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Combination of *Crotalaria spectabilis* with *Rhizophagus irregularis* MUCL41833 decreases the impact of *Radopholus similis* in banana

Abla Anene[a,b], Stéphane Declerck[b,*]

[a] Institut Togolais de Recherche Agronomique (ITRA), B.P. 1163 Lomé, Togo
[b] Université catholique de Louvain (UCL), Earth and Life Institute, Mycology, Croix du Sud 2, bte L7.05.06, 1348 Louvain-la-Neuve, Belgium

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ABSTRACT

The past decade has seen substantial progress in our knowledge on microorganisms (e.g. arbuscular mycorrhizal fungi—AMF) and push-pull plants (e.g. *Crotalaria spectabilis*) that contribute to the biocontrol of nematodes. However, the application of microorganisms together with push-pull plants has seldom been considered. Therefore, the objective of the study was to investigate the combined effect of AMF and *C. spectabilis* on the control of the nematode *Radopholus similis* in banana. Banana plants, pre-colonized or not with the AMF *Rhizophagus irregularis* MUCL 41833 were grown in 3 L pots in presence/absence of *Crotalaria spectabilis*. Above-ground parts were separated with a fixed talpa net to separate shoots and leaves of the two plants. Similarly, the pots were divided below-ground in two compartments by growing the banana roots in a pocket nylon mesh (30 μm), to avoid roots of both plants to intermingle. The banana plants were established first and were followed three weeks later by *C. spectabilis*. Inoculation of nematodes was done in parallel to the planting of *C. spectabilis*. Half of the banana plants received 1000 monoxenically produced juveniles and adults of *R. similis.* Eight treatments were set up with 6 replications: mycorrhizal banana plantlets with/without *C. spectabilis* and with/without nematodes (+M + C + N, +M + C – N, +M – C + N, +M – C – N) and non-mycorrhizal banana plantlets with/without *C. spectabilis* and with/without nematodes (–M + C + N, –M + C – N, –M – C + N, –M – C – N). Plant growth parameters, root colonization by the AMF and infection by the nematode were evaluated. *R. similis* did not impact banana root colonization by the AMF. Conversely, the fungal symbiont as well as *C. spectabilis* significantly decreased the total number of nematodes as well as their multiplication rate. The multiplication rate in the controls (i.e. –M – C + N) was 280.3, while it decreased to 176.5, 106.7 and 83.8 in the –M + C + N, +M – C + N and +M + C + N treatments, respectively. The root necrosis index (RNI) was significantly decreased in presence of the AMF, *C. spectabilis* and the combination of both. The RNI of the control (i.e. –M – C + N) was 61.7% while it was 33.7, 19.8 and 17.2 in the –M + C + N, +M – C + N and +M + C + N treatments, respectively. Banana root fresh weight was significantly increased in presence of *C. spectabilis* and shoot dry weight in presence of AMF, but no increase was noticed in presence of both organisms together. This study reaffirmed that AMF and *C. spectabilis* are effective in decreasing the pressure caused by *R. smilis* in banana roots. It demonstrated further, for the first time, that their combination decreased even more drastically the surface of necrotic cortical tissues caused by the nematodes, opening new avenues for their concomitant use in an integrated pest management strategy.

1. Introduction

Bananas and plantain are a major staple food for millions of people (Lassoudière, 2011; FAOSTAT, 2014) and an important source of revenues for many countries in the humid and sub-humid tropical regions of the world (Shobhana et al., 2014; CIRAD, 2014). Their production is under threat of several shoot, bunch and root pests (e.g. nematodes and weevil) and diseases (e.g. black Sigatoka, Xanthomonas wilt, Panama Disease) (Ploetz, 2003; AUGURA, 2009; Ngando et al., 2015), although figures on the loose are extremely hard to validate. It is admitted that farmers in the tropics losses much more of their crops due to pests than the EU and USA (estimated 50% and 25–30%, respectively) (Maxmen, 2013). The reason is that pests are a year-round problem in the tropical regions, and farmers are generally poorer and have limited

* Corresponding author.
E-mail address: stephan.declerck@uclouvain.be (S. Declerck).

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access to adequate control measures (e.g. pesticides, improved varieties).

The burrowing nematode *Radopholus similis* is the most devastating pest in banana around the world (Queneherve, 2009; Hölscher et al., 2014). It destroys roots, leaving plants with weakened soil anchorage, causing in the most severe case toppling, or reduced capacity to take up and translocate water and nutrients (Gowen et al., 2005). The control of *R. similis* is usually based on nematicides. Even if their utilization has been continuously reduced, their side-effects on the environment and farmers health have encouraged the development of alternative control measures such as fallow, paring and hot water treatment of the corms, the use of resistant cultivars and the planting of *in vitro* micro-propagated plantlets (Queneherve, 2009; CIRAD, 2014).

The application of biocontrol microorganisms (e.g. *Purpureocillium lilacinum*, *Trichoderma atroviride*, *Bacillus firmus,* arbuscular mycorrhizal fungi (AMF)) is another option that proved promising under greenhouse or *in vitro* conditions to control nematodes (Vos et al., 2012a,b; Koffi et al., 2013; Lopez and Sword, 2015; Panebianco et al., 2015). A number of studies have, for instance, reported the impact of AMF in decreasing the populations of *R. similis* in pre-mycorrhized banana (Anene et al., 2013; Koffi et al., 2013) and tomato (Vos et al., 2012a) plants as well as in excised root organs of carrot (Elsen et al., 2003).

In the last decade, several studies have mentioned the beneficial effects of push-pull plants (e.g. *Desmodium uncinatum*, *Tagetes erecta*, *Crotalaria spectabilis*.) to control pests (Hassanali et al., 2008; ICIPE, 2011). Push-pull plants refers to plants that produce repellents (known as "push" plants) or attractant (known as "pull" plants) semiochemicals (Hassanali et al., 2008; ICIPE, 2011; Pickett et al., 2014). *Crotalaria* spp. is a legume used as cover crop to control weeds (Wang and McSorley, 2012). Many species of *Crotalaria* are also grown to provide green manure to the soil and are thus used to increase nitrogen content via their symbiosis with rhizobium (Wang et al., 2002). Interestingly, these plants also produce secondary metabolites (i.e. allelochemicals) that are toxic or inhibitory to some pests and provide niches for antagonistic flora and fauna trapping nematodes (Wang et al., 2002). The alkaloid monocrotaline-pyrrolizidine is the main toxic principle of *C. spectabilis* with nematicidal, ovicidal and repellent effects on plant parasitic nematodes (Thoden et al., 2009; Marahatta et al., 2012). *Crotalaria* species are non-host for root-knot nematodes (e.g. *Meloidogyne incognita*) and poor-hosts for some migratory nematodes (e.g. *Pratylenchus penetrans*) (Wang et al., 2002; Thoden et al., 2009). The few nematodes that penetrate their roots do not develop beyond the penetration point, limiting their proliferation (Wang et al., 2002; Germani and Plenchette 2004). Interestingly, ground seeds of *Crotalaria* species incorporated into the soil at 2% of soil volume suppressed the whole population of the root-knot nematodes *Meloidogyne javanica* and *M. incognita* (Rich and Rahi, 1995).

Germani and Plenchette (2004) reported that *Crotalaria* species is a highly mycotrophic plant and suggested that these plants could be used as pre-crops for providing green manure while at the same time decreasing the level of detrimental nematodes and increasing the level of beneficial AMF in soil. The combination of *Crotalaria* species and AMF could thus represent an interesting option to control/decrease the impact of nematodes in banana. To the best of our knowledge, no study reported on such combination for the biocontrol of *R. similis* in banana plants.

The objective of the present study was to investigate the combination of an AMF, *Rhizophagus irregularis* MUCL 41833 with *C. spectabilis*, on the population of *R. similis* in banana (*Musa accuminata* cv. Grande Naine, AAA genome, Cavendish group) plantlets grown under greenhouse conditions. Root colonization by the AMF and infection by the nematode were simultaneously analyzed in presence/absence of *C. spectabilis* to evaluate the impact of the AMF, *C. spectabilis* and both in combination on the nematodes and the impact of the later on the fungal symbiont and push-pull plant.

2. Materials and methods

2.1. Biological material

Tissue-cultured banana plantlets (*Musa acuminata* Colla c.v. Grande Naine, clone CV902, AAA genome, Cavendish group) were provided by VITROPIC SA (Montpellier, France). The plant material was proliferated, regenerated and rooted on the Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), supplemented with 30 g L^{-1} sucrose and 2 g L^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA), and with pH adjusted between 6.12 and 6.15 before sterilization (Banerjee and de Langhe, 1985). The plantlets were incubated in a growth chamber at 27/25 °C (day/night) with a photoperiod of 16 h day^{-1} and under a photosynthetic photon flux of 300 μmol $m^{-2}s^{-1}$.

Seeds of *Crotalaria spectabilis* Roth (Wolfseeds, Brazil) were sown in 1 L pots containing 500 g of sterilized sand (2 × 8 h at 110 °C) (1–2 mm diameter; Euroquartz, Belgium) and maintained in a growth chamber at 28/24 °C (day/night) with a relative humidity of 80% during the first 2 weeks and 70% thereafter. The pots were watered with deionized water when needed. The seedlings were kept in the pots for 3 weeks reaching a size of 10 cm at the start of the experiment.

Seeds of *Medicago truncatula* Gaertn. c.v. Jemalong strain A17 (SARDI, Australia) were surface-disinfected by immersion in sodium hypochlorite (8% active chloride) for 10 min, rinsed in sterilized (121 °C for 15 min) deionized water and further germinated in 90 mm Petri plates filled with 35 mL of the Modified Strullu-Romand (MSR) medium (Declerck et al., 1998) solidified with 3 g L^{-1} Phytagel (Sigma-Aldrich, St. Louis, USA) following the method of Declerck et al. (1998). The Petri plates were incubated in the dark at 27 °C for 4 days.

A strain of *Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C. Walker & Schuessler as ['irregulare'] MUCL 41833 was supplied by GINCO (http://www.mycorrhiza.be/ginco-bel/index.php) on Ri T-DNA transformed carrot (*Daucus carota* L.) roots clone DC1 (with negative geotropism) grown in 90 mm Petri plates on the MSR medium (Declerck et al., 1998). *R. irregularis* was previously classified as *Glomus intraradices* N.C. Schenck & G.S. Sm. The strain MUCL 41833 was selected in the present study because it was used to develop the mycelium donor plant (MDP) *in vitro* culture system (Voets et al., 2009) that was recently adapted to banana by Koffi et al. (2013) and Anene et al. (2013) for interaction studies with nematodes. The Petri plates were maintained in the dark in an inverted position at 27 °C until thousands of spores were obtained.

A strain of *Bradirhizobium arachidis* Wang, Chang, Zheng, Zhang, Zhang, Sui, Wang, Hu, Zhang and Chen 2013 LMG 26795 was supplied by BCCM/LMG (http://bccm.belspo.be/about/lmg.php). The bacteria was cultured in 90 mm Petri plates on Yeast Malt Agar (YMA). A subsample was then isolated from a one week-old culture and poured in a flask containing 100 mL liquid Difco™ Yeast Malt (YM) Broth medium. The flask was maintained in agitation at 80 rpm during 10 days, at 28 °C in the dark for inoculum production.

A strain of *Radopholus similis* (Cobb) Thorne originating from Uganda (high virulent strain) was provided by the Nematology laboratory of K.U. Leuven (Belgium). The nematodes (juveniles and adults) were maintained under aseptic conditions on carrot discs (Pinochet et al., 1995) at 28 °C in the dark.