CONTROL OF *PHYTOPHTHORA* SEEDLING BLIGHT OF COCOA

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ABSTRACT

Phytophthora palmivora *(Butler) Butler causes occasional serious losses of cocoa (*Theobroma cacao *Linnaeus) planting material in the nursery. Experiments were conducted to find an effective but inexpensive chemical control measure. Metalaxyl was found to be very effective both as a foliar spray and as a seed treatment and the prophylactic use of either method at the rate of about 10g a.i./1000 seedlings raised production costs by less than 0.5%.*

INTRODUCTION

Seedling blight of cocoa (*Theobroma cacao* Linnaeus), caused by *Phytophthora* species, has received little attention from research workers who have rightly concentrated on the more devastating pod and bark diseases caused by the same pathogens. Most work with cocoa seedlings has used them as convenient host material for fungicide testing (Newhall 1971; Daguenet 1980), pathogenicity testing (Firman and Vernon 1970) or resistance testing (Lawrence 1978). The fungus naturally attacks and kills unhardened 'flush' leaves and young green stem tissue. Under exceptional conditions it also infects mature leaves but this is not normally regarded as being serious (Manço 1966; Gregory 1969). Infection of flush leaves and stems can, however, lead to death of the growing point, or of the whole plant in the case of seedlings, and where the fungus spreads down a chupon (chupon wilt) bark cankers can form (Prior and Smith 1981).

Until recently little thought has been given to the control of seedling blight in Papua New Guinea (PNG), caused by *P. palmivora* (Butler) Butler as most plantings were done 'at stake', several seeds being sown and only the most vigorous healthy seedling left to grow to maturity. The development of high yielding Trinitario clones and Trinitario x Amazonian hybrids has led to the more frequent use of cocoa nurseries. The cost of hybrid seed and the inherent expense of running a nursery has prompted growers and nurserymen to request cheap and reliable measures for controlling disease outbreaks.

Seedling blight has a sporadic incidence but large losses of expensive seedlings or buddings can occur during periods of very wet weather, which are rather unpredictable in many cocoa growing areas of PNG. In Nigeria Chant and Hall (1959) found sprays of copper fungicide at 6 day intervals gave effective control of the disease. Although this is an expensive and time consuming routine, similar schedules at Keravat failed to prevent disease outbreaks. Virtually all outbreaks involved young plants, less than three weeks from emergence or bud growth, whose lowest leaves had not yet hardened off. It is likely that most infection came from the soil and the flush leaves were infected by rain splashed spores before being sprayed with fungicide. Although establishment of nurseries close to mature cocoa is strongly discouraged, they are still common in PNG and severe seedling blight occurs in such situations.

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The suggested source of soil for planting bags is from beneath virgin or secondary forest but this is not always available to cocoa plantations which frequently have to use soil from old cocoa blocks.

The seedling blight problem, being sporadic in nature but having the potential for causing serious and expensive losses, requires an inexpensive and reliable control measure which can either be incorporated into routine nursery procedures as prophylaxis or which can effectively stop disease outbreaks when they occur. The aim of this work was therefore to develop disease control recommendations which fulfilled the above requirements and would be acceptable to growers.

Cocoa seedlings grow very rapidly in the first few months after planting and in the first three weeks after emergence produce 4-6 leaves all of which are highly susceptible to *P. palmivora* attack as is the green stem tissue. Thus it was considered that the newly available systemic fungicides active against Oomycetes might prove more effective than surface protectants which have to be applied frequently to keep up with new leaf production. These systemic fungicides were tried both as spray and seed treatments in a series of experiments spanning three wet seasons at Keravat from November 1979 to February 1982.

MATERIALS AND METHODS

SITE AND NURSERY MATERIALS

Experiments were carried out beneath the shade of a large rain tree (*Samanea saman* Merrill) 60 m from the nearest cocoa. Black polyethylene planting bags (38 × 18 cm flat) filled with free draining, black volcanic ash topsoil were used for growing the cocoa seedlings. Soil was obtained from beneath secondary forest for each of the experiments except 5 and 6 which used bags from the previous experiment 4 from which the top 4 cm of soil had been removed and replaced with *Phytophthora* infested top soil from beneath old cocoa. Bags were arranged in plots of 100 (20 × 5 lines) each plot at least 1 m from its neighbours and separated from them by bare ground.

EXPERIMENTS

Experiments 1-3 were carried out from November 1979 to April 1980, experiments 1 and 2 concurrently, experiment 4 in December 1980 and experiments 5 and 6 from December 1981 to February 1982. Treatments are summarised in *Table 1*. Results were statistically analysed by Analysis of Variance.

Experiment 1. Fungicide spray comparison

Three systemic fungicides were tried, metalaxyl as Ridomil 25% w.p. (Ciba Geigy), aluminium tris (ethyl phosphonate) as Aliette 80% w.p. (May & Baker) and propamocarb as Previcur N 70% e.c. (Schering). Seedlings at the 2 – 4 leaf stage were used. A fine spray of each fungicide was applied by slide pump in 700 ml water per plot. Four mm of rain fell 4 h after spraying.

Inoculations were carried out during that rainfall (day 0) and again 1, 6, 16 and 26 days after spraying on one plot of each treatment and on the untreated control per inoculation date. Seedling blight in each plot was recorded 10 days after each inoculation.

Experiment 2. Fungicide seed treatment comparison

The three fungicides from experiment 1 were tried as seed treatments. One inoculation was performed on all plots 26 days after sowing (d.a.s.) at the four leaf stage. The number of seedling mortalities was recorded 14 days after inoculation.

Table 1. — **Fungicide treatments tested for effectiveness in controlling seedling blight of cocoa**

Experiment No.	Seed preparation	Method of fungicide application	Fungicide	Dose rate (% a.i.)	Treatment time (h)	No. replicate plots (100 seeds/plot)
1	pregerminated	spray	metalaxyl	0.13	—	5
			aluminium tris (ethyl phosphonate)	0.40	—	5
			propamocarb	0.35	—	5
			nil control	—	—	5
2	pregerminated	seed soak	metalaxyl	0.13	2	1
			aluminium tris (ethyl phosphonate)	0.40	2	1
			propamocarb	0.35	2	1
			water control	—	2	1
3	pregerminated	seed soak	metalaxyl	0.025	16	3
			"	0.050	16	3
			"	0.125	16	3
			"	0.250	16	3
			"	0.500	16	3
			"	1.250	16	3
			"	2.500	16	3
			water control	—	16	3
4	pregerminated	seed soak	metalaxyl	0.31	16	8
			"	0.63	16	8
			"	0.94	16	8
			"	1.25	16	8
			water control	—	16	8
5	ungerminated	seed soak	metalaxyl	0.25	16	10
			"	0.25	2	10
			"	0.25	dip	10
			nil control	—	—	10
6	ungerminated	seed soak	metalaxyl	0.13	dip	8
			"	0.25	dip	8
			"	0.38	dip	8
			"	0.50	dip	8
			nil control	—	—	8

Experiment 3. Metalaxyl seed treatment dose rate trial No. 1

Metalaxyl was found effective and systemic (see *Figure 1* and Results Section) so further experiments were conducted to determine the effectiveness of various doses of seed treatment. Percentage germination and emergence were recorded for each treatment overall. Three inoculations were performed 27 (4 leaf stage), 40 and 46 d.a.s. on one plot of each treatment. The number of seedlings with *P. palmivora* infection symptoms were recorded 10 days after each inoculation.

Experiment 4. Metalaxyl seed treatment dose rate trial No. 2

Percentage germination and emergence were recorded for each treatment. Inoculations were carried out 25 (4 leaf stage), 30 and 37 d.a.s. on one plot of each treatment and at 50 d.a.s. on the remaining five plots of each treatment. Blight symptoms were recorded 10 days after each inoculation.

Experiment 5. Metalaxyl seed treatment duration

In commercial practice mucilage re-

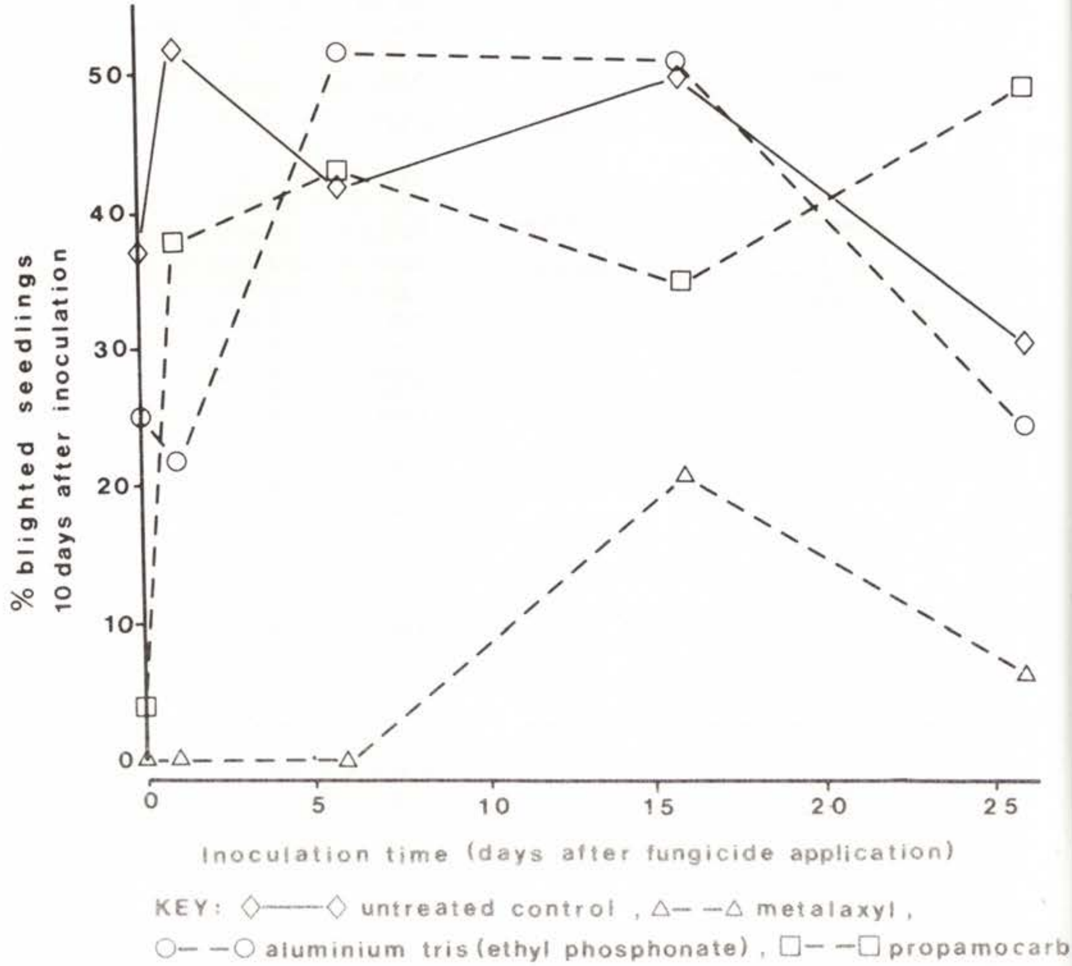


Figure 1. —Comparison of fungicide sprays for controlling seedling blight of cocoa (experiment 1).

moval and pregermination are seldom carried out. The retention of mucilage may affect fungicides' effectiveness so a replicated trial was performed with the aim of evaluating the effect of various treatment times on subsequent natural infection.

The 40 plots from experiment 4 were reused and the bags topped up with soil from a heavily black pod infected cocoa block. Percentage emergence was recorded for each plot and numbers of dead seedlings, as a proportion of emerged seeds, counted up to 50 d.a.s.

Experiment 6. Metalaxyl seed treatment dose rate trial No. 3

Merely dipping a batch of seed with mucilage intact and proceeding to sow the treated seed was found just as effective as an overnight soaking (see *Table 3* and Results Section). A final and replicated trial was therefore performed to re-evaluate the effectiveness of various rates of metalaxyl seed treatment under conditions of heavy natural infection. The 40 plots from experiments 4 and 5 were reused. The top 4 cm of soil from the bags was replaced with soil from a heavily black pod infected block. Percentage emergence was recorded for each plot and the numbers of dead seedlings, as a proportion of emerged seeds, recorded 40 d.a.s.

SEED SOURCE AND TREATMENT

For experiments 1,2,3 and 6 open pollinated seed from mixed Trinitario trees was used. To try to reduce variability in seedling growth rates seed from open pollinated pods of Trinitario clone KA2-101 was used for experiments 4 and 5. Pregermination of cocoa seeds produces an even stand of seedlings all bearing flush leaves for 2-3 weeks after emergence which was convenient for experimental purposes. Seeds were pregerminated by removing mucilage in sawdust and laying the seeds in damp hessian supported on a wire tray. Seeds were checked daily and planted as soon as the radicle was visible.

Seeds were soaked in fungicide suspension before pregermination or before sowing in the case of ungerminated seed which did not have mucilage removed.

INOCULUM PREPARATION AND SEEDLING INOCULATION

Zoospore suspensions were made by flooding 10 day old petri dish cultures of a local *P. palmivora* isolate on 5% V8 juice agar with 20 ml cold (*c.* 5°C) sterile distilled water. The plates were left in the dark for 40 min and the suspension decanted into a plastic bucket and diluted with rain water to a final concentration of about 5-8 $\times$ 10^3 spores/ml.

About 500 ml of the dilute zoospore suspension were sprinkled evenly over each plot of 100 seedlings using a plastic toy watering can during late afternoon or evening rainfall when the seedlings were expected to remain wet all night. Thus in all experiments inoculations were performed at irregular intervals when suitable weather conditions prevailed. This did not always coincide with leaf flushing particularly with older seedlings.

SYMPTOMS AND RECORDINGS

Germination was recorded as growth of the radicle beyond the testa. Emergence was defined as growth of the seedling above soil level to the stage of producing recognisable leaves within opened cotyledons.

Three symptoms of seedling blight were recognised; typical necrosis and withering of unhardened flush leaves (*Plate I* a, b & c), black 'V' shaped necrotic patches along margins or veins of 'hardened off' leaves (*Plate I* c & d) and dark brown necrotic patches on green stems or petioles. Infection of the stem at or just below the growing point, or infection spreading from flush leaves or petioles to that part of the stem produced the characteristic crook-neck appearance of dead seedlings (*Plate I* a & b). Infection of stems well below the growing point caused sudden death with the stem remaining erect bearing unwithered dead leaves. Any infection which killed the terminal bud rendered the seedling useless for transplanting or as rootstock but mature leaf infection alone was not damaging. In some cases infected flush leaves abcissed before infection reached the stem, particularly in dry weather, and where inoculations were performed between leaf flushes few seedling deaths resulted. Counts of mortality from successive inoculations were therefore not a reliable measure of the treatment effects. Consequently in experiments 1, 3 and 4 infection was recorded as any of the described symptoms. In experiments 2, 5 and 6, however, only one assessment was made and only death of the growing point was recorded as this is of greatest relevance to the grower.

RESULTS

Experiment 1. Fungicide spray comparison

The proportion of plants per plot with symptoms of *P. palmivora* infection is shown in *Figure 1* for the five successive inoculations. Metalaxyl gave complete control of the artificial inoculations up to 6 days after spraying and was better than the other treatments up to 26 days after spraying.

Some plants in three plots were infected before spraying. These were marked and their fate recorded. Of the 24 plants infected before treatment in a metalaxyl sprayed plot none subsequently died; the leaf and stem infections were completely arrested. Of the 34 plants infected before treatment in a propamocarb sprayed plot, 19 (55.9%) died of the infection and similarly in a control plot 26 of the initial 46 infected plants (56.5%) died of the infection. No pre-treatment infection occurred in the aluminium tris (ethyl phosphonate) sprayed plot. These results demonstrate a valuable curative effect of metalaxyl.

Experiment 2. Fungicide seed treatment comparison

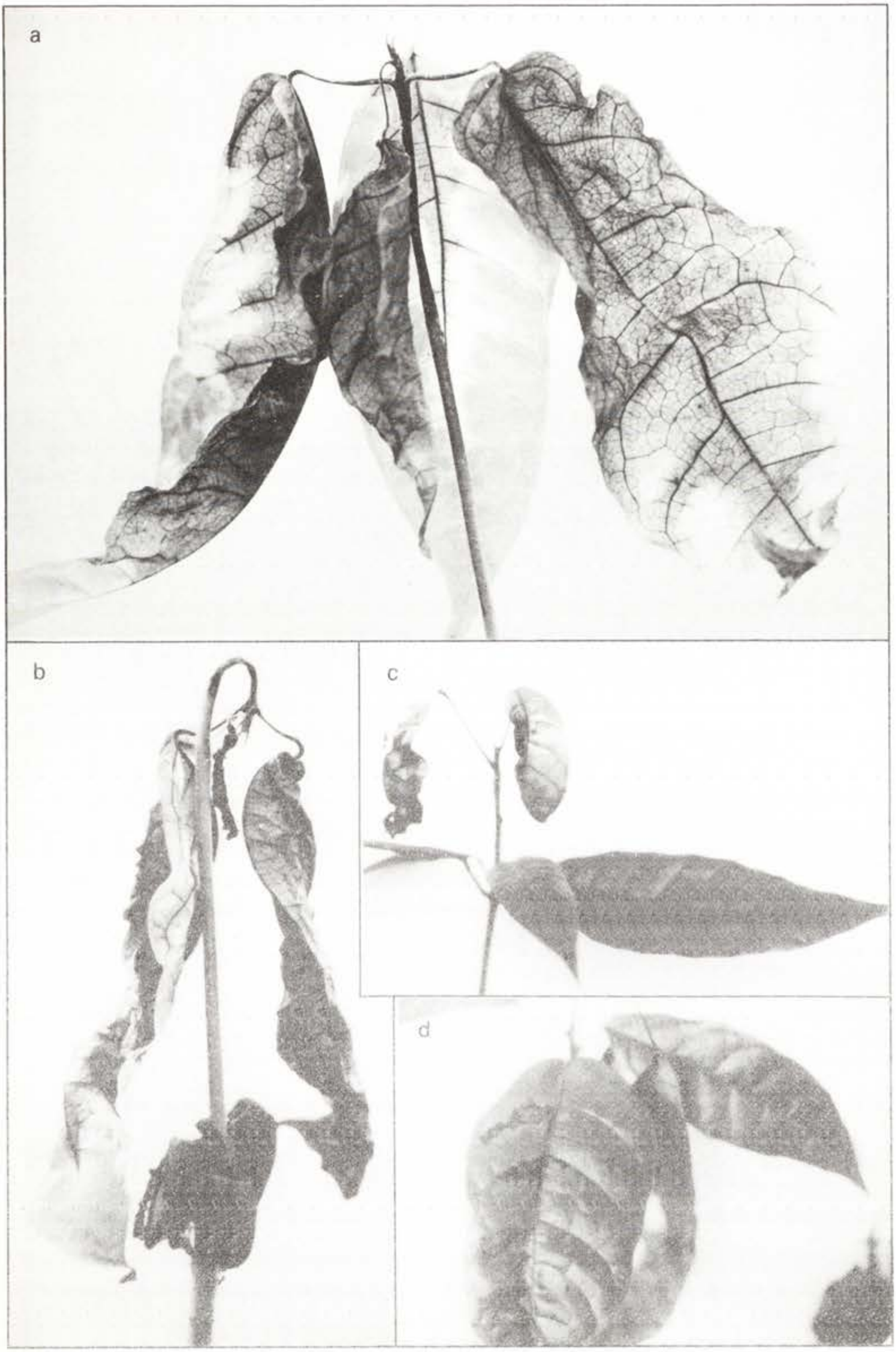
The proportion of dead plants in each plot 14 days after inoculation was metalaxyl 0, aluminium tris (ethyl phosphonate) 22%, propamocarb 34% and water control 43%.

This supports the conclusion from experiment 1, that metalaxyl gives better control of seedling blight than the other treatments and demonstrates its systemic activity.

Experiment 3. Metalaxyl seed treatment dose rate trial No. 1

Percentage germination and emergence for the eight treatments are given in *Table 2*. The results of the successive inoculations are given in *Figure 2*. Germination and emergence appears to be increased by metalaxyl treatment except for the highest dose 2.5% which appeared to be phytotoxic. All metalaxyl treatments averaged over the three inoculation dates significantly reduced blight compared

Plate I—Symptoms of cocoa seedling blight caused by *Phytophthora palmivora* (Butler) Butler.
- a Early symptoms of flush leaves. Note the lesion spreading down the stem which is starting to bend.
- b Crook-necked appearance of a seedling killed by progression of infection from flush leaves into the stem.
- c Early symptoms on flush leaves and 'v' shaped lesions at the margin of a mature seedling leaf.
- d 'V' shaped lesions along the veins of a mature seedling leaf.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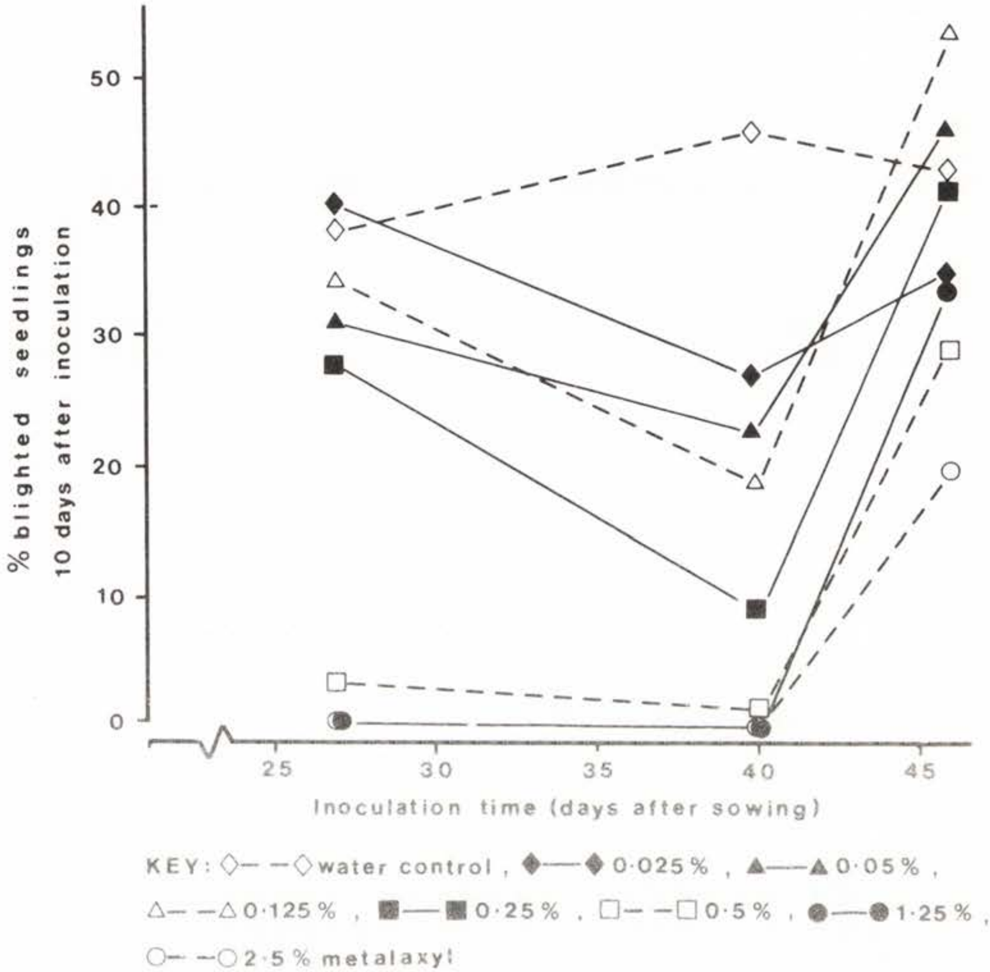

Figure 2.—Comparison of metalaxyl seed soaking dose rates for controlling seedling blight of cocoa: dose rate trial No. 1 (experiment 3).

Table 2.—**Effect of metalaxyl seed soaking treatment dose rates on germination and emergence of cocoa seeds (experiment 3)**

Treatment % metalaxyl	Germination %	Emergence %
0 (control)	86.52	61.46
0.025	89.84	97.87
0.050	89.22	96.94
0.125	89.75	97.83
0.250	90.90	100.00
0.500	88.20	98.98
1.250	86.31	98.94
2.500	81.73	82.29

with the untreated control ($P<0.01$). The three highest rates were, however, not significantly different and all gave significantly better control than all other treatments ($P<0.01$).

Experiment 4. Metalaxyl seed treatment dose rate trial No. 2

In contrast to experiment 3 the germination and emergence of untreated control seeds was over 94% and there was little difference between any metalaxyl treatment and the control. The results of the four successive inoculations are given in *Figure 3*. All metalaxyl treatments averaged over the first three inoculation dates significantly reduced blight compared with the untreated control ($P<0.001$). The two highest rates were not significantly different but were significantly better than the two lower rates ($P<0.001$). There was no significant difference between the control and any of the fungicide treatments 50 d.a.s.

Experiment 5. Metalaxyl seed treatment duration

Percentage emergence and percentage of seedlings infected for the four treatments are given in *Table 3*. All fungicide treatments gave significantly higher ($P<0.001$) emergence from heavily *P. palmivora* infected soil than the control and significantly lower ($P<0.001$) mortality but there was no significant difference between seed soaking times demonstrating that simply immersing a batch of seed in fungicide is adequate when mucilage is left intact.

Experiment 6. Metalaxyl seed treatment dose rate trial No. 3

The results of emergence and of natural mortality of seedlings grown in heavily *P. palmivora* infected soil are given in *Table 3*. All treatments were significantly better than the control ($P<0.001$) and the three higher doses gave significantly better control ($P<0.05$) than the lowest dose.

DISCUSSION

The results of the fungicide comparisons are in agreement with Daguenet (1980) and McGregor (1982) who also found metalaxyl more effective against *P. palmivora* than other systemic fungicides. The metalaxyl spray gave good control of artificially induced infection for three weeks after spraying and had a curative effect on established natural infection. Whilst this treatment cannot prevent early deaths of seedlings or damping off it is likely to be an effective control measure where disease outbreaks do occur.

In seed treatment experiments 5 and 6, where there was a large soil population of *P. palmivora* all doses of metalaxyl gave substantially increased emergence pre-

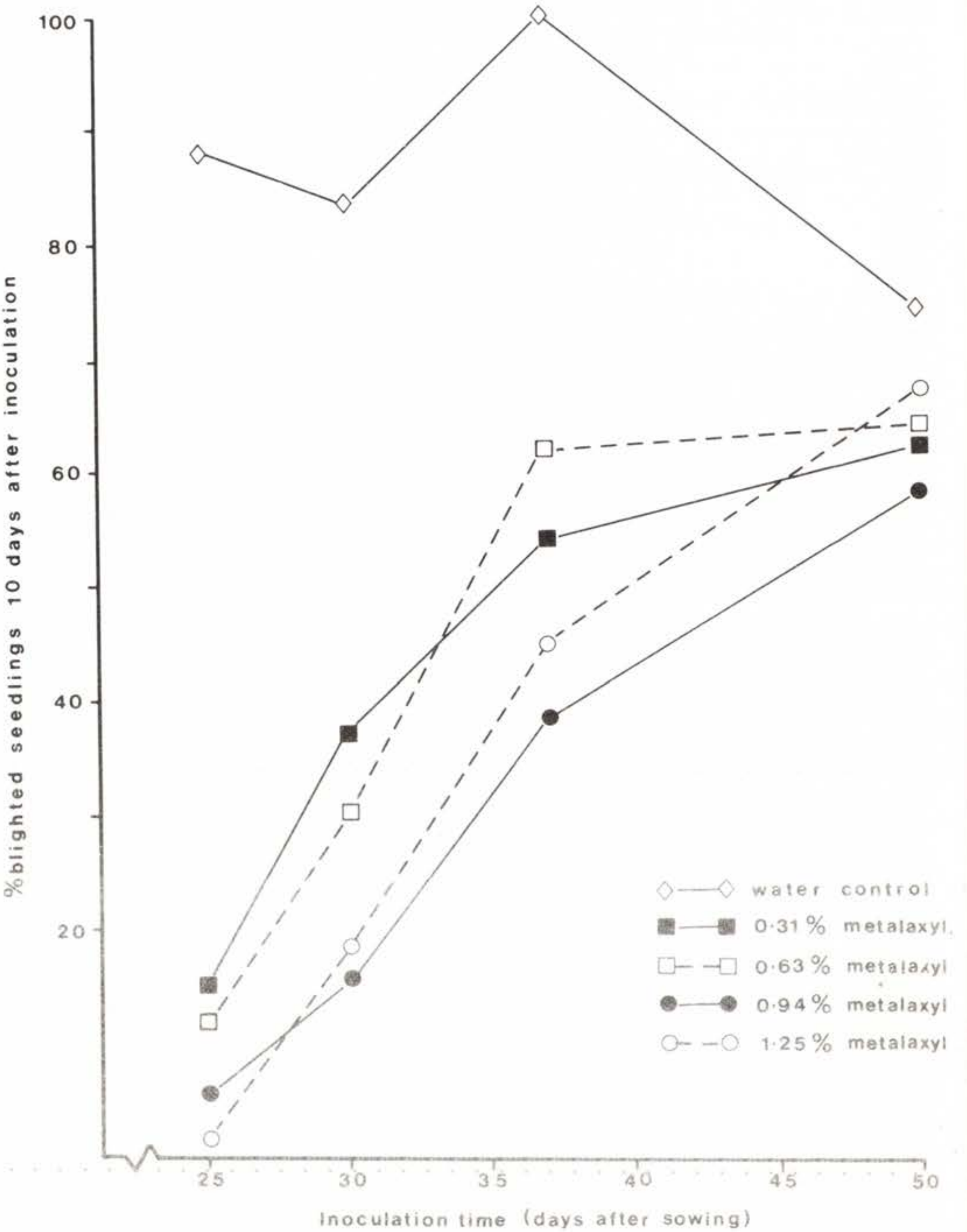

Figure 3.—**Comparison of metalaxyl seed soaking dose rates for controlling seedling blight of cocoa: dose rate trial No. 2 (experiment 4).**

Table 3. —**Effect of metalaxyl seed soaking treatment time (expt. 5) and dose rate (expt. 6) on cocoa seed emergence and seedling mortality in soil heavily infested with *Phytophthora palmivora***

Treatment	EXPERIMENT 5 Mean emergence[1]		Mean mortality[1]	
Time (h)				
Untreated control	21.7a[2]	(16.9)	68.5 a[2]	(81.4)
Dip	76.2 b	(93.7)	17.0 b	(9.5)
2	73.5 b	(91.6)	17.6 b	(11.4)
16	74.5 b	(92.6)	16.7 b	(9.8)
s.e.d. (32 d.f.)	2.15		5.25	
Treatment	**EXPERIMENT 6 Mean emergence[1]**		**Mean mortality[1]**	
% metalaxyl				
Untreated control	40.3 a[2]	(42.0)	51.8 a[2]	(61.4)
0.13	68.7 b	(86.9)	17.1 b	(9.3)
0.25	72.3 b	(90.1)	6.5 c	(1.9)
0.38	69.0 b	(88.0)	7.8 c	(2.0)
0.50	71.6 b	(90.8)	2.2 c	(0.5)
s.e.d. (45 d.f.)	5.24		1.88	

Key: 1 Statistical analyses and significance tests performed on Arc Sin transformed data, with original per-cent values shown in brackets.
2 Within the same column treatments followed by different letters are significantly different ($P < 0.001$).

sumably by controlling damping off. A dip in fungicide suspension was found to be just as effective as an overnight soak in controlling natural infection. In experiment 3, 0.5% metalaxyl gave good disease control up to 40 days after sowing but in experiment 4 infection rates were exceptionally high due to the use of uniform and highly susceptible planting material and the frequent coincidence of suitable inoculation conditions with leaf flushing. Even under these conditions 0.94% metalaxyl gave excellent control of infection up to 25 days after sowing and some control up to 40 days after sowing. None of the metalaxyl treatments were effective 50 days after sowing but by this time seedling blight rarely occurs naturally. In experiment 6, using a natural inoculum source, 0.25% metalaxyl was not significantly different from the two higher doses.

It is clear that the optimum dose of fungicide varies with seedling susceptibility and inoculum pressure. The recommended dose should be that which is likely to give good and reliable control under normal field conditions and, as 0.5% metalaxyl gave good control in experiments 3 and 6, that would seem to be a suitable general recommendation. The lower dose of 0.25% is likely to give good control where nursery soil is obtained from primary or secondary bush with a low *P. palmivora* population, whilst 1% might be required where the nursery is established under mature cocoa.

The cost of the 0.5% metalaxyl treatment was K1.00/1000 seeds. The planting bags cost K15.00/1000, hybrid seed K100.00/1000 and other nursery running costs raise total production costs to about K200.00/1000 seedlings. Buddings are even more expensive to produce. Thus an effective fungicide seed treatment costs only 0.5% of total production costs. The cost of the single metalaxyl spray in experiment 1 was similar to the seed soaking treatment, K0.90/1000 seedlings or buddings.

Metalaxyl tolerance has been reported in other pathogens (Davidse *et al*. 1981; Urech *et al*. 1981) but only where the fungicide had been used intensively. It would therefore be wise to restrict the use of metalaxyl to once only on any plant in the nursery and to destroy subsequently blighted plants as a precaution against the possibility of transporting metalaxyl tolerant *P. palmivora* to the field.

ACKNOWLEDGEMENTS

I thank Mr. J. Ban, Mr. T. Nevenimo and Mr. P. Potaisa for their technical assistance and Dr. C. Prior and Mr. A. Charles for reading the manuscript.

REFERENCES

CHANT, S.R. and HALL T.R. (1959). Investigations on a seedling dieback of *Theobroma cacao* L. in Nigeria. II Factors affecting the incidence of the disease and its control. *Tropical Agriculture (Trinidad)* **36**(2), 145-149.

DAGUENET G. (1980). Étude des fongicides systémiques anti-phycomycètes en vue de la lutte contre la pourriture brune des cabosses du cacaoyer due à *Phytophthora* sp. Recherche d'une méthode de tri sur plantules en laboratoire. *Café-Cacao-Thé* **24**(3), 195-202.

DAVIDSE, L.C., LOOIJEN, D., TURKENSTEEN L.J. and VAN DER WAL D. (1981). Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields. *Netherlands Journal of Plant Pathology* **87**, 65-68.

FIRMAN, I.D. and VERNON A.J. (1970). Cocoa canker caused by *Phytophthora palmivora. Annals of Applied Biology,* **65**, 65-73.

GREGORY, P.H. (1969). *Black pod disease project report*. London: Cocoa, Chocolate and Confectionary Alliance. 52 pp.

LAWRENCE, J.S. (1978). Evaluation of methods for assessing resistance of cacao (*Theobroma cacao* L.) cultivars and hybrids to *Phytophthora palmivora* (Butler) Butler. *Boletim Tecnico 62*. CEPEC-CEPLAC, Bahia, Brasil. 47 pp.

McGREGOR, A.J. (1982). A small scale screening technique for evaluating fungicides against *Phytophthora palmivora* pod rot of cocoa. *Annals of Applied Biology* **101**, 25-31.

MANÇO, G.R. (1966). *Phytophthora palmivora* in flower cushions, old infected pods and leaves of cacao plants. *Turrialba* **16**, 148-155.

NEWHALL, A.G. (1971). Some research bearing on the control of pod rot of cacao caused by *Phytophthora palmivora. Proceedings of the 3rd International Cocoa Research Conference,* Accra, Ghana, 1969, pp 462-468.

PRIOR, C. and SMITH, E.S.C. (1981). Association of *Phytophthora palmivora* bark canker and insect damage in cocoa in Papua New Guinea. *Annals of Applied Biology* **97**, 27-30.

URECH, P.A., MARGOT, P. and STAUB, T. (1981). Efficacy and use concept of metalaxyl for control of diseases in tobacco. CORESTA Phytopathology and Agronomy Study Group. Reports for 1981, Torgiano, Italy, 1981.