VOL. 8, NO. 3, MARCH 2013 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

USE OF FERMENTED COCOA PULP JUICE FOR THE CONTROL OF NON-VASCULAR EPIPHYTES ON COCOA

Kofi Acheampong, Samuel Tetteh Lowor, Frank Owusu-Ansah and Kwabena Opoku-Ameyaw
Cocoa Research Institute of Ghana, Akim Tafo, Ghana
E-Mail: oyacheampong@yahoo.com

ABSTRACT

On many other host plants epiphytes may be harmless but on cocoa they are generally considered obnoxious as they can compete with the reproductive structures of cocoa for points of attachment. The potential of fermented cocoa pulp juice and 4% aqueous NaCl (common salt) solution in controlling five epiphytes - *Platycerium stemaria*, *Bulbophyllum* spp., lichens (*Phyllopsora buettnei*), mosses (*Pilotrichella communis*,) and liverworts (*Blasia* spp.) - that are commonly found on cocoa plants in Ghana was studied at the Bunso substation of the Cocoa Research Institute of Ghana between April and July 2008. A pneumatic knapsack sprayer (Jacto X-15) was used to spray the two solutions separately onto the epiphytes at a rate of 20 ml/m^2. A significant epiphyte x solution interaction on epiphyte kill suggested that the choice of solution for epiphyte control should be influenced by the target epiphyte and that fermented cocoa pulp juice is a better option for the control of the non-vascular epiphytes than 4% aqueous NaCl but not effective against the vascular epiphytes that were tested.

Keywords: Cocoa, cocoa pulp juice, epiphytes, common salt.

INTRODUCTION

Epiphytes have been reported to be an important component of many ecosystems, playing a key role in ecosystem carbon uptake, water storage, nutrient recycling and creating unique habitats for vertebrates and invertebrates in the rainforest canopy (Nieder et al., 2001, Stundz et al., 2002, Joost et al., 2005). A report on non-vascular epiphyte infestations on cocoa in tropical agro-forestry systems indicates that their diversity on cocoa trees is comparable to that of rainforests trees (Anderson and Gradstein, 2005). Bryophytes, lichens, algae, ferns and small orchids are closely associated with cocoa but their effects have not been adequately investigated. This has sometimes led to contradicting recommendations regarding their control. However, they are commonly believed to have long-term deteriorative effects on cocoa trees (Becker and Wurzel, 1987, Zinsmeister and Mues, 1988, Adenikinju and Akinfenwa, 1991, Sporn et al., 2007). Obviously some climbing epiphytes, including *Bulbophyllum* spp., interfere with the supply of light available to host plants whilst mosses and lichens potentially support the growth of parasitic organisms (Thorold, 1975, Agbeniyi and Adenikinju, 2001). Moreover, Adenikinju and Akinfenwa (1991) reported bryophytes and lichens as obstructing the development of the cauliflorous flowers of cocoa, causing losses in the final bean yield. Therefore, removal of epiphytes has been recommended as a control measure in cocoa farms (Olaiya and Agbeniyi, 2005).

However, removing many epiphytes is arduous and impracticable as they may either be too brittle to pull or may not be easily accessed from ground level (Anderson and Gradstein, 2005). Therefore, Acheampong and Ofori-Frimpong (2004) used 4% aqueous NaCl to control some vascular and non-vascular epiphytes on cocoa. Against the background of a growing interest in the use of natural products as alternatives to synthetic pesticides (Lydon and Duke, 1987, Koul et al., 2008, Koul, 2008) this paper describes an attempt to control three non-vascular epiphytes [lichens (*Phyllopsora buettnei*), liverworts (*Blasia* spp., family Bryophyta) and mosses (*Pilotrichella communis*, family Bryophyta)] and two vascular epiphytes [*Bulbophyllum* spp., (family Orchidaceae) and *Platycerium stemaria*, (family Polypodiaceae)] that are commonly found on cocoa by the application of fermented cocoa pulp juice. Fermented cocoa pulp juice was used as an alternative to NaCl as it is a source of organic acids (acetic and lactic acids) (Copetti et al., 2012) reported to have pests control properties (Lynch, 1980, Trias et al., 2008, Ivany, 2010).

MATERIALS AND METHODS

Determination of lactic and acetic acid contents in the fermented cocoa pulp juice

The cocoa pulp juice was fermented for five days and then its lactic and acetic acid contents were qualitatively and quantitatively determined by HPLC. To 10ml of the fermented juice 10ml of HPLC grade water was added. Sample was then centrifuged at 10,000 rpm for 5 minutes. Supernatant was then collected for analysis. Prior to analysis, the extracts were filtered through a 0.45µm Millex filter (SLHV013SL, Millipore, Carrigtwahill, Ireland). The HPLC system comprised Waters 1525 binary HPLC pumps fitted with a 20µl sample loop, a Waters 2487 dual absorbance detector set at 210 nm and a Waters 2414 refractive index detector. An Aminex HPX-87H column (300 x 7.8 mm) maintained at 25°C was used to achieve the chromatographic separations. Compounds were eluted with an isocratic mobile phase of 0.01N H_2SO_4 at a flow rate of 0.6ml/min. Compounds were identified by matching retention times with authentic standards as well as co-eluting with the

standards. Concentrations of the lactic and acetic acids in the samples were obtained by calibrations obtained from pure standards of both acetic and lactic acids.

Epiphyte control

A three replicate experiment was conducted in a 21-year old experimental cocoa plot at the Bunso Substation (060 13' N, 000 22' W) of the Cocoa Research Institute of Ghana. In each replication thirty cocoa trees of heights ranging from 2.8 m to 3.6 m were grouped into 10 sets of three trees. Each of the trees in a set had a severe infestation by either one of the three non-vascular epiphytes - lichens, mosses and liverworts or one of the two vascular epiphytes - *Bulbophyllum* spp (mature) and *Platycerium stemaria* (with base diameter > 7.5 cm). Between April and July 2008 (the period of the major rainy season when relative humidity is high in the cocoa growing belt of Ghana) the dominant epiphyte on each of the trees in a set was sprayed with either 4% aqueous NaCl (common salt) solution or with fermented cocoa pulp juice (the organic fluid) at a rate of 20ml/m^2 by means of a calibrated 'Jacto X-15' pneumatic sprayer (Máquinas Agricolas Jacto, Pompéia, Brazil). Concurrently the experiment was repeated once in another block of the cocoa experiment. Since only non-mobile activity of the fluids was expected, complete epiphyte coverage was ensured.

Microclimate

On 14th May, 19th June and 23rd July 2008 'Tiny-tag' Data Loggers (Gemini Data Loggers, Chichester, UK) placed inside Stevenson screens were used to measure diurnal aerial temperature and relative humidity under the cocoa canopy of the treated plots.

Assessment of epiphyte kill

Post-treatment visual assessment of epiphyte survival or death was done every other day over a 60-day period. The effects of the two treatment solutions on the different epiphytes were assessed using the Cox proportional hazard regression model (Cox, 1972). There was a complete censoring of two groups of the solution combined with epiphyte. The model failed to converge whenever any of these groups and/or their interaction terms were included in the analysis. Two models based on subsets of the data were finally used to understand the effect of solution on epiphyte. The first model (Model-1) was based on analysing the solution and epiphyte combinations with the exception of the two 'complete censored' groups - fermented cocoa pulp juice on *Bulbophyllum* spp. and *Platycerium stemaria*. The second model (Model-2) on the other hand modelled epiphyte, solution and their interactions on a further subset of the data where data on *Bulbophyllum* spp. and *Platycerium stemaria* for both solutions were excluded. The proportional assumptions of the two models were assessed based on the Scheonfeld Goodness of fit test (residual analysis) (Schoenfeld, 1982). All computations were done using the Survival package of R statistical software (version R.2.11).

RESULTS

The diurnal temperature values ranged from 24°C to 26°C by 08:30 hours, 30°C to 33°C by 13:30 hours and 28°C to 30°C by 16:30 hours. The corresponding relative humidity values ranged from 81% to 83%, 64% to 65% and 69% to 72%.

The fermented cocoa pulp juice was found on analysis to contain 1.86% lactic acid and 0.96% acetic acid. In both experiments fermented cocoa pulp juice caused only slight injuries (apparent loss of chlorophyll and necrotic spots) along the leaf margins in *Platycerium stemaria*. In *Bulbophyllum* spp., injury (leaf chlorosis) was confined to the older leaves after application of the fermented pulp juice. The situation was different with aqueous NaCl. With aqueous NaCl leaf chlorosis and subsequent leaf senescence were observed in *Bulbophyllum* spp., while a brown, water-soaked rot developed at the base of leaves of *Platycerium stemaria*. The aqueous NaCl killed 50% of lichens, liverworts, mosses, *Platycerium stemaria* and *Bulbophyllum* spp. in 27, 23, 19, 25 and 29 days, respectively. In contrast, fermented cocoa pulp juice failed to kill *Platycerium stemaria* and *Bulbophyllum* spp. within the 60-day period of study but had an LT_{50} (lethal time for 50% of test organisms) of 17 for both lichens and liverworts and an LT_{50} of 3 for the mosses (Table-1).

Table-1. Median time to kill and Cox proportional hazard estimates for Models 1 and 2.

Treatment solution	Epiphyte	Median time to kill (days)	Hazard ratio	
			Model-1	Model-2
Cocoa pulp juice				
	Lichens (Baseline)*	17	1	1
	Liverworts	17	0.42^{NS}	0.53^{NS}
	Mosses	3	90.48	91.30
Aqueous NaCl				
	Lichens	27	0.09	0.09
	Liverworts	23	0.24	0.28
	Mosses	19	0.78^{NS}	0.78^{NS}
	Bulbophyllum	29	0.03	
	Platycerium stemaria	25	0.10	

NS means not significant at 5% level. Those without NS are significant at 0.05
*All estimates are compared to that of lichens treated with cocoa pulp juice.

Generally, fermented cocoa pulp juice was more effective against the non-vascular epiphytes than the aqueous NaCl. For both liquids the mosses died significantly faster ($p < 0.05$) than the liverworts which, in turn, died faster ($p < 0.05$) than the lichens. The effects of fermented cocoa pulp juice and aqueous NaCl applied on liverworts and mosses, respectively, did not differ significantly ($p > 0.05$) from that of fermented cocoa pulp juice on lichens (the baseline hazard) (see Model-1). While fermented cocoa pulp juice applied on the mosses gave a significantly high percentage (90.48%) multiplicative effect on the baseline hazard rate, aqueous NaCl showed significantly ($p < 0.05$) lower hazard rates on the non-vascular epiphytes than the baseline hazard ratio except on the mosses. Effect of NaCl on the mosses was non-significantly lower than the baseline hazard ratio. Model-2 showed a similar pattern to Model-1 for the effect of the fluids on the respective epiphytes. Similar to Model-1 it indicated that the efficacy of aqueous NaCl on mosses, as compared to fermented cocoa pulp juice, was significantly lower (91.30%). It further showed a significant ($p < 0.05$) interaction between solution and epiphyte. Similar to an earlier observation (Acheampong and Ofori Frimpong, 2004) cherelles that were unintentionally sprayed with either fermented cocoa pulp juice or aqueous NaCl remained intact but flowers that had already formed dropped. In all cases flower production resumed on affected flower cushions about three weeks after imposing the treatments.

DISCUSSIONS

The recorded temperature and relative humidity values fell within the range normally observed in the major rainy season of West Africa during which most epiphytes of cocoa experience rapid vegetative growth and thereby exert their greatest influence on the yield of cocoa (Adenikinju and Akinfenwa, 1991).

The observation in the current work which showed that all the epiphytes died after the application of a 4% NaCl solution confirms an earlier research in which distilled water (the control) killed none of the epiphytes tested whereas various aqueous concentrations of NaCl (ranging from 2 to 10%) killed responsive epiphytes including mosses, *Bulbophyllum* spp. and *Platycerium stemaria* by osmosis (Acheampong and Ofori Frimpong, 2004).

In the present work the mechanism by which fermented cocoa pulp juice killed the responsive epiphytes was not studied. However, natural products have previously been used to control the growth of various living organisms including emerging weed seedlings, insects and plant pathogens (Eguaras et al., 1996, Kremer and Kennedy, 1996, Eguaras et al., 2001, Abbott and Ingledew, 2004, Eguaras, 2005, Mahmood et al., 2011). For example, Agarry *et al*. (2005) found microbes in grated cassava to be antagonistic to the growth of *Aspergillus niger*, *Aspergillus fumigatus* and *Fusarium monoliforme*. Inhibition of the pathogenic microorganisms was suspected to have been caused by the ability of the microbes to produce organic acids and bacteriocins. Similarly enzymatic hydrolysis of corn gluten meal (a by-product from corn wet-milling) has been observed to produce a chemical that possesses strong 'herbicidal properties which are credited to the presence of root-inhibiting dipeptides' (Liu and Christians, 1994, 1996). Commenting on the effect of acids on sapling growth, Liu *et al*. (2007) explained that acids exhibit phytotoxicity by affecting the dynamics of potassium, calcium and magnesium in plants, the effect being species specific (Lynch, 1980). Similarly, Sarwar and Kremer (1995) observed that plant growth suppressive activity can be

obtained by deleterious rhizobacteria (DRB) which produce metabolites that are absorbed through plant roots. The authors observed that auxin derivatives such as indole-3-acetic acid and indole-3-lactic acid produced by *Enterobacter taylorae* enhanced phytotoxic activity against emerging weed seedlings and suggested that this may be a practical biological weed control strategy. Also Vescovo *et al*. (1996) used the lactic acid bacteria (*Lactobacillus casei*, L. plantarum and *Pediococcus* spp.) to control pathogens in ready-to-use vegetables while Gourama and Bullerman (1995) observed *Lactobacillus* species from a commercial silage inoculum to cause reduced mould growth and inhibited production of aflatoxin by the mould *Aspergillus flavus*. In that trial while the pH of the culture broth and supernatant from which the acid was obtained was 4.0, acidification of unfermented broth to pH 4.0 did not cause similar inhibition of spore germination. This suggested that in the case of the mould the observed effect was not solely due to the pH status.

In the current work we deduce that the injuries and/or death of the responsive epiphytes that were observed on application of the fermented cocoa pulp juice were, probably, caused by the presence of lactic and acetic acids produced by deleterious microbes during fermentation (Abdul et al., 1993). Our hypothesis is that the observed injury symptoms, including the apparent loss of chlorophyll, impeded photosynthesis and where severe enough, caused starvation of the responsive epiphytes until they died.

CONCLUSIONS

Whereas the results of this work show fermented cocoa pulp juice as possessing biocontrol properties, the observation that it gave a better control of the non-vascular epiphytes (liverworts, lichens and mosses) but failed to control the vascular epiphytes suggests that the choice of a chemical for epiphyte control should be influenced by the target epiphyte.

ACKNOWLEDGEMENTS

The authors are grateful to Messrs John Kwabena and Evans Aboagye of the Bunso Substation of the Cocoa Research Institute of Ghana (CRIG) for providing technical support. This paper is published with the kind permission of the Executive Director of CRIG.

REFERENCES

Abbott D. A. and W. M. Ingledew. 2004. Buffering capacity of whole corn mash alters concentrations of organic acids required to inhibit growth of *Saccharomyces cerevisiae* and ethanol production. *Biotechnology Letters*, 26(16): 1313-1316.

Abdul S. O., N. Ibrahim, H. Alimon, M. I. Abdul-Karim, O. A. Samah and M. I. A. Karim. 1993. Fermentation studies of stored cocoa beans. World Journal of Microbiology and Biotechnology. 9(5): 603-604.

Acheampong K. and K. Ofori Frimpong. 2004. Use of sodium chloride to control epiphytes on cocoa in Ghana. *Tropical Science*. 44(1): 51-53.

Adenikinju S. A. and F. O. Akinfenwa. 1991. Preliminary observations on the effects of bryophytes, lichens and other trunk infestations on flowering of cocoa. *Cafe Cacao Thé*. 35(1): 43-48.

Agarry O. O., F. A. Akinyosoye and F. C. Adetuyi. 2005. Antagonistic properties of microogranisms associated with cassava (*Manihot esculenta* Crantz) products African Journal of Biotechnology. 4(7): 627-632.

Agbeniyi S. O. and S. A. Adenikinju. 2001. Recent observation on the source of inoculum for the spread of cocoa black pod disease in Nigeria. In: Proceedings, 13th International Cocoa Research Conference. Kota Kinabalu, Malaysia. 689-694.

Anderson M. S. and S. R. Gradstein. 2005. Impact of management intensity on non-vascular epiphyte diversity in cacao plantations in western Ecuador. *Biodiversity Conservation*. 14: 1101-1120.

Becker H. and G. Wurzel. 1987. Sind lebermoose Arzncipflanzen? Pharmazie in Unserer Zeit. *Weinheim*. 16(3): 152-156.

Copetti M. V., B. T. Lamanaka, R. C. Mororó, J. L. Pereira, J. C. Frisvad and M. H. Taniwaki. 2012. The effect of cocoa fermentation and weak organic acids on growth and ochratoxin a production by Aspergillus species International Journal of Food Microbiology. 155(3), 16 April: 158-164, 155(3): 158-164.

Cox D. R. 1972. Regression Models and Life Tables (with Discussion). Journal of the Royal Statistical Society Series B. (34): 187-220.

Eguaras M., M. Del Hoyo, M. A. Palacio, S. Ruffinengo and E. L. Bedascarrasbure. 2001. A New Product with Formic Acid for *Varroa jacobsoni* Oud. Control in Argentina. I. Efficacy. Journal of Veterinary Medicine, Series B. 48(1): 11-14.

Eguaras M., S. Quiroga and C. Garcia. 1996. Organic acid in the control of Varroa jacobsoni Oud. *Apiacta*. 31.

Eguaras M. F., I., Satta A., Bolognesi A., Velis G., Mariani F. 2005. Organic management of varroasis in bee colonies with presence of permanent bee broods. *Vida Apícola*. 131: 44-47

Gourama, H. & Bullerman, L. B. 1995. Inhibition of growth and aflatoxin production of *Aspergillus flavus* by Lactobacillus species Journal of Food Protection 58, 1249-1256.

Ivany J. A. 2010. Acetic acid for weed control in potato (*Solanum tuberosum* L.) Canadian Journal of Plant Science. 90 (4): 537-542.

Joost F., A. V. Duivenvoorden and C. Ricardo. 2005. A first quantitative census of vascular epiphytes in rainforests of Colombian Amazonia. Biodiversity and Conservation. 14(3): 739-758.

Koul O. 2008. Phytochemicals and insect control: An antifeedant approach. Critical Reviews in Plant Sciences. 27: 1-24.

Koul O., S. Walia and G. S. Dhaliwal. 2008. Essential oils as green pesticides: potential and constraints. Biopesticide International. 4(1): 63-84.

Kremer R. J. and A. C. Kennedy. 1996. Rhizobacteria as biocontrol agents of weeds. Weed Technology. 10: 601-609.

Liu D. and N. Christians. 1994. Isolation and identification of root-inhibiting compounds from corn gluten hydrolysate. Journal of Plant Growth Regulation. 13: 227-230.

Liu D. and N. Christians. 1996. Bioactivity of a pentapeptide isolated from corn gluten hydrolysate on *Lolium perenne* L. Journal of Plant Growth Regulation. 15: 13-17.

Liu J. C., G. Y. Zhou and D. Q. Zhang. 2007. Simulated effects of acidic solutions on element dynamics in monsoon evergreen broad-leaved forest at Dinghushan, China. Part 1: dynamics of K, Na, Ca, Mg and P. Environmental science and pollution research. 14(2): 123-129.

Lydon J. and S. O. Duke. 1987. Progress toward natural herbicides from plants. Herbs, Spices and Medicinal Plants Digest. 5: 1-4.

Lynch J. M. 1980. Effects of organic acids on the germination of seeds and growth of seedlings. Plant, Cell and Environment. 3(4): 255-259.

Mahmood R., E. S. Wagchoure, S. Raja, G. Sarwar and M. Aslam. 2011. Effect of Thymol and Formic Acid Against Ectoparasitic Brood Mite Tropilaelaps clareae in Apis mellifera Colonies. Pakistan J. Zool. 43(1): 91-95.

Nieder J., J. Prosperi and G. Michaloud. 2001. Epiphytes and their contribution to canopy diversity. Plant Ecology. 153: 51-63.

Olaiya A. O. and S. O. Agbeniyi. 2005. Management of epiphytes and mistletoes infesting cocoa plantations in Nigeria. In: Proceedings, 14th International Cocoa Research Conference. Accra, Ghana. pp. 351-357.

Sarwar M. and R. J. Kremer. 1995. Enhanced suppression of plant growth through production of L-tryptophan-derived compounds by deleterious rhizobacteria. Plant and Soil. 172(2): 261-269.

Schoenfeld D. 1982. Partial residuals for the proportional hazards regression model. Biometrika. 69: 239-241.

Sporn G. S., M. M. Bos and S. R. Gradstein. 2007. Is productivity of cacao impeded by epiphytes? An experimental approach. Agriculture Ecosystems and Environment. 122(4): 490-493.

Stundz S., U. Simon and G. Zotz. 2002. Rainforest air conditioning: the moderating influence of epiphytes on the microclimate in the tropical tree crowns. International Journal of Biometeorology. 46: 53-59.

Thorold C. A. 1975. Parasites, saprophytes and epiphytes associated with cocoa. Edited by P. A. F. Clarendon: Oxford.

Trias R., L. Bañeras, E. Montesinos and E. Badosa. 2008. Lactic acid bacteria from fresh fruit and vegetables as biocontrol agents of phytopathogenic bacteria and fungi. December. 11(4): 231-6 4: 231-236.

Vescovo M., S. Torriani, C. Orsi, F. Macchiarolo and G. Scolari. 1996. Application of antimicrobial-producing lactic acid bacteria to control pathogens in ready-to-use vegetables. Journal of Applied Microbiology. 81(2): 113-119.

Zinsmeister H. D. and R. Mues. 1988. Bryophytes as a remarkable secondary component, a survey. In: Plant Research and Development, 27.