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A field investigation into delivery systems for agents to control *Moniliophthora roreri*

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Abstract

This paper describes a factorial trial designed to investigate the delivery systems of fungal biological agents (*Clonostachys rosea*), applied as single strain or as strain mixture, in comparison with copper hydroxide as a chemical standard against *Moniliophthora roreri* in cocoa. Application techniques compared were motorised mistblowers fitted with rotary atomisers and hydraulic sprayers fitted with cone nozzles giving a narrow angle of spray. The third factor was the presence or absence of an emulsifiable rape-seed adjuvant oil in the tank mixture. Copper fungicide was the best agent for moniliasis control and resulted in the highest yield. Both the mycofungicides reduced sporulation of the pathogen. Net reduction of inoculum was best for the hydraulic sprayer-applied copper fungicide and the motorised mistblower-applied single-strain biocontrol agent. Overall, directional hydraulic sprays were found to be somewhat superior to the motorised mistblower technique, with its dissipated cone of spray. Addition of the adjuvant oil was the least important of the factors, but efficacy depended on the agent used, with a significant enhancement in the efficacy of copper with oil but a detrimental effect on *C. rosea*.

Introduction

Moniliasis or frosty pod of cocoa (*Theobroma cacao*), caused by *Moniliophthora roreri* (Cif. & Par.; Evans et al.) is a major constraint to cocoa production in parts of Central and South America (Evans and Prior, 1987; Evans et al., 1998). The disease was first identified in Ecuador in the 1930s, but has since spread and currently ranges from southern Peru to Honduras. In Costa Rica, yield losses are estimated to range from 50% to 90%. The effects of this disease are compounded by the ubiquitous black pod (*Phytophthora* spp.; usually *Phytophthora palmivora* in Costa Rica). Recommended control measures include crop sanitation and regular harvesting, the objective being to limit disease spread by the highly mobile, powdery conidia of *M. roreri* (Soberanis et al., 1999). A number of chemical control agents have been tested (Cronshaw, 1979; Villegas and Enriquez, 1979), but are perceived not to be cost effective, especially when cocoa prices are depressed. Even at higher prices, only copper fungicides are generally thought to be cost effective, and may still be used for organic cocoa, for which producers sometimes enjoy 200% of the world market price. However, the use of copper is now disputed amongst proponents of organic production. The International Federation of Organic Agriculture Movements (IFOAM, 2002) states that: 'Mineral inputs should contain as few heavy metals as possible. Due to the lack of any alternative, and long-standing, traditional use in organic agriculture, copper and copper salts are an exception for the time being. The use of copper in any form in organic agriculture must be seen, however, as temporary and use must be restricted with regard to environmental impact.'

Mycofungicidal agents of cocoa diseases have shown great promise in Peru (Krauss and Soberanis, 2001). *Clonostachys rosea* (Link: Fr.; Schroers et al.)

reduced moniliasis by some 15–25% under field conditions. Although a mild reduction of black pod and witches' broom (*Crinipellis perniciosa*) failed to reach statistical significance, mixtures of up to five mycoparasites performed consistently better against all three diseases simultaneously than the best single strain at equal application rates. With the mixed inoculum, yields were increased by 16.7% and net returns by 24%. This was attributed to the fact that mixed inocula can cover a wider range of pathogen diversity as well as environmental fluctuations. However, not all antagonist combinations are compatible (Raupach and Kloepper, 1998) and we opted to include a single strain and a mixed inoculum in the present study.

With mycopesticides, the use of oils in formulations for spraying has shown great potential for enhanced efficacy. Originally demonstrated with mycoinsecticides (Prior et al., 1988), where the need for high humidity is also overcome (Bateman et al., 1993), this principle was later applied to mycofungicides (Hofstein and Chapple, 1998). Chapple and Bateman (1997) discuss technical issues relating to particles of the hyperparasitic mycofungicide *Ampelomyces quisqualis* Ces. ex Schlecht. and its emulsified oil adjuvant.

There are essentially two types of equipment commonly used for spraying cocoa trees. Efficient fungicidal control of both black pod (Gorenz, 1974) and moniliasis (Desrosiers and Suárez, 1974) has been obtained with either but direct comparisons are inconclusive to date.

1. Motorised knapsack mistblowers (or air blast sprayers): these have many uses, but they were originally developed for obtaining good droplet coverage for the control of Mirid (Capsid) bugs in the tall cocoa trees of West Africa (Clayphon, 1971). The most common design of nozzle is of the air-shear type, in which thin layers of liquid are introduced into a high-speed airstream and thus produce fine sprays.
2. The lower cost alternative is to use side-lever knapsack (SLK) (hydraulic) sprayers, and many authors (e.g. Pereira et al., 1996) believe these to be the only viable method for small-holder cocoa farmers. The spraying of tall trees can be achieved by the use of extended booms.

The choice of spraying equipment depends on a number of socio-economic and technical factors. Hydraulic, manual sprayers are usually used by small-holder farmers because of their simplicity and low capital and running costs. On the other hand, motorised mistblowers can often achieve higher work rates and better reach (typically between 5 and 10 m: Clayphon, 1971). Work rate is enhanced, not only by enabling treatment of a greater number of trees per hour, but also because complete canopy coverage can be achieved at low volume application rates (VARs). Operators typically need to transport 10–50 l ha^{-1} in contrast to the hundreds of litres needed with conventional hydraulic application. However, one of the advantages of hydraulic sprayers is that a more targeted application on trunks and pods can be achieved; Jollands and Jollands (1984) showed that the efficacy of metalaxyl, a systemic phenylamide fungicide, against *P. palmivora* could be enhanced by reducing VARs to as little as 65 l ha^{-1}. Hollow cone nozzles are normally considered most appropriate for three-dimensional targets of this nature (Matthews, 2000) and frequently fitted as standard to manual sprayers.

Although controlled droplet application techniques are theoretically more efficient, in practice it is often possible only to demonstrate equivalent (or even slightly reduced) efficacy at very low volume (VLV) rates. However, the practical advantage of low volume application includes the economic benefit that accrues from improved work rates. Bateman and Alves (2000) demonstrated that rotary atomisers, such as the 'Micronex' (Micron Sprayers Ltd., Three Mills, Bromyard, Herefordshire, UK), gave the narrowest droplet size spectra and maximised the volume of droplets (85%) in a size range that is believed to be optimal for fungicides. Addition of the oil-based adjuvant 'Codacide' (Microcide Ltd, Shepherds Grove, Stanton, Suffolk, UK) (a rape-seed oil with 5% emulsifier.) had little effect on droplet spectrum.

The development of application techniques appropriate for biological fungicides is very much in its infancy, and even with chemical control, there are few published articles describing the interactions between control agents and their application techniques. The objective of this study was to investigate delivery systems (application techniques and presence of an emulsified oil adjuvant) that may influence the effectiveness of agents to control *M. roreri* and other cocoa pathogens such as *P. palmivora*.

Materials and methods

Site and design

A site was selected in CATIE's La Lola field station in Costa Rica (altitude 70 m; access point: 10°5′18.7″N; 83°23′20.2″W) and the trial was conducted over a complete production cycle, from May 2000 to April 2001. The area was planted with hybrid seedlings in 1992, and trees had grown to ~3 m, spaced apart evenly in 3 × 3 m rows. Plots consisted of 8 × 8 trees (as illustrated), consisting of a 4 × 4 tree assessment zone surrounded by one row of trees that were sprayed but not assessed, with a perimeter row of unsprayed (guard) trees. Before the trial, preliminary spray tests were carried out, using the ultra-violet tracer technique (Staniland, 1959). Unless the wind is strong, air currents within the cocoa canopy are usually low, and it was established that droplets were unlikely to drift further than two tree rows, even with motorised mistblowers.

X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X
S	S	X	X	S	S	S	S	S	S	X	X	S	S
A	S	X	X	S	A	A	A	A	S	X	X	S	A
A	S	X	X	S	A	A	A	A	S	X	X	S	A
A	S	X	X	S	A	A	A	A	S	X	X	S	A
A	S	X	X	S	A	A	A	A	S	X	X	S	A
S	S	X	X	S	S	S	S	S	S	X	X	S	S
X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X
S	S	X	X	S	S	S	S	S	S	X	X	S	S
A	S	X	X	S	A	A	A	A	S	X	X	S	A

Key: A: Sprayed and assessed; S: Sprayed, not assessed; X: Guard rows. Plot size: 576 m^2 (0.0576 ha); (1111 trees ha^{-1}).

A total of 32 plots were marked out for a factorial, completely randomised plot design. With two replicates, the factors were:

- Four agents (including chemical standard and control; water only);
- Two Sprayers: SLK (hydraulic sprayer) applying a medium volume rate, and modified motorised mistblower (MMM) at a very-low VAR;
- Two tank mixtures: suspensions with and without adjuvant oil.

Agents and adjuvants

The primary factor in the trial consisted of a comparison between two biological agent regimes compared against a copper fungicide standard and control. Treatments were:

1. *C. rosea* isolate APP0023, in all cases applied at an equivalent to ~3 × 10^{12} conidia per hectare;
2. *C. rosea* cocktail consisting of approximately equal quantities of five isolates: AMR0007, AMR0009, AMR0048, APP0023 and APP0043, at the same total rate as above;
3. Chemical standard: copper hydroxide (Kocide, Abonos Superior, Costa Rica: distributor for Griffin Corp., USA, 101 WP) applied at 3 kg product (1.5 kg a.i.) per hectare;
4. Water (or water + oil) control.

C. rosea isolate APP0023 had been isolated from a cocoa flower collected in Suretka (Talamanca, Costa Rica) using the precolonised plate method (Krauss and Soberanis, 2001) with *P. palmivora* as isolation host. All other *C. rosea* isolates likewise originated from the surface of healthy cocoa pods or flowers in Costa Rica and had appeared promising in preliminary tests (not presented). Apart from the advantages listed in the introduction, the mixture selected (agents: 2) was considered appropriate for both *M. roreri* and *P. palmivora*.

The adjuvant oil selected was 'Codacide'; applied at an equivalent of 4 l ha^{-1} in all cases. The concentrations made up depended on VARs, thus for 30 l ha^{-1} sprays, 10% oil was added to the tank mixture, or 1% for the 200 l ha^{-1} treatments.

Application techniques

Two application strategies were assessed as a trial factor:

1. SLK sprayer: Carpi 'Spray-mec'(Agrosuperior S.A., San Jose, Costa Rica) fitted with an extended lance and a Spraying Systems(Spraying Systems Co., Wheaton IL, USA.) D2-45 hollow cone nozzle. This configuration was designed to deliver an equivalent of 200 l ha^{-1}, simulating (in a reproducible way) the type of directional spray that farmers often create with variable cone nozzles. At 300 kPa this nozzle had a measured flow rate of

765 ml min^{-1}, and typically produces a spray with a volume median diameter (VMD) of ∼400 μm in a 40° cone (Bateman, in prep.).

2. Modified motorised mistblower: Stihl SR 400 (Andreas Stihl AG & Co., Waiblingen, Germany) fitted with a 'Micronex' rotary atomiser via a No. 59 restrictor; when calibrated with the motor operating and the nozzle directed upwards at 45°, the measured flow rate was 118 ml min^{-1}, delivering an estimated equivalent of 30 l ha^{-1} (the objective was 20 l ha^{-1}, but the spray logs recorded a higher VAR – see below). At this flow rate, the 'Micronex' produces sprays with a VMD of ∼50 μm with water-based formulations (Bateman and Alves, 2000).

For each treatment (two replicates) the amount of tank mixture prepared exceeded the volume needed to treat the plots. After spraying, the excess was recorded and spray logs maintained throughout the trial. The control treatments were sprayed first to check equipment and calibration. A total of 10 persons sprayed the plots throughout the duration of the trial. These exhibited significant variability in application rates but were fully randomised across treatments. Applications were made in monthly intervals. Occasionally, spraying had to be postponed by 1 or 2 days because of rain. Other weather conditions posed no hindrance to applications.

Preparation of agents

Biological agents were prepared in a two step liquid/solid fermentation as described by Krauss et al. (2002). High-concentration suspensions were prepared in the afternoon, before application and stored at 4 °C. This did not affect viability of any isolate (data not shown). The following morning, conidial concentrations were adjusted and taken to the trial site (1.5 h drive) in PET bottles. Copper fungicide powders were pre-weighed into plastic sachets ready for mixing on site.

Approximately 10 ml of left-over microbial spray agent was collected in the field and taken back to the laboratory in McCartney bottles. Germination was assessed after 24 h at ambient temperature. According to preliminary experiments, germination levelled off at that time (data not shown). Additionally, selected individual strains were plated on potato dextrose agar (PDA, Difco, Sparks, MD, USA) after retrieval from the sprayer and germination was compared with controls (which had not passed through any sprayer).

Weather conditions

Meteorological data were measured on site. The average monthly temperature ranged from 22.3 to 25.7 °C with a minimum of 18.6 °C and a maximum of 31.5 °C. The average monthly relative humidity ranged from 88.8% to 92.7%. A total of 3342.1 mm of rain were received during the trial year with the monthly extremes ranging from 436.1 mm in June 2000 to 101.3 mm in March 2001. The long-term (∼50 years) averages for La Lola are as follows: average monthly temperature: 24.0–25.7 °C, average minimum: 20.4 °C, average maximum: 29.9 °C, average monthly relative humidity: 88.7–92.4%, annual precipitation: 3525.4 mm with a maximum in December (463.1 mm) and a minimum in March (169.9 mm). Thus, the trial year was within the normal range of conditions.

Data recording and statistical analysis

A total of 10 applications took place between May 2000 and March 2001. The evaluation period commenced eight weeks after the first application of biocontrol agent to avoid recording pods with a latent infection contracted prior to applications. Evaluation consisted of fortnightly harvesting and counting healthy, mature pods, and quantifying and removing any diseased pod of at least 8 cm in length. Dead pods shorter than 8 cm were attributed to cherrelle wilt, a physiological disorder, which affects young pods and cannot be distinguished with certainty from diseases at this stage.

Analysis of the spray logs and tank residues revealed that the following applications had effectively been made (Table 1) (spore concentrations are pooled data from both the microbial agent treatments).

The field trial and germination results exhibited binomial (percentage) data, which were analysed using the appropriate general linear model on Genstat 5, Release 2.2 (Genstat 5 Committee, 1993). The maximum model (Crawley, 1993) was fitted, using all three factors, together with their first and second order interactions. This rather rigid approach allowed the determination of the relative importance of each factor but resulted in over-dispersion (high residual mean deviance). This does not influence parameter estimates

Table 1. Analysis of the spray logs and tank residues

	SLK		Modified mistblower	
	Mean	SE	Mean	SE
Volume applied (l per 36 tree plot)	6.94	0.10	1.00	0.02
VAR ($l\,ha^{-1}$)	214.2	—	30.9	—
Spore concentration ($spores\,l^{-1}$)	5.9×10^{9}	1.2×10^{9}	5.9×10^{10}	1.3×10^{10}
Spores ha^{-1}	1.3×10^{12}	2.6×10^{11}	1.8×10^{12}	4.0×10^{11}

but inflates errors leading to a reduction of significant effects (Crawley, 1993), i.e. increases the risk of type II errors; which were not the primary concern here. The 'RPAIR' procedure of Genstat was used to allocate differences between means.

Results

Disease control

Moniliasis incidence was very high (>80%). *Phytophthora* incidence was found on ~0.6% of pods examined and largely unaffected by factors under investigation; therefore, percentage healthy pods behaved opposite to moniliasis trends. Agent and spray technique were the important main factors influencing control of *M. roreri* and there were significant interactions between the two (Table 2). The disease was reduced only by the copper fungicide ($P < 0.001$). Hydraulic sprayer (SLK)-applied fungicides achieved higher levels of control in comparison with modified mistblowers (MMM) ($P < 0.001$). However, the agent interacted with sprayers and, to some extent, formulation. Whereas moniliasis incidence, in control and biocontrol mixture plots, was higher with the hydraulic sprayer than the motorised mistblower, this trend was reversed for the single-strain biocontrol agent and the fungicide (Figure 1). All agents (except the control) were more effective in water. It is possible that the oil had a slight antifungal effect; which would have lowered moniliasis incidence in the control but also mycoparasite efficacy in the biological treatments.

Both microbial control agent (MCA) treatments significantly ($P < 0.001$) lowered the percentage of pods that reached the sporulation stage, whereas the copper fungicide did not reduce sporulation ($P = 0.115$) (Figure 2). In the reduction of inoculum, the reduction in sporulation was of equal importance to the reduction of moniliasis incidence as seen by the absolute number of sporulating pods (which is a function of both: see Table 3). The absolute number of sporulating

Table 2. Accumulated analysis of deviance for moniliasis incidence

Change	d.f.	Deviance	Mean deviance	Deviance ratio
Agent	3	602.1	200.7	4.69
Sprayer	1	95.9	95.9	2.24
Formulation	1	15.8	15.8	0.37
Sprayer · agent	3	330.2	110.1	2.57
Form. · agent	3	140.7	46.9	1.10
Sprayer · form	1	0.02	0.02	0.00
Sprayer · form · agent	3	16.2	5.4	0.13
Residual	16	684.9	42.8	
Total	31	1885.8	60.8	

pods, and therefore level of field inoculum, was best reduced by the SLK-applied copper fungicide and the MMM-applied, single-strain biological treatment. These contrasting assessments illustrate the need to evaluate various aspects of disease progression and subsequent testing of disease spread in the presence of MCAs.

Both relative and absolute yield was highest with the hydraulic sprayer-applied fungicide. The copper fungicide was significantly better than any other agent ($P < 0.002$). For the other treatments, the sprayer used did not make a significant difference and, overall, the effect of the factor sprayer was inconclusive ($P = 0.13$). Both MCAs performed better in water, whereas the fungicide and control gave higher yields in the oil formulation. Altogether, the formulation was the least important factor ($P = 0.877$). Nevertheless, absolute yield (Table 4, Figure 3) was by far the highest with the oil-formulated copper fungicide applied by hydraulic sprayer.

Microbial viability

Germination in the field-collected, nutrient-poor formulations was low (mean 7%). The oil in the formulation reduced germination significantly ($P = 0.015$). The addition of nutrients in the form of pod

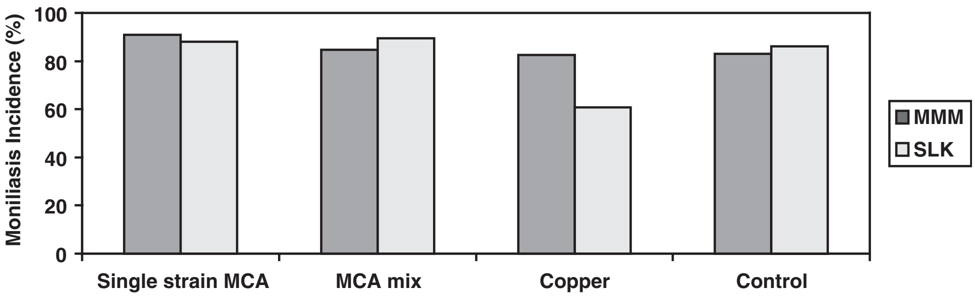

Figure 1. Moniliasis incidence (%). MMM – modified motorised mistblower, SLK – side-lever knapsack sprayer.

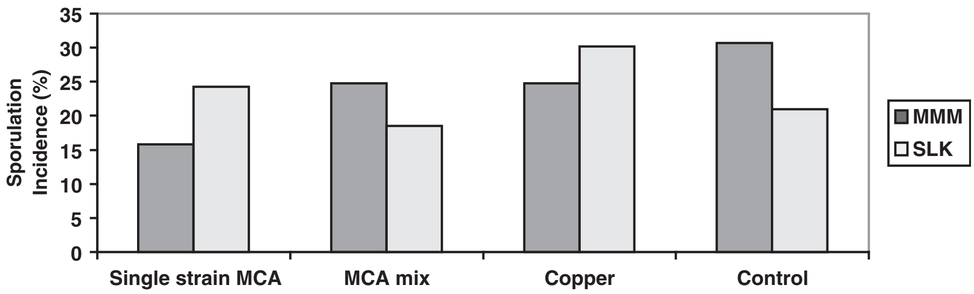

Figure 2. Percentage of pods infected with *M. roreri* reaching sporulation stage. MMM – modified motorised mistblower, SLK – side-lever knapsack sprayer.

Table 3. Accumulated analysis of deviance for absolute number of pods sporulating with moniliasis per tree

Change	d.f.	Deviance	Mean deviance	Deviance ratio
Agent	3	295.6	98.5	8.23
Sprayer	1	30.4	30.4	2.54
Formulation	1	0.3	0.3	0.02
Sprayer · agent	3	82.0	27.3	2.28
Form · agent	3	177.7	59.2	4.94
Sprayer · form	1	0.01	0.01	0.00
Sprayer · form · agent	3	44.9	15.0	1.25
Residual	16	191.7	12.0	
Total	31	822.5	26.5	

Table 4. Accumulated analysis of deviance for total healthy pods harvested per tree

Change	d.f.	Deviance	Mean deviance	Deviance ratio
Agent	3	576.3	192.1	6.46
Sprayer	1	74.8	74.8	2.51
Formulation	1	0.5	0.5	0.02
Sprayer · agent	3	55.8	18.6	0.63
Form · agent	3	387.3	129.1	4.34
Sprayer · form	1	4.7	4.7	0.16
Sprayer · form · agent	3	61.9	20.6	0.69
Residual	16	475.7	29.7	
Total	31	1636.9	52.8	

exudates increased germination significantly ($P < 0.001$) to 17%. Spores recovered from the motorised mistblower exhibited significantly impaired germination (5%) compared with spores collected from the hydraulic sprayer (11%) ($P < 0.001$). However, the addition of pod exudates restored germination to 16% and 18%, respectively, which was not significant for the factor sprayer. We therefore plated selected strains onto PDA where germination approached 100%. Both sprayers reduced viability slightly but significantly, the motorised mistblower more so than the hydraulic sprayer ($P \leq 0.036$). The oil formulation consistently exhibited lower germination than the suspension in water only ($P < 0.05$).

Discussion

Agents and adjuvants

The MCA biological control efficacy in this trial fell short of the 14.6–24.9% reduction of moniliasis

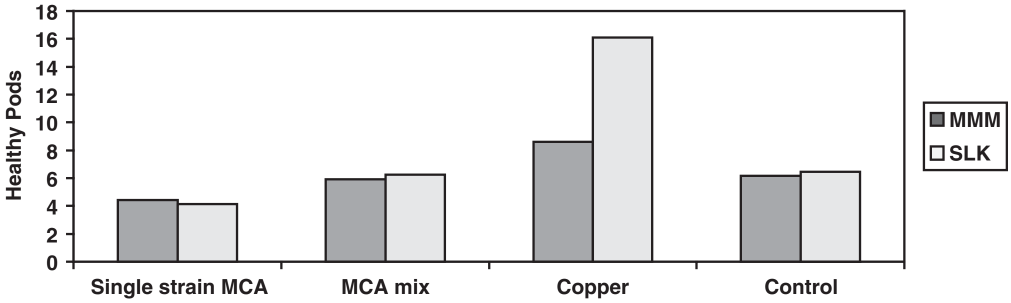

Figure 3. Number of healthy, mature pods harvested per tree. MMM – modified motorised mistblower, SLK – side-lever knapsack sprayer.

incidence reported by Krauss and Soberanis (2001) for a *C. rosea* based mycofungicide in Peru. Our group currently aims to analyse whether this is due to inferior agents, pathogen resistance and/or ecological differences. The statistical over-dispersion as indicated by a high residual mean deviance in this trial suggests that a relevant factor may have been overlooked (Crawley, 1993). One possibility is that a residual molasses component in MCA preparations may have nurtured *M. roreri* more than its antagonists and investigations are underway to test this hypothesis. Nevertheless, the MCAs did reduce sporulation, and thus might lead to a reduction of disease incidence if applied at a large enough scale (and also perhaps less frequently over a longer time period). We are only starting to appreciate the enormous diversity of potentially useful hyperparasitic MCAs; these must be evaluated in the field, as well as under controlled conditions: perhaps by using streamlined 'pre-field trial' techniques, rather than factorial trials such as this.

Unfortunately, there is very little literature on impartial research into pesticidal control that has been carried out in the past decade. It is probable that most farmers would resort to copper fungicides (or perhaps the chloronitrile fungicide: chlorothalonil) which are neither particularly efficacious nor environmentally sound. Since the days of high cocoa prices, much has happened to the pesticide market, including the introduction of whole new chemical classes of compounds. Perhaps of equal significance, the patents on useful molecules (such as triazole fungicides) have expired, raising the prospect of using products that were previously considered too expensive.

The use of an adjuvant oil in the tank mixture was the least important of the main factors tested. Previously, several stickers and UV protectants were tested with respect to their effect on antagonist establishment and survival on cocoa pods. However, none of them had a significant effect (Piper et al., 2000). Their use imposes an extra cost on treatments, and further field testing of adjuvants will take place only after detailed preliminary evaluation. The addition of adjuvant oil to the tank mixture had a mildly detrimental effect on hydrophilic conidia of *C. rosea*. Oils must always be used with care: some are fungicidal in their own right (e.g. against black and yellow Sigatoka disease of banana: *Mycosphaerella fijiensis* and *M. musicola*); while other oils may be phytotoxic (Wrigley, 1973). In contrast, the conidia of fungi such as *Metarhizium* spp. are highly lipophilic and the use of oils appears to confer substantial biological advantages, besides facilitating formulation (e.g. Bateman and Alves, 2000). Also, *Verticillium lecanii*, a mycoparasite with hydrophilic conidia, was not adversely affected by low concentrations ($\leq$0.5%) of various oil adjuvants while some conferred tolerance to low humidity (Verhaar et al., 1999).

The putative benefits of adjuvant oils, including improvements in rain-fastness applied only to the copper fungicides in this trial. Rudgard et al. (1990) discussed various aspects of the tenacity of copper deposits, and showed that the viscous, polyisobutene adjuvant 'Hyvis 150' reduced overall loss of copper deposits at low rates of application, with high levels of simulated rain. Biological activity was proportional to surface concentration, and avoiding loss on pods could improve disease control. However, redistribution of large dosages of copper, placed high in the canopy, can also be effective (Pereira et al., 1996), and the addition of materials imparting rain-fastness would only be beneficial with high dose transfer efficiencies to the pods themselves. However, since there was little apparent difference between the presence and absence of oil with the most efficacious agent (copper fungicide), we have assigned a low priority to this factor for trials in the near future.

Application

Both the SLK and the MMM are recommended for cocoa depending on the size of the holding, the height of trees, the yield, the disease losses and the labour cost (Mabbett, 1985). With hydraulic sprayers, hollow cone nozzles are suitable and widely available, with reproducible results most likely from fixed geometry rather than variable cone nozzles. Motorised mistblowers are recommended for tall trees and improved work rates.

The apparently reduced efficacy of the MMM can probably be attributed to the distribution of spray deposits in the crop. By implication therefore, the intrinsic quality of the droplet size spectrum is less important than maximising deposits of an efficacious agent (in this case copper) on the biological target. Fitting a 'Micronex' to the mistblower had two effects: it noticeably splayed out the droplet stream from the mistblower, besides modifying the droplet size spectrum. Whereas this might be useful for whole canopy treatments (e.g. for treatment of witches' broom or Mirid bugs), in this case, it diverted spray away from the biological target: flowers and growing pods. In our trial, trees were relatively short and accessible, whereas much higher canopies are often found in farmers' fields. Nevertheless, all trial participants found mistblowers easier to use than the manual knapsack sprayers and plots were treated in approximately half the time needed for high volume spraying. Any economic evaluation of spraying techniques should also consider the transportation of typically between 2 and 10 times less water (7 times in this trial) to the field with low volume techniques. Where labour costs are substantial, selecting motorised sprayers remains an important option for timely disease treatment.

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References

Bateman R, Carey M, Moore D and Prior C (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. Annals of Applied Biology 122: 145–152

Bateman RP and Alves RT (2000) Delivery systems for myco-insecticides using oil-based formulations. Aspects of Applied Biology 57: 163–170

Chapple AC and Bateman RP (1997) Application systems for microbial pesticides: Necessity not novelty. In: Microbial Insecticides: Novelty or Necessity? British Crop Protection Council Proceedings/Monograph Series No. 68: 181–190

Clayphon JE (1971) Comparison trials of various motorised knapsack mistblowers at the Cocoa Research Institute of Ghana. PANS 17(2): 209–224

Crawley MJ (1993) GLIM for Ecologists. Methods in Ecology In: Lawton JH and Likens GE (Series eds) Blackwell Scientific Publications, Oxford, p 379

Cronshaw DK (1979) Fungicide application together with cultural practices to control cocoa diseases in Ecuador. Tropical Agriculture (Trinidad) 56(2): 165–170

Desrosiers R and Suárez C (1974) Monilia pod rot of cacao. In: Gregory PH (ed) Phytophthora Disease of Cocoa (pp 273–277) Longman, London

Evans HC, Krauss U, Ríos R, Zecevich T and Arévalo E (1998) Cocoa in Peru. Cocoa Growers' Bull 51: 7–22

Evans HC and Prior C (1987) Cocoa pod diseases: Causal agents and control. Outlook on Agriculture 16(1): 35–41

Genstat 5 Committee of the Statistics Department of Rothamsted Experimental Station (1993) 'Genstat 5, Release 3. Reference Manual'. Oxford University Press, Inc., NY

Gorenz AM (1974) Chemical control of black pod: Fungicides. In: Gregory PH (ed) Phytophthora Disease of Cocoa (pp 235–257) Longman, London

Hofstein R and Chapple A (1998) Commercial development of biofungicides. In: Hall FR and Menn JJ (eds) Biopesticides: Use and Delivery, Ch. 6 (pp 77–102) Humana Press, Totowa, NJ, USA

IFOAM: International Federation of Organic Agriculture Movements (2002) http://www.ifoam.org/standard/ibs_draft2_2002_b.html (accessed 7/2/2002)

Jollands P and Jollands P (1984) Improved application methods for chemical control of black pod disease (*Phytophthora palmivora*) in redeveloped cocoa in Papua, New Guinea. British Crop Protection Conference – Pests and Diseases (BCPC) 10A-5 987–993

Krauss U and Soberanis W (2001) Biocontrol of cocoa pod diseases with mycoparasite mixtures. Biological Control 22: 149–158

Krauss U, Martínez A, Hidalgo E, ten Hoopen M and Arroyo C (2002) Two-step liquid/solid state mass production of *Clonostachys rosea*. Mycological Research 106: 1449–1454

Mabbett T (1985) An integrated approach to spray machines for cocoa. World Crops 37(5): 206–209

Matthews GA (2000) Pesticide Application Methods, 3rd edn. (432 p) Blackwell Scientific Publications, UK

Pereira JL, de Almeida LCC and Santos SM (1996) Witches' broom disease of cocoa in Bahia: Attempts at eradication and containment. Crop Protection 15: 743–752

Piper S, Martínez A, Hidalgo E and Krauss U (2000) Effect of formulation on population dynamics of mycoparasites on the surface of cocoa pods. In: Bong CL, Lee CH and Shari FS (eds) Proceedings of the INCOPED 3rd International Seminar on Cocoa Pest and Diseases (pp 21–26) Kota Kinabalu, Sabah, Malaysia, 16–17 October

Prior C, Jollands P and Le Patourel G (1988) Infectivity of oil and water formulations of *Beauveria bassiana* (Deuteromycotina; Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: Curculionidae). Journal of Invertebrate Pathology 52: 66–72

Raupach GS and Kloepper JW (1998) Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88: 1158–1164

Rudgard SA, Pettitt TR and Hadley P (1990) Tenacity, biological activity and redistribution of copper fungicides on cocoa in controlled environments. Crop Protection 9: 281–288

Soberanis W, Ríos R, Arévalo E, Zúñiga L, Cabezas O and Krauss U (1999) Increased frequency of phytosanitary pod removal in cacao (*Theobroma cacao*) increases yield economically in eastern Peru. Crop Protection 18: 677–685

Staniland LN (1959) Fluorescent tracer techniques for the study of spray and dust deposits. Journal of Agricultural Engineering Research 4: 42–81

Verhaar MA, Hijwegen T and Zadoks JC (1999) Improvement of efficacy of *Verticillium lecanii* used in biocontrol of *Sphaerotheca fuliginea* by addition of oil formulations. Biological Control 44: 73–87

Villegas C and Enriquez G (1979) Moniliasis: Bibliografía parcialmente anotada (Anotated literature review in Spanish). Documentación e Información Agrícola, No. 71 (45 pp) CATIE, Turrialba, Costa Rica

Wrigley G (1973) Mineral oils as carriers for ultra-low-volume (ULV) spraying. PANS 19: 54–61