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The effect of cane molasses amendment on biocontrol of frosty pod rot (*Moniliophthora roreri*) and black pod (*Phytophthora* spp.) of cocoa (*Theobroma cacao*) in Panama

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Abstract

Frosty pod rot (FPR), caused by *Moniliophthora roreri*, and black pod (BP), caused by *Phytophthora* spp., of cocoa (*Theobroma cacao*) cause combined pod losses of more than 80% in Panama. Biological control of both diseases appeared promising in Peru and is desired by certified organic producers in Panama. We evaluated both local and Peruvian fungal antagonists in participatory trials on smallholdings during two complete production cycles. Furthermore, we tested the influence of a 3% v/v cane molasses amendment on biocontrol efficacy, yield and population dynamics of mycoparasites on the cocoa pod. Significant variation was observed between the two years: FPR was more severe in the first year, BP in the second. FPR was significantly reduced by biocontrol agents (BCAs), but not by the molasses amendment. However, BCAs responded differently to molasses in both years. All BCAs reduced inoculum production by *M. roreri* with no consistent effect of molasses. BCAs had a lesser and more variable effect on BP, whereas molasses reduced BP slightly by increasing the efficacy of native antagonists. All BCAs and the molasses amendment enhanced the percentage of healthy pods. Molasses was beneficial to absolute yield, but only one inoculum improved yield significantly in the first year. Populations of a Peruvian *Trichoderma asperellum* isolate remained high for over two months after application to surface-sterilized pods. Molasses had no effect on establishment or survival of this antagonist or recolonization by any native mycoparasite. The reasons for enhanced biocontrol efficacy of the molasses formulation requires further investigation.

Keywords: Antagonism; Biological control; Black pod; *Clonostachys* spp.; Cocoa; Frosty pod rot; Molasses; *Moniliophthora roreri*; Mycoparasitism; *Phytophthora* spp.; *Theobroma cacao*; *Trichoderma asperellum*

1. Introduction

Frosty pod rot (FPR) and black pod (BP) of cocoa (*Theobroma cacao* L.) are caused by *Moniliophthora roreri* (Cif) Evans and several *Phytophthora* spp., predominantly *Phytophthora palmivora* (Butl.) Butl., respectively. In the Bocas del Toro Province, in the northwest of Panama, both diseases are devastating cocoa production, which is in the hands of mostly indigenous smallholders. Combined pod losses generally exceed 80% under traditional management. This represents further aggravation from the 35–75% reported by Somarriba and Beer (1999). Most of the area's production is certified organic, so that only cultural and biological approaches are viable disease management options. Germplasm with

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resistance to frosty pod, the main disease, is not yet available to growers, although a breeding program with regional trials exists (Phillips-Mora et al., 2005).

Biocontrol of FPR and BP has shown great promise in Peru. Krauss and Soberanis (2001, 2002) used native, broad host-range mycoparasites and found mixtures of them particularly effective, with some yield increases exceeding 15%. Similar attempts in Costa Rica yielded variable results (Bateman et al., 2005; Hidalgo et al., 2003; Krauss et al., 2003).

Very little is known about the effects of nutrient amendments on the biocontrol efficacy of fruit rots. Harman et al. (1996) reported increased biocontrol efficacy of *Trichoderma harzianum* against botrytis bunch rot of grape when the antagonist was formulated in 0.5% Pelgel, a mixture of carboxycellulose and gum arabic. Davis et al. (1992) found that *Chaetomium globosum* formulations containing cellulose improved biocontrol efficacy of pre-harvest apple fruit diseases. Nunes et al. (2001) observed that addition of ammonium molybdate enhanced biocontrol activity of *Candida sake* against post-harvest problems caused by *Penicillium expansum* on apple and pear. Somewhat more research has been done on leaf surfaces, and it may be possible to draw a comparison, although these play a fundamentally different role in plant carbon partitioning: a carbohydrate source, as opposed to the carbohydrate sink, represented by fruit. Even on leaves, the literature is divided (Sutton and Peng, 1993). Populations of desirable yeasts and bacteria can be encouraged effectively by the application of carbohydrates, amino acids or mixtures of nutrients (Andrews, 1992; Kokalis-Burelle et al., 1992). Filamentous fungal biocontrol agents (BCAs) may benefit from nutrients via enhanced germination, as postulated for *Trichoderma* spp. (Hjeljord et al., 2001) and *C. globosum* (Davis et al., 1992), or by boosting antibiotic production, as shown for *C. globosum* (Andrews, 1992). However, mycoparasitic activity tends to be inhibited by nutrients, especially simple sugars (Hjeljord et al., 2001), whereas for example the plant pathogen *Botrytis cinerea* is dependent on exogenous nutrients such as sucrose and glucose for germination and penetration (Guetsky et al., 2002). The effect of nutrients on competition between pathogen and antagonist, furthermore, depends on the infection strategy: only necrotrophic pathogens are responsive (Köhl and Fokkema, 1998).

In the present study, we attempted to repeat the Peruvian biocontrol success in Panama, initially with native, Central American antagonists, because we believed these to be best adapted to local agroecological conditions. Only moderate, albeit significant, control levels were obtained in early trials on a research station in Costa Rica (Hidalgo et al., 2003) and later in farmers' fields (Krauss et al., 2003). Therefore, collaborators opted to also test a Peruvian isolate, *Trichoderma asperellum* Samuels, Lieckfeldt & Nirenberg Tr-4,[2] of known effectiveness (Krauss and Soberanis, 2002), but unknown adaptability to Panamanian conditions, in addition to some mixed, native inocula. In further discussions, the question as to whether biocontrol effectiveness could be enhanced by the addition of nutrients to the formulation was prioritized.

The main objective of these trials was to evaluate Central American and Peruvian biocontrol agents (BCAs) for their effectiveness against FPR and BP under Panamanian smallholder conditions. Furthermore, we tested the effect of nutrients in the form of cane molasses on biocontrol efficacy and antagonist establishment on cocoa pods.

2. Materials and methods

2.1. Participatory on-farm field trials

Participatory field trials were conducted in collaboration with the *Cooperativa Cacaotera Bocatorreña* (*COCABO*), based in Almirante, Bocas del Toro Province, Panama. Certified organic member smallholder farmers with an interest in experimentation met with extensionists and researchers to discuss disease control options and prioritized realistic research objectives at the beginning of the trials. Most growers were Ngöbe-Bugle Indians, but descendents of Afro-Caribbean and European settlers were also represented.

Four farms in the communities Junquito and Rio Este Arriba were chosen to install trials in a randomized block design. Relatively large differences existed between trial sites in terms of cocoa germplasm, farmers' practice and local agroecology. No attempt to standardize these factors or deviate from diverse existing practices was made, because our aim was to arrive at robust recommendations of general applicability. Where within-farm variability was observed for an agroecological feature, treatments were installed along the gradient of this feature, so that all treatments experienced similar exposure. Each treatment was applied to a row of 20 trees per farm. Trials were carried out over two complete growing seasons (May 2001–March 2002 and May 2002–March 2003), with a complete removal of all pods at the beginning of each season. One of the three agents was replaced by another in the second season, resulting in an unbalanced factorial arrangement, as reflected in Figs. 1 and 2 and Tables 1 and 2.

Native *Clonostachys* spp. were applied in mixtures of up to six isolates per treatment. Mixture MSC contained isolates AMR07, AMR09, AMR48, APP23, and APP43; these were *Clonostachys rosea* or *Clonostachys byssicola*, two species that can only be distinguished by molecular means (Schroers, 2001). Mixture T2 contained *C. byssicola* isolates AMR37, AMR39, AMR41, AMR42, AMR43, and *Clonostachys* cf. *byssicola* AMR38. Mixture T12 contained *C. byssicola* AMR55, AMR56, AMR57 and again *Clonostachys* spp. APP23 and APP43. Inoculum was produced on Guata (Krauss et al., 2002). Only the Peruvian *T. asperellum* Tr-4 was applied as single strain. This fungus was produced on rice (Krauss et al., 2002). Both production

[2] The International Mycological Institute originally identified this isolate as *Trichoderma longibrachiatum* (IMI 382482). However, subsequently Gary Samuels (USDA) reclassified it as *T. asperellum*.