

Research Article

Cocoa (*Theobroma cacao*) yield increase in Costa Rica through novel stress management and fertilization approach

Ulrike Krauss^{1*}, Valex Adonijah², Claudio Arroyo², Mirjam Bekker¹, Jayne Crozier³, Arturo Gamboa², Chantal Steuten¹ & Keith Holmes³

¹ CABI Caribbean and Latin America - Costa Rica, c/o Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), 7170 Turrialba, Costa Rica

² CATIE, 7170 Turrialba, Costa Rica

³ CABI Europe – UK, Silwood Park, Buckhurst Road, Ascot, Berks., U.K.

* Corresponding Author's Email: ulrike.krauss@gmail.com, P.O.Box GM1109, Sunny Acres, Saint Lucia, West Indies

ABSTRACT

We assessed the effect of calcium-based anti-stress and fertilization products on cocoa diseases, yield and quality. Calcium levels in cocoa seeds were elevated by up to 25% w/w. Cocoa bean defects, pod index, pH and organoleptic parameters were unaffected by treatments. *Impaction* did not improve yield (average 179.6 kg ha⁻¹ yr⁻¹), but led to a reduction of cherelle wilt from 3.7% to 1.6% over two seasons. *Impaction* with *Speedo CaN* increased yield by over 40%, from 190.7 kg ha⁻¹ yr⁻¹ to 273.7 kg ha⁻¹ yr⁻¹, by stimulating pod production. Diseases, most notably frosty pod rot, claimed over 80% of pods and were unaffected by the above products. Thus, we combined them with the biocontrol agent *Trichoderma ovalisporum*. The agents proved to be compatible and augmented the number of healthy pods harvested, but not as much as a copper fungicide.

Keywords: calcium fertilization, cocoa, plant stress, *Theobroma cacao*, *Trichoderma ovalisporum*

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an under-storey tree crop of Amazonian origin, planted almost exclusively by smallholders in the tropics. The raw material for cacao, the dried cocoa bean, is a relatively imperishable product with a high value to weight ratio, rendering cocoa an ideal cash crop for remote communities (Krauss *et al.*, 2003). Cocoa-based agroforestry systems play an important role in buffer zones of protected areas in Latin America (Rice and Greenberg, 2000). They provide a refuge for many animal taxa as well as the cultural diversity of indigenous peoples (Gaudrain and Harvey, 2003; Toledo *et al.*, 2003). However, abandonment of traditional agroforestry systems as a result of low cocoa productivity and high disease losses seriously threatens the role these systems play in anthropological and biodiversity conservation. Certified organic cocoa production attracts a premium that renders production economically viable and even attractive. Thus, our aim is to develop an integrated crop management package that enhances productivity in a sustainable manner and is compatible with organic production standards.

Calcium has repeatedly been implicated in plant tolerance to diseases and abiotic stress factors (Goodman *et al.*, 1986) because of its role in cell wall structure and membrane stability (Richter, 1988). Mature cocoa requires approximately 373 kg Ca ha⁻¹ yr⁻¹ (Wessel, 1985) and cocoa roots, in particular, are recognized for their high calcium content (Falade *et al.*, 2005). *Alethea Technology* has been developed and patented by Plant Impact plc. While the detailed mechanism is proprietary, the novelty consists of employing an abiotic stress tolerance product, *Impaction*, together with co-applied plant nutrients, in order to improve plant growth during stressful climatic conditions. *Speedo CaN* is the controlled-release nitrogen/calcium fertiliser tested here. It improves calcium uptake and distribution and thereby combats physiological disorders and stress-related problems (www.plantimpact.com). After the study presented here was completed, the company produced a novel formulation, *Caln*, based on both products, as more advanced formulation (www.plantimpact.com).

The first objective of these trials was to evaluate the effect of *Impaction* and *Speedo CaN* on the dominant pod disease of cocoa, frosty pod rot (FPR), caused by *Moniliophthora roreri*, and black pod (BP), caused by *Phytophthora* spp., on cherelle wilt (CW), on cocoa yield and quality. After two trial seasons, it became clear that these products

increased the yield potential of the cocoa trees, but did not reduce pod diseases. In an attempt to better translate the improved yield potential into enhanced productivity, in one trial, these products were combined with the previously tested biocontrol agent *Trichoderma ovalisporum* TK-1 (Holmes *et al.*, 2005; Krauss *et al.*, 2010). All these agents are compatible with organic production.

MATERIALS AND METHODS

Germination assay

Germination tests were carried out to assess the compatibility of *T. ovalisporum* TK-1 with *Alethea Technology* products. For this, potato dextrose broth (PDB) with antibiotics (penicillin 30 $\mu\text{g ml}^{-1}$ and streptomycin 50 $\mu\text{g ml}^{-1}$) was amended with *Impaction* at 20% of its recommended concentration, the full concentration, four or ten times the recommended concentration, *Speedo CaN* at 10% its recommended concentration, the full concentration, four or ten times that amount, or the respective combination of both products. Conidia suspended in negligible volumes of water were added to Erlenmeyer flasks (400 ml medium in 1000 ml flasks) to yield a final concentration of approximately 10^6 ml^{-1} and incubated on a rotary shaker (Thermolyne, Big Bill) at 25 °C and 120 rpm.

Trial layout and installation

Field trails

All trials were conducted in Sections 13 to 15 of *La Lola* Research Station, *Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)*, Costa Rica (altitude 70 m; access point: 10° 5' 18.7" N; 83° 23' 20.2"W). The alluvial soils of *La Lola* present a range of textures. Stones and poor drainage can be a problem in some fields, but the nutrient status is regarded adequate for cocoa (Bazán, 1963). Disease pressure, particularly FPR, is high; no other stress factor for cocoa production has been reported for this site. The selected terrain was flat and uniform. Cocoa hybrids had been planted in 1992 at 3m \times 3m spacing. Meteorological data were measured on site.

For logistical reasons (availability of suitable field space, labour as well as time requirements to apply treatments and collect quantitative data) the hypotheses tested had to be addressed in three separate field trials, as follows. The *Impaction* Trail (IT) was carried out over two complete production cycles; the Combination Trial (CT) over three (Table 1). During the last season, the Biocontrol Trial (BT) was pioneered to form hypotheses for future in-depth testing.

In the IT and BT, the 8 \times 8 plots consisted of one surrounding border row, a 6 \times 6 plot of sprayed trees with an inner 4 \times 4 plot, i.e. the experimental unit, of sprayed trees that were assessed (Table 1). The CT was the original priority of the company. Thus, it was conducted throughout all three seasons and with slightly larger 9 \times 9 plots. They also consisted of one surrounding border row, a 7 \times 7 plot of sprayed trees with an inner 5 \times 5 plot of sprayed trees that were assessed. Thus, each assessed tree was surrounded only by sprayed trees, and treatments were separated by at least two non-treated border rows. The total number of available cocoa trees in suitable configurations was 725. Four hundred and five trees (5 \times 9 \times 9) were required for the CT, leaving 320 (5 \times 8 \times 8) for either the IT or BT.

Prior to the first application per trial season, all developing pods were removed from trees to be sprayed. Therefore, we regard disease incidence data from different seasons as independent from each other. Occasional phytosanitary removal of diseased pods was practised in border row trees, as in the non-experimental remainder of the plantation, according to local practice. Maintenance pruning was concentrated between seasons as much as possible.

We used hydraulic knapsack sprayers (Carpi 'Spray-Mec', Agrosuperior S.A., San Jose, Costa Rica), fitted with D2-45 hollow cone nozzles (Spraying Systems Co., Wheaton IL, USA). At 300 kPa, this nozzle has a measured flow rate of 765 ml min^{-1} , and typically produces a spray with a volume median diameter of 170 μm in a 40° cone, desirable characteristics for fungicide application to cocoa trees (Bateman, 2004).

An overview of applications and treatments is presented in Table 1. In each season, monthly applications were made from the onset of flowering to four weeks before the last harvest. These crop development parameters are largely determined by meteorological factor, i.e. flowering is triggered by the onset of rains, and thus resulted in seasons of different duration in different years, ranging from nine to eleven months. Thus, direct statistical comparison between trials would not be legitimate and each is interpreted individually here.

In all trials, *Impaction* (0.5% v/v) was applied at a rate of 122.8 l ha^{-1} , which was equivalent to 614 ml ha^{-1} a.i. In the CT, *Speedo CaN* (1 % v/v) was added to the *Impaction* treatment (tank mixture) at an equivalent of 1.23 l ha^{-1} a.i. per

TABLES

Table 1: Schedule of trials during three seasons, with corresponding meteorological data and long term (> 40 years) averages for *La Lola*, Costa Rica.

Dates	Trial Season		
	2004/2005	2005	2006/2007
Date of first application	15 April 2004	17 March	5 April 2006
Date of last evaluation	3 March 2005	8 December 2005	9 February 2007
Field trial	Treatment ¹	Plot size (trees assessed)	
Impaction Trial (IT), ,	<i>Impaction</i>	8 × 8 (4 × 4)	11 applications
Combination Trial (CT)	<i>Impaction + Speedo CaN</i>	9 × 9 (5 × 5)	11 applications
Biocontrol Trial (BT)	<i>Impaction + Speedo CaN + Trichoderma ovalisporum TK-1</i>	8 × 8 (4 × 4)	-
			9 applications
			9 applications
			11 applications

¹ *Impaction* was consistently applied at 0.5% v/v, *Speedo CaN* at 1% v/v, according to manufacturer's instructions. *Trichoderma ovalisporum* TK-1 was applied at 10⁶ conidia ml⁻¹

Table 2: Meteorological data and long term (> 40 years) averages for *La Lola*, Costa Rica, during three trial seasons from 2004 to 2007

Dates	Trial Season			Long term (> 40 yrs) annual average
	15 April 2004 – 3 March 2005	17 March - 8 December 2005	5 April 2006 – 9 February 2007	
Average monthly temperature (°C)	Min	21.4	23.2	24.1
	Max	26.3	26.2	26.5
Absolute temperature (°C)	Min	16.2	16.0	17.8
	Max	35.2	34.2	35.2
Average monthly relative humidity (%)	Min	88.8	87.9	85.8
	Max	95.0	91.0	92.3
Rain total (mm)		4,412	2,273	2,953
Driest month (mm)		61.8 (October)	41.1 (March ¹)	50.3 (October)
Wettest month (mm)		941.9 (May)	494.5 (April)	432.4 (December)

¹ For whole month; the second trial season started 17 March.

application. Treatments were compared with untreated control plots of the same size.

In the BT, the tank mixture contained 0.5% v/v *Impaction*, 1 % v/v *Speedo CaN*, and 10^6 conidia ml⁻¹ (1.23×10^{11} ha⁻¹) of *Trichoderma ovalisporum* TK-1, an endophytic and mycoparasitic biocontrol agent of FPR. Background information on TK-1 can be found in Holmes *et al.* (2005). Inoculum was produced in rice as described by Krauss *et al.* (2002), extracted using a Mycoharvester MH I (www.mycoharvester.info), weighed, pre-packed in sealed plastic bottles for transport to the field and suspended immediately prior to application. This treatment was compared with an absolute control as well as the best-proven chemical disease control, copper hydroxide (Kocide 70WP at 10 g l⁻¹, i.e. 1.23 kg ha⁻¹ a.i.).

Post-harvest processing

Seeds were extracted from healthy pods and healthy sections of pods only partly affected by BP. Seeds were subjected to locally typical post-harvest processing, which consisted of fermentation in wooden boxes and sun drying. They were then stored in sealed plastic containers at ambient temperature, until the end of each season, and the dry seed weight per plot was determined. For good fermentation, a minimum critical mass of cocoa is necessary, which the fortnightly harvest of individual plots could not consistently provide. Thus, samples from each plot were kept separate by placing them into individual cotton mesh bags. These were then put into fermentation boxes together with "bulk cocoa" from the same fields. While this method allowed to maintain the experimental structure, quality may somewhat blend, decreasing any possible treatment effect. During fermentation of such relatively low volumes, the temperature does not rise to the level it would in larger lots. Thus, a higher percentage of beans tends to be somewhat under-fermented (purple and purple turning brown in cut test) than in commercial-scale fermentations. This phenomenon, however, was equal across treatments and did not affect their comparison.

Experimental Design, Data Evaluation and Analysis

A completely randomized design was used throughout. In the germination experiment, three independently repeated experiments constituted the replicates. Five replicate plots per treatment were used in the three field trials and quality assessments.

Germination was assessed after 24h by counting 100 spores each in four samples. A spore was considered germinated when the germtube had a length equivalent to the diameter of the spore. Percentages germination was arcsine-transformed in order to normalize the error distribution prior to two-way analysis of variance (ANOVA) on Infostat (InfoStat, 2004). Protected means were separated using the Tukey test.

Field assessment took place in fortnightly intervals, starting two weeks after the first application and ending one month after the last application. Assessment consisted of harvesting and counting healthy, mature pods, and quantifying and removing any diseased pods. Two cocoa pod diseases were evaluated: FPR and BP. CW, a physiological disorder that affects young pods, was also quantified. Percentages (e.g. disease incidences) were arcsine-transformed and pod counts log-transformed prior to one-way (BT) or two-way (IT, CT) ANOVA, as appropriate, on Infostat. Whenever significant treatment effects were observed, means were separated using the Tukey test.

For quality assessment, random samples of 50 seeds each were taken from each treatment for quality analysis comprising the cut test, pH measurement (Whatman test paper), organoleptic assessment on an arbitrary scale from 0 to 6 by a panel of four to six people, and a calcium analysis, which was done by CATIE's soil laboratory. Samples were incinerated at 550°C for 8h. The ashes were suspended in an equal volume of HCl and the calcium content measured by atomic absorption (AAnalyst 100, Perkin Elmer). The frequency of quality defects was expressed as percentages, which were arcsine-transformed prior to ANOVA, followed by Tukey test for protected means. Nonparametric data were analysed using Kruskal-Wallis and orthogonal contrasts were employed to compare treatments and years, on Info stat.

RESULTS

Meteorological Data

A summary of meteorological data is presented in Table 2. For temperature and humidity, the three trial years were within the normal range of conditions. However, precipitation in September (87.2 mm) and October (61.8 mm) 2004 was below the long-term average for these months: 175.4 mm and 252.7 mm, respectively. This deficit was more than compensated for by rains in March (402.1 mm), May (941.9 mm), November (495.9 mm), December (695.9 mm) 2004, and January 2005 (866.9 mm), which were markedly higher than normal for these months:

171.7 mm, 335.9 mm, 272.8 mm, 467.6 mm, and 299.2 mm, respectively. Overall, annual rainfall in the first season was 25% higher than normal. This can lead to increased wash-off of applied products and thus reduce effectiveness. The second and third season, if extrapolated to 12 months, were on average within the normal range for *La Lola*, except that October 2006 was again markedly drier (50.3 mm) than normal for this month (256.3 mm).

Impaction Trial (IT)

Cocoa pod losses due to FPR were in the typical range for the region, with an average of 78% and no difference between the two seasons ($P = 0.491$). FPR was not influenced by *Impaction* ($P = 0.628$, Fig. 1). BP incidence was low (average 5.3%), with neither a seasonal ($P = 0.274$) nor a treatment effect ($P = 0.788$). Neither for FPR ($P = 0.836$), nor for BP ($P = 0.338$) an interaction of season with treatment occurred. CW decreased from an average of 4.0% in the first season to 1.3% in the second season ($P = 0.032$). Plots treated with *Impaction* showed less CW (1.6%) than the control (3.7%, $P = 0.050$). Again, there was no interaction between season and treatment ($P = 0.304$).

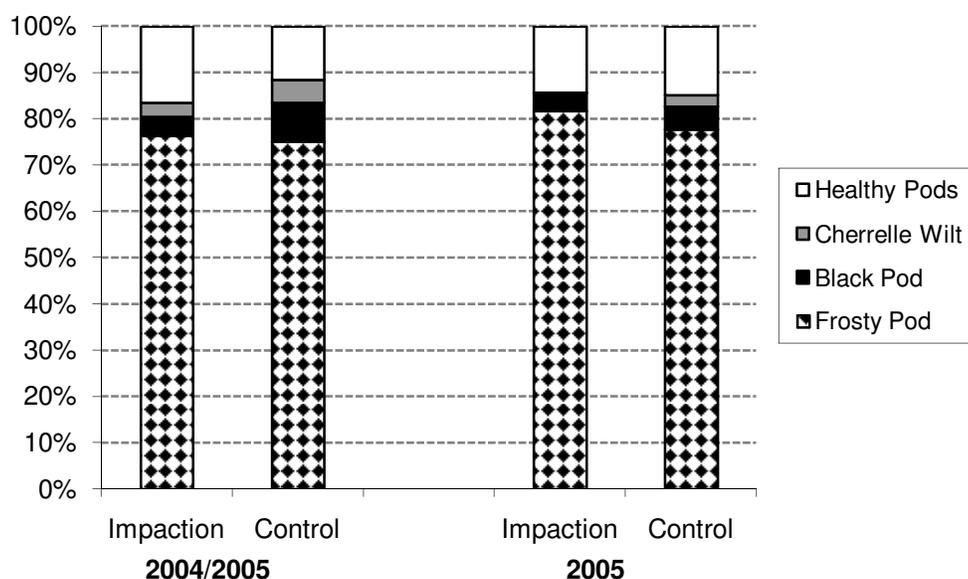


Figure 1: Disease incidence in cocoa Impaction Trial (IT) in Costa Rica, over two seasons: 2004/2005 and 2005.

The percentage of healthy pods averaged 14.3% with no significant difference between treatments ($P = 0.620$) or years ($P = 0.894$) and no treatment \times year interaction ($P = 0.477$, Fig. 1). A matching lack of significance was observed in absolute terms. The average yield in the IT was $179.6 \text{ kg ha}^{-1} \text{ yr}^{-1}$ without any significant effects: $P = 0.497$, $P = 0.858$ and $P = 0.280$, respectively. For the numbers of healthy pods, probabilities were: $P = 0.645$, $P = 0.690$ and $P = 0.516$, respectively. The IT had an average pod index¹ of 21.8, again with no significant effects ($P = 0.351$, $P = 0.177$ and $P = 0.428$, respectively) and an average thousand-seed-weight (TSW) of 1.33 kg, similarly without significant effects ($P = 0.681$, $P = 0.078$ and $P = 0.718$, respectively).

Combination Trial (CT)

FPR continued to be unaffected by treatment ($P = 0.804$), but increased with years ($P = 0.014$; Fig. 2). In the third season, significantly more FPR (91%) was recorded than in the two preceding years (average 80%). BP was also unaffected by treatment ($P = 0.161$), but, in contrast to FPR, decreased over time ($P < 0.001$): BP was higher in the first year (3.1%) than in the subsequent two seasons (average 0.8%). Similarly, CW was independent of treatment

¹ The pod index is the number of pods necessary to produce 1 kg of fermented, dried cocoa seeds. Thus, a low value is desirable.
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($P = 0.574$) and decreased over time ($P = 0.004$), with year three exhibiting significantly less wilt (0.1%), than years one and two (1.1%; Fig. 2). No treatment \times year interactions were observed for FPR ($P = 0.676$), BP ($P = 0.891$) or CW ($P = 0.589$).

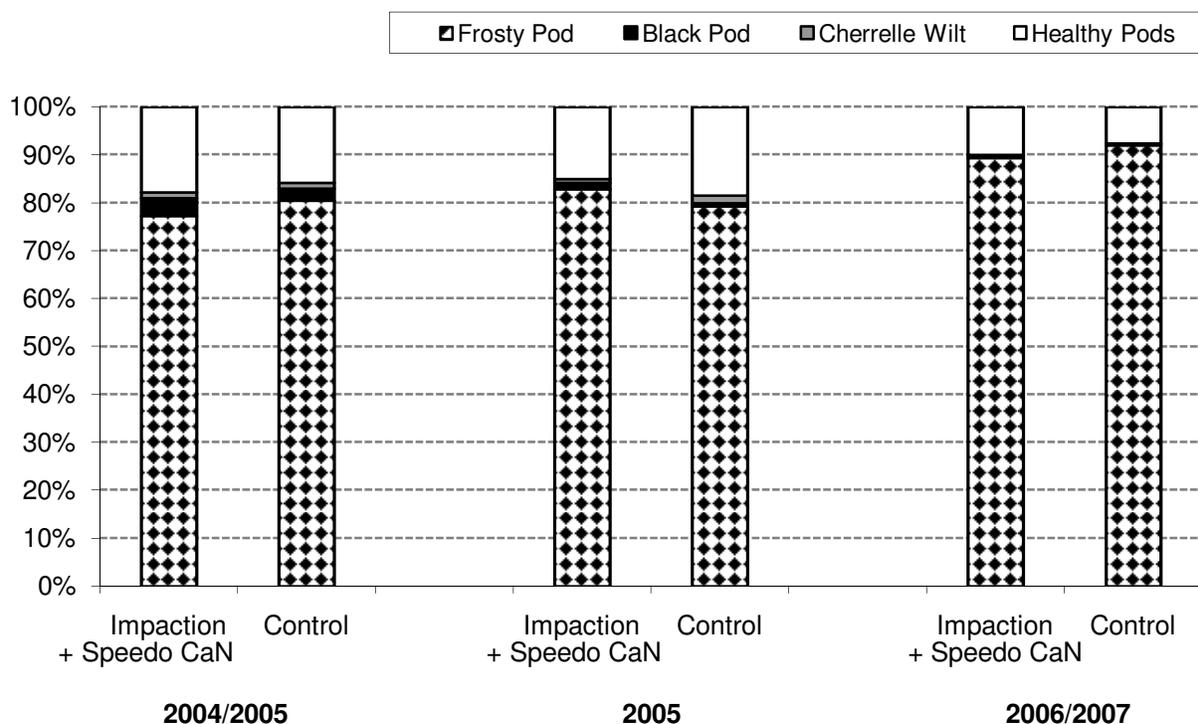


Figure 2: Disease incidence in Combination Trial (CT) in Costa Rica, over two seasons: 2004/2005 and 2005.

The percentage of healthy pods was unaffected by treatment ($P = 0.890$), but dropped from an average of 16.7% in the first two years to only 8.7% in year three ($P = 0.014$). No interaction was observed ($P = 0.676$; Fig. 2). Yield was consistently improved by the application of *Impaction* plus *Speedo CaN* ($P = 0.035$), with no effect of years ($P = 0.218$) or treatment \times time interaction ($P = 0.878$), from an average of $190.7 \text{ kg ha}^{-1} \text{ yr}^{-1}$ to an average of $273.7 \text{ kg ha}^{-1} \text{ yr}^{-1}$, i.e. by 43.5% (Table 3).

Table 3: Yield data in the Combination Trial (CT), carried out in Costa Rica over three growing seasons: 2004/2005, 2005, and 2006/2007.

Treatment	Yield ($\text{kg ha}^{-1} \text{ yr}^{-1}$)	Number of healthy pods per plot	Pod index	Thousand-seed- weight (kg)
<i>Impaction</i> + <i>Speedo CaN</i> +	273.7 ^b	117.6 ^b	20.4 ^a	1.38 ^a
Untreated control	190.7 ^a	82.4 ^a	20.9 ^a	1.34 ^a
Coefficient of Variance	43.8	8.4	11.4	4.4
F-value (DoF = 1)	5.0	7.3	0.3	2.9

^{a, b} Values within a column followed by the same letter do not differ at $P = 0.05$ (ANOVA)

Application of *Impaction* combined with *Speedo CaN* increased the number of healthy pods per plot significantly ($P = 0.013$; Table 3). The highest number of healthy pods per plot (123.0) was recorded in the second season. This

count differed significantly ($P = 0.037$) from the third year (76.5); year one (97.7) was intermediate, differing from neither extreme. This trend was paralleled by the pod index, which, with 22.2, was significantly higher in year two ($P = 0.006$) than in year three (18.6), while the first year (21.1) was intermediate. In the first year, a higher TSW

(1.45 kg) was measured than in the subsequent years (average 1.31 kg; $P < 0.001$). Treatment had no effect on either parameter ($P = 0.607$ and $P = 0.103$, respectively), nor did interactions occur ($P = 0.978$ and $P = 0.569$, respectively; Table 3).

Biocontrol Trial (BT)

Adjuvant type and concentration had a significant effect on *in vitro* germination of *T. ovalisporum* TK-1; both factors interacted with each other (all $P < 0.001$) (Fig. 3, Table 4). *Speedo CaN*, alone or in combination, reduced germination compared with *Impaction* alone. However, only at 10 times the recommended concentration, did these treatments differ from the bulk of other treatments. At the normal dosage, germination averaged 80% across adjuvants and was not significantly reduced by *Speedo CaN* (Fig. 3).

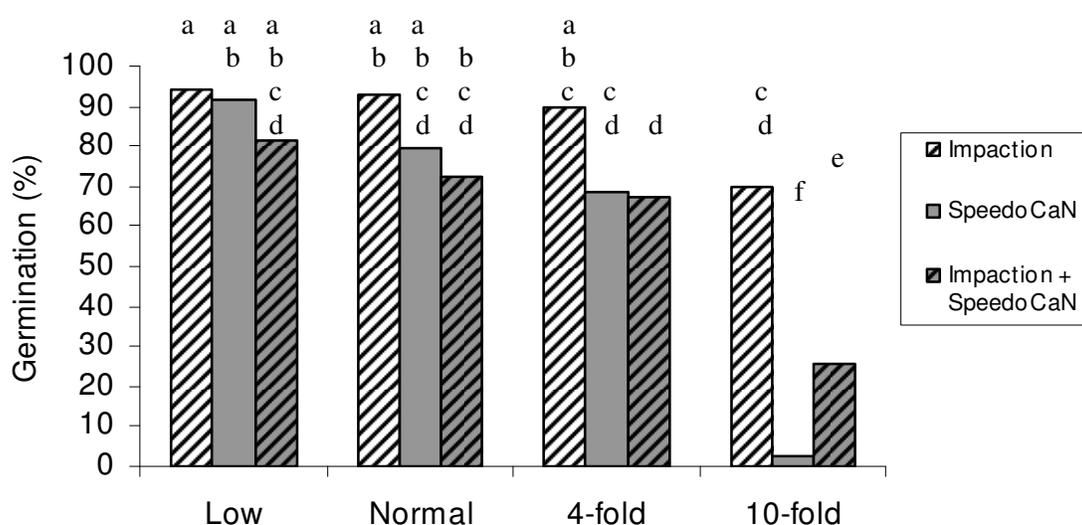


Figure 3: Germination (%) of *Trichoderma ovalisporum* TK-1 conidia in *Impaction* at 0.2 (low), 1.0 (full) 4.0x or 10.0x its recommended concentration, in *Speedo CaN* at 0.1 (low), 1.0 (full) 4.0x or 10.0x its recommended concentration, and the respective combination of both products.

Table 4: Analysis of variance (ANOVA) table of arcsine-transformed percentages of germinated spores of *Trichoderma ovalisporum* TK-1 after 24h exposure to *Impaction*, *Speedo CaN* or both products ("Treatment") at low, recommended or high concentration ("Conc").

Source of Variation	Sum of Squares	Degrees of Freedom (DoF)	Mean Square	F-value	P-value
Model	16,477.63	11	1,497.97	38.22	<0.0001
Treatment	3,524.37	2	1,762.18	44.96	<0.0001
Conc	10,603.13	3	3,534.38	90.18	<0.0001
Treatment × Conc	2,350.14	6	391.69	9.99	<0.0001
Error	940.63	24	39.19		
Total	17,418.27	35			

In the BT, cocoa pod losses due to FPR, with an average of 85.4%, were in the typical range for the region and year. The treatment effect narrowly failed to reach statistical significance ($P = 0.080$) (Fig. 4). The incidences of BP and CW were low and irresponsive to treatments ($P = 0.689$ and $P = 0.702$, respectively). However, the percentage of healthy pods exhibited a significant effect ($P = 0.018$) as a result of cumulative losses accrued by all three disorders. The copper hydroxide (Kocide) control had a higher percentage (18.0%) of healthy pods than the absolute control (6.3%); the combination of *Impaction*, *Speedo CaN* and *T. ovalisporum* TK-1 was intermediate (15.1%), differing from neither extreme (Fig. 4). This trend was similar to yield (Table 5), which was highest in the Kocide treatment ($P < 0.001$). A higher resolution was exhibited by the number of healthy pods, which differed between all treatments ($P < 0.001$). Again, the Kocide plots yielded best, followed by the *Impaction*, *Speedo CaN* plus TK-1 treatment, which both surpassed the absolute control. Pod indices appeared lower than in the other trials, but continued to be unaffected by treatments ($P = 0.326$). The TSW was lowest in the untreated control ($P = 0.004$), while Kocide and the organic treatment did not differ from each other (Table 5).

Calcium Levels and Quality

Calcium levels in cocoa seeds in the IT were unaffected by *Impaction* ($P = 0.574$; Fig. 5). However, in the CT, where a combination of *Impaction* with *Speedo CaN* was applied, calcium levels increased by 25% from 0.08% w/w to 0.10% w/w ($P = 0.029$). An even greater increase of at least 33% was measured in the BT; however, as only one season was assessed, no statistical analysis could be done.

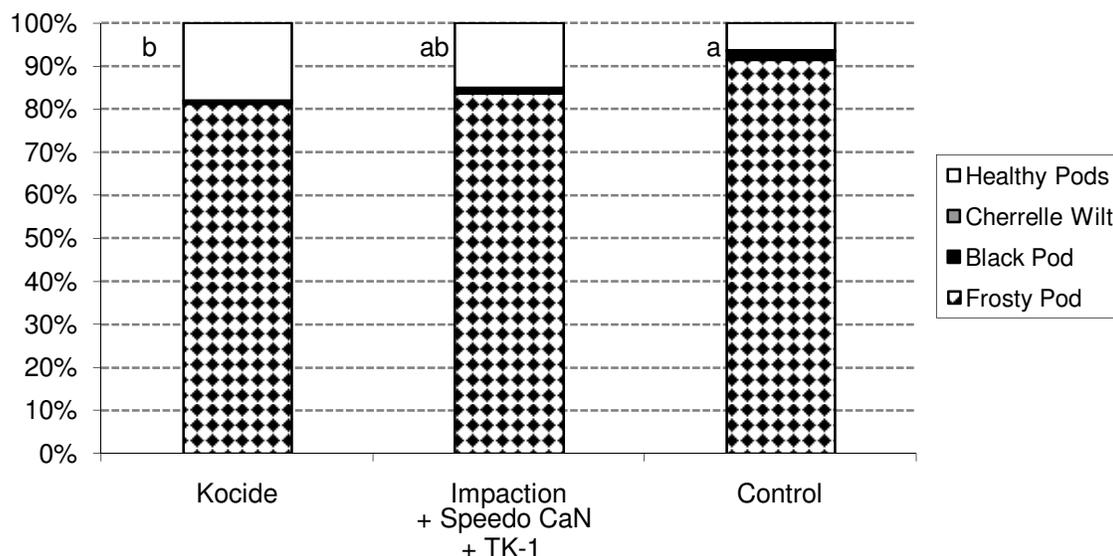


Figure 4: Disease incidence in cocoa Biocontrol Trial (BT) in Costa Rica, 2006/2007 season. Healthy pods: bar carrying the same letter do not differ from each other at $P = 0.05$.

Table 5: Yield data in the Biocontrol Trial (BT), carried out in Costa Rica in 2006/2007.

Treatment	Yield (kg ha ⁻¹ yr ⁻¹)	Number of healthy pods per plot	Pod index	Thousand-seed- weight (kg)
Kocide	560.8 ^b	153.1 ^c	19.4 ^a	1.32 ^b
<i>Impaction</i> + <i>Speedo CaN</i> + <i>T. ovalisporum</i> TK-1	277.0 ^a	77.4 ^b	19.8 ^a	1.31 ^b
Untreated control	177.0 ^a	43.4 ^a	17.9 ^a	1.25 ^a
Coefficient of Variance	24.0	7.5	10.7	2.1
F-value (DoF = 2)	30.0	18.4	1.7	8.8

^{a, b} Values within a column followed by the same letter do not differ at $P = 0.05$ (ANOVA)

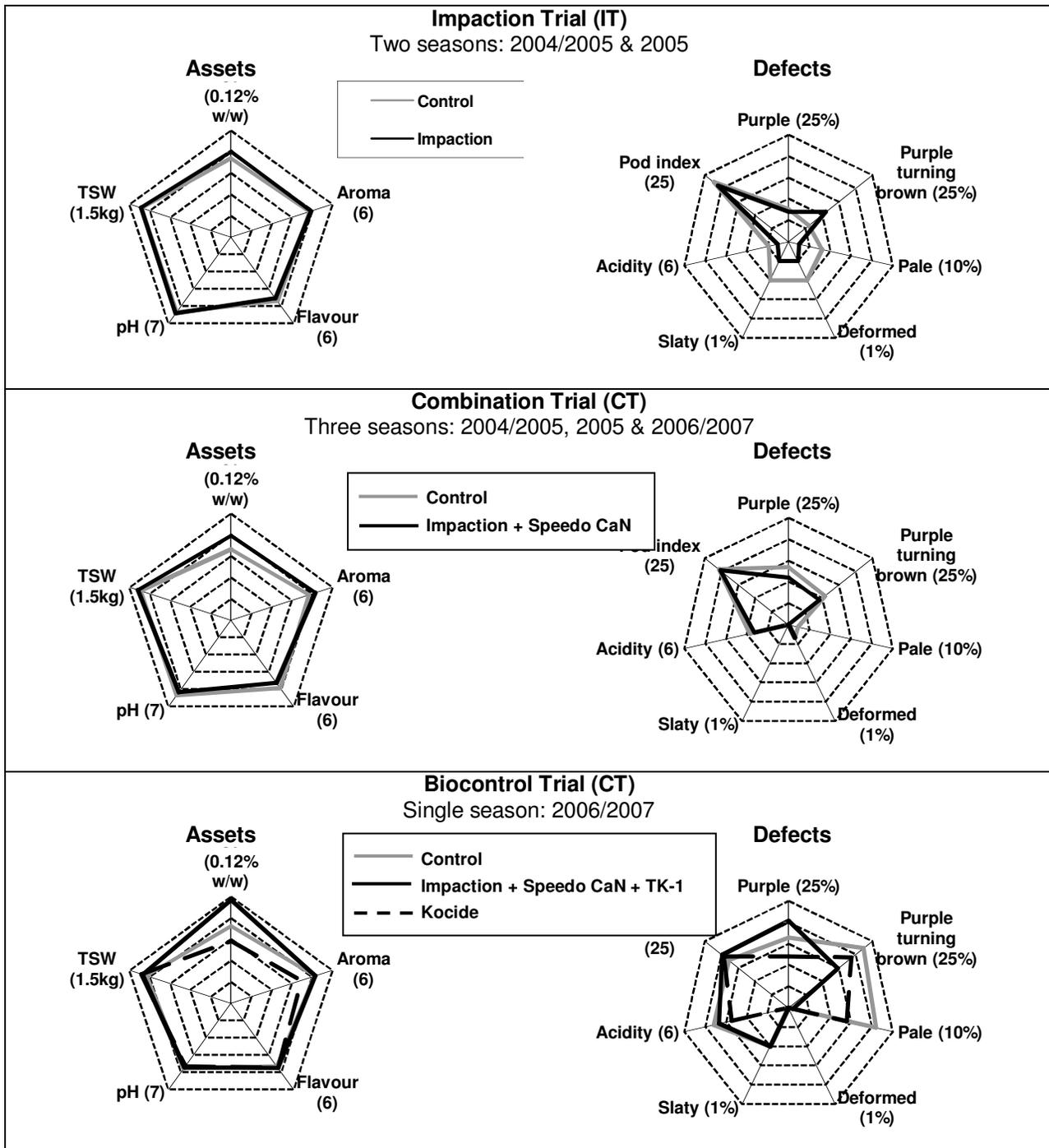


Figure 5: Calcium content and quality analysis of fermented and dried cocoa seeds. Axis lengths in spidergrams are scaled and the maximum level together with its unit of measurement is given in parentheses for each parameter. Data were averaged over one to three trial seasons, as indicated in the headings.

The pH (objective measure) and acidity (subjective assessment) were unaffected by the treatments ($P = 0.511$ and $P = 0.690$, respectively, in the IT; $P = 0.069$ and $P = 0.438$, respectively, in the CT; and $P = 0.872$ and $P = 0.347$, respectively, in the BT; Fig. 5). Year had no effect on pH ($P = 0.361$) or acidity ($P = 0.375$) in the IT, but in the CT, a lower pH of 5.50 ($P < 0.001$) and concomitant higher acidity ($P < 0.001$) was measured in the third year than in the preceding seasons (average pH=6.18). There was no significant treatment \times year interaction for pH in the IT

($P = 0.792$) or the CT ($P = 0.185$). The organoleptic qualities, aroma ($P = 0.481$ and $P = 0.389$) and flavour ($P = 0.420$ and $P = 0.701$), were unaffected by treatment and year, respectively, in the IT ($P = 0.093$ and $P > 0.051$) and the CT ($P = 0.070$ and $P > 0.084$). Similarly, treatment had no effect on aroma ($P = 0.175$) or flavour ($P = 0.982$) in the BT (Fig. 5).

No mouldy cocoa beans were detected throughout the trial series. For no quality parameter or trial did the treatment interact with season ($P \geq 0.185$). Deformed and slaty seeds were rarely (<0.5%) observed with no effect of variables under consideration (Fig. 5, statistics not shown). Purple grains in the IT ($P = 0.477$), the CT ($P = 0.327$), and the BT ($P = 0.150$) were not affected by treatments (Fig. 5). This defect decreased from the first to the second season from 12.0% to 2.6% in the IT and from 17.8% to 2.4% in the CT, where, in the third year, purple seeds rose again to 16.6% (both trials $P < 0.001$). The lesser defect "purple turning brown" was unreceptive to treatment ($P = 0.057$, $P = 0.165$, and $P = 0.477$ in the IT, CT and BT, respectively). In the IT, "purple turning brown" beans did not differ between seasons ($P = 0.422$). In the CT, this defect was significantly ($P = 0.013$) higher in the third year (15.2%), than in the second year (7.0%); the first year (8.0%) was intermediate.

DISCUSSION

Over two seasons *Impaction* alone did not improve cocoa yield or quality (Figs. 1 & 5). However, *Impaction* plus *Speedo CaN* increased yield by an impressive 43.5%, principally as the result of more healthy pods reaching maturity, and to a lesser extent to an increased TSW; the pod index played no role (Table 3). These observations coincide with those of Uthaiyah and Sulladmath (1980), who found that both foliar- and soil-applied calcium reduced CW and increased bean yield per tree, but not per pod.

Although diseases, most notably FPR, accounted for over 80% of cocoa beans being lost, the observed yield improvement was unrelated to disease incidence (Figs. 1 & 2). Similarly, Naundorf (1954) had reduced CW and increased both absolute yield and the percentage of healthy beans by foliar application of urea, calcium glycerophosphate and potassium chloride, without FPR being significantly affected. We therefore believe the products tested here strengthened the plant, resulting in a higher yield potential, which could not be fully exploited without complementary disease control. This was the rationale behind combining the *Impaction* plus *Speedo CaN* treatment with the biocontrol agent *T. ovalisporum* TK-1 in the BT and comparing this mixture also with copper hydroxide, the most cost-effective measure to date.

Despite detectable reduction of germination *in vitro*, the biocontrol agent proved compatible with *Plant Impact* products in tank mixture and can thus be applied without extra field labour. FPR incidence appeared closer to the copper standard than the untreated control; however, this trend discernible in Fig. 4 narrowly failed to reach statistical significance ($P = 0.080$) in the single season of observation. A yield improvement of over 50% paralleled, and may even exceed, observations made in the CT, but again failed to reach significance (Table 5). The increased TSW and number of healthy pods are in agreement with the CT, where the number of healthy pods was also the main determinant of enhanced yield, with TSW playing a secondary role, while the pod index appeared irrelevant.

Cocoa is known for its capricious behaviour (Lotodé and Muller, 1974), not least because it is usually planted as heterogeneous germplasm mixture, because of predominantly self-incompatible pollination. This was also illustrated in the CT, carried out in a mixed hybrid field, for which statistical differences were only confirmed after three years, although consistent and marked yield improvements were observed each season (data not shown). The composition of diseased pods also changed over time: while FPR increased, BP and CW decreased. This is in agreement with other trials at the same location (Krauss *et al.*, 2010). The percentage of healthy pods decreased in the third season, independent of treatment. In contrast to percentage healthy pods, their absolute number peaked in year two, followed by year three and then year one. A parallel numeric trend of pod indices means that the higher number of pods recorded was partly off-set by pods with a lower seed yield, which also explains the lack of comparably drastic yield fluctuations over time. Meteorological data (Table 2) provide no convincing explanation for this seasonal variation, except that above average rainfall in year one is consistent with a high BP incidence.

Plant Impact products work by increasing calcium supplies to the plant cell (www.plantimpact.com). A measurable increase of calcium in cocoa seeds was observed in the CT. Bailey *et al.* (2006) showed that some endophytic *Trichoderma* spp. from cocoa induced seven expressed sequence tags (ESTs), including one for the EF-calcium-binding protein (P29); however, strain TK-1 (=DIS 70a in their publication) had no effect on P29.

The key requisites for successful cocoa production are simultaneous accomplishments in productivity, disease management and quality. The combination of *Impaction* plus *Speedo CaN* plus *TK-1* tested in the BT has the potential to achieve these tenets better than either product without the biocontrol agent, as tested in the IT and CT. However, the single-season BT requires further field testing to ensure reproducible results. *Plant Impact* products had no adverse effect on quality, yield was markedly improved and, together with effective biocontrol, diseases can probably be managed successfully, but the latter hypothesis requires additional field testing.

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REFERENCES

- Bailey BA, Bae, H, Strem MD, Roberts DP, Thomas SE, Crozier J, Samuels GJ, Choi IY, Holmes, K.A. (2006). Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. *Planta* 224: 1449-1464.
- Bateman RP (2004). The use of narrow-angle cone nozzles to spray cocoa pods and other slender biological targets. *Crop Protection* 23: 989-999.
- Bazán SR (1963). Soil Survey of La Lola Cacao Farm. M.Sc. Thesis, IICA, Turrialba, Costa Rica, 127 pp.
- Falade OS, Otemuyiwa IO, Oladipo A, Oyedapo OO, Akinpelu BA, Adewusi SRA. (2005). The chemical composition and membrane stability activity of some herbs used in local therapy for anemia. *Journal of Ethnopharmacology* 102: 15-22.
- Gaudrain C, Harvey CA (2003). Caza y diversidad faunística en paisajes fragmentados del territorio indígena Bribri de Talamanca, Costa Rica. *Agroforestería en las Américas* 10: 46-51.
- Goodman RN, Király Z, Word KR (1986). *The Biochemistry and Physiology of Plant Disease*. Columbia, USA: University of Missouri Press.
- Holmes KA, Krauss U, Samuels G, Thomas SE, Bateman RP, Hidalgo E, García J, Schroers H-J, Brown NA, Arroyo C, Evans HC (2005). *Trichoderma ovalisporum*, a novel biocontrol agent of frosty pod rot (*Moniliophthora roreri*) of cocoa (*Theobroma cacao*): from discovery to field. Keynote at the 1st International Conference on Plant-Microbe Interactions: Endophytes and Biocontrol Agents 18-22 April 2005, Saariselkä, Lapland, Finland.
- Infostat, 2004. Infostat, versión (2004). Grupo Infostat, FCA, Universidad Nacional de Córdoba, Argentina.
- Krauss U, Martínez A, Hidalgo E, ten Hoopen GM, Arroyo C (2002). Two-step liquid/solid state mass production of *Clonostachys rosea*. *Mycological Research* 106: 1449-1454.
- Krauss U., Ten Hoopen M., Hidalgo E., Martínez A., Arroyo C., García J., Portugués A., Sánchez V. (2003) Manejo Integrado de la moniliasis (*Moniliophthora roreri*) del cacao (*Theobroma cacao*) en Talamanca, Costa Rica. *Agroforestería en las Américas*, 10, 52-58.
- Krauss U, Hidalgo E, Bateman R, Adonijah V, Arroyo C, García J, Crozier J, Brown NA, ten Hoopen GM, Holmes KA (2010) Improving the formulation and timing of application of endophytic biocontrol and chemical agents against frosty pod rot (*Moniliophthora roreri*) in cocoa (*Theobroma cacao*). *Biological Control* 54: 230–240.
- Lotodé R, Muller RA (1974). Problems of experimentation with cocoa trees. In: *Phytophthora Diseases of Cocoa*, P. H. Gregory (Ed.), London: Longman.
- Naundorf G. (1954). Aplicación de fertilizantes en forma de aspersiones foliares al cacao. V Reunión del Comité Técnico Interamericano del Cacao, Centro Interamericano del Cacao 3: 10.
- Rice RA, Greenberg R (2000). Cacao cultivation and the conservation of biological diversity. *Ambio* 29: 167-173.
- Richter, G. (1988) *Stoffwechselfysiologie der Pflanzen*. Georg Thieme Verlag, Stuttgart, Germany.
- Toledo VM, Ortiz-Espejel B, Cortes L, Moguel P, Ordonez MDJ (2003). The multiple use of tropical forests by indigenous peoples in Mexico: a case of adaptive management. *Conservation Ecology* 7: 9. Available on-line: <http://www.consecol.org/vol7/iss3/art9>
- Uthaiyah BC, Sulladmath UV (1980). Effect of boron, calcium, and magnesium on cherelle wilt in cacao. *Journal of Plantation Crops* 8: 24-28.
- Wessel M (1985). Shade and Nutrition. In *Cocoa*, 166-194 (Eds. G.A.R. Wood and A. Lass). Harrow, U.K.: Longman Scientific & Technical.