BioControl (2007) 52:547–558
DOI 10.1007/s10526-006-9052-2

Nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson is also a biological agent for control of greenhouse insects and mite pests

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Received 9 November 2005; accepted in revised form 11 October 2006

Abstract. Pathogenicity of nematophagous fungus *Paecilomyces lilacinus* (Thom) Samson in control of the most destructive greenhouse pests such as: greenhouse whitefly, *Trialeurodes vaporariorum*, glasshouse red spider mite, *Tetranychus urticae*, the cotton aphid, *Aphis gossypii* and western flower thrips, *Frankliniella occidentalis* was examined in laboratory and pot experiments. The fungus showed the greatest efficacy in controlling winged and wingless forms of the cotton aphid. The cotton aphid's population was almost totally eliminated. In controlling the greenhouse whitefly, *P. lilacinus* was most successful when applied against nymphal growth stages (L_3-L_4). Control of the western flower thrips was most efficient against prepupal and pupal stages when the fungus was applied as a water spore suspension to the soil. When the fungus was applied at temperatures below 10 °C, it was able to reduce a glasshouse red spider mite population by 60%.

Key words: biological control, greenhouse pests, *Paecilomyces lilacinus*

Introduction

For many years entomopathogenic fungi have been applied in the biological control of the most severe greenhouse pests, such as aphids, mites, greenhouse whiteflies and western flower thrips. However, their efficacy is dependent upon several abiotic factors and therefore, results are not always satisfactory. In Poland only one bioinsecticide is available based on fungus: *Paecilomyces fumosoroseus*. When applied together with the parasitoid *Encarsia formosa* whitefly populations can be sufficiently decreased (Sosnowska and Piątkowski, 1995, 1996). Currently, biological control agents that are commercially

available are based mainly on entomophagous species (van Lenteren, 2003). Nevertheless, there is a constant search for other types of biological control agents, including nematophagous fungi. Until now, there have been no reports on the use of nematophagous fungi in control of greenhouse arthropods. One of the candidate agents for arthropod control is the fungus *Paecilomyces lilacinus*, which is recommended for control of cyst and root-knot nematodes in greenhouse and the open field condition. *P. lilacinus* is a common soil hyphomycete with a cosmopolitan distribution (Samson, 1974) and has been most widely tested for the control of root-knot and cyst nematodes. This fungus has also been reported as a human pathogen (Takayasu et al., 1977). *P. lilacinusis* used mainly under tropical conditions, e.g., in the Philippines (Jatala, 1986) and the Republic of South Africa (Neethling, 2002). This species is effective against different species of plant pathogenic nematodes, and mainly infects eggs and females (Amancho and Sasser, 1995; Borisov, 1998; Pandey and Trivedi, 1990; Silva et al., 1992; Sosnowska, 2001; Zaki, 1994). Wang et al. (2001) observed some infection of larval stages in East China. There are large differences in pathogenicity among strains of *P. lilacinus* (Rodriguez-Kabana et al., 1984). In Poland, a domestic strain of this fungus species was investigated as a potential biological agent against root-knot nematodes in greenhouses (Sosnowska, 2003). At present, it is the only commercially available fungal formulation to control nematode pests in Europe, and commercial strain 251 is registered for sale in several countries (Atkins et al., 2005). Because it is classified as a microbiopesticide, it needs to go through full toxicological and ecological testing before it can be registered.

The main objective of the research presented in this paper was to evaluate the pathogenicity of the Polish strain Pl of *P. lilacinus* in control of the most common and destructive insect pests occurring in greenhouses, i.e., greenhouse whitefly, *Trialeurodes vaporariorum*; glasshouse red spider mite, *Tetranychus urticae*; the cotton aphid, *Aphis gossypii* and western flower thrips, *Frankliniella occidentalis*.

Materials and methods

Experiments were carried out under greenhouse and laboratory conditions. The fungus *P. lilacinus* was isolated from eggs of the sugar beet cyst nematode, *Heterodera shachtii* collected from fields near Toruń (West of Poland) and maintained in a collection at the Department of

Biological Control and Quarantine, Institute of Plant Protection, Poznan, Poland.

In laboratory tests, 10 individuals of each insect or mite pest species were placed on bean leaves covered with moistened filter paper (POCh S.A., Poland) in Petri dishes (9 cm diam.). These leaves were sprayed with a spore suspension in sterile water at a concentration of 10^6/ml with Triton X-100 (0.05%) (POCh S. A., Poland) until leaves were wet. The dishes were incubated for the duration of the assay at 25 °C in the dark. The spores were collected from a 12-day old fungus culture grown on potato-dextrose-agar medium (PDA, Difco ^{TM}). Spores were harvested by adding 20 ml of sterile distilled water and scraped off with a sterile cell scraper and then homogenized in a glass homogenizer. The concentration of conidial suspension was subsequently adjusted to 10^6 conidia ml^{-1} using a Goriaev haemocytometer. Observations were conducted 2, 5 and 7 days after a treatment and each time the number of live and dead pest individuals were recorded. There was one Petri dish with bean leaves with 10 individual insects or mites per treatment, with five replications. The degree of fungal infection was evaluated under a microscope.

In pot tests conducted in greenhouses of the Institute of Plant Protection, efficacy of the fungal species in controlling pests at different growth stages was investigated. Pots (14-cm in diam.) with bean plants of cv. Zlota Saxa were placed in glass cylinders (30 cm high, 16 cm diam.) covered with netting to maintain a sufficiently high humidity. Next, plants were infested with a particular pest species. Prior to spray treatment five leaves from each plant were detached for evaluation of the population density of the pest arthropod. Plants were sprayed with a spore suspension at a dose of 10^6/ml (with Triton X-100, 0.05%) using a hand sprayer until the leaves were wet. Observations were performed 2, 5 and 7 days after the spray and each time 25 leaves from each treatment were evaluated (5 leaves from each plant, one pot with bean plants infected with pests, 5 plants/treatment with different numbers of pest arthropods, 25 leaves per treatment). At the observations, the number of live and dead individuals was recorded. In populations of *A. gossypii*, mortality of winged and wingless forms was determined, and in populations of *T. vaporariorum*, mortality of eggs and nymphs at L_1-L_2 and L_3-L_4 stages was evaluated. In populations of *T. urticae*, mortality of larvae and adults was recorded.

During laboratory trials the temperature was constant and optimal for fungus development (25 °C). However, trials were conducted in

greenhouses during different months and temperatures varied. During trials, temperatures were recorded at 8 p.m., 12 p.m. and 15 p.m. in the greenhouse using contact thermometers. Trials with *T. urticae* were conducted during May and mean daytime temperatures (±SD) averaged 14.9 °C (±3.7 °C) over the 7 days of the experiment. Trials with *T. vaporariorum* were conducted during June and mean daytime temperatures averaged 22.4 °C (±2.4 °C) over the 7 days of the experiment. Trials with *A. gossypii* were conducted during July and mean daytime temperatures averaged 26.7 °C (±3.1 °C) over the 7 days of the experiment.

Evaluation of the effect on *F. occidentalis* in the laboratory and greenhouse was done by counting insect populations on leaves before treatment and 7 and 14 days after spray and also by sampling the density of prepupal stages on blue sticky traps (5 cm × 5 cm) (Biobest) in pots on the soil surface 7 and 14 days after treatment. The effect of the fungus on *F. occidentalis* was evaluated using spray treatments as described above and, additionally by watering plants with a spore solution of 10^6/ml applied at 20 ml/pot. These treatments were performed jointly or separately. The experimental design was a randomized complete design with five replications. This experiment was conducted in July when greenhouse daytime temperatures during the experiment averaged 27.4 °C (±2.3 °C) over the 14 days of the experiment.

Trials were also carried out on cucumber plants cv. Colonel in a commercial greenhouse in Szamotuly (near Poznan) during July and under normal commercial production conditions. Cucumber plants (1.7 m high) were naturally infested with *F. occidentalis*. Pest populations were assessed by leaf counts from three leaves per plant and 10 plants per row. On 10 randomly selected and marked plants (one plant per replication) and three randomly selected leaves per plant (from the top, middle and bottom of plant) were examined for each sample in the laboratory before treatments were made. The number of live larval and adult thrips was counted before and 7, 14 and 21 days after treatment. The number of thrips were also counted on blue sticky traps (size: 5 cm × 5 cm), which were placed at the soil surface near plants and on the top of plants (size: 10 cm × 25 cm) a week before treatments and 7, 14 and 21 days after treatments. *P. lilacinus* was applied as a suspension of conidia. Spore suspensions were prepared by washing spores grown from cultures on PDA using tap water plus Triton X-100 (0.05%). The concentration of the spore solution was 10^6/ml. Cucumber plants were sprayed to run-off and

special care was taken to cover the leaves. In one treatment spore suspension was applied to the soil (100 ml/plant). Nothing was done to the plants in the control treatment. The following treatments were evaluated: *P. lilacinus* (soil application), *P. lilacinus* (spraying plants) and control (untreated plants).

The collected data were subjected to an analysis of variance with Freeman-Tukey's. Only in the case of *T. urticae* mortality, collected data were analyzed using Student's tests.

Results

For all studies, controls were always statistically different from treatments ($p < 0.05$). For studies with whiteflies, mites and aphids, mortality of controls ranged from 0–6% (data not presented). For thrips studies, where numbers of thrips on plants were counted for each treatment, control data are presented.

In laboratory studies *P. lilacinus* showed high efficacy in control of whitefly nymphs of all stages, especially against L_3-L_4 stages. The fungus caused 84% mortality 7 days after application (Table 1). Significantly lower mortality was recorded for the L_1-L_2 stages (46%) while eggs were infected the least (to 22%). Pot tests carried out under greenhouse conditions also showed the greatest efficacy of the fungus in controlling L_3-L_4 nymphs. Here, the mortality of whiteflies 7 days after fungus application was 72% on bean leaves (Table 1).

Table 1. Comparison of percentage mortality (mean ± sd) of whitefly, *Trialeurodes vaporariorum*, on *Paecilomyces lilacinus* – treated and control bean leaves under laboratory and greenhouse conditions

Treatment	After 2 days	After 5 days	After 7 days
P. lilacinus, Laboratory conditions			
Eggs	12.1 ± 1.8 a	21.0 ± 5.1 a	22.2 ± 3.2 a
L_1-L_2	34.2 ± 7.4 b	40.0 ± 3.2 b	46.1 ± 5.1 b
L_3-L_4	73.1 ± 9.4 c	62.1 ± 4.8 c	84.0 ± 6.2 c
P. lilacinus, Greenhouse conditions			
Eggs	10.0 ± 2.1 a	11.2 ± 1.6 a	14.1 ± 2.1 a
L_1-L_2	26.2 ± 1.8 b	41.4 ± 5.3 b	42.2 ± 5.3 b
L_3-L_4	67.3 ± 6.1 c	68.0 ± 7.4 c	72.0 ± 7.1 c

Within each time interval and type of experimental condition, means with different letters are significantly different at $p < 0.05$ (Tukey's test).

The results revealed that spraying plants with conidia was not sufficient for thrips control compared with soil application. Fourteen days after treatment, we observed double the insects on bean leaves sprayed with fungal spores compared with soil application, although differences were not significantly different. At the same time we found six times more thrips on sticky traps when fungus was applied as a spray treatment comparing with soil application and these results were significantly different (Table 2).

For trials against *F. occidentalis* in the commercial greenhouse, on sticky traps the numbers of thrips increased when plants were sprayed with the fungus (Table 3). In contrast, numbers of *F. occidentalis* significantly decreased after 21 days when the fungus was applied to the soil. Population numbers decreased on the sticky traps on tops of plants, from 2.7 thrips per trap before to 1.8 after fungal application. Sticky traps on the soil demonstrated a much greater change, with numbers decreasing from 3.4 thrips per trap before to 0.6 after fungal application (Table 3).

Table 2. Mean number (mean ± sd) of *Frankliniella occidentalis* on bean leaves and sticky traps after application of *Paecilomyces lilacinus* (greenhouse experiment with plants covered with glass cylinders)

Treatment	Mean number of thrips/leaf			Mean number of thrips/sticky trap	
	Before treatment	After 7 days	After 14 days	After 7 days	After 14 days
P.lilacinus-soil application	2.2 ± 0.2 a	0.4 ± 0.3 bc	0.2 ± 0.2 bc	1.4 ± 0.3 c	0.8 ± 0.3 c
P.lilacinus-spray application	2.2 ± 0.3 a	0.8 ± 0.3 b	0.4 ± 0.4 b	5.4 ± 0.5 b	4.8 ± 0.7 b
P.lilacinus-soil + spray application	2.1 ± 0.3 a	0.2 ± 0.2 c	0.1 ± 0.01 c	1.9 ± 0.1 c	0.9 ± 0.3 c
Control without spraying and watering	2.1 ± 0.2 a	2.3 ± 0.2 a	2.5 ± 0.3 a	8.8 ± 1.1 a	6.8 ± 0.5 a

Means marked by the same letter in each column are not significantly different, $p < 0.05$ (Tukey's test).

Table 3. Mean number (mean ± sd) of *Frankliniella occidentalis* on cucumber plant leaves and sticky traps after application of *Paecilomyces lilacinus* (commercial greenhouse experiment)

Treatment	Mean number of thrips/sticky trap				Mean number of thrips/leaf	
	Top sticky trap		Bottom sticky trap			
	Before treatment	After 21 days	Before treatment	After 21 days	Before treatment	After 21 days
P. lilacinus – soil application	2.7 ± 0.1 a	1.8 ± 0.2 a	3.4 ± 0.6 a	0.6 ± 0.2 b	2.0 ± 0.3 a	0.8 ± 0.1 b
P. lilacinus – spray application	2.2 ± 0.4 a	2.6 ± 0.6 a	2.5 ± 0.2 a	2.7 ± 0.1 a	1.8 ± 0.1 a	2.0 ± 0.2 a
Control (without spraying and watering)	2.1 ± 0.2 a	3.8 ± 0.5 a	2.7 ± 0.4 a	4.2 ± 0.4 a	1.7 ± 0.3 a	3.5 ± 0.5 a

Means marked by the same letter in each column are not significantly different, $p < 0.05$ (Tukey's test).

In tests with the red spider mite, efficacy of spray application of *P. lilacinus* was lower under greenhouse conditions compared to the laboratory conditions. In the greenhouse we observed 60% *T. urticae* mortality while in the laboratory mortality reached 78% (Table 4). However, during this experiment the greenhouse temperature was very low, dropping to 10 °C on one day. We hypothesize that the effi-

Table 4. Mean percentage mortality (mean ± sd) of *Tetranychus urticae* caused by the fungus *Paecilomyces lilacinus* under laboratory and greenhouse conditions

Mortality (%)			
Treatment	After 2 days	After 5 days	After 7 days
Greenhouse	50.2 ± 7.6 a	58.1 ± 6.4 a	60.0 ± 5.1 a
Laboratory	54.0 ± 3.9 a	68.2 ± 4.2 b	78.0 ± 7.3 b

Means followed by the same letter in each column are not significantly different (Student's *t*-test).

Table 5. Mortality (mean ± sd) of *Aphis gossypii* (winged and wingless stages) caused by the fungus *Paecilomyces lilacinus* under laboratory and greenhouse conditions

Mortality (%)			
Treatment	*After 2 days*	*After 5 days*	*After 7 days*
P. lilacinus, Laboratory conditions			
Winged,	23.3 ± 8.2 b	88.2 ± 5.3 a	90.0 ± 4.1 a
Wingless	38.2 ± 4.8 b	92.4 ± 3.1 a	96.0 ± 3.6 a
P. lilacinus, Greenhouse conditions			
Winged	70.0 ± 5.6 a	95.2 ± 3.7 a	100.0 ± 0.0 a
Wingless	80.0 ± 3.6 a	95.0 ± 3.6 a	95.4 ± 2.4 a

With in columns, values followed by the same letter are not significantly different at $p < 0.05$ (Tukey's test).

cacy of this fungus in the greenhouse would have been even better under higher temperatures.

The fungus showed promising results for control of *A. gossypii*. We observed mortality of 90–96% of winged and wingless aphids during laboratory conditions and 95–100% mortality under greenhouse conditions (Table 5).

Discussion

Until now the fungus *P. lilacinus* has rarely been applied in the control of insect pests, although Russian researchers observed significant suppression in population of the tomato leaf-miner, *Liriomyza bryoniae* (Borisov and Ushchekov, 1997) when fungus was applied to the soil. Our results showed that *P. lilacinus* kills L_3-L_4 nymphs of whitefly. This is the first report on efficacy of this fungus in whitefly control in Poland, and one of the first anywhere. In Turkey Gökce and Er (2005) reported that *P. lilacinus* was pathogenic to glasshouse whitefly nymphs, but mortality of second instar nymphs only reached 10% 6 days after application of the fungus in conidia concentration 10^7 ml^{-1}.

Until now no fungus bioproduct has been registered in Poland to control *T. urticae*. However, there are products based on the fungi *P. fumosoroseus*, *Beauveria bassiana* and *Lecanicillium lecanii* that are commercially available in Europe (Sosnowska, 2005). The red spider mite is one of the most serious pests of vegetables. Chemical control is very difficult due to mite resistance to numerous insecticides and acaracides. In our experiments the nematophagous fungus *P. lilacinus*

was effective against this pest even at low temperature. It is known that this strain starts growing at a temperature of 10 °C and is the most efficient in control of root-knot nematode at 15 °C (Sosnowska, 2003). This feature makes it possible to apply the fungus at low temperatures.

Numerous parasitoids, predators, and different fungal species attack aphids. However, entomophthoralean fungi are the most frequently isolated species from aphid colonies. They often cause epizootics (Balazy, 2000). Due to technical difficulties with mass rearing of these species there is no bioproduct available yet. Using the natural enemies of aphids like the parasitic wasp *Aphidius colemani* and predator *Aphidoletes aphidimyza* provides the best results in control of the cotton aphid in greenhouse production present. Our results showed that *P. lilacinus* could also be recommended for control of aphids.

Two thrips species occur regularly in greenhouses i.e., *Thrips tabaci* and *F. occidentalis*, the second species occurring most frequently. Successful control of thrips is very difficult to achieve due to its life cycle where some stages are not available to the insecticides. Thrips can develop in flowers, lay eggs under leaf epidermis and prepupal and pupal stages develop in soil. There are several fungal pathogens that reduce thrips populations under natural conditions, but they cannot infest all stages, in particular stages living in the soil. Commercial bioproducts available for the thrips control are based on *L. lecanii* and *B. bassiana* (Benuzzi and Santopolo, 2001; Goodwin et al., 2002; Jacobson et al., 2001), and *Metarhizium anisopliae* (Azaizeh et al., 2002). Because of its activity in the soil, *P. lilacinus* may support biological control of other bioproducts. The water application at a dose of 10^6/ml spores of *P. lilacinus* to the soil resulted in satisfactory control of the thrips. The same dose used in a commercial greenhouse with cucumbers sufficiently reduced soil stages of thrips. This fungus is already recommended for the control of nematodes in the soil, so such soil treatments might result in control of other pests such as western flower thrips. The results from combined spray and soil treatments did not differ from those of the soil treatment alone, thus one can conclude that the spray application was not effective.

Microbiological control of arthropod pests using entomopathogenic fungi is not a new idea. But the use of a microbial control agent that is already recommended for nematodes and is now also proposed for arthropod control is a new idea. Serman and Smiths (2000) used *L. lecanii* for control of *F. occidentalis* and they observed that foliage stages of thrips were more susceptible to the fungus than soil stages.

Similar results were obtained when *B. bassiana* was applied (Shipp et al., 2002). There is no information in the literature about using *P. lilacinus* against *F. occidenatlis*.

In practice, entomopathogenic fungi are mostly used in control of insect pests of greenhouse crops because environmental factors such as temperature and humidity are optimal for their development and efficacy. However, application of entomophagous and entomopathogenic nematodes is still the most common biological control method. Chemical products are applied in cases of failure of the biological methods or their insufficient effect. According to the Polish Act of Plant Protection issued on December 18th 2003, biological methods should be used prior to any application of chemical products. Thus, biological control is a priority in plant protection, and *P. lilacinus* seems to be an excellent candidate to be used in greenhouse biocontrol programs, together with parasitoids and predators.

It is known that *P. lilacinus* can be a human pathogen (Takayasu et al., 1977), but there is a large differentiation in host infection between strains of this fungus. Several strains are registered as a biopesticides against plant parasitic nematodes. No adverse effects on humans, animals, beneficial organisms or on the ecosystem have been reported so far (www.biocontrol.co.za/pl; www.prophyta.com), but we advise particular care when handling the fungus.

Acknowledgement

The authors want to thank Prof. J.C. van Lenteren for his comments about the manuscript and for language correction.

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