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Pathogenicity and its mode of action in different sedentary stages of *Heterodera glycines* (Tylenchida: Heteroderidae) by *Verticillium lecanii* hybrid strains

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Abstract
The current study was conducted to investigate the pathogenicity and its mode of action by *Verticillium lecanii* hybrid strains in different sedentary stages of *Heterodera glycines*. Three different sedentary stages (pale yellow female, yellow brown cyst, and dark brown cyst) of *H. glycines* were treated with *V. lecanii* and incubated for 3 weeks on water agar. After 3 weeks of incubation, eggs were investigated for the following parameters: (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 *H. glycines* treated with *V. lecanii* was investigated in greater detail. Consequently, some strains had a greater ability to infect eggs and this significantly reduced the number of eggs laid and mature eggs in pale yellow females. This study indicates that *V. lecanii* is more effective on females rather than cysts of *H. glycines* and that *V. lecanii* may act on *H. glycines* in multiple ways.

Key words: Soybean cyst nematode; protoplast fusion; fecundity; egg laying; biological control

INTRODUCTION

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is one of the most important pests of soybean (Riggs, 1977; Wrather et al., 2001). The basic management strategy for SCN control, such as resistant cultivars and crop rotation, has been practiced, but soybean yield losses persist. In combination with biological control agents, this basic management strategy may be used more effectively.

One potentially useful agent for biological control is the fungus *Verticillium lecanii* (A. Zimmermann) Viegas. It is known that *V. lecanii* has a broad host range (Hall, 1981) and is effective against insects and fungi (Hall, 1980, 1982; Allen, 1982; Jackson et al., 1985). It has previously been used commercially for the control of aphids and whiteflies (Hall, 1984). Further, *V. lecanii* is one of the fungi known to colonize cyst nematodes and root-knot nematodes (Hänssler and Hermanns, 1981; Gintis et al., 1983; Meyer et al., 1990; Uziel and Sikora, 1992; Meyer, 1994; Eapen et al., 2005).

Aiuchi et al. (2004) carried out protoplast fusion among three strains of *V. lecanii* in order to obtain new hybrid strains. As a result of this experiment, 174 hybrid strains were obtained. Lalithakumari (2000) reported that protoplast fusion is a valuable tool for strain improvement by facilitating the recombination of whole genomes and the development of hybrid strains in fungal biological control agents. Furthermore, improvement in the antagonistic activity of hybrid strains was observed when compared with parental strains (Pe'er and Chet, 1990; Couteaudier et al., 1996; Prabavathy et al.,

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2006).

In a preliminary experiment, some hybrid strains of *V. lecanii* suppressed damage to soybean plants caused by SCN more effectively in greenhouse pot tests in comparison with their parental strains, suggesting that these strains have potential as biological control agents against SCN. However, field-scale applications would be more difficult owing to competition with other soil organisms. Thus, before field trials can be conducted, there is a need for detailed information about the key factors affecting the pathogenicity of *V. lecanii* and its modes of action. This information would be valuable when considering the most effective mode of application in field trials.

Some reports have indicated that immature eggs are more susceptible to fungal attack than mature eggs containing second-stage juveniles (J2) (Irving and Kerry, 1986; Kim and Riggs, 1991; Chen and Chen, 2003). Furthermore, Meyer et al. (1990) demonstrated that one strain of *V. lecanii* decreased the number of viable 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 eggs; however, no such effect was observed in other strains of *V. lecanii*.

Therefore, the current study was conducted in order to investigate the pathogenicity in different sedentary stages of SCN by *V. lecanii* hybrid strains by using more strains and by studying the mode of action of SCN in greater detail.

MATERIALS AND METHODS

Fungal strains. Twelve strains of *V. lecanii* were tested in the current study (Table 1). Of these, 2 strains, Vertalec® and Mycotal® (Koppert UK Ltd., Wadhurst, East Sussex, UK), have been exploited commercially as biological control agents against aphids and whiteflies, respectively. A third strain, B-2, was isolated from a green peach aphid in Obihiro. The remaining 9 strains were all obtained by protoplast fusion (Aiuchi et al., 2004, 2008). We used AaF17, 23, 42, 80, and 103 as good antagonists to SCN; AaF11, 49, BbF17, and 2aF26 as moderate antagonists; and Vertalec®, Mycotal®, and B-2 as poor antagonists to SCN (Table 1).

Nematodes. SCN (*H. glycines*) was cultured on soybean and *Glycine max* (L.) Merr. cv. Kurosengoku was planted in plastic pots (diameter, 16 cm; height, 20 cm) containing sterilized field soil (loamy soil; 66.3% sand, 31.1% silt, 2.6% clay, pH 5.8) in a greenhouse. Pale yellow females (PYFs) and yellow brown cysts (YBCs) were obtained from the roots of 10-week-old soybean by carefully washing rhizosphere soil from the roots through a 710-μm-pore sieve onto a 250-μm-pore sieve. However, only dark brown cysts (DBCs) were collected from the soil using a method described by Yoshihara and Kegasawa (1989). This soil had been maintained at 4°C for 3 months before being used.

Table 1. *Verticillium lecanii* strains used in this experiment, corresponding parental combinations, and evaluation of antagonism to *Heterodera glycines*, the soybean cyst nematode (SCN)

Strains	Combinations of each parent[a]	Evaluation of antagonism to SCN[b]
AaF11	Vertalec×Mycotal	±
AaF17	Vertalec×Mycotal	++
AaF23	Vertalec×Mycotal	+
AaF42	Vertalec×Mycotal	++
AaF49	Vertalec×Mycotal	±
AaF80	Vertalec×Mycotal	+
AaF103	Vertalec×Mycotal	+
BbF17	Vertalec×Mycotal	±
2aF26	Mycotal×B-2	±
Vertalec®	—	−
Mycotal®	—	−
B-2	—	−

[a] Combination of each parent according to Aiuchi et al. (2004).

[b] ++: The strain suppressed both SCN population and plant damage in third screening, +: the strain suppressed only SCN population in third screening, ±: the strain did not suppress SCN population in second screening, −: the strain did not suppress SCN population in first screening.

The evaluation of antagonism to SCN is based on the suppression of damage to soybean plants and the SCN population in three screening tests using greenhouse pots.

Experiment 1: Pathogenicity to eggs of PYFs, YBCs, and DBCs on water agar. Females and cysts were surface-disinfested with 0.5% sodium hypochlorite (NaOCl) for 3 min and placed on water agar. Females and cysts that exhibited no signs of either fungal or bacterial growth after 3 d were used (Chen et al., 1996).

Twelve strains of *V. lecanii* were cultured on potato dextrose agar (PDA; Difco Laboratories) at 25°C for 2 weeks. Plugs (5 mm diameter) cut from

these cultures were transferred to the center of Petri dishes (90 mm diameter) containing 1.5% water agar with streptomycin sulfate (0.1 mg/ml), kanamycin sulfate (0.05 mg/ml), and benzylpenicillin potassium (0.1 mg/ml) and incubated at 25°C.

After 3 weeks of incubation, any of 4 fungus- and bacteria-free PYFs or 4 YBCs or 3 DBCs were placed 2 cm away from the center plugs on each Petri dish, and two replicate Petri dishes per fungal treatment per SCN stage were prepared (two Petri dishes per fungal treatment for each of PYFs, YBCs and DBCs). One group of PYFs, YBCs and DBCs was plated on water agar without fungus as a control. The results of two replicates were combined for analysis. These females and cysts were further incubated at 25°C for 3 weeks.

After incubation, the females and cysts were picked up with forceps, placed in water, and squeezed with the forceps to obtain eggs. These eggs were observed with an inverted light microscope at ×100–400 magnification. The percentages of eggs that were colonized by fungi and whether they were mature or immature were recorded by observing the first 100 eggs seen from 2 females and 2 cysts treated with each strain of *V. lecanii*. An egg was considered to be infected when either penetrating hyphae were visible or when hyphae emerged from the egg (Segers et al., 1996). Empty eggs from which the juvenile had already hatched and eggs containing J2 individuals were considered to be mature eggs. It was confirmed that there was a exit slit through which the juvenile had already emerged from the empty egg. Further, in PYFs and YBCs, eggs containing an embryo or first-stage juvenile were considered to be immature. However, immature eggs were not counted in DBCs, since most of the eggs had already been laid and matured before fungal treatment. In addition, the number of eggs in PYFs and YBCs were determined.

Experiment 2: Number of eggs laid in PYFs after fungal treatment. AaF42 significantly reduced the number of eggs in PYFs, whereas 2aF26 had no effect on SCN in experiment 1. Since the difference in fecundity caused by these 2 strains needs to be examined in detail, hybrid strains, AaF42 and 2aF26, were used in experiment 2.

AaF42 and 2aF26 were cultured and incubated using the same procedures as described in experiment 1. After 3 weeks of incubation of each strain on water agar, 28 fungus- and bacteria-free PYFs were placed 2 cm away from the center plugs on each Petri dish, and three Petri dishes per fungal treatment were prepared. One group of PYFs was plated on water agar without fungus as a control. PYFs were collected and surface-disinfested by the aforementioned method.

After 3, 7, and 14 d incubation at 25°C, females were picked up with forceps, placed in water, and squeezed with the forceps to obtain eggs. Then, the number of eggs laid per 6 females was counted in each Petri dish. Concomitantly, the percentage of tan discoloration of the PYF bodies was determined per Petri dish per day. A PYF was considered to be discolored when its color became the same color as DBC.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) and when the *F*-test was significant at $p<0.05$ or 0.01, the treatment means were compared using Tukey's honestly significantly different (HSD) test.

RESULTS

Pathogenicity to eggs of different stages of SCN on water agar

PYFs contained few eggs (mean=10 eggs/female) in the body, all of which were immature (eggs fertilized but without J2 development). However, these females had already been inseminated, since most females produced eggs containing an embryo and had transformed into a brown cyst by the time of the preliminary examination (Triantaphyllou and Hirschmann, 1962). Among the eggs in the YBCs, 72% were immature and 28% were mature (with development of J2). YBCs had not completed egg laying at that point in time (mean=84 eggs/cyst). Among the eggs in DBCs, 6.5% were immature and 93.5% were mature. DBCs had completed egg laying (mean=184 eggs/cyst).

The infection frequencies of the various fungal strains with regard to the eggs of SCN varied and were affected by the different sedentary stages of SCN (Table 2). In most strains, a higher egg infection rate tended to be observed in females rather than cysts. AaF103 infected the highest percentage of eggs in females. On the other hand, BbF17 exhibited the lowest percentage of egg infection in both females and cysts.

At the same time, a large number of immature

Table 2. Infection frequencies of eggs of *Heterodera glycines* by *Verticillium lecanii* strains on water agar

Strains	Rate of infection of eggs (%) (mean±SD)		
	Nematode developmental stages		
	In pale yellow females	In yellow brown cysts	In dark brown cysts
AaF103	84.3±2.9 a	46.0±12.1 a	36.8±38.6
AaF80	79.5±7.7 ab	44.3±12.5 a	52.2±33.5
AaF17	77.5±2.6 abc	46.8±16.2 a	45.5±37.1
AaF23	74.8±7.1 abcd	51.3±5.2 a	29.7±35.7
AaF11	70.0±2.4 abcd	41.3±12.3 a	43.0±38.8
Mycotal®	68.5±8.8 abcd	48.3±14.8 a	25.8±30.1
AaF42	55.5±19.5 bcde	46.5±5.8 a	51.8±42.3
Vertalec®	51.8±15.1 cdef	37.8±9.3 a	13.3±11.4
AaF49	49.8±3.4 def	55.3±9.2 a	22.3±21.9
2aF26	29.3±13.4 efg	27.8±16.9 ab	16.8±20.7
B-2	27.8±10.1 fg	30.0±4.1 ab	7.7±6.8
BbF17	6.0±6.7 gh	2.5±5.0 b	0.3±0.8
Control	0.0±0.0 h	0.0±0.0 b	0.0±0.0
	$p<0.01$	$p<0.01$	NS

The values are the mean±standard deviation of 8 replicates in pale yellow females and yellow brown cysts and 6 replicates in dark brown cysts. Different letters in the same column indicate significant difference ($p<0.01$, Tukey's test). NS: not significant at $p=0.05$.

eggs and a high immature egg rate were observed in both PYFs and YBCs treated with BbF17 and 2aF26, although hyphae were not observed on/in the eggs. BbF17 significantly ($p<0.01$) increased the ratio of immature eggs in PYFs, and BbF17 and 2aF26 also significantly ($p<0.01$) increased the ratio in YBCs as compared to the control (Table 3).

Furthermore, AaF42, AaF17, AaF103, and AaF23 significantly ($p<0.01$) reduced the number of eggs in PYF as compared to the control (Table 4). AaF23 also reduced the number in YBC; however, the difference in the number of eggs laid among strains was obviously greater than YBC in PYF.

Subsequently, the number of mature eggs was determined (Figs. 1a and b). In some strains, mature eggs hardly remained in PYF. Moreover, all strains significantly ($p<0.01$) reduced the number of mature eggs in PYFs and YBCs when compared with the control, although no significant difference was observed among individual strains ($p>0.05$) (Figs. 1a and b).

Table 3. The rate of immature eggs of *Heterodera glycines* in pale yellow females and yellow brown cysts treated with *Verticillium lecanii* strains after 3 weeks of incubation on water agar

Strains	Rate of immature eggs (%) (mean±SD)	
	Nematode developmental stages	
	In pale yellow females	In yellow brown cysts
AaF80	6.0±3.5 a	21.0±12.2 ab
AaF103	7.8±3.1 a	8.8±3.7 a
AaF49	8.3±4.5 a	15.3±6.2 a
AaF17	10.5±1.9 a	16.5±5.4 a
AaF23	10.5±5.0 a	12.8±2.2 a
Mycotal®	11.0±8.3 a	16.5±8.7 a
Vertalec®	13.0±7.0 ab	8.3±1.7 a
AaF11	16.3±2.6 ab	16.3±3.8 a
B-2	22.3±10.2 abc	16.8±4.9 a
AaF42	34.0±18.1 bc	9.0±3.2 a
2aF26	41.5±6.8 cd	34.5±2.9 bc
BbF17	60.3±5.5 d	49.8±6.1 c
Control	21.0±2.2 abc	13.5±4.8 a

The values are the mean±standard deviation of 8 replicates. Different letters in the same column indicate significant difference ($p<0.01$, Tukey's test).

Table 4. The number of eggs per 2 pale yellow females and 2 yellow brown cysts of *Heterodera glycines* treated with *Verticillium lecanii* strains after 3 weeks of incubation on water agar

Strains	Number of eggs per two females or cysts (mean±SD)	
	Nematode developmental stages	
	In pale yellow females	In yellow brown cysts
AaF42	34.5±19.1 a	276.5±73.3 ab
AaF17	56.3±20.5 ab	274.5±83.6 ab
AaF103	60.8±22.3 ab	219.3±43.7 ab
AaF23	90.0±28.3 ab	175.3±25.6 a
AaF80	132.3±27.5 abc	258.3±105.6 ab
AaF11	145.8±29.5 abc	196.5±55.4 ab
Mycotal®	158.3±49.8 abc	237.0±65.3 ab
Vertalec®	160.3±66.0 abc	224.5±38.5 ab
BbF17	162.0±34.1 abc	209.3±44.8 ab
B-2	176.0±39.9 abc	189.8±31.1 ab
AaF49	210.0±99.3 abc	213.5±21.3 ab
2aF26	271.0±102.0 bc	243.5±21.2 ab
Control	310.5±201.0 c	367.8±109.0 b

The values are the mean±standard deviation of 4 replicates. Different letters in the same column indicate significant difference ($p<0.01$, Tukey's test).

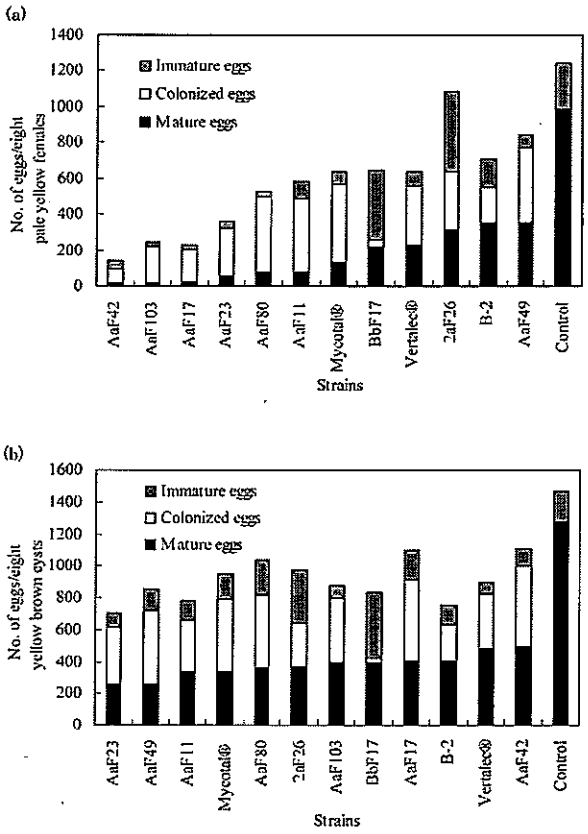

Fig. 1. Number of eggs (immature, colonized, and mature) in (a) pale yellow females and (b) yellow brown cysts treated with *Verticillium lecanii* strains after 3 weeks of incubation on water agar.

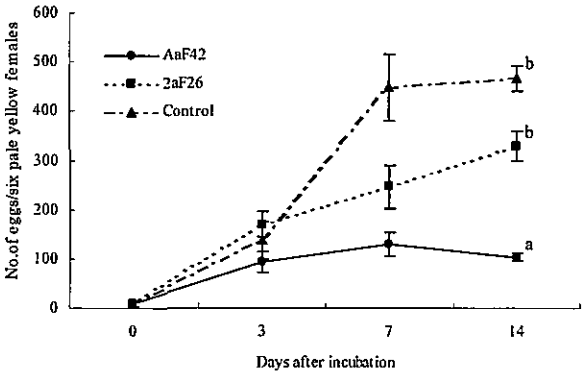

Fig. 2. Number of eggs in 6 pale yellow females of *Heterodera glycines* treated with *Verticillium lecanii* strains after 3, 7, and 14 d incubation on water agar at 25°C. Different letters indicate significant difference ($p<0.05$, Tukey's test) on day 14. There was no significant difference at days 3 and 7 ($p>0.05$, Tukey's test).

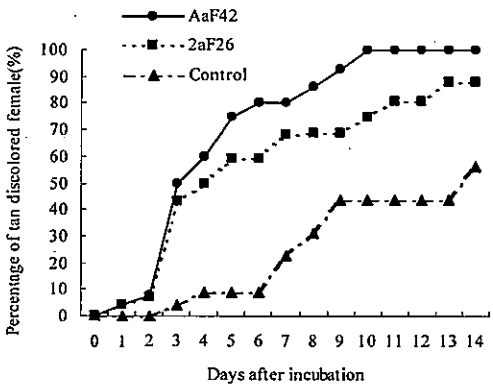

Fig. 3. Percentage of tan discolored females incubated on water agar at 25°C.

Detailed evaluation of ability to reduce the number of eggs laid

The number of eggs laid in PYFs treated with fungi was determined after 3, 7, and 14 d incubation on water agar at 25°C (Fig. 2). After 3 and 7 d subsequent to fungal treatment, no significant difference was observed in the number of eggs laid in PYFs. After 14 d, however, AaF42 significantly reduced the number of eggs laid in PYFs as compared to 2aF26 and the control ($p<0.05$ and $p<0.01$, respectively).

In addition, the tan discoloration of PYF bodies treated with AaF42 or 2aF26 was accelerated as compared to the control (Fig. 3). In particular, this change rapidly appeared in PYFs treated with AaF42 or 2aF26 approximately 3 d after incubation. In addition, all PYFs treated with AaF42 had changed into brown cysts by 10 d after incubation.

DISCUSSION

Most *V. lecanii* strains examined appeared to have higher infection rates of PYF eggs than those of YBCs and DBCs (Table 2). Meyer and Wergin (1998) reported that cysts tended to be more rapidly colonized by *V. lecanii* 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 *V. lecanii* infects more eggs that have not completed their embryonic development than mature eggs containing J2 individuals.

Moreover, infection with some strains of *V. lecanii* reduced the number of eggs in PYF (Table 4). Egg laying by females treated with AaF42 ter-

minated approximately 3 d after incubation. The body wall of these females rapidly tanned and the individuals subsequently encysted (Figs. 2, 3). 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* reduced the fecundity of *H. schachtii*; infected individuals forming small cysts containing few healthy eggs. In this study, four *V. lecanii* hybrid strains (AaF42, AaF17, AaF103, and AaF23) that suppressed SCN population and damage to soybean plants in a preliminary greenhouse test tended to reduce the number of eggs (Table 4) and also the number of mature eggs (Fig. 1a) in PYFs; however, no significant difference was observed in the effect on YBCs among individual strains in YBCs (Table 4), and AaF42, which caused remarkable suppression of SCN population in a greenhouse test, did not exhibit a high percentage of egg infection in cysts (Table 2). This suggests that *V. lecanii* 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.

The ratio of immature eggs in PYFs and YBCs treated with BbF17 was significantly higher than that of the control, and 2aF26 also significantly increased that in YBCs as compared to the control (Table 3). In particular, a large number of eggs in females and cysts treated with BbF17 remained immature after 3 weeks of incubation (Fig. 1), although BbF17 was a weak parasite with regard to the eggs of SCN (Table 2). In other strains, the ratio of immature eggs in PYFs and YBCs was low. The reason behind this low ratio is that other strains have high colonization ability, and thus these strains might already have infected immature eggs. Additionally, several abnormal eggs (including eggs with vacuoles and deformities) were observed in PYFs and YBCs treated with fungi, although these eggs were included in immature eggs in this experiment (data not shown). These results indicate that fungal enzymes or other active compounds secreted into females and cysts of SCN might prevent embryonic development or kill the eggs without the need for the eggs to be colonized. This suggestion was supported by our previous study. Our previous study demonstrated that fungal culture filtrates of some hybrid strains of *V. lecanii* exhibited high toxicity against embryonated eggs of *H. glycines* (Shinya et al., 2008).

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 to nematode eggs of *V. lecanii*. 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 *V. lecanii* is 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. From this viewpoint, the ability to attack females and the ability to colonize on soybean root surface, from which females emerge, may be important to control SCN by *V. lecanii*, and at least these two abilities should be high in potentially useful strains of *V. lecanii*. It is quite likely that AaF42 exhibited that high reduction of the fecundity of SCN has high potential as a biological control agent against SCN, though further studies in non-sterilized soil are necessary.

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REFERENCES

Aiuchi, D., M. Koike, M. Tani, K. Kuramochi, M. Sugimoto and H. Nagano (2004) Protoplast fusion, using nitrate non-utilizing (*nit*) mutants in the entomopathogenic fungus *Verticillium lecanii* (*Lecanicillium* spp.). *IOBC/wprs Bulletin* 27: 121–127.

Aiuchi, D., K. Inami, M. Sugimoto, R. Shinya, M. Tani, K. Kuramochi and M. Koike (2008) A new method for producing hybrid strains of the entomopathogenic fungus *Verticillium lecanii* (*Lecanicillium* spp.) through protoplast fusion by using nitrate non-utilizing (*nit*) mutants.

Micol. Apl. Int. 20: 1–16.

Allen, D. J. (1982) *Verticillium lecanii* on the bean rust fungus, *Uromyces appendiculatus*. *Trans. Br. Mycol. Soc.* 79: 362–364.

Chen, S. Y. and F. J. Chen (2003) Fungal parasitism of *Heterodera glycines* eggs as influenced by egg age and precolonization of cysts by other fungi. *J. Nematol.* 35: 271–277.

Chen, S. Y., D. W. Dickson and D. J. Mitchell (1996) Pathogenicity of fungi to eggs of *Heterodera glycines*. *J. Nematol.* 28: 148–158.

Couteaudier, Y., M. Viaud and G. Riba (1996) Genetic nature, stability, and improved virulence of hybrids from protoplast fusion in *Beauveria*. *Microb. Ecol.* 32: 1–10.

Eapen, S. J., B. Beena and K. V. Ramana (2005) Tropical soil microflora of spice-based cropping systems as potential antagonists of root-knot nematodes. *J. Invertebr. Pathol.* 88: 218–225.

Gintis, B. O., G. Morgan-Jones and R. Rodriguez-Kabana (1983) Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama soybean field soil. *Nematropica* 13: 181–200.

Hall, R. A. (1980) Laboratory infection of insects by *Verticillium' lecanii* strains isolated from phytopathogenic fungi. *Trans. Br. Mycol. Soc.* 74: 445–446.

Hall, R. A. (1981) The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. In *Microbial Control of Pests and Plant Disease* (H. Burges ed.). Academic Press, London, pp. 483–498.

Hall, R. A. (1982) Control of whitefly, *Trialeurodes vaporariorum* and cotton aphid, *Aphis gossypii* in glasshouses by two isolates of the fungus, *Verticillium lecanii*. *Ann. Appl. Biol.* 101: 1–11.

Hall, R. A. (1984) Epizootic potential for aphids of different isolates of the fungus *Verticillium lecanii*. *Entomophaga* 29: 311–321.

Hänssler, G. and M. Hermanns (1981) *Verticillium lecanii* as a parasite on cysts of *Heterodera schachtii*. *J. Plant Dis. Protec.* 88: 678–681.

Irving, F. and B. R. Kerry (1986) Variation between strains of the nematophagous fungus, *Verticillium chlamydosporium* Goddard. II. Factors affecting parasitism of cyst nematode eggs. *Nematologica* 32: 474–485.

Jackson, C. W., J. B. Heale and R. A. Hall (1985) Traits associated with virulence to the aphid *Macrosiphoniella sanborni* in eighteen isolates of *Verticillium lecanii*. *Ann. Appl. Biol.* 106: 39–48.

Kerry, B. R. (1990) An assessment of progress towards microbial control of plant-parasitic nematodes. *J. Nematol.* 22: 621–631.

Kerry, B. R. (2001) Exploitation of the nematophagous fungus *Verticillium chlamydosporium* Goddard for the biological control of root-knot nematodes (*Meloidogyne* spp.). In *Fungi as Biocontrol Agents* (T. M. Butt, C. Jackson and N. Magan eds.). CABI Publishing, Wallingford, pp. 155–167.

Kim, D. G. and R. D. Riggs (1991) Characteristics and efficacy of a sterile hyphomycete (ARF18), a new biocontrol agent for *Heterodera glycines* and other nematodes. *J. Nematol.* 23: 275–282.

Lalithakumari, D. (2000) Applications of protoplast fusion in filamentous fungi. In *Fungal Protoplast—A Biotechnological Tool* (D. Lalithakumari ed.). Science Publishers, Enfield, pp. 129–155.

Meyer, S. L. F. (1994) Effects of a wild type strain and a mutant strain of the fungus *Verticillium lecanii* on *Meloidogyne incognita* populations in greenhouse studies. *Fundam. Appl. Nematol.* 17: 563–567.

Meyer, S. L. F. and W. P. Wergin (1998) Colonization of soybean cyst nematode females, cysts, and gelatinous matrices by the fungus *Verticillium lecanii*. *J. Nematol.* 30: 436–450.

Meyer, S. L. F., R. N. Huettel and R. M. Sayre (1990) Isolation of fungi from *Heterodera glycines* and *in vitro* bioassays for their antagonism to eggs. *J. Nematol.* 22: 532–537.

Niblack, T. L. (2005) Soybean cyst nematode management reconsidered. *Plant Dis.* 89: 1020–1026.

Pe'er, S. and I. Chet (1990) *Trichoderma* protoplast fusion: a tool for improving biocontrol agents. *Can. J. Microbiol.* 36: 6–9.

Prabavathy, V. R., N. Mathivanan, E. Sagadevan, K. Murugesan and D. Lalithakumari (2006) Self-fusion of protoplasts enhances chitinase production and biocontrol activity in *Trichoderma harzianum*. *Biores. Technol.* 97: 2330–2334.

Riggs, R. D. (1977) Worldwide distribution of soybean cyst nematode and its economic importance. *J. Nematol.* 9: 34–39.

Segers, R., T. M. Butt, B. R. Kerry, A. Beckett and J. F. Peberdy (1996) The role of the proteinase VCP1 produced by the nematophagous *Verticillium chlamydosporium* in the infection process of nematode eggs. *Mycol. Res.* 100: 421–428.

Shinya, R., D. Aiuchi, A. Kushida, M. Tani, K. Kuramochi and M. Koike (2008) Effect of fungal culture filtrates of *Verticillium lecanii* (*Lecanicillium* spp.) hybrid strains on *Heterodera glycines* eggs and juveniles. *J. Invertebr. Pathol.* 97: 291–297.

Triantaphyllou, A. C. and H. Hirschmann (1962) Oogenesis and mode of reproduction in the soybean cyst nematode, *Heterodera glycines*. *Nematologica* 7: 235–241.

Uziel, A. and R. A. Sikora (1992) Use of non-target isolates of the entomopathogen *Verticillium lecanii* (Zimm.) Viegas to control the potato cyst nematode, *Globodera pallida* (Stone). *Nematologica* 38: 123–130.

Wrather, J. A., T. R. Anderson, D. M. Arsyad, Y. Tan, L. D. Ploper, A. Porta-Puglia, H. H. Ram and J. T. Yorinori (2001) Soybean disease loss estimates for the top ten soybean-producing countries in 1998. *Can. J. Plant Pathol.* 23: 115–121.

Yoshihara, T. and K. Kegasawa (1989) A method for the extraction of cyst of soybean cyst nematode from large volumes of wet soil. *Jpn. J. Nematol.* 19: 52–55 (in Japanese).