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The Effects of a Fungal Biocontrol Agent and a Chemical Adjuvant on the Efficacy of Cuprous Oxide Sprays for Controlling Witches' Broom Disease in Cocoa

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تأثير أحد العوامل الفطرية للوقاية الحيوية و عامل مساعد كيميائي على كفاءة الرش بأكسيد النحاس لمقاومة مرض مكنسة الساحرة في زراعات الكاكاو

الملخص: أجريت تجربة حقلية في مزرعة كاكاو في البرازيل لدراسة إمكانية قيام عامل مقاومة حيوي (ترايكوديرما) و عامل كيميائي مساعد بتحفيز فاعلية رشات أكسيد النحاسوز في مكافحة مرض مكنسة الساحرة. احتوت التجربة على ١٦ وحدة تجريبية تحتوي كل منها على حوالي ٥٠ شجرة كاكاو بالغة. كانت مساحة كل وحدة حوالي ١٠٠٠ متر مربع. تم توزيع المعاملات عشوائيا. و كانت المعاملات كالآتي: معاملة حاكمة (بدون رش)، ورش بأكسيد النحاسوز بالإضافة إلى العامل الكيميائي المساعد، ورش بأكسيد النحاسوز بالإضافة إلى العامل الكيميائي المساعد زائدا الفطر. تم إعطاء الرشات شهريا من أبريل إلى سبتمبر تماشيا مع التوصيات المعمول بها لمحاربة مرض مكنسة الساحرة. تم الرش باستخدام آلة رشاش الرذاذ (الضباب) المحمولة على الكتف و المزودة بموتور. تم إجراء فحص تقيمي شهري للكشف عن معدل حدوث الإصابة بالمرض بين شهري مارس و نوفمبر. تمت الفحوصات على خمس شجرات مختارة عشوائيا من بين خمس عشرة شجرة معلمة موجودة في منتصف كل وحدة تجريبية. شملت الفحوصات عدد البراعم السليمة و المريضة من كل شجرة عند الحصاد. أوضحت النتائج أن معدل الإصابة كانت أعلى في المجموعة الحاكمة حيث تم جمع أقل عدد من البراعم السليمة. تراوح معدل حدوث الإصابة بالمرض بصورة عامة (كل المعاملات) بين ٢٥-٤٥%. أوضحت نتائج التحاليل الإحصائية أنه ليس هناك فروق معنوية بين المعاملات، بما في ذلك المعاملة الحاكمة. لم يلاحظ تحسن معنوي في تأثير الرشات ذات القاعدة النحاسية كنتيجة لإضافة الفطر أو العامل المساعد كما باستخدام أكسيد النحاسوز وحده. ولذلك توصلنا إلى خلاصة مفادها أن رشات النحاس ليست ذات تأثير مفيد في السيطرة على المرض. قمنا بمناقشة نتائجنا في ضوء التوصيات الرسمية لمحاربة المرض.

ABSTRACT: A Field experiment was carried out in a cocoa plantation in Brazil to evaluate whether a fungal biocontrol agent (*Trichoderma* spp.) and a chemical adjuvant could enhance the efficacy of cuprous oxide sprays that are applied to control witches' broom disease. The experimental design was comprised of 16 plots containing approximately 50 mature cocoa trees each. Each plot measured approximately 1000 m^2. Treatments were allocated to plots randomly. Treatments were: control (no spray), cuprous oxide, cuprous oxide plus adjuvant, and cuprous oxide plus adjuvant plus fungus. Sprays were applied monthly from April to September in line with official recommendations for the control of witches' broom disease. Sprays were applied using a motorized backpack mistblower. Monthly assessments of disease incidence were made from March to November. Assessments were made on five randomly selected trees from 15 marked trees located in the center of each plot. Assessments were comprised of the numbers of healthy and diseased pods observed on trees, the numbers of green and necrotic axillary and terminal brooms, and the numbers of healthy and diseased pods taken from trees at each harvest. The results show that disease incidence was the highest in the control plots where the fewest healthy pods were harvested. The overall incidence of disease varied from 25 to 45% across all treatments. Statistically, the results show that there were no significant differences among treatments, including the control. No significant improvements in the efficacy of copper-based sprays were measured as a result of adding the fungus or the adjuvant to the spray. Also, no significant improvements in disease control were recorded when using cuprous oxide on its own. We concluded that copper sprays were ineffective in controlling the disease. This paper discusses our results in the light of official recommendations for the control of the disease.

The region of Bahia in Brazil was the second largest producer of cocoa in the world until the accidental introduction, in 1989, of the fungal pathogen (*Crinipellis perniciosa* [Sahel] Singer) that causes witches' broom

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disease (Pereira *et al.,* 1990). *C. perniciosa* is a fungus which is indigenous to the Amazon region (Evans, 1981) and attacks the pods as well as the canopy causing both direct (attack of pods) and indirect yield reductions (reduced photosynthesis) (Orchard and Hardwick, 1988; Pereira, 1999).

The methods of disease control used to date include crop sanitation, chemical control, and the planting of tolerant material (Baker and Crowdy, 1941; Cronshaw, 1979; Evans, 1981; Rudgard, 1987; Fulton, 1989; Laker *et al.,* 1990; Laker, 1991; Laker, 1992; Anon., 1993). The chemical control product recommended by the Brazilian government organization, Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), is cuprous oxide which is a protective fungicide. Despite this recommendation, yields in Bahia are continuing to decline. The scientific literature regarding the efficacy of cuprous oxide for disease control is, at present, mixed (Thorold, 1953; McGregor, 1984; Laker *et al.,* 1988; Anon., 1993).

The approach taken in the experiment described in this paper was to assess the efficacy of cuprous oxide sprays and to ascertain whether the use of a strain of *Trichoderma* and/or an organosilicone/latex-based adjuvant could improve the effectiveness of the spray. Fungi in the genus *Trichoderma* are known to inhibit other fungi (Samuels, 1996) and the species used in this trial had been shown to be effective against *C. perniciosa* in small-scale studies (Bastos, 1996). The adjuvant used in the experiment was comprised of a novel sticker-spreader formulation designed to enhance both retention and resistance to weathering. Environmental loss of product is a problem with copper as there is a large amount of wash-off that occurs during tropical downpours (McGregor, 1984).

Materials and Methods

The field trial was carried out at São Jorge farm, near Itabuna, Bahia, Brazil. Plots were marked out so as to each contain approximately 50 mature cocoa trees (Thacker *et al.,* 1998). Four different treatments were applied, each being replicated four times. The treatments included a control (no spray), cuprous oxide (Copper Sandoz), cuprous oxide plus an adjuvant (Tactic, a novel spreader-sticker comprising an organosilicone and latex-based formulation), and cuprous oxide plus adjuvant plus a fungus (*Trichoderma* spp. - strain isolated from the Amazon basin, not yet identified). These treatments were allocated randomly to the plots.

The treatments were applied at five week intervals from April to August. Applications were made using a motorized backpack sprayer operating at a rate of 400 ml/tree. The concentrations for cuprous oxide and Tactic were 3 and 0.75%, respectively. Tap water was used for diluting the spray. The treatment mixtures were made up prior to spraying and were thoroughly stirred before application to re-suspend the cuprous oxide. *Trichoderma* was cultured at the Almirante Cacau Research Center in 20L fermentors using molasses yeast extract as the substrate (Papavizas *et al.,* 1984). The cultures were composed of clamydospores (10^7/ml) and conidia (10^8/ml). The presence of clamydospores was important as these are environmentally-resistant structures (Lumsdem and Lewis, 1989; Papavizas *et al.,* 1984).

Assessments were carried out on 15 marked trees from the center of each plot. On each occasion, five trees from the marked 15 were randomly chosen. Disease assessments were carried out each month, from March to November. The assessments entailed counts of the number of necrotic brooms, green axillary and terminal brooms, vegetative cushion brooms, healthy pods, and pods infected with *C. perniciosa*. The pods assessed were a minimum of 6 cm in length. This length was picked as it was highly likely that small pods (those less than 6 cm in length) would be missed when assessments were made. Assessments of disease incidence were also carried out at each harvest (Table 1).

The schedule for the experiment is given in Table 1. The field trial ran from March 1998 to November 1998. The schedule was structured so as to be repeated at 5-week intervals. The first week was a disease assessment followed by a spray week. After a week's interval, the harvest was performed which was then followed by another week's interval prior to the start of the cycle once again.

The raw data were analyzed using two-way analysis of variance. Means' separation were carried out using 95% confidence limits. Percentage disease incidence in plots was calculated based on pooled data for individual treatments.

TABLE 1

Disease assessment and spraying cycle[1].

Disease	Spray Application	Harvest
30 March 1998	6 April 1998	20 April 1998
08 May 1998	11 May 1998	25 May 1998
08 June 1998	5 June 1998	29 June 1998
13 July 1998	29 July 1998	03 August 1998
17 August 1998	24 August 1998	08 September 1998
21 September 1998		13 October 1998
26 October 1998		16 November 1998
30 November 1998[2]		

[1]Each activity took about one week to complete for the experimental area.
[2]Field trial completed.

TABLE 2

Results of the two-way analysis of variance.

Assessment Parameter	Source of Variation	Degrees of Freedom	F-ratio	P-value
Infected pods observed on trees	Time	8	14.71	p<0.001
	Treatment	3	3.15	p<0.05
	Interaction	24	0.72	NS[1]
Green brooms observed on trees	Time	8	2.24	p<0.05
	Treatment	3	3.08	p<0.05
	Interaction	24	0.70	NS[1]
Necrotic (dry) brooms observed on trees	Time	8	5.07	p<0.001
	Treatment	3	2.39	NS
	Interaction	24	0.94	NS
Harvested pods	Time	6	25.34	p<0.001
	Treatment	3	4.91	p<0.01
	Interaction	18	1.06	NS

[1]NS = not significant.

Results

Figure 1 shows the mean number of infected pods that were observed on trees for each treatment and sampling occasion. The number of observed infected pods remained relatively low (< 1 per tree) until August. Thereafter, the number of observed infected pods increased to approximately 3 per tree. Although the number of infected pods was the highest in control plots from August to the end of the trial in November, this difference was statistically significant on only one sampling occasion. Statistical analyses of these data are given in Table 2. The number of infected pods varied significantly with time and among treatments (most notably for the August assessment). However, these significant differences were inconsistent. No significant differences were observed between any of the cuprous oxide-based treatments.

The harvest data for infected pods (Figure 2) supports the above results. Most infected pods were harvested from control plots. However, no consistent statistical differences, and no differences among any of the cuprous oxide-based treatments were recorded. Statistical analyses of these data are given in Table 2. The September harvest was significantly more infected in control plots, presumably following the assessment data collected in August (Figure 1). However, this was the only obvious difference between the control and fungicide-treated plots.

The mean number of green and dry (necrotic) brooms assessed are shown in Figures 3 and 4, respectively. Statistical analyses of these data are given in Table 2. The number of green brooms remained fairly constant across treatments at 2 to 4 per tree per sampling occasion (Figure 3). The number of green brooms was the highest in the control plots but this difference was statistically insignificant. No differences among cuprous oxide-based treatments were apparent. The number of necrotic brooms (Figure 4) slowly increased as the experiment progressed. Once again, although the number was the highest in the control plots, statistical differences among treatments were not recorded.

The total numbers of healthy and infected pods harvested from trees are shown in Figure 5. Overall disease incidence, calculated from these data, varied from 45% in the control plots to 25% in the plots that

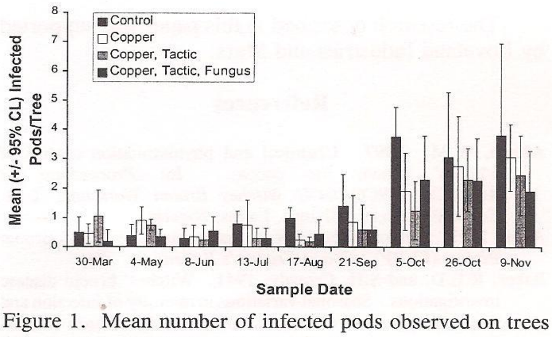

Figure 1. Mean number of infected pods observed on trees for the different treatments through time.

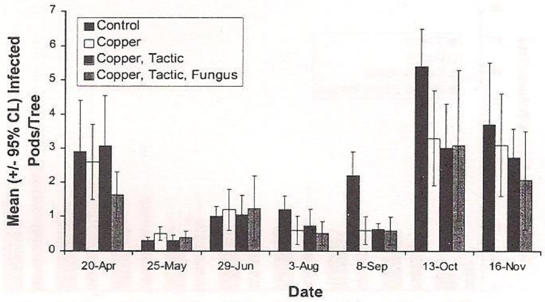

Figure 2. Mean number of infected pods havested from the different treatments through time.

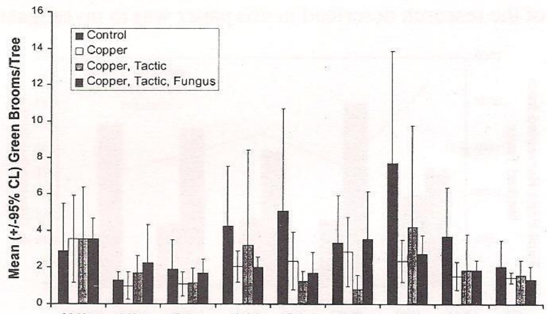

Figure 3. Mean number of green auxiliary and terminal vegetative brooms observed on trees for the different treatments through time.

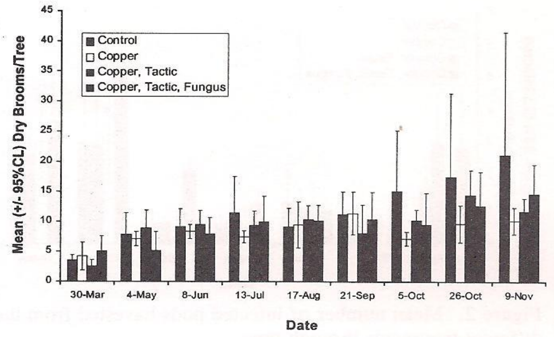

Figure 4. Mean number of dry, brown, necrotic brooms observed on trees for the different treatments through time.

were treated with cuprous oxide plus adjuvant plus *Trichoderma*. All of the plots that were treated with cuprous oxide-based sprays gave a higher yield of disease-free pods than the control plots. However, there were virtually no differences in the number of diseased pods harvested from the plots that received cuprous oxide-based sprays.

Overall the data presented in Figures 1 to 5 indicate that more disease was present in the control plots than in any of the plots sprayed with a copper-based formulation. In addition, the highest yields were recorded in plots that had been sprayed with a copper formulation, of any type. Also, no differences in disease incidence on yield were recorded among copper-based treatments. Finally, there were no statistical differences between the control plots and any of the treatment plots that could be identified as a definite trend.

Discussion and Conclusions

Yields for cocoa growers in Bahia, Brazil have steadily decreased following the arrival of the aetiological agent of witches' broom disease. The aim of the research described in this paper was to investigate methods for improving the efficacy of the fungicide that is recommended for use in the control of the disease. Unfortunately, the data collected indicate that the official recommendations that growers receive may not be sound. Although disease incidence was highest and crop yield lowest in control plots, these results were not statistically significant. Indeed, a simple economic analysis concerning the cost-effectiveness of cuprous oxide sprays has indicated that it would be better not to spray at all (Laine *et al.*, 2000).

These results may not be altogether surprising. Aitken (1997) reported that most farmers do not make the required number of fungicide applications recommended by CEPLAC. In his paper, Aitken cites economic constraints as the reason why these applications are not made. However, it may also be the case that farmers are not convinced concerning the efficacy of the fungicide. Indeed Thorold (1953), questioned whether copper-based sprays were effective against *C. perniciosa*. What is surprising, therefore, is that the official recommendations still suggest the use of these products.

Given that we were unable to show that cuprous oxide, on it's own, worked effectively against witches' disease, it is not surprising that our spray additives did little to improve product efficacy. In other situations, both the adjuvant and the biocontrol agent assayed have been shown to improve pesticide efficacy. That these products did not improve the efficacy of the fungicide sprays used has more to do with a lack of active ingredient efficacy than with a lack of additive efficacy.

Clearly, the situation for cocoa farmers in Bahia will continue to worsen. What is required immediately are trials to evaluate cost-effective fungicides that can be used as part of an integrated disease management program for cocoa crops. Such an integrated program would include cultural (phytosanitation), biological, and chemical approaches to disease management. If such work is not undertaken, we can only imagine the worst for farmers attempting to grow cocoa in Brazil.

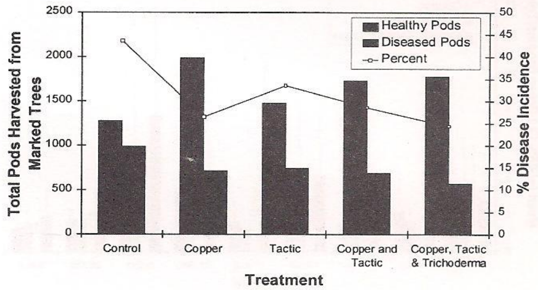

Figure 5. Total number of healthy and infected pods harvested from marked trees in the different treatments through time.

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