University near Valdivia and on the Fundo Quechuco near San José de la Mariquina. The larvae enter trunks of live robles at the base of the tree and bore in the live wood below the soil level and up to several meters above the ground. Young trees of roble

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commonly are riddled with *H. chilensis* galleries, rendering the wood useless for lumber. This species prefers *N. obliqua*, but one adult was captured as it emerged from the bole of *N. dombeyi*.

Lautarus concinnus (Philippi) — One adult was extracted from a gallery in the wood of a live *N. dombeyi* in the Cordillera Pelada, south of Valdivia*.

Planopus laniniensis Bosq — Two adults were extracted from galleries in the live wood of large limbs of *N. dombeyi* on the Fundo Quechuco near San José de la Mariquina. A wood rotting fungus appears to be associated with this cerambycid species.

Platinocera lepturoides Blanch. — The larvae of this species initially bore in the wood of live branchlets of *N. dombeyi*. Infested branches often break and fall to the ground, where the insects complete their development within the severed branch. Several adults were reared from branchlets collected beneath a large opengrown coigüe near the city of Valdivia.

Discussion

As published reports of larval and adult host records for Chilean cerambycid beetles are brief and incomplete, conclusions discussed here can be only preliminary. Linsley (27) stated that species in the more primitive Prioninae and Lepturinae groups, with the exception of some specialized forms having restricted host ranges, are generally more polyphagous and the polyphagous species are usually associated with dead or decomposing wood. Conversely, cerambycid species developing in live wood are generally believed to be more host specific. As shown in Table 1, the Chilean Prioninae species, *Acanthinodera cummingi* Hope and *Microptophorus castaneus* Blanch. are both polyphagous and develop in dead wood. Both of these cerambycids were collected in this study from the dead wood in stumps of *Pinus radiata*. This is a new host record for the latter species and both are examples of a polyphagous indigenous insect adapting to an exotic host plant.

With the exception of *Callisphyris semicaligatus*, all species of Lepturinae found in association with *Nothofagus* are reported to develop exclusively in

live trees belonging solely to this host genus. This dependency would appear to contradict the general polyphagous habits of the Lepturinae, but these species are associated with hosts restricted to limited ranges in Chile and Argentina.

The species in the unique subfamily Oxypeltinae, recently created by Duffy (16) and revised by Cerda (11), develop exclusively in live *Nothofagus* trees. The Cerambycinae are reported to develop in dead or dying *Nothofagus* host material and several also develop in other host genera. One notable exception is *Holopterus chilensis* which appears to be almost exclusively restricted to the live wood of *N. obliqua*. No conclusions can be drawn for the Lamiinae due to the scarcity of host records for species in this subfamily.

More cerambycid species are associated with coigüe than with any of the other *Nothofagus* species. Coigüe has a wide geographical distribution in Argentina and Chile and it grows on a variety of sites in association with many other tree species (7, 37). The wide variety of habitats in which coigüe grows probably accounts for the large number of cerambycids which utilize it as a host. Conversely, the relatively limited distribution of raulí may account for the small number of cerambycid beetles reported to develop in this species.

The record of *Callisphyris semicaligatus* in young stands of raulí could be of major significance to the forest industry in Chile since plans to reforest large areas of cut-over native forests with raulí currently are under consideration. *C. semicaligatus* has been reported to cause mortality to young trees of *N. dombeyi* in Argentina (23, 38). Another species, *C. vespa* Fairm. and Germain, severs large branches of fruit trees, (*Prunus* and *Malus* spp.) in Chile and Argentina (3, 32, 34). Gara (19) observed extensive wood boring damage by an unknown cerambycid larva in vigorous stands of raulí in the Fundo Jauja, 45 km east of Collipulli in Chile. It is possible that this damage also was caused by *C. semicaligatus*.

Many cerambycid beetles have been reported to develop in the wood of *Nothofagus* species in Argentina and Chile, and some of these beetles cause considerable damage. Clearly, as the value of *Nothofagus* timber steadily increases, there is a need for further research to document additional host associations and to study the biologies of the more damaging cerambycid species.

* Specimen collected by Pedro Real, Universidad Austral de Chile, Valdivia, Chile.

Table 1. Species of Cerambycidae associated with the genus *Nothofagus* in Chile and Argentina*.

Subfamily, genus and species	Larval host and host condition					Possible <i>Nothofagus</i> association	Other host genera
	Distribution	<i>N. alpina</i>	<i>N. antarctica</i>	<i>N. dombeyi</i>	<i>N. obliqua</i>		
Prioninae							
<i>Acanthinodera cummingi</i> Hope	C				NS-13, 17, 33, 34		NS-2, 13, 17 33, 34 D-32, 40
<i>Microplophorus castaneus</i> Blanch.	A, C		D-6, 13, 23 NS-26	D-6, 13, 23 NS-22, 24		D-6, 13	D-3, 13, 23, 28, 40 NS-32
<i>Microplophorus magellanicus</i> Blanch.	A, C		D-13			D-13	
Lepturinae							
<i>Adalbus crassiconis</i> Fairm. & Germain	A, C		L-23			L-23	NS-18
<i>Callisphyris semicaligatus</i> Germain & Fairm.	A, C	L-40**	L-23	L-23, 28			NS-18 L-23, 28
<i>Lautarus concinnus</i> Philippi	A, C		L-12, 23	L-39**			NS-25
<i>Planopus laniniensis</i> Bosq	A, C			L-40**			
<i>Planopus octaviobarrosi</i> Cerde	C			L-38 NS-10			
<i>Platynocera lepturoides</i> Blanch.	C			L-40**			
<i>Sibylla coemeterii</i> Thomson	A, C			NS-6	L-6, 28		
<i>Sibylla flavosignata</i> Fairm. & Germain	A, C			NS-12			NS-18
<i>Sibylla integra</i> Fairm. & Germain	A, C			L-23			
<i>Sibylla livida</i> Germain	C			L-31 NS-32			NS-12
Oxypeltinae							
<i>Cheloderus childreni</i> Gray	A, C			L-6, 8, 11, 23, 24 NS-21, 32 D-28	L-8, 31		NS-20
<i>Cheloderus peñai</i> Kuschel	A, C					NS-32	NS-11, 29
<i>Oxypeltus quadrispinosus</i> Blanch.	A, C	L-6, 11	L-11	L-6, 11, 23 NS-3		L-6, 11 NS-3, 20	NS-18

Table 1 (Cont.)

Subfamily, genus and species	Larval host and host condition					Possible <i>Nothofagus</i> association	Other host genera
	Distribution	<i>N. alpina</i>	<i>N. antarctica</i>	<i>N. dombeyi</i>	<i>N. obliqua</i>		
Cerambycinae							
<i>Callideriphus laetus</i> Blanch.	A, B, C			D-23 NS-17	D-23 NS-17	NS-22	D-23 NS-2, 17
<i>Calydon globithorax</i> (Fairm. & Germain)	A, C**			NS-6			
<i>Calydon havrylenkoi</i> Bosq	A		NS-6	D-6, 23			
<i>Calydon submetallicum</i> Blanch.	A, C		D-23	D-23, 28 L-38	D-40 NS-18, 22	D-32 NS-24	NS-24, 34
<i>Chenoderus bicolor</i> Fairm	C				D-40**		
<i>Chenoderus octomaculatus</i> Fairm.***	A, C			D-23			
<i>Chenoderus testaceus</i> Blanch.	A, C			D-40**	NS-16		D-1, 38
<i>Chenoderus tricolor</i> Fairm & Germain	A, C					NS-6, 18	
<i>Grammicosum Flavofasciatum</i> Blanch	A, C			D-28 NS-22	NS-3, 6		D-28
<i>Xenocompsa flavonitida</i> Fairm. & Germain	A, C			NS-15			
<i>Holoptera chilensis</i> Blanch.	A, C			L-40 NS-32	L-40**		
<i>Maripanus decoratus</i> Germain	A, C			NS-4, 23			
<i>Phymatoderus bizonatus</i> Blanch.	A, C			D-23			NS-16, 35
Lamiinae							
<i>Azygocera picturata</i> Fairm. & Germain	A, C					NS-18, 38	
<i>Paroectropsis decoratus</i> Cerde	C					NS-9	
<i>Tuberopeplus chilensis</i> Breun.	C					NS-29	

* A - Argentina, B - Brazil, C - Chile, L - live, D - dead or dying, NS - host condition not specified; numbers refer to literature citations with the following additions: 38 - Ernesto Kraemer, Valdivia, Chile, personal communication; 39 - Pedro Real, Universidad Austral, Valdivia, Chile, personal communication; and 40 - This study.

** New record.

*** Since the submission of this article, a new genus, *Achenoderus*, has been proposed for *Chenoderus octomaculatus*; NAPP, D. S. Revisao do gênero *Chenoderus* Fairmaire et Germain, 1859 (*Coleoptera. Cerambycidae*). Rev. Brasil, Biol. 39(3):571-585. 1979.

Summary

Several tree species in the genus *Nothofagus* are of major importance to the timber industry in Chile. Serious defects caused by cerambycid beetles are commonly found in the wood of some *Nothofagus* species. This paper summarizes the widely scattered information on cerambycids which develop in *Nothofagus* species in Chile and Argentina. Original observations of host plants are presented for the following nine cerambycid species: *Acanthinodera cummingi* Hope, *Microplophorus castaneus* Blanch., *Callisphyrus semicaligatus* Fairm. and Germain, *Lautarus concinnus* (Philippi), *Planopus laniniensis* Bosq., *Platynocera lepturoides* Blanch., *Calydon submetallicum* (Blanch.), *Chenoderus testaceus* (Blanch.) and *Holopterus chilensis* Blanch. *Nothofagus* is the reported host for the larvae of 27 cerambycid species; 22 species develop in *N. dombeysi* (Mirb.) Oerst., 5 in *N. pumilio* (Poepp. et Endl.), 10 in *N. antarctica* (Forst.) Oerst., 2 in *N. alpina* (Poepp. et Endl.) Oerst., and 6 in *N. obliqua* (Mirb.) Oerst.

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COMUNICACIONES

Evaluation of *Phaseolus coccineus* Lam. germplasm for resistance to common bacterial blight of bean.

Resumen. Fueron evaluados once materiales provenientes de la especie *Phaseolus coccineus* Lam., para conocer su resistencia al anubio bacterial común causado por *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye. Las líneas M 7701, G 35044, N. I. 520 y N.I. 15 fueron altamente resistentes y pueden servir como fuentes de resistencia en mejoramiento genético de frijol para anubio bacterial común.

The common bacterial blight of bean caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye is one of the important seed-transmitted diseases of dry bean (*P. vulgaris* L.) for which efficient chemical control measures are not available and also within the dry bean germplasm very few materials possess acceptable levels of resistance to this disease. In search of adequate sources of resistance to this disease several related *phaseolus* species were tested for blight resistance and the present report is related to the evaluation of *P. coccineus* Lam. germplasm for common blight resistance.

Materials and Methods

Eleven materials of *P. coccineus* obtained from the germplasm bank of CIAT, Colombia were planted in the green house pots in 3 replications along with two blight susceptible local cultivars of *P. vulgaris* as controls. In the third week after germination two pairs of trifoliolate leaves per plant were inoculated with isolate 822A-1 of *X. campestris* pv. *phaseoli* (Smith) Dows, at an approximate concentration of

10^6 cells/ml utilizing modified multiple needle inoculation method. The blight resistance of the inoculated material was recorded 15 days after inoculation on a scale 1-5 as described earlier(2).

Results and discussion

The blight reaction of the eleven *P. coccineus* materials and the susceptible *P. vulgaris* controls are presented in Table 1. As seen from the results M

Table 1. Levels of resistance of *P. coccineus* germplasm for common blight (*X. campestris* pv. *phaseoli* (Smith) Dye.

Identification of Material	Level of Resistance*
<i>P. coccineus</i>	
MITA 46-1	4
N.I. 520 (P.I. 201304)	2-
G 35022 (P.I. 165421)	2
N.I. 2 (P.I. 176672)	3
N.I. 229	2
G 35075	4
M 7701	2-
G 4834 (Puebla 56-C)	3
G 35044 (P.I. 201297)	2-
N.I. 15 (Blanc No. 5)	2-
G 35078 (P.I. 273-448)	2
<i>P. vulgaris</i> controls	
CV. 'Iguaçu'	5
CV. 'Carioca'	5

* Scale of evaluation 1-5 representing resistant to susceptible reaction.

7701, G 35044, N.I. 520 and N.I. 15 possessed high levels of resistance followed by G 35022, N.I. 229 and G 35078 which presented acceptable levels of resistance but with more distinct chlorotic border around the lesion than the earlier group of materials. The rest of the materials showed intermediate to susceptible reaction. Considering the efforts involved in obtaining useful hybrids with high fertility by interspecific crossing, only the first group of material possessing high levels of resistance are adequate for use in the breeding program aiming at incorporation of blight resistance in the commercial cultivars belonging to *P. vulgaris*.

Tolerance to common blight was reported in *P. coccineus* P.I. 165421 (3) and also in populations of *P. coccineus*. The advanced interspecific hybrid progenies of *P. vulgaris* x *P. coccineus* cross showed disease reaction varying from high tolerance to susceptibility (personal correspondence, D. P. Coyne, Univ. Nebraska, USA) and the preliminary results of our selfed and backcrossed progenies of *P. vulgaris* x *P. coccineus* cross confirm the above results. It is important to bring together the genes conferring resistance to common blight originating from different species in order to have higher and stable levels of resistance and as *P. vulgaris* and *P. coccineus* are considered to be closely related species (1) such transference is relatively easier as compared to the other interspecific crosses of the genus *phaseolus*.

Resumo. Onze materiais provenientes da espécie *Phaseolus coccineus* Lam. foram avaliados para resistência à bacteriose comum causada por *Xanthomonas compestris* pv. *phaseoli* (Smith) Dye. As linhas M 7701, G 35044, N.I. 520 e N.I. 15 foram altamente resistentes e poderiam servir como fontes de resistência no melhoramento do feijoeiro para resistência à bacteriose comum.

Summary

Eleven materials of *Phaseolus coccineus* Lam. were evaluated for resistance to common blight caused by *Xanthomonas compestris* pv. *phaseoli* (Smith) Dye. The accessions M 7701, G 35044, N.I. 520 and N.I. 15 were highly resistant and can serve as sources of resistance in breeding dry beans for common blight resistance.

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Peso e teor de óleo de sementes de Mamoneira (*Ricinus communis* L.): Efeito da posição de amostragem do rácameo

Summary. The seed weight and oil content of the castor-bean cultivar Guarani and the introduction IPP, were evaluated at the top, middle and lower parts of the raceme. The evaluations were done in order to verify differences along the raceme.

Significant variations were observed for such characteristics among plants, especially on the apex of the raceme. In general, the seed weight and oil content decreased from the lower to the upper part of the raceme. Significant differences were found in the IPP introduction in relation to "Guarani." IPP introduction has a long raceme with a greater number of fruits than the Guarani cultivar, and could increase the competition for plant metabolites among fruits on the top, middle and lower part of the raceme.

The oil contents must be considered as average values since they were obtained by solvent extraction of several seeds. Individual seed evaluations should present greater differences than the solvent extraction method. Therefore, a standard sampling procedure should be used to obtain representative measurements. The middle and lower part of the raceme appears to be the most suitable for seed sampling.

Despite the tendency of seed weight and oil content to decrease from the bottom to the top part of the raceme, no significant correlation was found between such measurements.

No processo de seleção e melhoramento, a quantificação da variabilidade ambiental em relação a variação genotípica é de importância crítica.

Em plantas oleaginosas, além da variabilidade natural entre plantas em relação ao peso e teor de óleo das sementes, é conhecida a existência de variação dentro da própria planta. Assim, o peso e o teor da semente em soja (2) varia em função da posição desta na planta. No milho, estas características variam em função da posição de inserção da espiga na planta (4) e da localização do grão na própria espiga (5). Para o girassol (3), considerável variação é observada, dependendo da posição da semente no capítulo. Também para a momoneira, já é conhecida a existência de variação em peso, teor e qualidade do óleo, em função da posição do ráculo na planta (6). Como a análise para teor de óleo pode ser feita em sementes individuais, por exemplo, através de ressonância nuclear magnética (NMR), grande variação ambiental poderá ser detectada, a qual poderia mascarar a variabilidade genotípica. Desta maneira, procurou-se verificar a existência de variação em peso e teor de óleo da semente, em função da sua localização no ápice, na parte mediana ou basal do ráculo na momoneira.

Material e métodos

Foram utilizadas duas variedades de mamoneira (*Ricinus communis* L.), o cultivar comercial Guarani e uma introdução coletada no município de Pirapó-zinho – SP (IPP). O primeiro cultivar possui cerca de 60 a 70 frutos e 45 a 55 cm de comprimento do ráculo primário. Já na segunda, o número de frutos pode ultrapassar a 200, medindo cerca de 70 a 90 cm de comprimento o ráculo primário.

Foram amostradas cinco plantas de cada cultivar, dispostas no mesmo local, do Centro Experimental

de Campinas. Os cachos foram subdivididos em três partes, aproximadamente iguais, em número de frutos, para fins de avaliação: apical, mediana e basal. No cultivar Guarani, foram estudados os cachos primários e secundários, já para a introdução IPP, apenas o cacho primário.

As sementes foram avaliadas em peso e teor de óleo. Os teores de óleo bruto e umidade, foram feitos segundo os métodos da American Oil Chemists' Society (1). Os resultados das determinações de óleo bruto foram calculados na matéria seca.

Resultados e discussão

Os resultados obtidos em avaliações de peso e teor de óleo das sementes, são vistos no Quadro 1. Observou-se que tanto o peso quanto o teor de óleo das sementes apresentou maior variação na introdução IPP que no cultivar Guarani, em relação à posição de amostragem no cacho. Para o cacho primário, notou-se que o peso das sementes da parte apical foi menor, da parte basal maior e, da parte mediana intermediária, semelhantemente ao que é conhecido um milho (5). Para o cacho secundário do cultivar Guarani, este fato não foi observado. Contudo, apresentou valores inferiores em relação ao cacho primário, como já era sabido (6).

Apesar de ser ter observado variação significativa entre plantas para o peso de sementes, esta característica apresentou maior variação na parte apical do que na parte mediana ou basal do ráculo.

Com relação ao teor de óleo, verificou-se diferenças significativas apenas na introdução IPP, para as diferentes posições de amostragem do ráculo. Esta introdução além de possuir maior número de frutos, possui também maior comprimento do ráculo primário do

Quadro 1. Peso de 100 sementes e teor de óleo em mamoneira, amostradas na parte apical, mediana e basal do ráculo.

Cultivar	Peso de 100 sementes					Teor de óleo				
	Apical	Mediana	Basal	DMS 5%	CV	Apical	Mediana	Basal	DMS 5%	CV
	g	g	g	g	%	%	%	%	%	%
Guarani R ₁ *	46.0 ± 11.0	47.1 ± 7.8	50.5 ± 8.5	5.0	5.0	52.1 ± 1.9	52.3 ± 2.1	51.7 ± 2.7	2.2	2.3
Guarani R ₂ *	43.5 ± 5.1	41.3 ± 5.6	43.5 ± 5.0	3.1	4.0	51.8 ± 1.9	52.6 ± 1.7	51.8 ± 2.2	1.6	1.7
IPP R ₁	43.3 ± 2.2	45.9 ± 1.6	46.2 ± 1.9	1.9	2.4	50.3 ± 3.3	51.4 ± 4.1	52.5 ± 3.8	1.4	1.5

* R₁ e R₂ = ráculo primário e secundário, respectivamente

que o cultivar Guarani. Uma possível explicação para este fato poderia estar ligada à competição entre frutos do mesmo ráculo, por metabolitos elaborados na planta. Daí a razão para menor peso e menor teor de óleo para as sementes da parte apical. Esta hipótese foi também sugerida para o caso do milho (5). No caso da cultivar Guarani, a competição por metabolitos seria menos intensa, pelo fato do cacho ser menor e apresentar menor número de frutos no ráculo.

Apesar do peso e do teor de óleos das sementes tenderem a diminuir da base para o ápice, foi observada uma correlação muito baixa ($r=0.17$ NS) entre estas medidas, indicando a independência entre elas.

Os resultados obtidos de análise do teor de óleo por extração com solvente, representam valores médios de grupos de sementes. A variação do teor de óleo de semente para semente deverá ser maior e a variação ambiental poderá mascarar possíveis diferenças genotípicas. O critério de amostragem de sementes deverá ser padronizado, evitando coletar sementes na parte apical do cacho, preferindo-se a parte mediana ou basal.

Resumo e conclusões

O peso e o teor de óleo de sementes do cultivar Guarani e da introdução IPP, foram avaliadas visando-se verificar a existência de variação entre a parte apical, mediana e basal do ráculo. No cultivar Guarani, foram avaliadas os ráculos primários e secundários, enquanto na introdução IPP, apenas o primário.

Observou-se acentuada variação para o peso e o teor de óleo das sementes entre plantas, principalmente na parte apical do ráculo. Observaram-se também diferenças entre as três posições amostradas, mais acentuadamente na introdução IPP. De modo geral, tanto o peso quanto o teor de óleo das sementes tendem a diminuir da base para a extremidade apical do ráculo. A introdução IPP apresenta um cacho primário com maior número de frutos e de maior comprimento, do que o cacho do cultivar Guarani, o que acentuaria a competição por metabolitos entre sementes ao longo do ráculo no primeiro material.

A variação no teor de óleo de sementes individuais ao longo do ráculo poderá ser maior que a variação observada apenas nas três posições medidas, uma vez que os resultados em teor de óleo extraído com solvente, foram obtidos a partir de grupos de sementes, representando assim um valor médio. Para a análise do teor de óleo em sementes individuais, pela técnica de NMR por exemplo, se faz necessário uma padronização de amostragem, sugerindo-se evitar a coleta de

sementes da parte apical, preferindo-se as da parte mediana ou basal.

Observou-se também uma baixa correlação entre o peso de sementes e o teor de óleo ($r=0.17$ NS), apesar destas duas medidas diminuírem da base para a parte apical do ráculo, sugerindo a independência entre elas.

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Yield response of yellow yam (*Dioscorea cayenensis*) after disinfesting planting material of *Pratylenchus coffeae*¹.

Resumen. En Jamaica se encuentran varios nematodos dañinos asociados al ñame (*Dioscorea* spp.); sin embargo solo se ha encontrado el *Pratylenchus coffeae* infectando el ñame amarillo (*D. cayenensis*) afectado por la pudrición seca. llamada "quemazón". La pudrición seca puede estar asociada con herida del tallo y del primordio radical; si la herida es severa, en algunos casos la germinación y el vigor de la planta pueden verse seriamente afectados.

En un experimento se obtuvieron plantas provenientes de trozos de ñame desinfectados de *P. coffeae* por inmersión durante 30 minutos en una solución de 2 000 ppm de Oxamyl o por 45 minutos en agua a 45°C. Los rendimientos con estos tratamientos fueron, respectivamente, 36% y 23% superiores y produjeron tubérculos con menos nivel de "quemazón" que los obtenidos de plantas provenientes de tubérculos no desinfectados. Los resultados sugieren que los agricultores deberían usar, para siembra material con baja incidencia de "quemazón". Se recomienda establecer una dependencia cuya inmediata responsabilidad sea desinfestar el material de ñame que se vaya a usar para la siembra; eventualmente se encargaría de suministrar semilla "limpia".

Yams (*Dioscorea* spp) have traditionally constituted the staple root crop in the Jamaican diet. The yellow yam (*D. cayenensis*) is the most popular variety and in 1978 constituted 33% of total yam production of 164 500 tons. Between 1970 and 1979, average tuber yield of yellow yam ranged from 10.8 to 12.8 tons/ha which is considerably below the yield potential of this cultivar.

In Jamaica, several parasitic nematodes are associated with yam plants in the field (5, 6). *Pratylenchus coffeae*, *Scutellonema bradys* and *Hoplolaimus* sp. are involved in the etiology of a dry rot of yam tubers (2, 3, 4, 7, 8, 10). *P. coffeae*, considered to be the most noxious of the nematodes affecting yams (7, 8), is the only nematode found infesting yellow yam tubers which are affected by the dry rot, called "burning" in Jamaica. This condition is characterised by cracking in the skin underlaid by a brown, corky rot in the storage tissues (3, 10). This rot progresses deeper into the yam tissues following harvest and prior to planting or consumption and is generally more pronounced towards the stem end of yam tubers. When a yellow yam tuber is harvested, the stem end ("head") is cut off and retained for planting and the remainder consumed. On heavily "burnt" heads, stem primordia appear to be damaged or destroyed by the dry rot resulting in such heads not sprouting or vines growing from them being less

thrifty than those from less affected heads. The term "less affected" is used as a yellow yam tuber which was not infested by *P. coffeae* and affected to some extent by the dry rot. It has never been observed by the senior author.

When yam tubers affected by the dry rot were disinfested in previous investigations, populations of the invading nematode were reduced and development of the dry rot suppressed. There was a high incidence of sprouting and vines were more vigorous than those from untreated yams (2, 3, 4, 6, 7, 8).

This trial was conducted to investigate qualitative and quantitative yield response after disinfesting yellow yam planting material of *P. coffeae*.

Materials and methods

This trial was carried out at the Allsides Pilot Development Project (1, 9, 11, 12, 13), on recently-terraced plots which had been cropped for two successive years to yellow yam. Recently-harvested yellow yam planting material showing distinct symptoms of the nematode-related dry rot was used. Examination of random samples from the selected heads showed them to be infested with *P. coffeae* (avg 400/10 gm peeling). The following treatments were used: 1) no treatment (control); 2) heads dipped for 45 min. in water at 45°C and 3) heads dipped for 30 min. in a 2000 ppm solution of Oxamyl (Methyl N'N'-dimethyl-N-(methylcarbamoil)oxy)-1-thiooxamide).

Six days after being dipped, the heads were planted, 12 per plot, 0.67 m apart into two continuous mounds 1.5 m apart giving a plant density of 10 000/ha. The three treatments were replicated three times in a randomised complete block design. The soil, an Ultisol classified locally as Wire Fence Clay Loam, Map No. 32, is highly acidic (pH 4.9), and levels of available N, P and K are medium, low and very low respectively.

Cultural practices were those normally followed at Allsides. Weeds were controlled manually. Plants were supplied with a mixture of 200, 300 and 150 kg/ha of N, P₂O₅ and K₂O respectively divided equally at planting and at 16 and 25 weeks thereafter.

Forty weeks after planting, counts were made of *P. coffeae* in soil from each plot. At 43 weeks when the plots were harvested, every tuber was rated for the nematode-related dry rot on a 1-5 scale where 1 = 1-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80% and 5 = 81-100% of the tuber's surface having the dry rot. The weightsof heads (the top portion of the harvested

¹ Part of studies conducted jointly by the Plant Protection Division, Ministry of Agriculture (MINAG), Jamaica and the Inter-American Institute for Co-operation on Agriculture (IICA), at Allsides, Trelawny on the Project titled "Hillside Farming Development Project".

Table 1. Quantitative and qualitative yields of yellow yam (*Dioscorea cayenensis*) tubers harvested 43 weeks after planting *Pratylenchus coffeae*-infested planting pieces (heads) or infested heads disinfested by hot water or nematicide dips.

Treatment	Levels of dry rot on tubers ³	Wt. of table yams harvested from 36 plants (kg)	Wt. of heads harvested from 36 plants (kg)	Total wt. of tubers harvested from 36 plants (kg)	Wt. of yams harvested per kg planted (kg)	Calculated gross yields (tons/ha)
Untreated heads	2.6	58.0	46.8	104.8	3.82	29.1
Hot water-dipped ¹ heads	2.2	75.1	54.6	129.7	4.95	36.0
Oxamyl-dipped ² heads	2.0	91.1	51.7	142.8	5.05	39.7
LSD 5%	—	2.8	—	5.2	—	—

1. Heads dipped for 45 min. in water at 45C.
2. Heads dipped for 30 min. in a 2 000 ppm solution of Oxamyl.
3. Tubers rated for the nematode-related dry rot on a 1 - 5 scale where 1 = 1-20%, 2 = 21-40%, 4 = 61-80% and 5 = 81-100% of the tuber's surface having the dry rot.

tuber which is retained for planting) and table yams (the rest of the tuber) were taken separately. Counts were made of *P. coffeae* in the skin of harvested tubers.

Results and discussion

There was 100% germination of heads and every plant produced a tuber. Presented in Table 1 are the tuber yields and levels of dry rot for the different treatments. Tubers borne by plants growing from heads disinfested by Oxamyl or hot water showed lower levels of the nematode-related dry rot than tubers borne by plants from untreated heads. Plants from disinfested heads produced greater quantitative yields of heads and significantly greater weights of table yams than those from untreated heads. Overall, plants from disinfested heads bore significantly greater weights of tubers than plants from undisinfested heads. Substantially higher numbers of *P. coffeae* were found in the skin of tubers borne by plants arising from untreated heads but three weeks before harvest, there was no difference between treatments in the numbers of this nematode in the soil from plots (Table 2).

Results from this trial confirm previous findings that disinfesting yellow yam planting material of *P. coffeae*, the most noxious of the nematodes affecting *Dioscorea* spp, will result in significantly

Table 2. Numbers of *Pratylenchus coffeae* in soil and in the skin (peeling) of yellow yam (*Dioscorea cayenensis*) tubers harvested from plots in which nematode-infested planting pieces (heads) or infested heads disinfested by hot water or nematicide dips were planted.

Treatment	No. <i>P. coffeae</i> / 100 cc soil at 40 weeks	No. <i>P. coffeae</i> / 10 gm tuber skin at harvest (43 weeks)
Untreated heads	27	48
Hot water-dipped ¹ heads	29	2
Oxamyl-dipped ² heads	13	5

1. Heads dipped for 45 min. in water at 45C.
2. Heads dipped for 30 min. in a 2 000 ppm solution of Oxamyl.

increased tuber yields (7, 8). Disinfesting heads with hot water or Oxamyl resulted in increased quantitative yields of 23% and 36% respectively. Disinfesting yam heads can be costly (estimated at over \$ 100 per ton for Oxamyl treatment of yellow yam heads) but the high initial expenditure is easily recovered from the increased yields (estimated to result in revenue exceeding \$ 600 from each ton of planted yellow yam heads).

Tubers borne by plants arising from disinfested heads showed less of the nematode-related dry rot than those from plants growing from untreated heads. It has been observed in an on-going trial that plants growing from yellow yam heads with low levels of the *P. coffeae* related dry rot sprouted earlier and are more vigorous than plants growing from heavily dry rotted heads. It appears that levels of *P. coffeae* infestation and dry rotting of yellow yam heads have a direct bearing on the performance of plants arising from such heads. Any treatment that will reduce populations of an invading nematode and levels of the dry rotting of yam planting material should therefore be beneficial.

Recommendations

It is recommended that growers should use yellow yam planting material with the least evidence of the dry rot. However, in Jamaica, yams are always infested with noxious nematodes and those involved in the dry rot, especially *P. coffeae*, are ubiquitous. In any event, good yam planting material is generally unavailable and costly, and growers are generally forced to plant what they have or can obtain. Given this situation, it would be beneficial to the yam industry if an agency were established to see to the disinfestation of available planting material in the first instance and eventually be responsible for providing "clean" planting material. In this context, it is hoped that the Ministry of Agriculture would take the necessary steps which would assure increased production of this staple food crop.

Abstract

Several noxious nematodes are associated with yams (*Dioscorea* spp.) in Jamaica but *Pratylenchus coffeae* is the only one found infesting yellow yam (*D. cayenensis*) tubers affected by a dry rot called "burning". The dry rot appears to be associated with injury to stem and root primordial and in cases where the injury is severe germination and plant vigour are seriously impaired.

Plants growing from yellow yam heads disinfested of *P. coffeae* by dipping for 30 min in a 2 000 ppm solution of Oxamyl or for 45 min in water at 45°C produced 36% and 23% greater quantitative yields of tubers which showed lower levels of the nematode-related dry rot than tubers borne by plants arising from undisinfested heads. Results suggest that growers should use yellow yam planting material with the least evidence of the dry rot. It is recommended that an agency be established whose immediate responsibility would be to disinfest available yam

planting material and eventually be responsible for providing "clean" planting material.

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Behaviour of ^{14}C -metalaxyl in Brazilian soils.

Resumo. Estudou-se em laboratório o comportamento do ^{14}C -metalaxyl em três tipos de solos brasileiros (Gley Húmico, Latossolo Roxo e Latossolo Vermelho Escuro). O ^{14}C -metalaxyl apresenta alta mobilidade nas três cromatografias de solo e baixos coeficientes de sorção, mesmo em solos com alto teor de matéria orgânica (Gley Húmico e Latossolo Roxo) e foi persistente nos três solos pelo período estudado de 8 meses. A degradação do metalaxyl no solo Latossolo Roxo correspondeu após esse tempo à 29% , representada por um metabolito não identificado detectado pela cromatografia em camada delgada. A população bacteriana foi maior no Latossolo Roxo e provavelmente associada à esse processo de degradação

Metalaxyl is a new systemic fungicide (CGA 48988) (DL-methyl-N (2, 6-dimethyl-phenyl)-N-(2-methoxyacetyl) alaninate) with specific activity against pathogens belonging to the order Peronosporales (1, 2, 6, 7). The systemic activity of metalaxyl in azalea by applying it to the soil has been demonstrated (1). The structure of systemic fungicides applied to the soil can be altered by

chemical breakdown process and by action of the microorganisms. Also uptake of fungicides by plants from the soil can be very inefficient if there is a tight adsorption of the fungicide to the soil.

The purpose of this investigation was to obtain information on the behaviour of this new compound when applied to samples of Brazilian soils.

Materials and methods

Chemicals

Metalaxyl WP 25 was provided by Ciba Geigy, Brazil and ^{14}C -metalaxyl by Ciba Geigy, Basel, Switzerland. The ^{14}C -metalaxyl uniformly ring labelled had a specific activity of 46.5 $\mu\text{Ci}/\text{mg}$.

Soils

The three soil types used, consisted of Humic Gley, Red Latosol and Dark Red Latosol, differing in their properties (Table 1). Ten gram samples of each dried soil were added to wide necked screw capped jars, followed by addition of water to 2/3 field capacity. A week after, 1.0 ml of a mixture of ^{14}C -ring labelled metalaxyl (140.000 dpm/ml) and unlabelled metalaxyl (10 ppm) in acetone, was added on each soil sample. During the test, water was added periodically to maintain the moisture content of the soil. This was achieved by monthly quantity of distilled water to attain the desired soil water content. Duplicate samples of each soil were incubated at 20 – 25°C and the entire flask content were extracted at monthly intervals over an 8-month period.

Extraction of ^{14}C -metalaxyl

Each sample was extracted with 50 ml of methanol, by shaking the mixture for 4 hr. When necessary a sequence of two extractions with methanol was realized. The soil was separated by

Table 1. Properties of the soils

Soil Type	Organic matter (%)	Clay (%)	Sand (%)	pH	Microbial population after 8 months (g of soil) 10^3	
					Bacteria	Fungi
Humic Gley	4.3	32	57	5.7	34	4.7
Red Latosol	3.8	7.72	62	5.3	136.7	6.7
Dark Red Latosol	2.0	63	24	4.8	11.4	7.7

allowing to stand overnight. The extracted products in methanol were quantified by scintillation counting and thin-layer chromatography.

Scintillation counting

A Nuclear Chicago Liquid Scintillation Spectrometer, model Mark 1, was used for counting. Aliquots of each sample were transferred to scintillator vials, and after evaporation of methanol the scintillation counting solution was added (5). Recovery of ^{14}C -metalaxyl is presented as percent ^{14}C representing the ^{14}C recovered on each extraction over the ^{14}C -metalaxyl added.

Thin-layer chromatography (tlc)

Silica gel thin-layer plates (Merck F 256) were used for separation of extracted samples: 10 ml samples of soil extracts were concentrated and spotted on the plates, developed in the solvent systems either benzene:ether (BE) (50:50) or acetone: ether: acetic acid (Ace Eth AcoH) (79:20:1). ^{14}C -metalaxyl was chromatographed at the same time as reference.

After drying, the plates were exposed to X-ray films. The amount of degradation was determined by dividing the plates in 1 cm zones, scraping the silica into scintillation vials and assaying by scintillation counting (4).

Values were calculated as percent degradation representing the dpm per spot over the sum of dpm's in the plates.

Combustion technique

Residues of ^{14}C -metalaxyl, together with any ^{14}C -labelled degradation products in the soils not recovered after a sequence of two extractions by methanol, were determined by soil combustion (8).

Adsorption experiment

^{14}C -metalaxyl solution (10 ml = 2.5 μg) in 0.01 M calcium chloride was added to air dried soils (1.0 g) in wide necked screw capped jars. After shaking for 4 hr the soil was allowed to stand, and 1.0 ml aliquot of the supernatant solution taken for ^{14}C assay. The soil/water distribution coefficient (K) was calculated as concentration on soil (dpm/g) over the equilibrium solution concentration (dpm/ml).

Soil thin-layer chromatography

Mobility of the fungicide in the soils was determined by soil tlc techniques, and ^{14}C -metalaxyl in the plates was located by autoradiography (3, 4).

Soil microbial population

Soil microorganisms were enumerated at the end of the study by soil dilution. Soil dilutions were plated in triplicate on Czapek-Dox medium agar, and the plates incubated at 28°C for one week. The Czapek-Dox medium consisted of (g/l): saccharose: 30, NaNO_3 : 3, K_2HPO_4 : 1, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.5, KCl 0.5, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.1 and agar 15. Bacteria

Table 2. Recovery of radiocarbon (%) from soils treated with ^{14}C -metalaxyl

Soil Type	Methods for determining radioactivity	Time (days)							
		0	30	60	90	120	150	210	240
Humid Gley	1 st Extraction	95	92	71	72	47	62	75	66
	2 nd Extraction	—	—	27	25	12	15	10	13
	Wet Combustion	—	—	—	—	21	17	14	11
	Total	92	92	98	97	80	94	99	90
Red Latosol	1 st Extraction	92	82	56	62	47	51	55	55
	2 nd Extraction	—	—	30	33	13	11	15	14
	Wet Combustion	—	—	—	—	35	26	25	18
	Total	92	82	86	85	95	88	95	87
Dark Red Latosol	1 st Extraction	95	95	69	70	51	66	73	67
	2 nd Extraction	—	—	25	19	11	10	13	9
	Wet Combustion	—	—	—	—	18	16	13	10
	Total	95	95	94	89	80	92	99	86

were counted after 3 days and fungi colonies after 7 days. Populations are expressed as colonies per gram of soil.

Results and discussion

Recovery of metalaxyl from soils as a function of incubation time is presented on Table 2. After a 30 days incubation of ^{14}C -metalaxyl in soils, one solvent extraction was sufficient to recover most of the radiocarbon added to the three soils. After 60 days a second extraction of the samples with methanol was necessary to recover the same amounts of radiocarbon. As ^{14}C -metalaxyl aged in the soils, it was necessary to combust the samples to recover the amount of radiocarbon not extracted by methanol (Table 2). During the period of time from 120 to 240 days, values of radiocarbon adsorbed to the soils and detected by wet combustion were in the range of 10 to 30%. Red Latosol was the soil that showed the higher percentage of radiocarbon not recovered by the solvent and these amounts can be considered as bound residues or degradation of metalaxyl to more polar compounds, not extracted by methanol (Table 2). Comparing with results obtained in the same soils with a benzimidazole fungicide like carbendazim (5) metalaxyl showed to be less adsorbed to the soils and easily extracted by the organic solvent.

Thin-layer chromatography of soil extracts showed that most of the recovered radiocarbon chromatographed was ^{14}C -metalaxyl; however a metabolite

was detected in higher amounts in Red Latosol extracts. The percentage of this metabolite (R_f 0, 10 in BE), varied from 14% in the 60 days to 29% in 240 days extracts (Table 3).

When this compound was eluted from plates developed in BE, followed by further chromatography in Ace Eth AcoH the activity was located in a zone of tlc at R_f 0.3 to 0.4; ^{14}C -metalaxyl had an R_f 0.67 in this solvent. The second methanolic extraction from soils incubated over an 8 month period with ^{14}C -metalaxyl showed after chromatography the same pattern of degradation, as assessed by tlc.

Red Latosol supported a steady increase in the degradation. This observation may be related with the greater bacterial population noted in the Red Latosol and therefore the reduced degradation in Dark Red Latosol, a consequence of a much less active microbial population (Table 1). Though in less amount this metabolite is also detected in Humic Gley soil; however the variation at each sampling time is probably a consequence of the fluctuating microbial population with time.

Sorption coefficients of ^{14}C -metalaxyl in Humic Gley, Red Latosol and Dark Red Latosol were respectively of 0.80; 0.56 and 0.36. Sorption results are in agreement with the high mobility of ^{14}C -metalaxyl on soil thin-layers of Dark Red and Red Latosol soils and to an intermediate mobility of the fungicide on Humic Gley soils (Figure 1).

Table 3. Percent of metalaxyl and a metabolite detected in extracts from three soils incubated with ^{14}C -metalaxyl.

Soil Type	Compounds	Days after treatment					
		30	60	90	120	210	240
Humic Gley	Metalaxyl	82	80	78	79	72	72
	Metabolite	4.0	5.0	6.3	4.6	2.0	10
Red Latosol	Metalaxyl	83	80	63	74	64	68
	Metabolite	6.7	14	17.4	12.6	13	29
Dark Red Latosol	Metalaxyl	85	82	89	77	72	75
	Metabolite	2.0	5.5	5.1	5.3	3.4	4.2

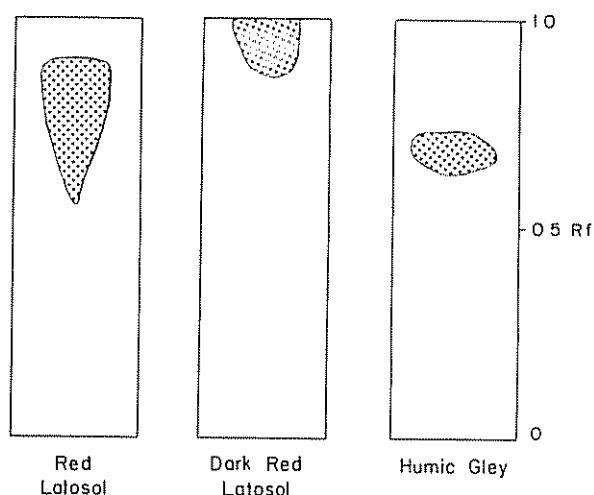


Fig 1. Movement of ^{14}C -metalexyl on soil thin-layer plates.

The data obtained in laboratory tests indicate a persistence of ^{14}C -metalexyl in soils and therefore its low sorption coefficients associated with its systemic activity (2, 6) indicate a long term availability for the control of soil plant pathogens. However, its low sorption coefficient and the high mobility also indicated a facility for leaching, and this must be considered in field applications

Over an 8-month period, only one metabolite was detected from ring - labelled metalexyl, indicating a high stability of the benzene ring to microbial attack and that degradation in these soils proceeded probably by hydrolyzation in the side chain of the molecule.

Persistence of metalexyl showed to be dependent on soil type and the degradation process probably associated with a bacterial population on soils.

Summary

Behaviour of ^{14}C -metalexyl in three types of Brazilian soils (Humic Gley, Red Latosol and Dark Red Latosol) was studied in the laboratory. Metalexyl had high mobility on the three soils thin-layers and low sorption coefficients, even in soils with high organic matter content (Humic Gley and Red Latosol). Metalexyl was persistent on the three soils over an 8-month period. Degradation of metalexyl in Red Latosol was 29 percent after this time, represented by a non identified metabolite detected by tlc analysis. Bacterial population was higher in Red Latosol and probably associated with this degradation process.

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