

Pathogenicity of *Paecilomyces* spp. to the Glasshouse Whitefly, *Trialeurodes vaporariorum*, with Some Observations on the Fungal Infection Process

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Abstract: This study was carried out to determine the infection rates of 9 *Paecilomyces fumosoroseus* isolates and 1 *Paecilomyces lilacinus* isolate on the glasshouse whitefly (GWF), *Trialeurodes vaporariorum*. Furthermore, the infection progress of *P. fumosoroseus* was observed on the insect using light and scanning electron microscopes. Bioassays were carried out on second instar nymphs by the application of a spore suspension of each isolate. The mortality data were recorded 3 and 6 days after the application. The effect of *Paecilomyces* isolates increased as the incubation time was extended. Seven isolates caused significant mortality to the GWF nymphs. The most virulent isolates, 2658, 4400, 4406, 4408 and 4415, killed over 70% of the inoculated nymphs 6 days after inoculation. The development of infection was monitored on artificially infected nymphs at 12-h intervals. Infection by the fungus on the GWF was similar to those reported for other fungus-insect cases. The results of this study showed that the fungus was pathogenic to *T. vaporariorum*, rapidly killing its host, and thus further studies are required to determine the potential use of the fungus as a biocontrol agent against GWF in greenhouses.

Key Words: *Paecilomyces fumosoroseus*, *Paecilomyces lilacinus*, Entomopathogenic fungus, *Trialeurodes vaporariorum*, Glasshouse whitefly, biological control.

Paecilomyces spp.'nin Sera Beyazsineğine, *Trialeurodes vaporariorum*, Patojenitesi ve Fungal Enfeksiyon Gelişimi Üzerine Bazı Gözlemler

Özet: Bu çalışma dokuz *Paecilomyces fumosoroseus* ile bir *Paecilomyces lilacinus* izolatının sera beyazsineği (*Trialeurodes vaporariorum*)'ni enfekte etme oranlarını belirlemek için yürütülmüştür. Ayrıca, fungal etmen *P. fumosoroseus*'un *T. vaporariorum* üzerinde gerçekleştirdiği enfeksiyon aşamaları ışık ve elektron mikroskopları kullanılarak gözlemlenmiştir. Biyotestler 2. dönem nimfler üzerine fungal spor süspansiyonu inokule edilerek yürütülmüştür. Spor süspansiyonunun inokule edilmesini takip eden 3. ve 6. günlerde ölümler kaydedilmiştir. Bütün *Paecilomyces* izolatlarının etkinlikleri inkübasyon süresi uzatıldıkça artmıştır. Test edilen izolatlardan 7 tanesi sera beyazsineği nimfleri üzerinde önemli oranda ölümlere neden olmuştur. İzolatlar arasında en yüksek virulens gösteren 2658, 4400, 4406, 4408 ve 4415 nolu izolatlar inokulasyondan altı gün sonra % 70'i aşan ölümlere neden olmuştur. Enfeksiyonun gelişim aşamaları suni olarak enfekte edilmiş nimfler üzerinde 12 saat aralıklar ile takip edilmiştir. Fungusun sera beyazsineğini enfekte etme aşamaları diğer böceklerde funguslar tarafından gerçekleştirilen enfeksiyonlarla benzerlik göstermektedir. Çalışmanın sonuçları fungusun konukçusunu kısa sürede öldürdüğünü, sera beyazsineğine karşı patojenik olduğunu ve bu nedenle sera beyazsineğinin seralarda mücadelesinde biyokontrol etmeni olarak bu fungus üzerinde daha fazla çalışmanın gerekliliğini göstermiştir.

Anahtar Sözcükler: *Paecilomyces fumosoroseus*, *Paecilomyces lilacinus*, entomopatojenik fungus, *Trialeurodes vaporariorum*, sera beyazsineği, biyolojik mücadele.

Introduction

The glasshouse whitefly (GWF), *Trialeurodes vaporariorum* (Westwood) (Aleyrodidae: Homoptera), is

an important polyphagous pest of various crops (Vet et al., 1980). They cause damage to plants by sucking the phloem sap, encouraging the growth of sooty molds on

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leaves and transmitting some plant viruses (Coffin and Coutts, 1995; Guzman et al., 1997). The extensive use of pesticides against potential pests has resulted in the appearance of pesticide resistance in many glasshouse pests, including *T. vaporariorum*, and phytotoxicity and pesticide residue problems on vegetables (Quinlan, 1988). The demand for a reduction in pesticide use and the requirement for residue-free food have resulted in an increasing interest in biological control systems for use in glasshouses.

Fungi seem to be the only entomopathogens that may be used to control GWF because they are able to penetrate their hosts, while other pathogens must be ingested. Fransen (1990) and Lacey et al. (1996) reported the use of entomopathogenic fungi against whiteflies. Most of fungal pathogens of GWF belong to the genera *Aschersonia*, *Beauveria*, *Verticillium* and *Paecilomyces*.

Paecilomyces fumosoroseus (Wize) Brown & Smith (Moniliales: Moniliaceae) has been isolated from most regions of the world and has been reported to infect several insects belonging to many different orders (Smith, 1993; Sterk et al., 1996). *P. fumosoroseus* was reported to be one of the most common fungi attacking nymph and adult *Bemisia* in fields and glasshouses and *T. vaporariorum* in glasshouses (Humber, 1992; Lacey et al., 1996). Several isolates of the fungus were screened against whiteflies and successful isolates were tested in the field and glasshouses (Smith, 1996; Vidal et al., 1997; Wraight et al., 2000; Gökçe and Er, 2005). *Paecilomyces lilacinus* (Thom) Samson (Moniliales: Moniliaceae) is a typical soil-borne fungus that appears to be common in the tropics and subtropics (Saxena et al., 1991). Although the fungus has been isolated from many insects, in recent years it has been mainly tested as a biological control agent for soil nematodes (Saxena et al., 1991; Humber, 1992).

In our study, the infection rates of 9 isolates of *P. fumosoroseus*, and 1 isolate of *P. lilacinus* were examined against GWF to assess the pathogenic potential of each isolate of the fungus and to identify the most effective isolate for possible use as a biological control agent. Additionally, the infection progress of *P. fumosoroseus* isolate 4415 was observed on GWF nymphs using scanning electron and light microscopes.

Materials and Methods

Insect culture

GWF was reared on tomato, *Lycopersicon esculentum* Mill., cv. Moneymaker, throughout this research. Plants with 4 to 6 partially expanded leaflets were used for producing the required insect instars. Before commencing the transfer of GWF, each tomato leaflet was covered with a mesh bag. About 40 adults, randomly selected from a single population, were transferred into each bag. They were then allowed to lay eggs on the leaflet for 24 h at 24 ± 1 °C under 16 h light / 8 h dark 13 days prior to the experiment and this time interval resulted in second instar nymphs at the time of fungal inoculation.

Preparation of spore suspension

Detailed information about the origin of the fungal isolates is presented in Table 1. The fungal isolates were routinely grown on Sabouraud dextrose yeast agar (Sigma) at 24 ± 1 °C under a 16-h photoperiod. Conidia were harvested from 3-week-old cultures by adding 10 ml of sterile distilled water supplemented with 0.02% Tween 80. The conidial suspension was filtered through 2 layers of sterile muslin into a sterile 25 ml plastic universal bottle (Sterilin) and then shaken for 5 min using a horizontal shaker with 40 mm horizontal movement and 300 oscillations per min. The concentration of conidial suspension was subsequently adjusted to 10^7 conidia ml⁻¹ using a Neubauer haemocytometer. The viability of the conidia of each isolate was tested by spraying 2 ml of conidia suspension containing 10^7 conidia ml⁻¹ onto tap water agar. An examination of these cultures after 24 h showed that about 99% of the conidia were viable.

Inoculation and incubation

For each treatment 25 ± 5 second instar nymphs were used on a tomato leaflet for each isolate. The conidia suspensions were reshaken for 5 min and 2 ml of conidia suspensions containing 10^7 conidia ml⁻¹ was applied to the underside of each leaflet using a Potter spray tower with fine droplet spray nozzle (Potter, 1952). This procedure resulted in the deposition of approximately 38 ± 2 ($n = 30$) spores mm². A control leaflet was treated with 2 ml of sterile distilled water (SDW) containing 0.02% Tween 80.

A randomised complete block design was used in this study. Nine *P. fumosoroseus* isolates, *P. lilacinus* and

Table 1. The geographical origin, insect hosts and host plants of *Paecilomyces fumosoroseus* and *Paecilomyces lilacinus* isolates obtained from ARSEF.

ARSEF CULTURE NUMBER	SPECIES	GEOGRAPHICAL LOCATION	INSECT HOST	HOST PLANT
2658	<i>P. fumosoroseus</i>	Florida/USA	<i>T. vaporariorum</i>	Not specified
3458	<i>P. fumosoroseus</i>	Not specified	<i>Myzus persicae</i>	<i>Jasmine</i> spp.
4205	<i>P. fumosoroseus</i>	Kuala Terla/Malaysia	<i>T. vaporariorum</i>	<i>Sonchus</i> spp.
4400	<i>P. fumosoroseus</i>	Kuala Terla/Malaysia	<i>T. vaporariorum</i>	<i>Solanum melongena</i>
4401	<i>P. fumosoroseus</i>	Kuala Terla/Malaysia	<i>T. vaporariorum</i>	<i>S. melongena</i>
4406	<i>P. fumosoroseus</i>	Kuala Terla/Malaysia	<i>T. vaporariorum</i>	<i>Sonchus</i> spp.
4408	<i>P. fumosoroseus</i>	Kuala Terla/Malaysia	<i>T. vaporariorum</i>	<i>Sonchus</i> spp.
4411	<i>P. fumosoroseus</i>	Kuala Terla/Malaysia	<i>T. vaporariorum</i>	<i>Sonchus</i> spp.
4415	<i>P. fumosoroseus</i>	Kuala Terla/Malaysia	<i>T. vaporariorum</i>	<i>Sonchus</i> spp.
3846	<i>P. lilacinus</i>	Rampur/India	<i>T. vaporariorum</i>	<i>Sonchus</i> spp.

SDW were sprayed on whitefly infested tomato plant leaflets on the same day. The experiment was repeated 4 times.

After treatment, the leaflets were left to dry at room temperature for about 5 min. The cut end of the leaflet petiole was covered with a 30 x 55 mm piece of sterile cotton wool moistened with 2 ml of water containing 1% NPK (20-20-20) fertilizer. This treatment ensured that the excised leaflet remained green and turgid for at least 7 days. Sterile 140 mm plastic petri dishes were lined with three 90 mm No. 1 Whatman filter papers wetted with 3 ml of SDW to create a near 100% relative humidity. A spacer, made from a solid plastic rod with a 7 mm cross section welded to form a ring 65 mm in diameter, was placed on the filter papers to ensure the leaflet did not come into contact with the wet surface. The dish was incubated in an incubator at 24 ± 1 °C set for a 16-h photoperiod. Following incubation for 24 h, a 45 mm diameter hole was cut in the centre of the lid of the plastic petri dish and the Whatman filter papers were removed to adjust the relative humidity to about 70%. The petri dishes were transferred into a sealed perspex chamber. The chamber was maintained at $70 \pm 10\%$ relative humidity using KOH solution, and was kept at 24 ± 1 °C with 16-h photoperiod (Solomon, 1951).

Whole leaflets were used in these experiments and the distribution of GWF eggs was generally found to be relatively uniform over the leaf surfaces. Second instar nymphs on each tomato leaflet were mapped on paper to

monitor the development of mycosis on each individual insect. Insects that were either covered by dense hyphal growth or whose colour changed from transparent greenish to opaque white or light yellow were considered dead. The mortality was recorded 3 and 6 days after inoculation using a dissection microscope at 4 x – 64 x magnification.

Infection progress

Conidial suspension preparation, inoculation and incubation of tomato leaflets were carried out using the procedure above with *P. fumosoroseus* isolate 4415. After 12, 24, 36, 48, 60 and 72 h of inoculation, 3-5 samples were taken from the treated leaves. Each sample comprised around a 4 mm² small piece of leaf bearing second instar GWF nymphs. The samples were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 5 ± 1 °C for 24 h and then dehydrated by passing them through an ethanol series (10%, 25%, 50%, 75%, 80%, 90% and 100% concentrations). The samples were kept for about 10 min in each concentration. The samples were then dried in a Samdri 780 apparatus (Tousimis Research Corp., USA) and 4 samples were mounted on a scanning electron microscope stub using conductive double-sided carbon tape (Cellotape®). The samples were sputter coated with gold palladium. The progress of fungal infection of *P. fumosoroseus* isolate 4415 on nymphs was then examined using a scanning electron microscope (Cambridge S240, Leo Ltd, UK).

In addition to the SEM investigation, samples were prepared for light microscopy. At 12, 24, 36, 48, 60 and

72 h after inoculation, 3-5 second instar nymphs were taken from the treated leaflets and transferred into water on a slide. A coverslip was placed over the nymphs and pressed until all the contents of the nymph body cavity were expelled. The samples were then examined using an Olympus BH-2 (Olympus, Tokyo Japan) phase-contrast light microscope at 400 x magnification.

Data analysis

Mortality data were normalised using arcsine transformation (Anscombe transformation) (Zar, 1999). After this, the effect of incubation time was analysed using the one-tailed paired-sample t-test ($P = 0.05$). Transformed mortality data at day 3 and day 6 were analysed separately using analysis of variance (ANOVA) ($P = 0.05$) and Tukey test ($P=0.05$). All statistical analyses were carried out using SPSS software version 10.1 (Norusis, 1993).

Results

Screening of *Paecilomyces* isolates

Although the fungal conidia suspension was applied to a specific instar of GWF nymphs, due to the length of the incubation period some insects had metamorphosed into the following stage before they died. It was often difficult to determine the precise developmental instar of cadavers when they were covered with dense hyphae and thus it was decided to present the data with respect to the instar of development when the conidia suspension was applied,

rather than to the one they had reached when they died.

Most of the *Paecilomyces* isolates caused a significant increase in mortality between day 3 and day 6, but a dramatic increase was recorded with *Pfr*4408, whose effectiveness increased nearly 4-fold over this period (Figure 1). *Pfr*4401, *Pfr*3458 and *P. lilacinus* were the least virulent fungal isolates among those tested. The mortality induced by the fungi after 3 days of incubation varied from 3.3% (*P. lilacinus*) to 51.4% (*Pfr*4406) and only mortality caused by isolates 2658, 4400, 4406, 4408 and 4415 was significantly higher than the control ($F = 36.36$, $df = 10, 30$, $P < 0.005$). Isolates 2658, 4400, 4406 and 4415 were not significantly different from each another, but isolate 4408 presented significant differences from this group. After 6 days of incubation, mortality varied significantly among the tested isolates, and 5 isolates (2658, 4400, 4406, 4408 and 4415) killed significant number of second stage nymphs of GWF ($F = 40.98$, $df = 10, 33$, $P < 0.000$). Among the tested isolates, *P. fumosoroseus* isolate 4406 caused 82.3% mortality 6 days after inoculation, while only 7.3% were killed by *P. fumosoroseus* isolate 4401. The most successful isolates, 2658, 4400, 4406, 4408 and 4415, were ultimately able to kill over 70% of the initial GWF population 6 days after inoculation (Figure 1).

Isolates 4415 and 4406 showed extensive sporulation on cadavers of GWF after 6 days of incubation. Other effective isolates, however, sporulated poorly. *P. lilacinus* appeared to sporulate better than some of the *P.*

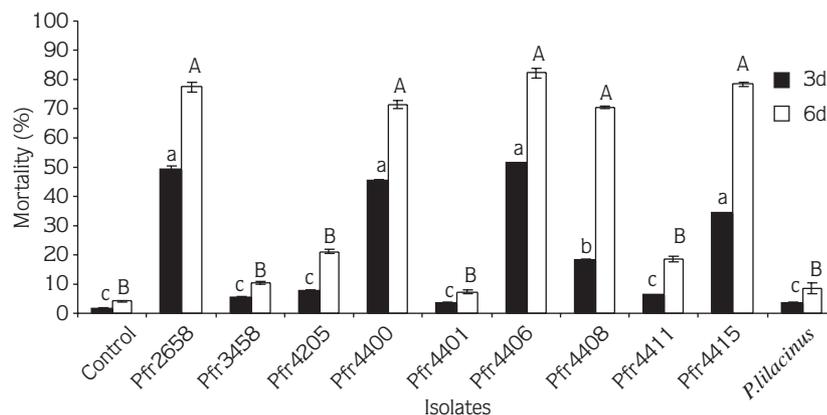


Figure 1. Mortality (mean \pm SEM) of second instar *Trialeurodes vaporariorum* nymphs caused by *Paecilomyces fumosoroseus* isolates and *Paecilomyces lilacinus* as assessed after 3 days and 6 days at 24 °C. Bars with different letters at each time interval are significantly different according to the Tukey test ($P < 0.05$).

fumosoroseus isolates on the dead GWF nymphs. All isolates of *P. fumosoroseus*, except for 2658, showed a high degree of uniformity in that there were only small variations between their effectiveness levels on replicate leaves (Figure 1).

Infection progress

In visual observations, the first morphological change in GWF nymphs infected by *P. fumosoroseus* was a colour change from transparent greenish to opaque white or light yellow due to mycelial development of fungus in the infected nymphs. This was followed by the appearance of several aerial fungal hyphae that soon became much more abundant as infection progressed. Finally, the nymphs turned milky white or yellow and sporulation of the fungus was seen on the cadavers.

The examination of infected nymphs at various time intervals after inoculation with the fungus allowed the tracing of different stages of the infection progress on and in the host body. The electron microscopic examinations revealed that the conidia of *P. fumosoroseus* adhered to the cuticle, germinated and produced germ tubes regardless of where they had adhered (Figure 2). Germinated conidia produced appressorium in 2 distinct manners; they formed near the conidium (Figure 3), or the conidium produced a long germ tube at the end of which the appressorium was

formed (Figure. 3). Although the conidia produced appressoria all over the insect, their formation appeared to be concentrated on the intersegmental areas of the abdomen (Figure 4). The conidia on the head or thorax generally produced the second type of appressoria. These were possibly the result of the cuticle flexibility on different parts of the nymph. The head and thorax are more rigid than the intersegmental areas on the abdomen. After the penetration, the fungus was observed under a light microscope to propagate inside the host's haemocoel as blastospores (Figure 5). At this time the colour of the infected insects began to change from greenish yellow to milky yellow. This was possibly due to hyphal growth, which totally colonized the inside of the host. Finally, the mycelium of the fungus appeared to cover the whole insect cuticle. If the infected nymphs were kept at high humidity, fungal mycelial development continued until the nymphs were entirely covered.

Discussion

Mapping all the insects on every leaflet allowed us to monitor the same insects repeatedly over time and thus to record the progress of infection in the population. The incubation procedure employed for maintaining the cut leaflets proved to be relatively effective in that the leaf material began to show signs of senescence from the 7th

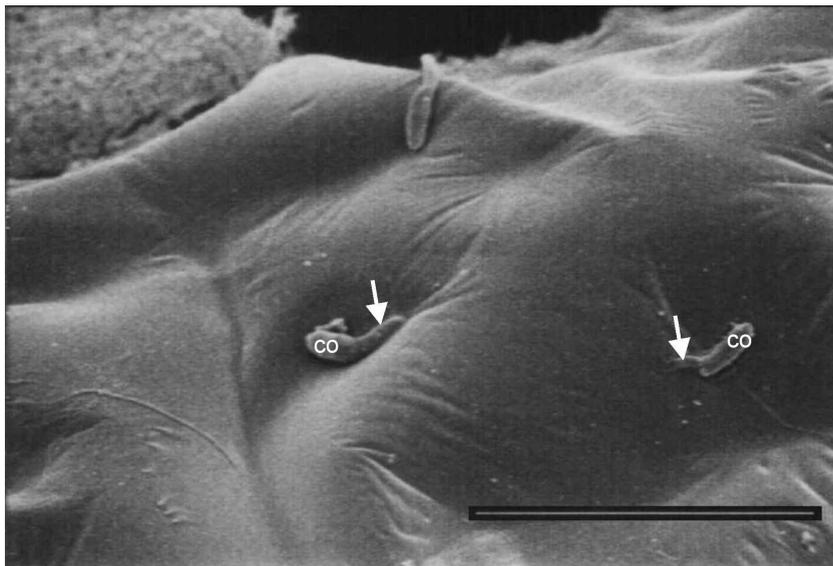


Figure 2. Conidia of *Paecilomyces fumosoroseus* on a second instar nymph at 24 °C and 100% RH after 24 h. Conidia were seen to swell and one had begun to germinate (scale: 20 µm).

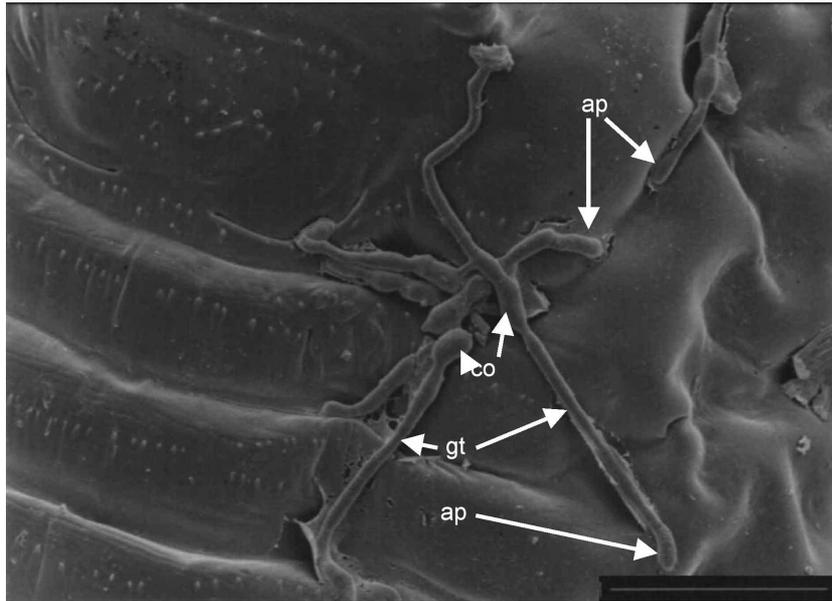


Figure 3. Appressoria forming at the end of long germ tubes on the intersegmental area of a second instar *Trialeurodes vaporariorum* nymph 36 h after inoculation with *Paecilomyces fumosoroseus*; co: conidium, gt: germ tube, ap: appressorium (scale: 20 μ m).

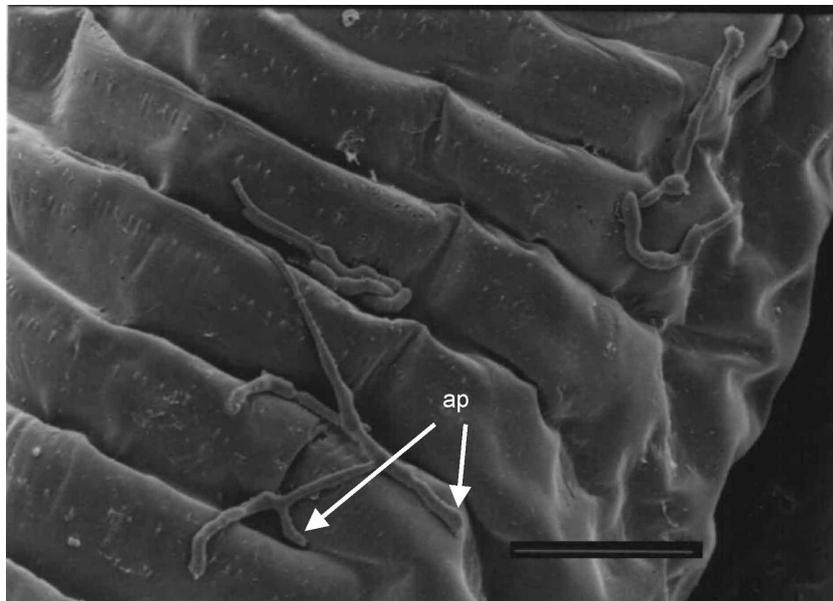


Figure 4. Appressorium formation concentrated on the intersegmental areas of the abdomen of a second instar *Trialeurodes vaporariorum* nymph 36 h after inoculation with *Paecilomyces fumosoroseus*; ap: appressorium (scale: 20 μ m).

day onwards. This suggested that insects were unlikely to have been stressed during the 6 days of incubation.

These experiments showed that all the *P. fumosoroseus* isolates tested killed second stage nymphs of the GWF used here. In addition, one culture of *P.*

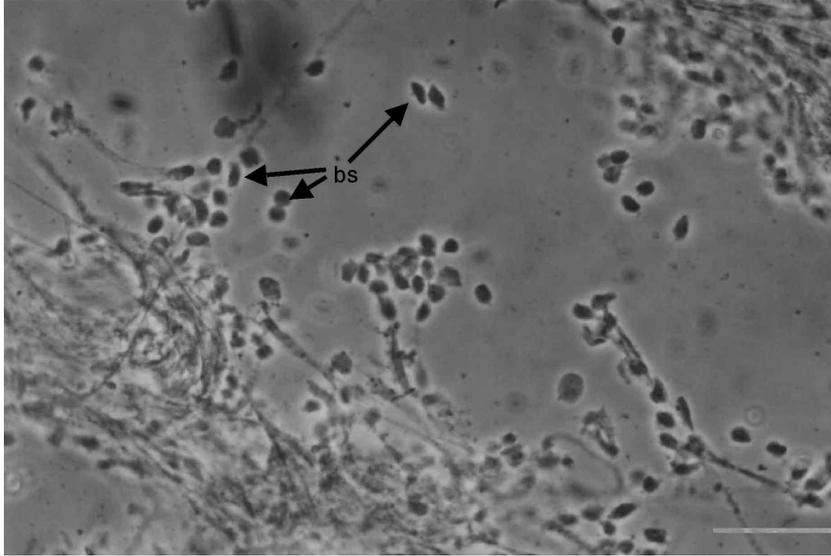


Figure 5. Blastospore formation by *Paecilomyces fumosoroseus* in a second stage *Trialeurodes vaporariorum* nymph 48 h after inoculation; bs: blastospore (scale: 50 μ m).

lilacinus was also shown to be pathogenic to GWF nymphs. This study thus confirms and extends previous work showing that these fungi were entomopathogenic against whiteflies (Bolckmans et al., 1995; Sterk et al., 1996; Vidal et al., 1997; Gökçe and Er, 2005).

A relatively long period of incubation is needed to achieve full effectiveness of most of the tested fungal isolates for killing the GWF. Although there was some indication that the fungi affected the GWF nymphs after 3 days of incubation, the number of dead nymphs continued to increase until the 6th day after inoculation. These results suggest that field and laboratory experiments should employ a similarly long incubation period before assessing their outcomes. These results would therefore validate work done on the same fungus by Wraight et al. (2000), who allowed 8 days to elapse before recording the mortality of *B. argentifolii*.

The screening experiment using second stage nymphs revealed that there was strong intraspecific variation between the isolates tested. There was also a clear difference between the 2 species of *Paecilomyces* examined. Interspecific and intraspecific variation in entomopathogenic fungi has been previously observed in different insects (Hayden et al., 1992) and similar variations have been reported in related Hyphomycetes parasitising other hosts (Ferron et al., 1991). The

existence of such variation in *Paecilomyces* species was reported by Vandenberg (1996), who found that the pathogenicity of 14 *P. fumosoroseus* isolates towards wheat aphids varied 12-fold, and by Wraight et al. (1998), who reported that 22 isolates of this fungus tested against silverleaf whitefly had LC_{50} values that varied 8-fold. Amongst the fungi tested in the present study, *P. fumosoroseus* isolates 2658, 4400, 4406, 4408 and 4415 were promising for GWF control on tomato in greenhouses, and thus these isolates demand further investigation if they will be considered as biopesticides against GWF in greenhouses.

The infection progress of *P. fumosoroseus* on the second instar GWF nymphs was similar to that recorded for other entomopathogenic fungi (Fransen, 1990). However, this study provided evidence of appressorium production of *P. fumosoroseus* on hosts.

P. fumosoroseus conidia were observed to produce appressoria on the second instar nymph integument. This contrasted with the findings of Altre and Vandenberg (2001), who reported no formation of appressoria on *Plutella xylostella*. Formations of appressoria were observed to be concentrated on intersegmental areas. Similar appressorial formations on intersegmental areas by other entomopathogenic fungi were reported by Charnley (1989). McCauley et al. (1968) reported that

M. anisopliae penetrated only thinner intersegmental regions of larvae of *Limonius californicus*, *Hypolithus bicolor*, *Ctenicera aeripennis* and *Ctenicera destructor*. Appressoria production on intersegmental areas could result from differences in the thickness of the insect's cuticle (Boucias and Pendland, 1991). The entomopathogenic fungi penetrate their hosts either using penetration pegs from appressoria or occasionally by direct hyphal penetration (Vestergaard et al., 1999; Altre and Vandenberg, 2001). *P. fumosoroseus* probably used penetration pegs developed beneath the appressoria to penetrate into its host cuticle similar to the production of appressorial pegs from the germ tube of germinated *Paecilomyces farinosus* conidia as reported by Madelin et al. (1967). They found that the fungus produced penetration pegs on *Tenebrio molitor* larvae 48 h after inoculation.

The present work provided a basis for comparing the variation between different isolates of *P. fumosoroseus* screened on second instar nymphs of GWF. The results indicated that the fungus was pathogenic to GWF and it

rapidly killed its host. Therefore, it has the potential to control the pest on glasshouse grown tomatoes. However, dose-mortality bioassays of promising fungal isolates against different development instars of GWF are necessary to differentiate between isolates and define those best suited for further development. In addition, the effects of environmental factors, temperature and humidity, should also be tested as factors that might limit to the practical use of some isolates as microbial control agents.

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References

- Altre, J.A. and J.D. Vandenberg. 2001. Penetration of cuticle and proliferation in hemolymph by *Paecilomyces fumosoroseus* isolates that differ in virulence against lepidopteran larvae. *J. Invertebr. Pathol.* 78: 81-86.
- Bolckmans, K., G. Sterk, J. Eyal, B. Sels and W. Stepman. 1995. PreFeRal (*Paecilomyces fumosoroseus* strain Apopka 97), A new microbial insecticide for the biological control of whiteflies in greenhouses. *Meded.Fac.Landbouwwet. Rijksuniv. Gent* 60: 707-711.
- Boucias, D.G. and J.C. Pendland. 1991. Attachment of mycopathogens to cuticle. In: *The Fungal Spore and Disease Initiation in Plants and Animals* (Eds.: G.T. Cole and H.C. Hoch). Plenum Press, New York. pp. 101-127.
- Charnley, A.K. 1989. Mechanisms of fungal pathogenesis in insects. In: *Biotechnology of Fungi for Improving Plant Growth* (Eds.: J.W. Whipps and R.D. Lumsden). Cambridge University Press, Cambridge, UK. pp. 85-125.
- Coffin, R.S. and R.H. A. Coutts. 1995. Relationships among *Trialeurodes vaporariorum*-transmitted yellowing viruses from Europe and North America. *J. Phytopathol.* 143: 375-380.
- Ferron, P., J. Fargues and G. Riba. 1991. Fungi as microbial insecticides against pests. In: *Handbook of Applied Mycology* (Eds.: D.K. Arora, L. Ajello and K.G. Mukerji). Marcel Dekker Inc, New York, USA. pp. 665-706.
- Fransen, J.J. 1990. Natural enemies of whiteflies:fungi. In: *Whiteflies: Their Bionomics, Pest Status and Management* (Ed.: D. Gerling). Intercept Ltd., Andover, UK. pp. 187-210.
- Gökçe, A. and M.K. Er. 2005. Virulence of *Paecilomyces fumosoroseus* and *Paecilomyces lilacinus* on *Trialeurodes vaporariorum*. *IOBC/WPRS Bull.* (In Press)
- Guzman, P., C.R. Arredondo, D. Emmatty and R.L. Gilbertson. 1997. Partial characterization of two whitefly-transmitted geminiviruses infecting tomatoes in Venezuela. *Plant Dis.* 81: 312.
- Hayden, T.P., M.J. Biodockha and G.G. Khachatourians. 1992. Entomopathogenicity of several fungi toward the English grain aphid (Homoptera: Aphididae) and enhancement of virulence with host passage of *Paecilomyces farinosus*. *J. Econ. Entomol.* 85: 158-164.
- Humber, R.A. 1992. Collection of entomopathogenic fungal cultures, USDA. Ithaca, New York, USA.
- Lacey, L.A., J.J. Fransen and R. Carruthers. 1996. Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. In: *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management* (Eds.: D. Gerling and R.T. Mayor). Intercept, Andover, UK. pp. 401-433.
- Madelin, M.F., R.K. Robinson and R.J. Williams. 1967. Appressorium-like structures in insect-parasitising deuteromycetes. *J. Invertebr. Pathol.* 9: 404-412.

- McCauley, V.J.E., R.Y. Zacharuk and R.D. Tinline. 1968. Histopathology of green muscardine in larvae of four species of Elateridae. *J. Invertebr. Pathol.* 12: 444-459.
- Norusis, M.T. 1993. SPSS for Windows; Base system user's guide. SPSS Inc., Chicago, Illinois, USA.
- Potter, C. 1952. An improved laboratory apparatus for applying direct sprays and surface films with data on the electrostatic charge on atomised spray fluids. *Ann. Appl. Biol.* 39: 1-28.
- Quinlan, R.J. 1988. Use of fungi to control insects in glasshouses. In: *Fungi in Biological Control Systems* (Ed.: M.N. Burge). Manchester University Press, Manchester, UK. pp. 19-36.
- Saxena, G., N. Mittal, K.G. Mukerji and D.K. Arora. 1991. Nematophagous fungi in the biological control of nematodes. In: *Handbook of Applied Mycology* (Eds.: D.K. Arora, L. Ajello and K.G. Mukerji). Marcel Dekker Inc, New York. pp 707-733.
- Smith, P. 1993. Control of *Bemisia tabaci* and the potential of *Paecilomyces fumosoroseus* as a biopesticide. *Biocontrol News & Information* 14: 71-78.
- Smith, P. 1996. Development of *Paecilomyces fumosoroseus* (Hyphomycete: Deuteromycotina) Conidia for Control of *Bemisia tabaci* (Homoptera: Aleyrodidae). PhD thesis. Department of Pure and Applied Biology, University of London, UK. 201 pp.
- Solomon, M.E. 1951. Control of humidity with potassium hydroxide, sulphuric acid, or other solutions. *B. Entomol. Res.* 42: 543-554.
- Sterk, G., K. Bolckmans and J. Eyal. 1996. A new microbial insecticide, *Paecilomyces fumosoroseus* strain Apopka 97, for the control of the greenhouse whitefly. In: *Proceedings of Brighton Crop Protection Conference: Pests and Disease.* 18-21 November 1996, Brighton, UK., pp. 461-466.
- Vandenberg, D.J. 1996. Standardised bioassay and screening of *Beauveria bassiana* and *Paecilomyces fumosoroseus* against the Russian wheat aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 89: 1418-1423.
- Vestergaard, S., T.M. Butt, J. Bresciani, A.T. Gillespie and J. Eilenberg. 1999. Light and electron microscopy studies of the infection of the western flower thrips, *Frankliniella occidentalis* by the entomopathogenic fungus *Metarhizium anisopliae*. *J. Invertebr. Pathol.* 73: 25-33.
- Vet, L.E.M., J.V. van Lenteren and J. Woets. 1980. The parasite-host relationship between *Encarsia formosa* and *Trialetrodes vaporariorum* IX. A review of the biological control of the greenhouse whitefly with suggestion for future research. *Z. Angew. Entomol.* 90: 26-51.
- Vidal, C., L.A. Lacey and J. Fargues. 1997. Pathogenicity of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) against *Bemisia argentifolii* (Homoptera: Aleyrodidae) with a description of a bioassay method. *J. Econ. Entomol.* 90: 765-772.
- Wraight, S.P., R.I. Carruthers, C.A. Bradley, S.T. Jaronski, L.A. Lacey, P. Wood and S. Galaini-Wraight. 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. *J. Invertebr. Pathol.* 71: 217-226.
- Wraight, S.P., R.I. Carruthers, S.T. Jaronski, C.A. Bradley, C.J. Garza and S. Galani-Wraight. 2000. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly, *Bemisia argentifolii*. *Biol. Control* 17: 203-217.
- Zar, J.H. 1999. *Biostatistical Analysis*. Prentice-Hall Inc., New Jersey, USA.