Effects of bean maturity on seedling vigour in cacao (*Theobroma cacao*)*

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COMPENDIO

Estudios llevados a cabo desde 1965 en la Estación Experimental de Gambari, del Cocoa Research Institute of Nigeria, han mostrado que el estado de madurez de las almendras de cacao tiene efectos definidos sobre su desarrollo y viabilidad. La madurez de las almendras afecta también el crecimiento de las plántulas mientras que las mazorcas verdes que han alcanzado su tamaño máximo pueden suplir almendras capaces de producir plántulas vigorosas o más vigorosas que aquellas de mazorcas fisiológicamente maduras. Usando plántulas más vigorosas, es posible reducir el período en el almácigo hasta en tres meses, mientras que las mazorcas que han alcanzado el tamaño máximo y color antes de la madurez fisiológica pueden ser también usadas para obtener esas plántulas para plantación en el campo.

Introduction

ATTEMPTS have been made in the past to measure the effects of pod maturity on bean viability and growth of cacao seedlings (1, 2, 3, 10). The results obtained since 1965 when the first studies were initiated at the Gambari Experiment Station of the Cocoa Research Institute of Nigeria (CRIN) have shown that the maturity of cacao pods affects both bean viability and seedling growth. No justification has so far been found for selecting only ripe cacao pods for raising seedlings for large scale planting as previously recommended (7, 8). On the other hand beans from mature green pods or pods that were only partially ripe have been found to produce seedlings which were as vigorous as or more vigorous than those raised from ripe pods (4).

This report deals with the results obtained in two previous experiments on the influence of bean maturity on total germination, seedling height and foliage production. The results on pod and bean development as well as bean viability obtained from the same experiments were reported earlier (4).

Materials and Methods

Pods of three Crosses, C77 × C23, C67 × C77 and C75 × C14 were used in the experiments. Clones C67, C75 and C77 are Upper Amazon selections while C23 and C14 are local Trinitario selections referred to earlier by Toxopeus in his reports on establishmentability (11, 12). Hand pollination was carried out in all cases to enable the age of pod from the time of pollination to be used as the measure of pod and bean maturity. There were 5 treatments represented by the symbols T1, T2, T3, T4 and T5 in each of the two experiments carried out.

Each treatment was replicated 4 times with 25 beans sown per replicate. A randomized block design was used in both experiments. The beans were sown in 25.4 × 12.7 × 5.1 cm, 0.005 gauge black polythene pots filled with top soil. Only Cross C67 × C77 was used in Experiment 1 while a mixture of the 3 Crosses was needed in Experiment 2 to provide enough samples.

In Experiment 1 the flowers were pollinated on the same day and the pods for the first treatment were harvested on January 21st 1975 with other treatments harvested subsequently at forthnight intervals to provide the 5 maturity treatments of 106, 120, 134, 148 and 162 days from pollination.

In Experiment 2, the Flowers were pollinated at fortnight intervals to provide the same 5 treatments as in Experiment 1. The pods were however harvested

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the same day on February 24, 1975 when they were 110, 124, 138, 152 and 166 days old. The maturity treatments in Experiment 2 were therefore produced by reversing the pollination and harvesting procedure in Experiment 1. This was done purposely to ensure that differences observed between maturity treatments in previous experiments where the pods in the different treatments developed over different but overlapping periods were not due mainly to differences in the climatic conditions during pod development. The pollination procedure and details about the treatments in both experiments are shown on Table 1. Sowing of the beans took place the day following each harvest.

In Experiment 1, leaf number and seedling height were recorded as from 10 weeks after sowing and subsequently at forthnight intervals till 24 weeks, the end of the nursery period. The same records were taken in Experiment 2 but these commenced as from 4 weeks after sowing.

The data on leaf number and seedling height were analysed statiscally at the end of the nursery period to detect any differences between the treatment means.

Due to unequal number of seedlings resulting from differences in bean viability per treatment as reported earlier (4), the analysis was carried out on 5 randomly selected data from each replicate. In Experiment 1 comparison was limited to only 4 of the 5 original treatments because the beans from 106 day old pods, i.e. Treatment T1, were not viable.

Results and discussion

Foliage production

In both Experiments 1 and 2 the differences between the effects of bean maturity treatments on foliage production were apparent as from the first records (Tables 2 and 3). Although leaf number per seedling tended to increase slightly with increase in bean maturity initially in Experiment 1, this trend did not persist and by the end of the nursery period the seedlings raised from the most mature beans (T5) recorded fewer leaves than the other treatments (Table 2). Only the difference between treatments T3 and T5 was however significant at that stage ($P = 0.05$). The pattern was somewhat different in Experiment 2 where leaf number continued to increase wits increase in bean maturity throughout the nursery period (Table 3). Only the difference between the least mature (T1) and the most mature beans (T5) was significant ($P = 0.05$) at 24 weeks.

The difference observed in the two experiments might be attributed to the different procedures adopted for producing the treatments as described earlier (Table 1). Seasonal factors probably exerted a greater influence on the results obtained in Experiment 1 where a greater part of the growth of cacao pods in each treatment covered different periods of time as was also the case for the seedlings, unlike in Experiment 2. This resulted from the fact that pollination for each treatment took place the same date while

Table 1.—Details of pollination date and the bean maturity treatments

Treatment*	Pollination date		Pod growth period		Harvesting date	
	Expt. 1	Expt. 2	1	2	1	2
T1	Oct. 10, 1974	Nov. 6, 1974	Oct. 10, 1974 to Jan. 21, 1975	Nov. 6, 1974 to Feb. 24, 1975	Jan. 21, 1975	Feb. 24, 1975
T2	"	Oct. 23, 1974	Oct. 10, 1974 to Feb. 4, 1975	Oct. 23, 1974 to Feb. 24, 1975	Feb. 4, 1975	Feb. 24, 1975
T3	"	Oct. 9, 1974	Oct. 10, 1974 to Feb. 18, 1975	Oct. 9, 1974, to Feb. 24, 1975	Feb. 18, 1975	Feb. 24, 1975
T4	"	Sept. 25, 1974	Oct. 10, 1974 to March 4, 1975	Sept. 25, 1974 to Feb. 24, 1975	Mar. 4, 1975	Feb. 24, 1975
T5	"	Sept. 11, 1974	Oct. 10, 1974 to March 18, 1975	Sept. 11, 1974 to Feb. 24, 1975	Mar. 18, 1975	Feb. 24, 1975

* T1, T2, T3, T4 and T5 represent beans obtained from cacao pods at 106, 120, 134, 148 and 162 days from pollination for Experiment 1 and at 110, 124, 138, 152 and 166 days, respectively for Experiment 2 in all tables

Table 2: Leaf number per seedling at different stages in Experiment 1.

Treatment*	Weeks			After		Sowing		
	10	12	14	16	18	20	22	24
T2	7.1	8.7	10.4	12.3	14.7	15.1	16.1	16.6
T3	7.2	9.3	10.1	12.8	14.1	15.6	16.7	17.6
T4	8.2	10.5	12.4	13.6	15.0	14.5	16.2	16.2
T5	10.9	10.9	12.0	13.1	12.3	14.5	15.4	15.0

L.S.D. (P = 0.05) at 24 weeks = 1.8

* T2, T3, T4 and T5 represent 120, 134, 148 and 162 days respectively after pollination

sowing of the beans took place on different dates in Experiment 1, unlike in Experiment 2. The effects of season and other factors besides that being tested would appear to have been reduced to the minimum in Experiment 2 where the pods were harvested on the same date for all treatments and raised in the nursery simultaneously over the same period.

Seedling height

Seedling height increased with increasing bean maturity in both experiments throughout the nursery period (Tables 4 and 5). While in Experiment 1 differences between treatments T2 and T3 on one hand T4 and T5 were significant at the end of the

Table 3: Leaf number per seedling at different stages - Experiment 2.

Treatment*	Weeks				After				Sowing		
	4	6	8	10	12	14	16	18	20	22	24
T1	3.4	4.5	5.6	7.1	8.7	9.9	11.4	11.4	11.4	12.5	12.5
T2	4.2	5.0	6.0	7.5	9.1	10.4	11.7	12.7	12.7	13.8	14.1
T3	4.2	5.1	6.0	7.6	9.2	10.7	12.2	13.5	13.5	14.2	14.2
T4	4.2	5.3	6.6	8.0	9.7	11.2	12.4	13.6	13.6	14.6	14.6
T5	4.2	5.6	7.6	8.1	10.4	11.4	12.5	14.1	14.1	14.8	15.4

LSD (P = 0.05) at 24 weeks = 2.2

* T1, T2, T3, T4 and T5 represent 110, 124, 138, 152 and 166 days respectively after pollination.

Table 4: Seedling height (cm) at different estages - Experiment 1

Treatment	Weeks After Sowing							
	10	12	14	16	18	20	22	24
T2	12.9	13.8	15.0	19.3	23.3	24.1	27.3	30.9
T3	15.8	20.0	20.5	26.3	28.0	27.1	31.4	33.5
T4	19.8	23.9	27.9	29.6	32.6	34.3	37.3	40.5
T5	22.1	26.3	28.6	32.7	32.8	39.8	42.9	46.1

LSD (P = 0.05) at 24 weeks = 5.5

nursery period (Table 4), only the difference between T1 and T5 was significant (P = 0.05) in Experiment 2 (Table 5). Unlike in foliage production it is noteworthy that the relative effects of bean maturity on seedling height were similar in the two experiments in spite of the differences in the procedure used for treatment application as described earlier.

It is clear from the results obtained here and from earlier studies (2, 3) that the more mature the cacao beans are the taller the seedlings produced (Fig. 1). This cannot be considered as an asset by itself, as casual observations have revealed a tendency for the taller seedlings to possess longer taproots which usually outgrow the 25.4 cm tall polythene pots into the nursery bed by the end of the nursey period of 20-24 weeks (Fig. 2). This usually results in deformation of the taproot and often the death of its tip. This is therefore a factor that counts against the use of fully ripe or overripe pods for raising seedlings. And the experience in these studies have shown that ripening or overripening can occur any time after about 162-166 days from pollination (2, 4).

The seedlings produced by the different bean maturity treatments used here were all cansidered vigorous enough for field planting (Fig. 3). And according to Freeman (9), provided a seedling is big enough to transplant, its original size has no bearing on its subsequent growth in the field. One can therefore assume that initial differences observed in growth of seedlings tend to disappear in the field. This

Table 5: Seedling height (cm) at different stages Experiment 2

Treatment	Weeks After Sowing										
	4	6	8	10	12	14	16	18	20	22	24
T1	9.1	11.6	12.4	12.3	14.1	18.4	18.3	21.8	23.3	25.3	27.2
T2	11.8	14.9	14.9	17.1	18.5	21.3	21.5	25.5	26.6	29.5	32.9
T3	13.0	15.4	15.9	17.9	18.6	22.5	22.8	27.5	28.7	30.6	33.5
T4	14.7	17.5	18.1	20.5	21.2	25.9	26.3	31.7	32.4	34.1	38.0
T5	15.4	16.6	17.5	20.6	22.2	25.4	27.4	30.9	31.6	32.8	36.8

LSD (P = 0.05) at 24 weeks = 10.3

would explain why previous experiments at the Station have shown that the largest plants at transplanting time were not necessarily the largest two years later (9)

The results obtained here also revealed that as high as 79 - 93 per cent germination were recorded from February sowing of beans obtained from 134-152 day old unripe immature pods (Table 6). Even sowing as late as March 5 recorded 95 per cent in Experiment 1 In contrast, annual sowing is usually carried out from November to December. Good bean germination can therefore still be obtained with late sowing. The nursery period in both experiments therefore commenced 2-3 months later than usual. Inspite of this late sowing the seedlings produced by Treatments T4 and T5 at 18 weeks from sowing were as vigorous as those produced by Treatments T1 and T2 at 24 weeks, the end the normal nursery period (Tables 3 and 4). The later seedlings from T1 and T2 were earlier considered vigorous enough for field planting. There is therefore no need to fear that seedlings raised over a shorter nursery period will not be vigorous enough for field planting In fact seedlings that are smaller than usual have been transplanted at the Station in the past when circumstances had not permited prompt sowing and no unusual perfomance was noticed. Freeman (9) has in fact recorded superior perfomance in surviving trees in the field when sowing was done in February 1961 under shaded nursery conditions followed by field planting in June. It is therefore suggested that the current nursery period of 5 to 6 months (20 to 24 weeks) recommended by Breeman can be reduced by up to 3 months and still successfully raise seedlings that

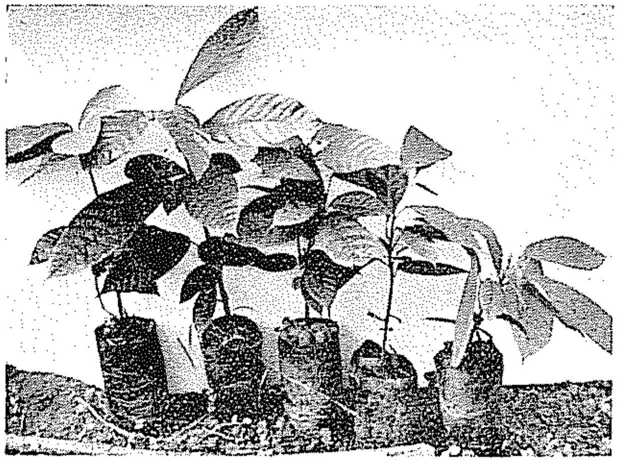

FIG 1

One representative seedling on height basis alone from treatments T5. T4. T3 T2 and T1 (Left to Right) - Expt 2

can be transplanted to the field by June. This could be achieved by sowing beans in February as opposed to November or December, using pods described earlier as varying from mature unripe and green to those showing signs of ripening now known to produce the most vigorous seedlings at the CRIN nursery (3). These pods are the equivalents of treatments T4 and T5 Some of the benefits from a shorter nursery period and the use of pods of lesser maturity than the traditional ripe pods would include savings in precious nursery time and labour as well as the production of better seedlings for transplanting at the end of the

Table 6: Sowing date, nursery growth period and final germination.

Treatment	Sowing date		Nursery growth period		% germination	
	Expt 1	Expt 2	1	2	1	2
T1	January, 22 1975	Feb 25, 1975	(Not viable)	Feb 25 to Aug, 12, 1975	—	18
T2	February 5, 1975	Feb 25, 1975	Feb 5 to July 23. 1975	Feb 25 to Aug. 12, 1975	42	75
T3	February 19, 1975	Feb 25, 1975	Feb 19 to Aug 6, 1975	Feb. 25 to Aug, 12, 1975	93	79
T4	March 5, 1975	Feb 25, 1975	Mar 5 to Aug 20, 1975	Feb 25 to Aug. 12, 1975	95	82
T5	March 19, 1975	Feb 25, 1975	Mar 19 to Sept 3, 1975	Feb 25 to Aug, 12, 1975	91	85

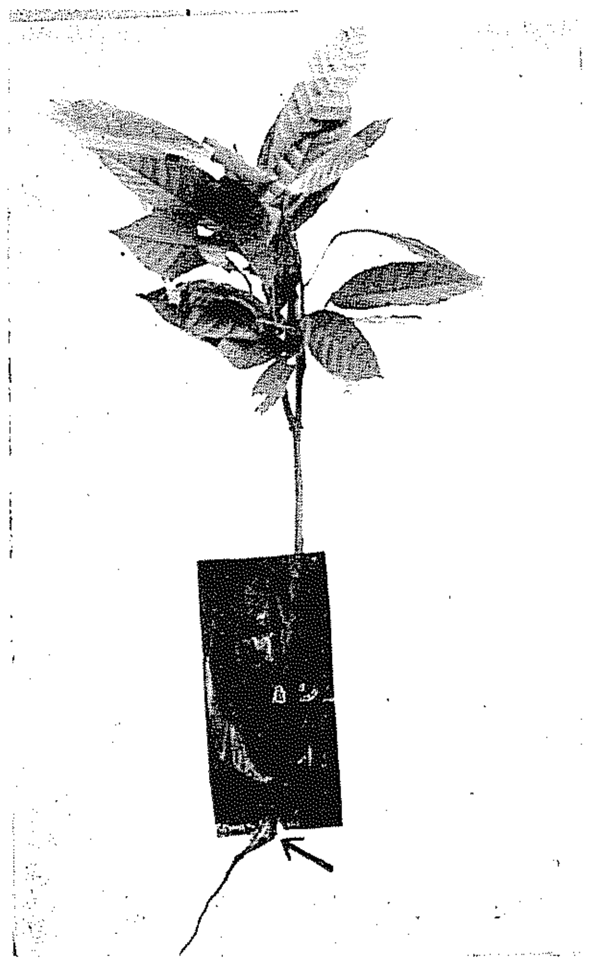

FIG 2.

Cacao seedling with taproot bent at point of contact with nursery bed (Indicated by arrow) after outgrowing 25.4 cm deep polythene pot

nursey period. Better in the sense that the taproot development will be limited in growth to the 25.4 cm polythene pots at the time of field planting in May or June because of the shorter nursery period. This will facilitate removal of the potted seedlings from the nursery with their taproots still intact and healthy. This aspect of nursery practice at the Station however still deserves further investigation.

In conclusion, the results discussed here and those obtained from previous studies carried out at the Station on this subject show that seedlings raised from relatively immature beans obtained from unripe cacao pods are vigorous enough for field planting. Beans obtained from green mature pods harvested just prior toripening in fact produce more vigorous seedlings than those of traditional ripe pods. There is therefore no by work done at Tafo in Ghana (6). Therefore when ever pods are required for raising seedlings on a large scale pods varying from mature green to those showing signs of ripening would also be adequate.

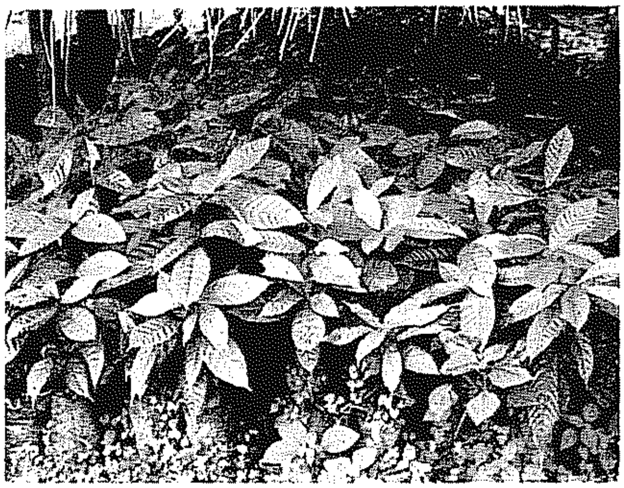

FIG 3

Seedlings of Expt 1 two rows each from Left to Right, from Treatments T2, T3, T1 and T5 at the end of the nursery period

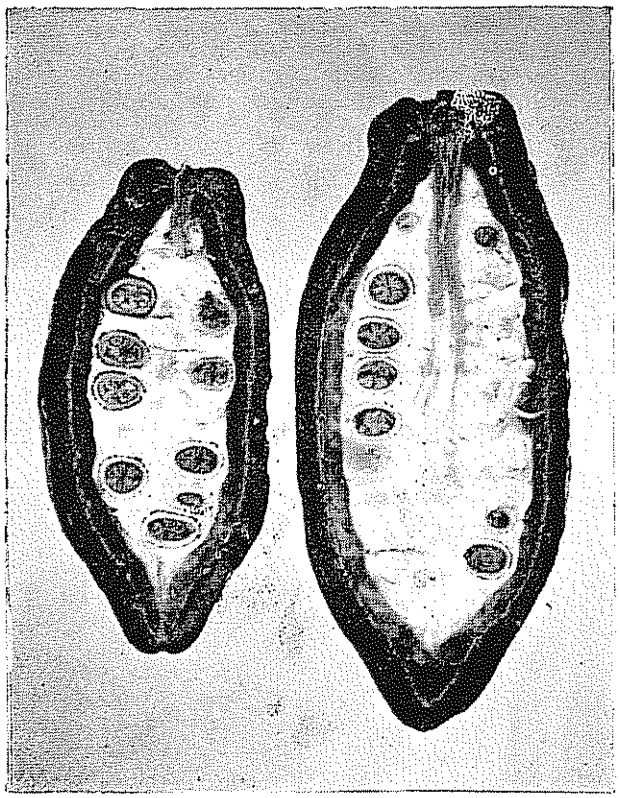

FIG. 4

Immature pods 119 days old with bean still firmly embedded in the pulp and difficult to separate for sowing.

Infact the only pods to be avoided would be those that have not attained that stage of maturity before which the beans still prove difficult to separate (Fig 4). Beans sown in February, i e., 2-3 months latter than usual still produced seedlings that were vigorous enough for field planting at the usual time in May or June.

Summary

Studies carried out at the Gambari Experiment Station of the Cocoa Research Institute of Nigeria since 1965 have shown that the stage of maturity of cacao beans has definite affects on their development and viability. The maturity of the beans also affects the growth of seedlings while mature green pods can supply beans capable of producing seedlings that are as vigorous or more vigorous than those of ripe pods. By using more vigorous seedlings it is possible to reduce the nursery period by up to 3 months while pods which have atained the mature size and colour prior to ripening could also be used for raising such seedlings for field planting.

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Notas y Comentarios

Papel de las lectinas en el organismo vegetal

Una proteína vegetal llamada lectina que podría estar involucrada en el mecanismo mediante el cual las bacterias simbióticas reconocen a sus hospedantes ha sido aislada de las raíces del trébol blanco por un equipo de científicos de la Universidad de Wisconsin. Frank Dazzo, W Yanke y W Brill han bautizado a su hallazgo "trifoliin" por la bacteria *Rhizobium trifolii* que infecta, forma nódulos, y entra en una relación simbiótica con el trébol (*Biochimica et Biophysica Acta* vol. 539, p 276).

Las lectinas vegetales son proteínas que se ligan a carbohidratos y que pueden aglutinar células Se conocen principalmente como herramientas para aislar proteínas y polisacáridos y para explorar la arquitectura molecular de las superficies celulares, las relaciones anticuerpo-antígeno y los cambios inducidos por la transformación de células normales a un estado maligno. Hasta hace poco se había prestado poca atención a la cuestión de lo que podrían estar haciendo las lectinas en la planta misma.

Los resultados del poco trabajo que se ha hecho hasta ahora sugieren que las lectinas están involucradas en la manera cómo las plantas reconocen el tipo apropiado de polen, y la forma cómo son invadidas por una enfermedad. Pero la evidencia más convincente ha emergido del estudio de la relación simbiótica entre las bacterias fijadoras del nitrógeno y las raíces de sus plantas hospedantes Cada planta es muy exigente acerca de las bacterias con quienes vive; por ejemplo, la bacteria *Rhizobium trifolii* es capaz de entrar en simbiosis con el trébol pero no con otras leguminosas como la alfalfa y la soya Los bioquímicos están interesados en la base molecular de este reconocimiento específico entre las bacterias y sus hospedantes.

En el caso de simbiosis del trébol con *R trifolii*, se ha demostrado que la atracción de la superficie de las bacterias a los pelos radicales del hospedante (el primer paso necesario para la infección) es inhibida por la 2-deoxiglucosa, lo que sugiere que el receptor ligador al carbohidrato está involucrado. Esta y varias líneas de evidencia anteriores, condujeron a la sugerencia de que una lectina podría estar involucrada en el proceso de reconocimiento, y es esta lectina la que ahora ha sido aislada.

Los investigadores purificaron la proteína y probaron su capacidad para aglutinar varias cepas de *Rhizobium* De 34 cepas probadas, sólo *R* trifolii fue aglutinado, aún en concentraciones muy bajas La lectina podía ser lavada de las raíces intactas con soluciones de 2-deoxiglucosa, lo que sugiere que está localizada en la superficie de las células adherida por un ligazón de carbohidrato.

Las técnicas de inmunofluorescencia confirmaron que el trifoliin estaba presente en las superficies radicales del trébol blanco, pero no en las de otras leguminosas como alfalfa y vicia. Las concentraciones más altas de trifoliin ocurrieron en las regiones de los pelos radicales donde se sabe que se fija el *R trifolii*.

Dazzo y sus colegas sugieren que el trifoliin es un determinante codificado para el hospedante que juega un papel importante en el reconocimiento de las células de *Rhizobium* y en el desarrollo simbiótico subsecuente

Las cucarachas, un refugio para los virus

Resultados sorprendentes, obtenidos por un grupo de la Universidad de Liverpool, muestran que, tanto la cucaracha americana (*Periplaneta americana*) como la común (*Blatta orientalis*), toleran en alto grado fumigaciones de formaldehido de doble fortaleza y cuatro veces la duración de la dosis recomendada para la desinfección de un cuarto (*Journal of Hygiene*, vol 80, p 125)

El grupo, que está formado alrededor de C. Bartzokas y K. McCarthy, descubrió que estos insectos no eran tampoco afectados cuando el agua que bebían fue reemplazada con formalina al cuatro por ciento y glutaraldehido al dos por ciento.

La solución de formol o formalina es un preservativo ampliamente usado para especímenes biológicos Por años, el vapor de formol ha sido usado también como biocida para una amplia variedad de bichos, especialmente para combatir bacterias y virus Es recomendado por las principales farmacopeas como fumigante para habitaciones en los que se sospecha pueda existir la viruela.

El grupo de Liverpool sabe que los expertos en control de plagas reconocen los grandes poderes de supervivencia de que gozan las cucarachas Otro hallazgo de este proyecto reveló, sin embargo, puede no ser tan ampliamente apreciado Los virus que son destruidos satisfactoriamente por el gas formaldehido pueden sobrevivir la fumigación mientras están protegidos en el intestino de una robusta cucaracha Mientras que el grupo admite que la trasmisión de enfermedades viróticas por las cucarachas no está bien probada, existe evidencia de que los virus, incluyendo los del polio y la fiebre amarilla, pueden ser transportados por los insectos

Bartzokas y sus colegas también descubrieron que aunque las cucarachas que bebían formalina al uno por ciento no sufrían inconvenientes, este trago erradicaba la flora de su intestino La inclusión de una inmersión en un baño sónico en glutaraldehido al dos por ciento(una prueba que destruye los virus superficiales, pero que las cucarachas pasan sin molestia) produce insectos estériles.

Pero el principal mensaje de este trabajo es que aquellos que usan la fumigación con formaldehido deberían estar advertidos de que los virus pueden sobrevivir en el interior de unas pocas cucarachas robustas.