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Future Biological Control for Soybean Cyst Nematode

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1. Introduction

The soybean cyst nematode (SCN) *Heterodera glycines* Ichinohe, is widely distributed in soybean-producing countries. The losses in total yield caused by SCN are greater than those for any other pest of soybean (Wrather et al., 2001). These nematodes have generally been controlled by rotating soybeans with nonhost crops, planting of resistant cultivars, application of effective nematocides and organic materials, and physical control techniques such as solarisation. The combination of biological control with above methods will enhance the effectiveness of nematode control. Recently, numerous studies have been conducted on the fungal antagonist of SCNs (Chen and Dickson, 1996; Kim and Riggs, 1991, 1995; Liu and Chen, 2001; Meyer and Huettel, 1996; Meyer and Meyer, 1996; Timper et al., 1999); however, few biological control agents have been commercialized to date.

Lecanicillium spp. (formally, *Verticillium lecanii*) have been studied as potential biological control agents for SCN. Entomopathogenic *Lecanicillium* spp. are ubiquitously distributed in soils, although these fungi are mainly isolated from insects. Numerous strains have been commercialized worldwide as biopesticides namely of aphids, thrips and mites (Faria and Wraight, 2007; Kabaluk et al, 2010) . In addition, it is known that *Lecanicillium* spp. have a broad host range, e.g., insects, phytopathogenic fungi, and plant-parasitic nematodes (Hall, 1981; Meyer et al., 1990; Goettel et al., 2008) providing the possibility that strains could be found that could be developed for simultaneous control of multiple pest problems. For instance, a strain of *L. longisporum* was found to effectively control both cucumber powdery mildew and aphids (Kim et al, 2007, 2008, 2010).

One strain of *Lecanicillium* sp was found to exhibit high virulence to SCNs, although it was found to be a poor colonizer of the soybean rhizosphere (Meyer and Wergin, 1998). However, it is quite likely that other strains are more aggressive rhizosphere colonizers because *Lecanicillium* spp. (*V. lecanii*) possess varied abilities among different strains

(Sugimoto et al., 2003). The objective of this chapter is to review the development of entomopathogenic *Lecanicillium* hybrid strains with effects on the SCN, and discuss the future prospects for its use in the biological control of the SCN.

2. Genus *Lecanicillium*, as pathogen of plant parasitic nematodes

Until recently, the form genus *Verticillium* contained a wide variety of species with diverse host ranges including arthropods, nematodes, plants and fungi (Zare and Gams, 2001). The genus has been recently redefined using rDNA sequencing, placing all insect pathogens into the new genus *Lecanicillium* (Zare et al., 2000; Gams and Zare, 2001; Zare and Gams, 2001). These include *L. attenuatum*, *L. lecanii*, *L. longisporum*, *L. muscarium* and *L. nodulosum*, which were all formerly classified as *V. lecanii*. These recent reclassifications bring forth the possibility that several different species were actually involved in previous studies. There is also evidence that in recent literature, some authors have simply replaced the genus name *Verticillium* with *Lecanicillium* without conducting the necessary rDNA sequencing, adding to the confusion (Sugimoto et al., 2003; Koike et al., 2007a). In this review, we refer to the former name, *Verticillium lecanii*, as *Lecanicillium* spp. unless it is specifically known that the species in question was verified using the new nomenclature.

Species of *Lecanicillium* are well known and important nematophagous fungi with potential for development as biopesticides against plant-parasitic nematodes. For instance, *L. psalliotae*, *L. antillanum*, and other *Lecanicillium* spp. infect the eggs of the root-knot nematode *Meloidogyne incognita* (Gan et al., 2007; Nguyen et al., 2007). *Lecanicillium* spp. infect females, cysts and eggs of *Heterodera glycines*, the soybean cyst nematode (SCN), reducing nematode populations in laboratory and greenhouse studies (Meyer et al., 1997). Mutant strains of an SCN active strain were induced through UV radiation which resulted in increased efficacy against this nematode (Meyer and Meyer, 1996).

Some reports indicated that immature eggs are more susceptible to fungal attack than the mature eggs containing second stage juveniles (J2) (Chen and Chen, 2003; Irving and Kerry, 1986; Kim and Riggs, 1991). Furthermore, Meyer et al. (1990) demonstrated that one strain of *Lecanicillium* sp. (as *V. lecanii*) decreased the number of viable SCN eggs from yellow females, whereas the viability of eggs from cysts was not affected. This strain also reduced the viability of SCN eggs without colonization of the egg; however, no such effect was observed in other strains. This suggested that *V. lecanii* produced a natural substance that could affect egg viability and there was a remarkable variation in the ability for producing such a substance among strains.

3. Genetic improvement of entomopathogenic *Lecanicillium* spp. using protoplast fusion

Mycotal® (*L. muscarium*) and Vertalec® (*L. longisporum*) are strains commercialized by Koppert, The Netherlands, for insect control. Strain B-2 of *L. muscarium*, which was isolated from the peach aphid (*Myzus persicae*) in Japan, has high epiphytic ability on cucumber leaves (Koike et al., 2004). Protoplast fusion was performed using three strains of *Lecanicillium* spp. (as *V. lecanii*) to obtain new strains possessing useful characteristics as biological control agents (Aiuchi et al. 2004, 2008). From the combination of Vertalec-Mycotal, B-2 -Mycotal, and B-2-Vertalec, many hybrid strains were detected. Nit (nitrate non-utilizing) mutants (Correll et al., 1987) were used for visually selecting protoplasts (Fig.1).

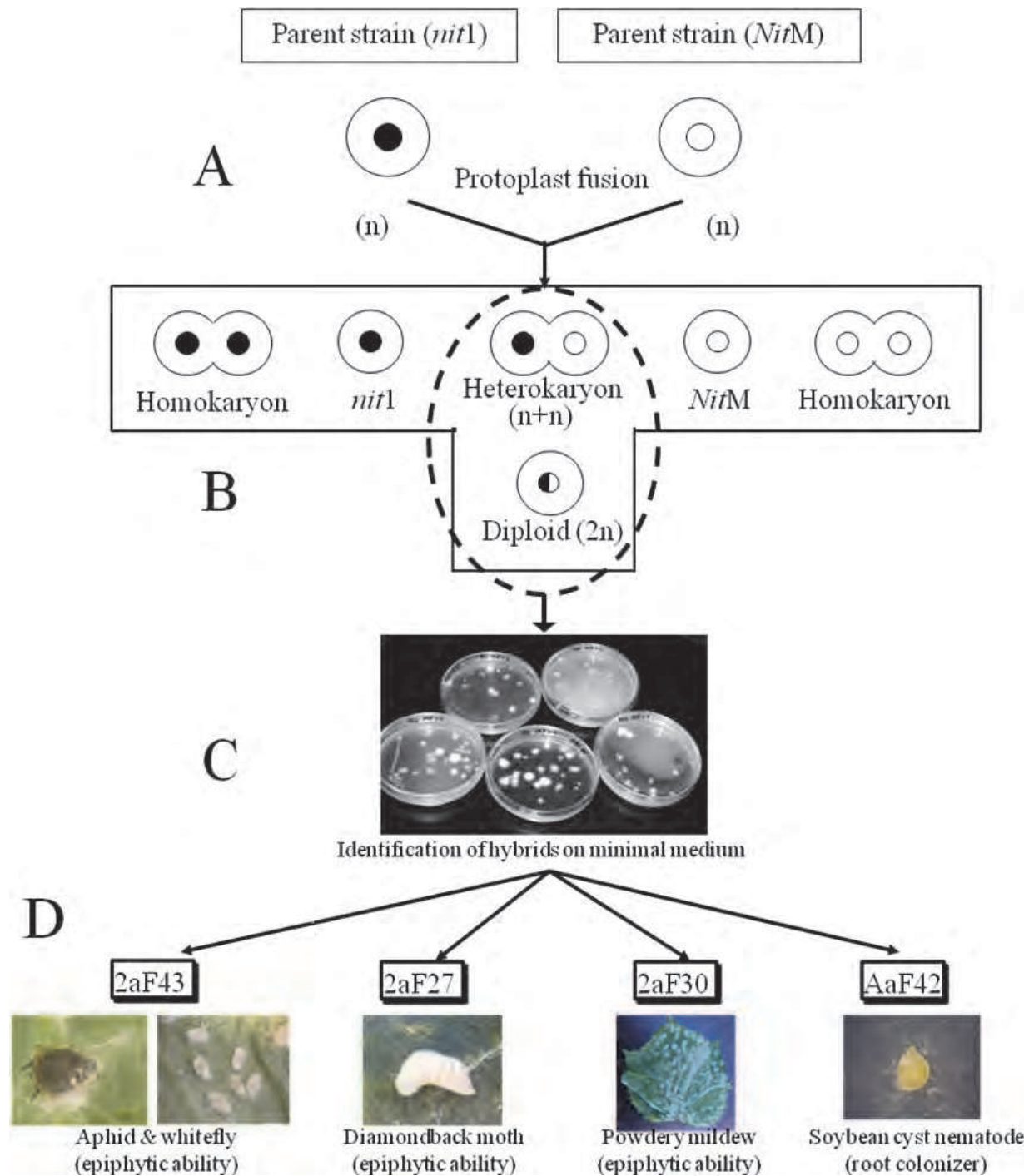


Fig. 1. Model for identification of hybrids on protoplast fusion procedure and selection sequence for hybrid strains of *Lecanicillium* spp. A) Protoplast fusion was conducted on complementary combination of *nit* mutants. B) Protoplast suspension after fusion treatment contains heterokaryon, diploid, homokaryon (self-fusing) and *nit* mutant (non-fusing). C) Only heterokaryon and diploid could develop the colony as prototrophic growth on minimal medium. D) Screening procedure based on various parameters and candidates of hybrid strains as BCAs.

The morphological characteristics of the hybrid strains differed from those of their parental *nit* mutants. Furthermore, genomic analyses were done to ascertain the success of protoplast

fusion. These confirmed protoplast fusions were in genomic DNA but not in mitochondrial DNA (mtDNA). In both analyses, they observed a uniform biased tendency of the banding pattern, depending on the combination of the parental strains. Some of these genomic analyses confirmed successful fusion and/or genetic recombination. These results demonstrated the usefulness of conducting genomic analyses such as polymerase chain reaction-restriction fragment length polymorphism, arbitrarily primed-PCR and genome profiling for discovering nucleotides that exhibit high polymorphism in order to ascertain success of protoplast fusion (Aiuchi et al., 2008, Kaibara et al., 2010).

Further studies were conducted to screen desirable *Lecanicillium* hybrid strains that have a wide host range or increased efficacy (Aiuchi et al., 2007). Initially, 43 hybrid strains were used in bioassays against the cotton aphid, *Aphis gossypii*. Of these, 30 strains induced mortality equal to or higher than Vertalec (42%). Secondly, 50 hybrid strains were used in bioassays against the greenhouse whitefly, *Trialeurodes vaporariorum*. Of these, 37 strains exhibited an equal or higher infection rate as compared to that of Mycotol (36.2%). Finally, 50 hybrid strains were applied to cucumber leaves in order to test strain viability under low humidity conditions (ca.13% RH). Two weeks after application, 17 hybrid strains exhibited viabilities equal to or higher than B-2 (1.5×10^3 cfu/cm²). These results identified hybrid strains whose parental characteristics had not only recombined but also whose pathogenicity or viability had improved, with a hybrid isolate even producing conidia on a leaf hair. Finally, 13 candidate hybrid strains were selected that exhibited improved qualities, and these hybrid strains can be expected to be highly effective as biological control agents (Fig.1).

3.1 Selection of *Lecanicillium* hybrid strains against the SCN

Shinya et al. (2008a) investigated whether the protoplast fusion technique was an effective tool for development of more efficient nematode control agents. Three parental strains (Vertalec, Mycotol, and B-2) and their 162 hybrid strains were screened in greenhouse pot tests against the soybean cyst nematode *H. glycines*. Some of these hybrid strains reduced the density of SCN in the soil and suppressed damage to soybean plants. In particular, one hybrid strain, AaF42 (Vertalec: *L. longisporum* × Mycotol: *L. muscarium*), reduced nematode egg density by 93% as compared with the control providing excellent protection to soybean plants. Furthermore, this strain significantly reduced cyst and egg densities compared to the parental strains (Fig.2, Table 1).



Fig. 2. *Lecanicillium* hybrid strain AaF42 (Vertalec × Mycotol) protected soybean plants from soybean cyst nematode (*Heterodera glycine*) 4, 6 and 8 weeks after treatment in SCN infested soil (Shinya et al. 2008a) .

Strains ¹	Cysts/50 g soil	Eggs/g soil	Eggs/cyst	Fresh root weights (g)
AaF17	12.3 ± 4.4 ^{de}	18.7 ± 7.5 ^d	73.3 ± 17.0 ^e	1.10 ± 0.09 ^{bc}
AaF23	26.7 ± 9.2 ^{cde}	39.3 ± 15.6 ^d	78.2 ± 16.6 ^e	0.93 ± 0.08 ^{ab}
AaF42	11.9 ± 3.6 ^e	16.8 ± 8.3 ^d	70.9 ± 29.4 ^e	1.52 ± 0.21 ^c
AaF80	27.8 ± 7.3 ^{cd}	65.0 ± 22.3 ^{cd}	114.9 ± 13.5 ^{cd}	0.85 ± 0.22 ^{ab}
AaF103	13.0 ± 4.6 ^{de}	27.0 ± 11.6 ^d	100.9 ± 19.0 ^{de}	0.83 ± 0.14 ^{ab}
Mycotal	39.4 ± 9.4 ^{bc}	106.0 ± 29.3 ^{bc}	133.3 ± 12.3 ^{bc}	0.62 ± 0.08 ^{ab}
Vertalec	47.9 ± 11.6 ^b	157.4 ± 54.5 ^b	161.1 ± 20.6 ^{ab}	0.72 ± 0.03 ^{ab}
Control 1 (without fungus) ²	69.1 ± 17.2 ^a	248.6 ± 75.7 ^a	179.2 ± 25.5 ^a	0.60 ± 0.09 ^{ab}
Control 2 (untreated) ³	ND ⁴	ND	ND	1.00 ± 0.10 ^{ab}

The values are the means ± standard deviation of three replicates. The different letters in the columns indicate significant differences ($P < 0.01$, Tukey’s HSD test).

¹ The hybrid strains, AaF were derived from protoplast fusion of Vertalec × Mycotal.

² Control 1: SCN was inoculated but fungus was not.

³ Control 2: Neither SCN nor fungus was inoculated.

⁴ ND: not detected.

Table 1. The effects of selected strains of *Verticillium lecanii* on the density of *Heterodera glycine* cysts and eggs, and the growth of soybean roots in pots (Shinya et al., 2008a).

3.2 Effects of culture filtrates of the *Lecanicillium* hybrid strains to SCN

Shinya et al. (2008b) also evaluated the effects of fungal culture filtrates of the *Lecanicillium* hybrid strains on mature eggs, embryonated eggs (eggs fertilized but without development of juveniles), and J2 of SCN and compared these effects to those of their parental strains. The fungal culture filtrates of some hybrid strains inhibited egg hatch of mature eggs. Furthermore, the fungal culture filtrates of two hybrid strains, AaF23 and AaF42 (Vertalec: *L. longisporum*× Mycotal: *L. muscarium*), exhibited high toxicity against embryonated eggs. However, most of the fungal culture filtrates did not inactivate J2.

These results suggested that the enzymes or other active compounds in the fungal culture filtrates exhibit activity against specific stages in the SCN life cycle. In addition, based on a visual assessment of the morphological changes in eggs caused by filtrates of each strain, there were differences between the hybrid strains and their respective parental strains with regard to the active substances produced by *Lecanicillium* spp. against the embryonated eggs (Fig. 3). It is known that some entomopathogenic fungi produced nematicidal and insecticidal metabolites, for example entomopathogenic *Verticillium* sp. FKI-1033 (*Lecanicillium* sp.) produced Verticilide (Shiomi et al., 2006). As a result of promoting recombination of whole genomes via protoplast fusion, several hybrid strains may have enhanced production of active substances that are different from those produced by their parental strains. It was concluded that natural substances produced by *Lecanicillium* hybrids are important factors involved in the suppression of SCN damage.

3.3 Parasitism of the *Lecanicillium* hybrid strains to SCN

Shinya et al., (2008c) also investigated the pathogenicity and mode of action of the *Lecanicillium* hybrid strains to the sedentary stages of SCN. Three different sedentary stages (pale yellow female, yellow brown cyst, and dark brown cyst) of SCN were treated and incubated on water agar. After 3 weeks incubation, eggs were investigated for the following:

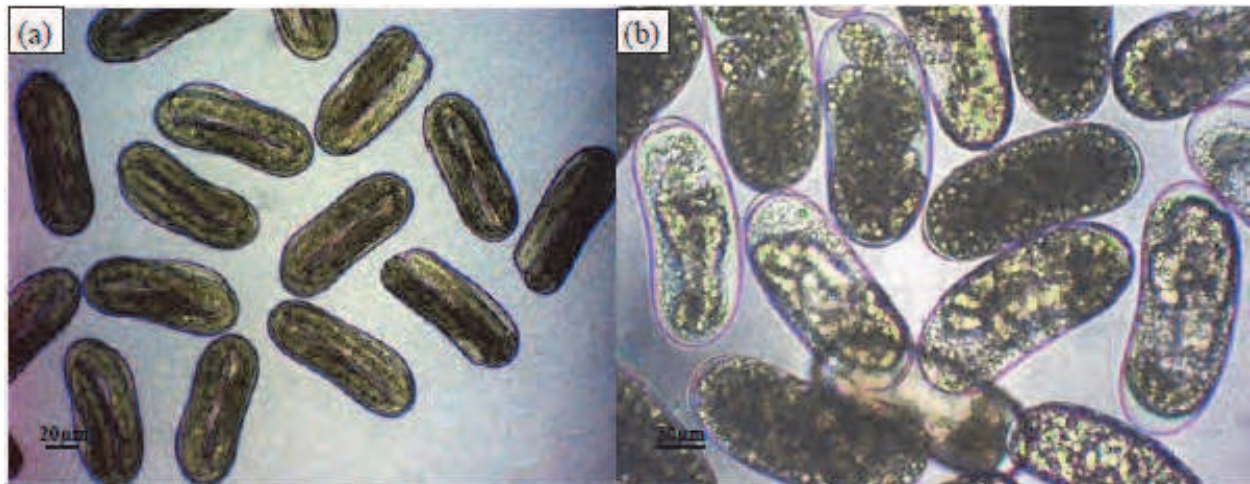


Fig. 3. Effect of fungal culture filtrates on the development of embryonated eggs. (a) Mature eggs containing J2 in the control well after 10 days incubation. (b) Abnormal eggs treated with fungal culture filtrates of *Lecanicillium* hybrid strain AaF23 after 10 days incubation (Shinya et al., 2008b).

(i) the infection frequencies of eggs, (ii) the number of eggs laid, and (iii) the number of mature and healthy eggs. Subsequently, the fecundity of SCN treated with the *Lecanicillium* hybrids was investigated in greater detail.

Most *Lecanicillium* hybrid strains examined appeared to have higher infection rates of pale yellow female (PYF) eggs than those of yellow brown cysts (YBCs) and dark brown cysts (DBC). Meyer and Wergin (1998) reported that cysts tended to be more rapidly colonized by *V. lecanii* (*Lecanicillium* sp.) than females and also described that the cyst wall apparently was not a barrier to *V. lecanii*, so it is possible that these results show differences in egg development. PYFs contained more immature eggs than cysts. It is thought that *Lecanicillium* hybrid strains infected more eggs that had not completed their embryonic development than mature eggs containing J2 individuals.

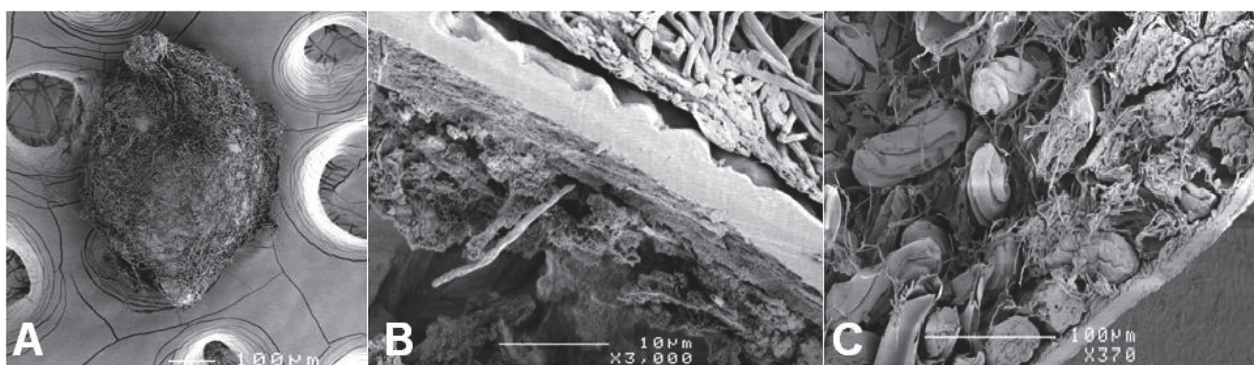


Fig. 4. Scanning electron micrographs of *Lecanicillium* hybrid strain AaF42 infected soybean cyst nematodes. A: Colonized mature female, B: Penetration of cyst wall, C: Infected eggs of SCN.

Moreover, infection with some *Lecanicillium* hybrid strains reduced the number of eggs of PYFs. Egg laying by females treated with AaF42 terminated approximately 3 d after incubation. The body wall of these females rapidly tanned and the individuals subsequently encysted. A cyst can be considered a dead female (Niblack, 2005); therefore, the formation of

cysts indicated that females treated with AaF42 died before the completion of egg laying. Meyer and Wergin (1998) observed that some females colonized by *V. lecanii* contained few eggs and hypothesized that *V. lecanii* infected and killed some females before a full complement of eggs was produced. Our results also support this hypothesis. In addition, Kerry (1990) indicated that *V. chlamydosporium* (*Lecanicillium chladosporia*) reduced the fecundity of *Heterodera schachtii* infected individuals forming small cysts containing few healthy eggs. In this study, four *Lecanicillium* hybrid strains (AaF42, AaF17, AaF103, and AaF23) that suppressed SCN populations and damage to soybean plants in a preliminary greenhouse test tended to reduce the number of eggs and also the number of mature eggs in PYFs; however, no significant difference was observed in the effect on YBCs among individual strains in YBCs, and AaF42, which caused remarkable suppression of SCN populations in a greenhouse test, did not exhibit a high percentage of egg infection in cysts (Shinya et al., 2008c). This suggests that *Lecanicillium* hybrid strains may have colonized and rapidly weakened or killed SCN females before the completion of egg laying and reduced the number of mature and healthy eggs in soil.

Since the evaluation method using estimates of the number of mature and healthy eggs is largely accurate over several modes of action, it appears that this method is an appropriate and simple *in vitro* test to evaluate the pathogenicity of *Lecanicillium* hybrid strains to nematode eggs. However, testing the efficacy of these fungi in soil is essential, since fungi that perform well in laboratory tests may not be effective under field conditions (Kerry, 2001).

Based on the results of this study, we conclude that *Lecanicillium* hybrid strains are more effective against female SCN than against cysts, and the following could be its modes of action: (i) the colonization of females and the reduction of their fecundity, (ii) the prevention of embryonic development or the killing of immature eggs, and (iii) the infection of immature or dead eggs (Fig.4). From this viewpoint, the ability to attack females and the ability to colonize soybean root surfaces, from which females emerge, may be important to control SCN by *Lecanicillium* hybrid strains, and at least these two abilities should be high in potentially useful strains. It is quite likely that AaF42 which exhibited a high reduction of fecundity has high potential as a biological control agent against SCN.

3.4 *Lecanicillium* hybrid strain AaF42 as rhizosphere colonizer and endophyte

There has been little unequivocal evidence of true rhizosphere competence (growth of the fungus within the root zone utilizing plant carbon) in entomopathogenic fungi. The mechanisms of interaction between fungus and plant root needs to be elucidated (Vega et al., 2009). Gaining an understanding of the population structure of rhizosphere colonizers and how they change throughout the season is imperative for development of strategies for controlling plant parasitic nematodes, root diseases and improving root health. The current soil treatment with methyl bromide: chloropicrin can improve plant growth and yield even in the absence of known soilborne pathogens (Martin, 2003).

The ecology of fungal entomopathogens in the rhizosphere is an understudied area of insect pathology. The rhizosphere is the region of soil in which the release of root exudates influences the soil microbiota, and may provide a favorable environment for fungal entomopathogens (Bruck, 2010). We performed studies to determine the persistence of *Lecanicillium* hybrid strain AaF42 as soybean root colonizer. It was found that AaF42 was a better root colonizer compared with parental strains (Vertalec & Mycotol, Fig. 5).

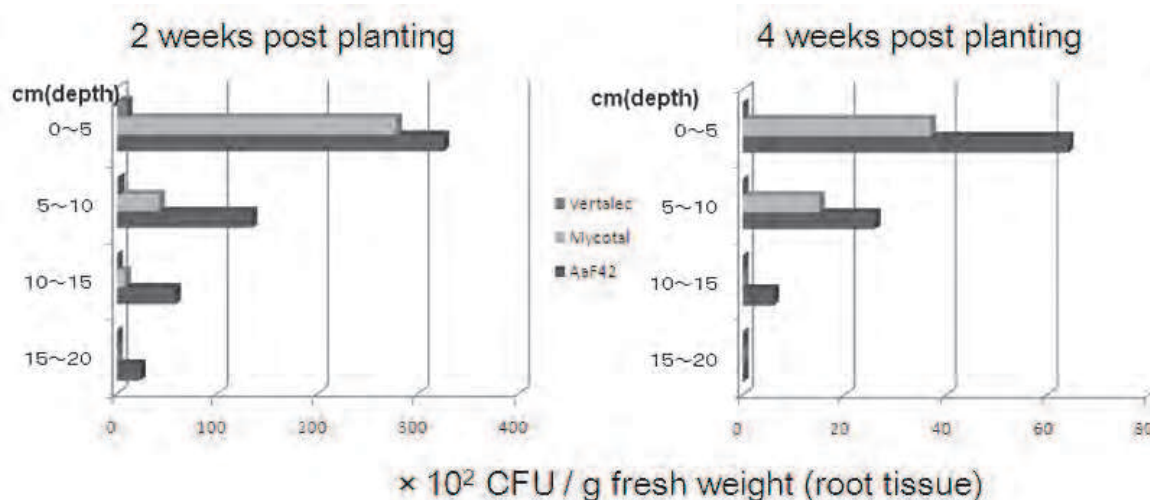


Fig. 5. Fungal populations on soybean roots (cfu g⁻¹ fresh weight of root tissue) of *Lecanicillium* hybrid strain AaF42 and the parental strains (Vertalec & Mycotol) using a cylinder pot (height 50mm, ϕ 85mm). Dilution plate method was done on the surface of soybean roots thus avoiding propagules within the root tissues

Two weeks after planting soybeans into pots pretreated with the fungi, Mycotol and AaF42 could be detected ca. 3×10^4 cfu per g root fresh weight and there were no significant differences at the soil depth 0~5cm. However, as the soil depth increased, more AaF42 was detected than Mycotol. At four weeks after planting, there was one order difference in detection between AaF42 and Mycotol. In contrast, the detections of Vertalec were nil or very low. Bruck (2010) described the role of fungal entomopathogens in the rhizosphere for controlling root-feeding insects. Currently, data on the pest management potential of rhizosphere competent fungal entomopathogens are scant. However, the prospective ramifications of this relationship are tremendous. A simple calculation of the economic benefits that can be realized by utilizing rhizosphere competent fungal entomopathogens yields savings significant enough to warrant further investigation (Bruck, 2010). It can be said that *Lecanicillium* hybrid strain AaF42 with high culture filtrate toxicity, pathogenicity and parasitism to SCN, and a good root-colonization ability, shows considerable promise for development as a biological control agent for SCN.

Recently, molecular and micro-ecological trials with *Lecanicillium* hybrid strain AaF42 were designed to do elucidate the tritrophic interactions among the fungi, SCN and soybean root (unpublished data). This was accomplished by employing a gfp gene driven by a constitutive promoter which strongly labeled the fungus with no impact on fungal growth or pathogenicity (Fig. 6). Preliminary results indicated that AaF42 might act as an endophyte, however, further studies are required before firm conclusions can be made.

3.5 Stage specificity of *Lecanicillium* against SCN and its importance in the control of SCN

As described above, the *stage* in the SCN life cycle attacked by *Lecanicillium* hybrid strain AaF42 has a profound effect on the viability of SCN and damage to soybean crops. This is a very significant point in the control of plant parasites, especially cyst nematodes. The cyst nematodes generally have a high reproductive potential, producing approximately 200-500 eggs per cyst (female), and they can survive for several years at least in the soil without a host plant. Therefore, several thousand nematodes appear in the next generation even if

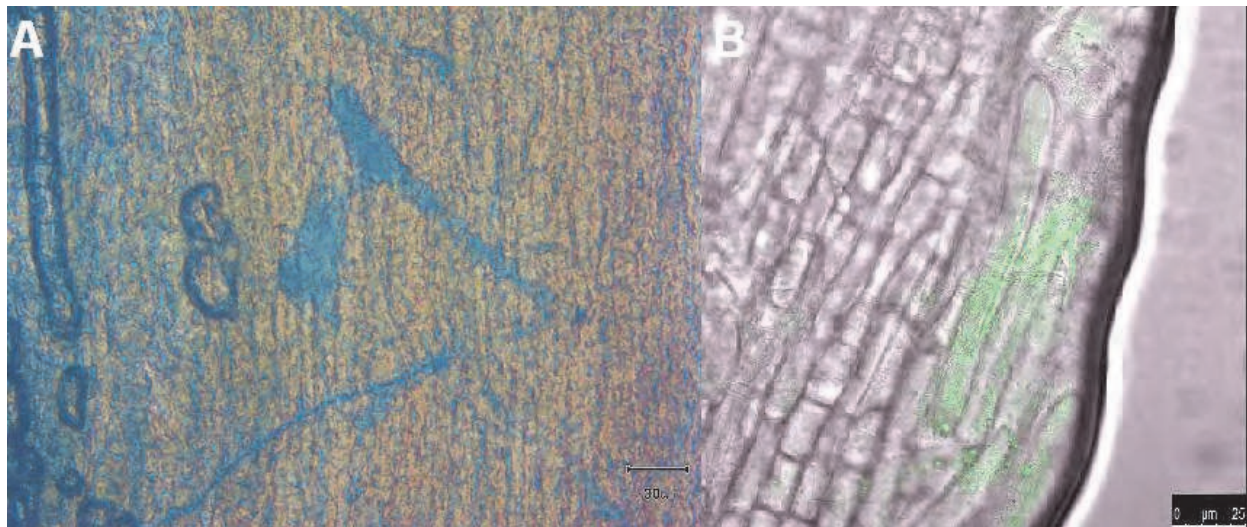


Fig. 6. *Lecanicillium* hybrid strain AaF42 as possible endophyte (A: AaF42 mycelium with normal Lactophenol Cotton Blue Stain, B: Recombinant AaF42 with GFP gene within soybean root tissue)

only several J2 nematodes successfully invade the root of a host plant. The J2 of cyst nematodes which emerge from the eggs can quickly invade roots near the root tip of a host plant. Thus, the sedentary stage, especially immature female or immature cyst, would be the most appropriate target stage in the biological control using nematophagous fungi. The nematode-trapping fungi, e.g., *Arthrobotrys* spp., are the well known group of nematophagous fungi, probably owing to their remarkable morphological adaptations and their dramatic infection of nematodes. However, these fungi are known as a poor colonizers of eggs and sedentary stages of cyst nematodes (Chen et al., 1996). From this view point, the nematode-trapping fungi seemed to be unsuitable as biological control agents against cyst nematode. We demonstrated that *Lecanicillium* hybrid strain AaF42 has a distinguished infectivity against sedentary stages of SCN, especially immature females and eggs, and a high ability as root colonizer and endophyte. It would be inferred from these exceptional talents that hybrid strain AaF42 has high potential as a biological agent against SCN.

4. Future prospect (potential of biological control agents for SCN and other complex diseases)

Fungi traditionally known for their entomopathogenic characteristics, such as *Beauveria bassiana* and *Lecanicillium* spp., have recently been shown to engage in plant-fungus interactions (Vega, 2008; Vega et al., 2008), and both have been reported to effectively suppress plant disease (Goettel et al. 2008; Ownley et al., 2004, 2008). Biological control of plant pathogens usually refers to the use of microorganisms that reduce the disease causing activity or survival of plant pathogens. Several different biological control mechanisms against plant pathogens have been identified. The biocontrol organism is directly involved in some mechanisms such as antibiosis, competition, and parasitism. With other modes of biological control, such as induced systemic resistance and increased growth response, endophytic colonization by the biocontrol organism triggers responses in the plant that reduce or alleviate plant disease (Ownley et al., 2010).

Lecanicillium spp. have activity against numerous phytopathogenic fungi including powdery mildews (Verhaar et al., 1997, 1998; Askary et al., 1997, 1998, 1999; Dik et al., 1998; Miller et al., 2004), rusts (Spencer and Atkey, 1981; Leinhos and Buchenauer, 1992) green molds (Benhamou and Brodeur, 2000) and *Pythium* (Benhamou and Brodeur, 2001). Fungi that may control phytopathogenic fungi can act through antibiosis and mycoparasitism (Kiss, 2003). Some *Lecanicillium* isolates act as mycoparasites, attaching to powdery mildew mycelia and conidia, producing enzymes such as chitinase, that allow penetration of the mildew spores and hyphae, killing the pathogen (Askary et al., 1997). Leinhos and Buchenauer (1992) demonstrated that several *Lecanicillium* spp. were able to penetrate and colonize uredial sori of *Puccinia coronata*. In *Penicillium digitatum*, the mode of action was attributed to changes in host cells prior to contact by the *Lecanicillium* spp. (Benhamou and Brodeur, 2000) while in *P. ultimum*, in addition to mycoparasitism of the plant pathogen, the mode of action was linked to colonization of host plant tissues, triggering a plant defense reaction (Benhamou and Brodeur, 2001). Hirano et al. (2008) found that applying *L. muscarium* blastospores to cucumber roots induced systemic resistance. *L. muscarium* pre-inoculated plants suffered significantly fewer lesions and reduced disease severity compared with non-inoculated plants. Kusunoki et al. (2006) and Koike et al. (2007b) found that root treatment with *L. muscarium* reduced disease incidence and wilting score in other soil-borne disease combinations such as tomato – *Verticillium dahliae*, Japanese radish – *V. dahliae*, and melon – *Fusarium oxysporum* f.sp. *melonis*.

In the case of soilborne pathogens, further opportunities exist for interactions with other microorganisms occupying the same ecological niche. The significant role of nematodes in the development of diseases caused by soilborne pathogens has been demonstrated in many crops throughout the world. In many cases, such nematode–fungus disease complexes involve root-knot nematodes (*Meloidogyne* spp.), although several other endoparasitic (*Globodera* spp., *Heterodera* spp., *Rotylenchulus* spp., *Pratylenchus* spp.) and ectoparasitic (*Xiphinema* spp., *Longidorus* spp.) nematodes have been associated with diseases caused by soilborne fungal pathogens (Back et al., 2002). In the case of SCN, Sudden Death Syndrome (SDS) caused by *F. solani* is a major disease of soybean which, among other symptoms, induces root rot, crown necrosis, interveinal chlorosis, defoliation and abortion of pods (Rupe, 1989; Nakajima et al., 1996). Recent research on SDS has focused on identifying genes for dual resistance against both nematode and fungus (Chang et al., 1997; Meksem et al., 1999; Prabhu et al., 1999).

It is known that entomopathogenic *Lecanicillium* spp. have antagonistic effects to soil-borne fungi such as *Fusarium oxysporum*, *F. solani*, *Pythium* spp. and *Verticillium dahlia* (Koike et al., 2006, Goettel et al., 2008). Therefore, it might be possible to develop *Lecanicillium* hybrid strains with potential for biological control of a complex of plant diseases, plant parasitic nematodes and insect pests.

5. Conclusion

Much research is still needed to fully understand the role that rhizosphere competent fungal entomopathogenic *Lecanicillium* hybrid strains play in regulating SCN populations and how we can use this knowledge to design and implement more effective SCN biological control programs. Questions of particular importance to consider are highlighted by Vega et al. (2009) and include the following: (1) Do plants benefit from a rhizosphere association with

fungus entomopathogens? (2) Is the 'bodyguard' concept relevant in soil? If so, what is the signaling mechanism between trophic levels? (3) Do different phylogenetic groups of fungal entomopathogens display different strategies in their association with plants? (4) How do soil-borne fungal entomopathogens interact between above and below ground ecosystems? (5) What is the mechanism of yield increases in biological control target plant? (6) Does plant diversity impact fungal entomopathogen diversity at the landscape or local level, and what is its impact on natural pest control? In addition to the basic scientific questions posed above, there are a number of questions that require further investigation as well: (1) What is the most effective approach for inoculating roots with rhizosphere competent isolates? Approaches will need to be identified for plants propagated via seed treatment, because there are a lot of problems in the direct treatment of soil such as costs & labor requirements. (2) How long do rhizosphere competent isolates persist on the root system of soybean or other host plants of plant parasitic nematodes? (3) Will the use of rhizosphere competent isolates provide consistent and acceptable levels of pest including plant parasitic nematode control?

At present there has been only limited success with field applications of biological controls against SCN. Chen (2004) pointed out factors involved in their biological control, 1) stage of nematode infected, 2) ability to colonize soil, roots, cysts and gelatinous matrices, 3) competition with other fungi, 4) cropping systems and tillage, and 5) edaphic and environmental factors. In our research, all experiments were done *in vitro* and in glasshouses. Although there is still much to be learned at the field level, it has been demonstrated that *Lecanicillium* hybrid strains have multiple effects (toxic and parasitism) for SCN and soybean plant roots (as root colonizer and endophyte) as well as on plant pathogens and insect pests, making these strains promising for development as broad spectrum biopesticides that include SCN.

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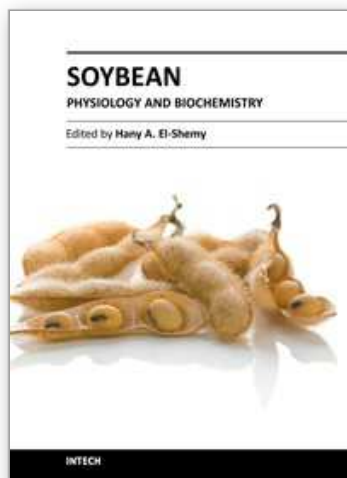
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