Purpureocillium lilacinum 251
Microbial pest control agent against plant parasitic nematodes

Dossier according to OECD dossier guidance for microbial agents and microbial pest control products, August 2006

Summary documentation, Tier II
Annex IIM, Section 5

Point IIM 7: Fate and Behaviour Studies on the Microbial Pest Control Agent in the Environment

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Introduction

The company Bayer CropScience AG is submitting a dossier for the re-approval of the microorganism *Purpureocillium lilacinum* 251 as an active substance under regulation (EC) 1107/2009.

The Microbial Pest Control Agent *Paecilomyces lilacinus* strain 251 was included into Annex I of Directive 91/414/EEC on 01/08/2008 (Commission Directive 2008/44/EC) and then approved according to the Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011, implementing Regulation (EC) No 1107/2009 of the European Parliament. *P. lilacinus* strain 251 was notified and defended by Prophyta GmbH. The active ingredient has been evaluated in Belgium according to Uniform Principles. The representative formulated product for the initial evaluation was the experimental formulation PBP-01001-I, containing \(2 \times 10^9\) spores/g. PBP-01001-I, is comparable to the commercial formulation BioAct WG, containing \(1 \times 10^{10}\) spores/g, and the only changes between both formulations were slight adjustments of the content of two co-formulants, without any impact on the performance or physical properties of the formulated product. The recommended rate in terms of spores per hectare remained exactly the same. The data on PBP-01001-I can therefore be extrapolated to the formulated product BioAct WG, a wettable granule formulation (WG), the representative formulation in the present application for the renewal.

In 2013 Bayer CropScience AG acquired Prophyta Biologischer Pflanzenschutz GmbH, now named Bayer CropScience Biologics GmbH. Bayer CropScience AG is the notifier for the renewal of *P. lilacinus* strain 251 in the procedure of AIR 3.

The microorganism has been previously classified as *Paecilomyces lilacinus* until 18S rRNA gene, internal transcribed spacer (ITS) and partial translation elongation factor 1-α (TEF) sequencing revealed that *P. lilacinus* is not related to *Paecilomyces*. The new genus name *Purpureocillium* has been proposed for *P. lilacinus* and the new species name was assigned: *Purpureocillium lilacinum*. Therefore the strain is now identified as *Purpureocillium lilacinum*. In this dossier *Paecilomyces lilacinus* 251 and *Purpureocillium lilacinum* 251 are used as synonyms: *Paecilomyces lilacinus* = *Purpureocillium lilacinum*.

It has to be taken into account that data on *Paecilomyces lilacinus* from the open literature stated before 2011 may not necessarily provide reliable information due to insufficient classification methods used in these studies, especially, if the strain identification is not provided and/or identification methods used were based solely on morphological characteristics. However, they may provide relevant information transferrable to *Purpureocillium lilacinum*.

*Purpureocillium lilacinum* 251 is a ubiquitous, saprobic filamentous fungus commonly isolated from soil, decaying vegetation, insects and nematodes. Strains of *P. lilacinum* are used in plant protection products due to their nematicide activity. The mode of action against plant pathogenic nematodes of *P. lilacinum* strain 251 is principally based upon parasitism of nematode eggs as well as the vermiciform stages of the nematodes, leading eventually to their death. With regard to the results of toxicity and ecotoxicity studies of the active substance *P. lilacinum* strain 251, it can be concluded that *P. lilacinum* strain 251 shows no risk for exposed humans, animals and environment.

*P. lilacinum* 251 is intended to be used in plant protection products to control plant pathogenic nematodes. The representative use presented in this dossier comprises applications of the formulation BioAct WG in protected and non-protected vegetable crops to control root knot nematode, *Meloidogyne* spp.

Here we submit data that were previously evaluated by RMS Belgium as well as new data and information based on literature searches and studies.

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IIM 7  Fate and Behaviour Studies on the Microbial Pest Control Agent in the Environment

IIM 7.1  Sufficient information on the origin, properties, survival and residual metabolites of the microorganism to assess its fate and behaviour in the environment.

Viability/population dynamics, persistence, multiplication and mobility

New data 2015

Strain 251 was first described as *Paecilomyces lilacinus*. *P. lilacinus* is a common soil saprophyte and several strains belonging to this genus are aggressive parasites of plant parasitic nematodes. Strain 251 of *P. lilacinus* is the most well studied of the nematophagous strains and is commercialized for biocontrol in several countries. A new species, *Purpureocillium lilacinum*, was proposed in 2011 (*Ze et al., 2011, M-534512-01-1) as a result of phylogenetic analyses of 18S rRNA gene, internal transcribed spacer (ITS) and translation elongation factor 1-ɑ (TEF) sequences. The phylogenetic data showed that *P. lilacinus* is not related to *Paecilomyces* and a new genus *Purpureocillium* was proposed with the type species *P. lilacinum*. Please refer also to Section 1, Point xx.

To gain sufficient information on fate and behaviour of *P. lilacinum* in soil, water and air, a literature search was performed using STN database (shëkpGJgz, 2015, M-542801-01-1). 14 databases were considered in this search: Agricola, BIOSIS, MEDLINE, CAB Abstracts, SCISEARCH and Chemical Abstracts, DRUGU, EMBASE, Esiobase, IPA, Pascal, POSciTech, Toxcenter and FSTA. After full text assessment, 15 articles published in the last ten years were determined to be relevant and were included in the dossier.

Cited references (abstracts):


FEMS Microbiology Letters 321: 141-149

Abstract: *Paecilomyces lilacinus* was described more than a century ago and is a commonly occurring fungus in soil. However, in the last decade this fungus has been increasingly found as the causal agent of infections in man and other vertebrates. Most cases of disease are described from patients with compromised immune systems or intracocular lens implants. In this study, we compared clinical isolates with strains isolated from soil, insects and nematodes using 18S rRNA gene, internal transcribed spacer (ITS) and partial translation elongation factor 1-ɑ (TEF) sequences. Our data show that *P. lilacinus* is not related to *Paecilomyces*, represented by the well-known thermophilic and often pathogenic *Paecilomyces variotti*. The new genus name *Purpureocillium* is proposed for *P. lilacinus* and the new combination *Purpureocillium lilacinum* is made here. Furthermore, the examined *Purpureocillium lilacinum* isolated grouped in two clades based on ITS and partial TEF sequences. The ITS and TEF sequences of the *Purpureocillium lilacinum* isolates used for biocontrol of nematode pests are identical to those causing infections in (immunocompromised) humans. The use of high concentrations of *Purpureocillium lilacinum* spores for biocontrol poses a health risk in immunocompromised humans and more research is needed to determine the pathogenicity factors of *Purpureocillium lilacinum*.


The report summarizes the search and selection process of open peer-reviewed literature for *Purpureocillium lilacinum* strain 251.

IIM 7.1.1 Persistence and mobility in soil

EU-Dossier: Doc M-IIB, Point 7.1.1

Persistence of *P. lilacinus* in the agricultural soil environment, into which it is delivered, is desired to accomplish efficacy and also is to be anticipated, since the soil is the original habitat of this saprophytic fungus.
For *P. lilacinus* and related species following information regarding persistence and survival in the soil environment can be derived from literature:

- Persistence of entomopathogenic Hyphomycetes in soil varies considerably on strain level, with *P. fumosoroseus* conidia being substantially degraded after 6 months (Sä8J(xa & öcCx-l, 1985, M-489363-01-1).
- Following application to soil some authors observed a substantial decline of several log in the content of viable spores of *P. lilacinus* per gram of soil, or complete clearance from spores within months after application (Ükä ズJ, 1991, M-477445-01-1), but residual spores from a last year’s application were also found at levels of 10^2 to 10^3 CFU/g of soil for different types of formulation employed (Wcj:? et al., 1999, M-477445-01-1).
- After planting tomatoes in a field which had been treated with *P. lilacinus* as a biocontrol agent for parasitic nematodes a year ago, residual populations of *P. lilacinus* were shown to increase by ~1 log within the growing season, up to harvest of these tomatoes (W?jjä et al., 1999, M-477445-01-1), which apparently provided a source of parasitic nematodes for the fungus.
- The species *P. lilacinus* is considered a rather competitive organism in agricultural soils, based on development and research for biocontrol agents, and is assumed to readily establish in the micro-flora of soils, which also is supported by its adaptability to a wide range of soil pH (P9lhljh キ & ジ5, 1996, M-477546-01-1; l/sääz, 1986, M-477553-01-1; j?jszc et al., 1981, M-477590-01-1).
- Survival of *P. lilacinus* is assumed to be as a saprophyte (W?zil et al., 1999, M-477445-01-1), and the competitiveness was found not to be dependent on soil texture or composition of soils (RョtteJcg?: et al. 1989, M-489356-01-1).
- However, *P. lilacinus* may still be subject to soil mycostatic factors, inhibiting germination (イ ä9 ディ Va7 et al., 1980; M-476530-01-1), as also evidenced for the zoopathogenic *Paecilomyces* species *P. fumosoroseus* and *P. farinosus* (イ & パ`q & j, 1984, M-477420-01-1).

**Persistence of strain 251 of *P. lilacinus* in the agricultural soil environment**

In the scope of her thesis on *P. lilacinus* strain 251 performed three field trials on persistence of the fungus in natural soils, one of which also is presented in k/ehÖi (1998, M-477414-01-1), showing a graph of decline in spore counts/g of garden soil within the first month after application: Initially high levels of strain 251 of *P. lilacinus* in the range of 10^6 colony forming units/mL garden soil gradually declined within 18 days to <1 CFU/mL and after ~ five months the population had diminished to zero (see Fig. 7.1.1-01; based on data from 2000, M-490110-01-1). Data on trial design and results for all three trials are presented in Table 7.1.1-01.

![Persistence of *P. lilacinus* in garden soils](image-url)
Table 7.1.1-01: Summary of persistence trials performed with *P. lilacinus* strain 251 (commercial product Paecil, $2 \times 10^9$ CFU/g) at different locations in Australia (itcb, 2000, M-490110-01-1).

<table>
<thead>
<tr>
<th>Location</th>
<th>Trial design</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soil type, area</td>
<td>Application</td>
</tr>
<tr>
<td>#1. Garden at Macquarie University, Sydney</td>
<td>Undisturbed garden soil, 2x2m</td>
<td>18 g on 4 m² = 4.5 g/m²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>applied as soil drench in early December (additional irrigation)</td>
</tr>
<tr>
<td>#2. ‘Fauna Park’ at Macquarie University, Sydney</td>
<td>Not specified garden soil 4.5 × 3.5m</td>
<td>15 g on 15.75 m² = 1.05 g/m²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>applied as soil drench in mid-June</td>
</tr>
<tr>
<td>#3. Vineyard near Broke, NSW</td>
<td>Not specified vineyard soil</td>
<td>2 g/vine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>applied as soil drench at budburst</td>
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</table>

The results from the Fauna Park (#2) basically were in line with the findings at the garden plot (#1), with no colonies recovered ~4 months after treatment, but some discrepancy is seen after 28 and 53 days, with 6 colonies found after 49 days but a low level after 7-8 weeks. This variation in CFU counts can be explained by uneven distribution of fungal spores among the sampled area and by the additive systemic fault due to the low numbers of colonies found on agar plates (itcb, 2000, M-490110-01-1). Recovery of *P. lilacinus* in the vineyard (#3) was much higher after 4-5 months following treatment, but showed no sign of a decline. According to itcb (2000, M-490110-01-1) this is presumably due to favorable weather conditions with light rain at the time of application, persisting for several days thereafter. Thus, spores found better starting conditions here, maybe also due to the root-knot nematode present in the vineyard, being potential hosts for *P. lilacinus*. The deviation from the results of the soil sampling studies can also partly result from the different sampling site in the vineyard trial, which was the rhizosphere, a micro-habitat offering optimum conditions for growth of numerous saprophytic micro-organisms, including *P. lilacinus*. Itcb (2000, M-490110-01-1) also stresses that the employed traditional method of serial dilution plating on semi-selective agar does not provide differentiation among *P. lilacinus* isolates, i.e. the biocontrol strain and native strains. In fact genetic analysis of some obtained cultures with differences in gross morphology showed that those isolates were definitely not strain 251. Therefore, the actual survival of strain 251 may be overestimated in these trials.
New data 2015

For *P. lilacinum* and related species following information regarding persistence and survival in the soil environment can be derived from literature search covering last 10 years, 2005-2015:

- **S.** 2006; M-534735-01-1 described biological control of *Meloidogyne incognita* in laboratory experiments in tomato. The authors provide data on the survival of *P. lilacinum* strain 251 (PL251) after soil application, indicating an average density decrease of *P. lilacinum* strain 251 of 55% during the test period. Neither the presence of glucose, if applied as formulated product, nor the spore concentration had any effect on the persistence of *P. lilacinum* strain 251 in soil. This confirmed previous studies of the authors showing a drastic decline of *P. lilacinum* strain 251 in soil 14-21 days after application (et al., 2004, cited in et al., 2006).

- **&,** 2006, M-534361-01-1 investigated on the effects of plant species on the persistence of *P. lilacinum* strain 251 in soil. 12 plant species, growing in soil pre-treated with *P. lilacinum* strain 251, were analysed. According to the results the tested plants had no effect on the behaviour of *P. lilacinum* strain 251 in soil. Furthermore, they showed a strong decline of the population density during testing, indicating a low potential for persistence and consequently for adverse environmental impacts. It was concluded that multiple applications of *P. lilacinum* strain 251 are necessary to maintain a high density for sufficient, long-term biocontrol (et al., 2006, M-534361-01-1). Furthermore, it could be demonstrated that the host plant is not the primary factor affecting the persistence of the fungus.

- **&** 2005a, M-534360-01-1 evaluated the potential of strain PL251 to establish and survive in the environment after broadcast field application of a commercial water dispersible granule formulation (BIOACT WG) (4kg/ha). Within the first 90 days past application the density of *P. lilacinum* strain 251 decreased by more than 90%. At harvest, the *P. lilacinum* could no longer be isolated from the rhizosphere soil. It could be demonstrated that the decline in the population density of PL251 was independent from the spatial distribution and the population dynamic of the nematode *Heterodera schachtii*.

- **&** 2005b, M-535175-01-1 evaluated the effects of the environment on the persistence of *P. lilacinum* strain 251 in soil in tomato and concluded that the strain shows relatively low persistence. The authors presented results that proved low environmental exposure to *P. lilacinum*, based on low field recovery after application (with 4 × 10^{13} conidia/ha applied and 4 × 10^5 expected, the recovery ranged between 10-50%); low persistence in soil and no growth after 144 h at 36°C.

- **&** 2006, M-534359-01-1 concluded from their experiments on *P. lilacinus* 251 that persistence and consequently the biocontrol efficacy of PL251 was not, unlike other nematophagous fungi, linked to the presence of the target nematode nor the host plant and that rhizosphere competence is not a key factor for the biocontrol efficacy of PL251. Multiple applications did increase the persistence of the fungus in soil which was correlated with excellent control of root-knot nematodes (*Meloidogyne incognita*) under field conditions.
• et al. (2008, M-534367-01-1) investigated the survival of *P. lilacinus* strain 251 (PL251) and the effect of application rate, substrate type, as well as the presence of the nematode host on its dynamics after application to the soil under controlled conditions. A graduate CFU decline was observed after application. The decline was independent from the application rates as well as from the presence of nematodes (Fig. 7.1.1.-02). However, the substrate type had a significant effect on *P. lilacinum* persistence in soil. Clay soils favored fungus survival in comparison to sandy soils but also addition of organic matter to sandy soils (Fig. 7.1.1-03).

![Fig. 7.1.1-02. Persistence of *P. lilacinum* strain 251 in the presence or absence of tomato plants and nematode host. Values are given as the mean of two applications rates for each sampling (n=6) (after et al., 2008, M-534367-01-1).](image1)

![Fig. 7.1.1-03. Persistence of *P. lilacinum* strain 251 in different soil substrates (after et al., 2008, M-534367-01-1).](image2)
• The above results were confirmed in another study on *P. lilacinum* strain 251 persistence using dilution plating techniques as well as molecular biology methods (nested PCR) for detection of fungus spores in the soil. In addition the interaction between *P. lilacinum* strain 251 and plants, nematodes, mutualistic fungal endophytes, and mycorrhiza was investigated. The studies showed that the initial density of the fungal antagonist after application was significantly lower than predicted and the spatial distribution was very heterogeneous. Already in the first year the density of PL251 already decreased by more than 90% within 90 days after application. After 120 days the fungus was no longer detected in soil. The observed decline was independent from the initial spatial distribution and not altered by the population dynamics of the host nematode. Two years after application the fungus was detected at equal level in treated and untreated plots; and the density of *P. lilacinum* strain 251 was far below the background level of other filamentous fungi. Furthermore, no adverse effects on mutualistic fungal endophytes, mycorrhiza, fungal antagonists or entomopathogenic nematodes were observed demonstrating the absence of competition under field conditions.

• **De* et al. (2005, M-534724-01-1), presented data on establishment of *P. lilacinum* on roots and soil in a period of 2 years, in carnation and gerbera plants. They found that the colonization with *P. lilacinum* increased significantly when the soil was pre-treated with dazomet, most likely due to reduction of competitor fungi.

• **Gc* et al. (2012, M-534851-01) showed that root-colonization of *P. lilacinus* in carrot field infested with *M. incognita* and *Erwinia carotovora* was very efficient. After seed treatment (4 × 10^6 CFU/g seeds) application 10^5 CFU/g soil, and roots were recovered. Additionally the authors reported that root colonization by *P. lilacinus* and *Pseudomonas putida* was higher when they were applied together in comparison to individual treatments. *Pseudomonas putida* and *P. lilacinus* co-existed without affecting root colonization by either, which indicates that there is no antagonism between these two biocontrol species.

• **Bo* et al. (2015, M-534211-01-1) monitored the population of another strain of *P. lilacinus* (strain PL1210) in a field experiment for 60 days, and showed that after an initial slight decrease from 1.2 × 10^5 spores/g soil to 0.3 × 10^5 spores/g soil, the fungus reached 0.99 × 10^5 after 60 days, which proves, it was capable of colonizing the rhizosphere under the tested conditions (Fig. 7.1.1-04).

**Fig. 7.1.1-04**: Real-time quantification of *P. lilacinus* PL1210 collected at 1, 30, and 60 days after inoculation. The number of PL1210 spores was determined by plotting CT values against the standard curve.

**Summary of persistence and mobility in soil - Persistence**

*Paecilomyces lilacinus* is a saprophytic fungus naturally occurring in soil. Thus, its viability is naturally adapted to soil compartments. However, numerous studies have demonstrated that the survival of different strains of *P. lilacinus* in soil after application is limited in time. **Bi**; 2000; M-490110-01-1 investigated the persistence of *P. lilacinus* strain 251. In two sites, in a garden area and in a Fauna Park, they performed a quantitative examination of the fungus applied as the formulated product (WG formulation). Before application they could not detect any *P. lilacinus* viable spores. The strain 251 was then applied onto the surface of each a defined area and soil was sampled at several intervals until the level of viable fungi fell under the detection limit. It could be observed that the
number of *P. lilacinus* colony forming units (CFU) declined constantly within the first 2-3 weeks to less than 2% of the initially applied spores: from 1.3 × 10⁶ down to 2.5 × 10⁴ CFU/mL of soil in the garden area and from 2.4 × 10⁶ down to 1.3 × 10⁴ CFU/mL of soil in the Fauna Park, respectively. These findings were supported by earlier studies. R.K.; M-489356-01-1 conducted laboratory experiments to examine the survival of a peruvian strain of *P. lilacinus*. When the spores were applied to soil and incubated at room temperature for up to 56 days the decline was first slow but after 14 days the authors determined a fast decline.

During the 2 to 3 weeks, spores of *P. lilacinus* strain 251 were expected to show a fast decline to very low percentages within a few weeks. As a result, the population density of *P. lilacinus* strain 251 may possibly approach a balance at a clearly lower population density compared to the initial number of viable cells or spores of *P. lilacinus* applied to soil the number of viable cells or spores of *P. lilacinus* expected to show a fast decline to very low percentages within a few weeks. However, depending on the prevailing environmental conditions of the relevant soil ecosystem, they may possibly approach a balance at a clearly lower population density compared to the initial concentration, in response to limiting abiotic and also counteracting biotic factors. On a long-term scale,
without further applications of *P. lilacinum* strain 251, this saprophytic fungus may diminish completely, indicating the need for more than a single application to achieve nematode control. Therefore, since *P. lilacinum* strain 251 is naturally occurring in soil. Neither an unlimited multiplication nor an accumulation is expected.

**Summary of persistence and mobility in soil - Mobility**

Dispersal of spores of *P. lilacinum* strain 251 under conditions of use is limited, since it is intended to be applied directly onto the soil surface and incorporated by drench, or drip irrigation. Therefore dispersal via drift or via aerosols is not anticipated. Exposure to natural UV-light will restrict germination and survival of applied *P. lilacinum* 251 into other environmental compartments, based on the results by **Zelmer**, et al. (2000; MB89366-07-1) for strain 251 tested for its UV sensitivity among other *P. lilacinus* strains. Since *P. lilacinus* strain 251 is a fungus dependent on aerobic respiration as well as its natural food, plant-parasitic nematodes, it is dependent on the upper aerobic zone of the soil. In deeper soil layers no survival of viable cells is expected. Due to their hydrophobicity the spores are expected to adsorb to soil particles and not to leach to lower zones. In conclusion, there is no risk for uncontrolled growth of *P. lilacinus* strain 251 in soil. Neither an unlimited multiplication nor an accumulation is expected.

Cited references (abstracts):


**Abstract:** The fungal biocontrol agent, *Paecilomyces lilacinus* strain 251 (PL251), was evaluated for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato. In growth chamber experiments, a pre-planting soil treatment reduced root galling by 66%, number of egg masses by 74% and the final nematode population in the roots by 71% compared to the inoculated control. Significant dose–response relationships were established when conidia were applied to soil either with or without the glucose-based formulation. The effective concentration 50 (EC50) values for the commercially formulated product ranged between 0.097 g and 0.08 g/500 cm³ soil, equivalent to an EC50 of 1.29 × 10⁶ and 9.88 × 10⁵ colony forming units (CFU)/g soil for the parameters gall index and final population per root, respectively. For the number of egg masses per root, the EC50 was 0.007 g product or 2.64 × 10⁵ CFU/g soil. Similarly, EC50 values for conidia applied without formulation were 0.068 g or 0.103 g/500 cm³ soil (EC50 of 8.10 × 10⁵–1.40 × 10⁶ CFU/g soil) for gall index and final population per root. In contrast, the EC50 was 0.096 g (EC50 of 1.28 × 10⁵ CFU/g soil) for the number of egg masses per root. We demonstrated that a single pre-plant application at a concentration of 1 × 10⁶ CFU/g soil is needed for sufficient biocontrol of *M. incognita* in PL251.


**Abstract:** The effect of 12 plant species on the persistence of *Paecilomyces lilacinus* strain 251 in soil was investigated. After incorporating formulated conidia into non-sterile soil followed by transplanting different test plants, the population dynamic of the fungus was determined over 100 days. Determination of the experiment, the fungal population in the planted soil was compared to the density of *P. lilacinus* in the rhizosphere and the percent increase or decrease was calculated for each crop. In addition, the potential of *P. lilacinus* strain 251 to colonize roots endophytically was investigated. Comparison of the slopes describing the population dynamics of the fungus showed no significant differences between soil without plants and soil from the root zone of the majority of the test plants. Bean was the only plant species consistently exerting a negative effect on the persistence of *P. lilacinus* strain 251 in the soil. For the first time, *P. lilacinus* strain 251 was
isolated in significant numbers from healthy root tissue of barley plants.


Abstract: The egg pathogenic fungus Paecilomyces lilacinus strain 251 (PL251) can be used in an integrated approach to control the sugar beet cyst nematode Heterodera schachtii. To evaluate the potential of PL251 to establish and persist in the environment after broadcast application, a field experiment was conducted using a commercial water dispersible granule formulation (BIOACT WG). The fungal antagonist was applied at a rate of 4 kg product per ha and incorporated into the soil prior to planting sugar beets. At day zero, 50 and 90 past application and at harvest soil samples were collected to determine the population density of PL251. It was found that the spatial distribution after application was quite heterogeneous and that the density of the fungal antagonist directly after application was much lower than expected. Within the first 90 days past application the density of PL251 decreased more than 90 percent. At harvest, the antagonist could no longer be isolated from the rhizosphere soil. It could be demonstrated that the decline in the population density of PL251 was independent from the spatial distribution and the population dynamics of H. schachtii. Due to the fact that the fungal antagonist was not able to persist long under field conditions, the potential for PL251 to pose a risk to the environment is likely to be low.


Abstract: The development of a biological control product faces many obstacles before the final goal, successful commercialization, is achieved. Although several biopesticides have already proven their ability to efficiently control pests and diseases without causing any adverse effects to the environment, there are still concerns about the fate of a microorganism after its release. Besides the monitoring of a specific biocontrol agent in the environment, models to appropriately assess the operator or bystander exposure need to be developed or modified for microbial pesticides. The egg pathogenic fungus Paecilomyces lilacinus (strain 251), was chosen as a model organism to identify the parameters needed to predict its fate in the environment. To monitor the long-term survival, a semi-selective medium was developed to enable monitoring of the population densities of P. lilacinus in the soil and rhizosphere. Monitoring was conducted with root knot nematodes (Meloidogyne spp.) as target and tomato as host plant. The population development of P. lilacinus was monitored, depending on the application rate, formulation, temperature, method of application and the presence or absence of the target pest as well as the host plant. Initial results demonstrated that P. lilacinus was not able to multiply in soil or the rhizosphere of tomato plants and consequently showed a relatively low persistence...


Abstract: An emerging organic citrus industry in Florida could benefit greatly from effective, non-conventional methods to mitigate losses from pests and diseases. We studied part of a soil food web in an organic orchard to learn ways to conserve and enhance biological control of insect pests by native entomopathogenic nematodes (EPNs). We evaluated two OMRI (Organic Materials Review Institute) approved cultural practices: (i) a mulch of commercially pelleted chicken manure, (ii) a commercial formulation of Purpureocillium lilacinus, and (iii) an un-amended control. Several soil nutrients (i.e. nitrogen, phosphate, and potassium) were affected by the amendments, but initial equilibrium values (T0) were restored by the last sampling time (T12). The plant parasitic nematode Tylenchulus semipenetrans increased in both treatments compared to the untreated control at T3 (P < 0.05). The oomycete Phytophthora nicotianae increased in the P. lilacinus plots at T1, marginally at T12, but decreased at T6 and T9. Steinernema diaprepsi, Heterorhabditis indica and Heterorhabditis zealandica were the only EPNs regularly detected in the orchard. Mulch increased numbers of H. zealandica at T6 and T9 (P < 0.05) and free living nematodes at T12 (P < 0.01). The nematophagous fungus (NF) P. lilacinus persisted in plots where it was augmented (P < 0.05), reaching a maximum level at T3 that was 17.5-fold greater than that in controls. Numbers of Paenibacillus sp. were directly related to both those of S. diaprepsi and...
Acrobeloides-group nematodes (P < 0.01), but inversely to the FLN counts (P < 0.05). The application of these two amendments did not produce strong changes in the EPN community but decreased the emergence from soil of adult Diaprepes abbreviatus, a root weevil pest. Thus, both amendments might contribute to citrus pest management under organic production.

Report: KIIM 7.1.1/20 – S., 2006b Multitrophic interactions of Paecilomyces lilacinus strain 251 in the rhizosphere of host and non-host plants.

Abstract: The facultative egg pathogenic fungus Paecilomyces lilacinus is one of the most widely tested biocontrol agents for control of plant parasitic nematodes. The commercial strain 251 (PL251) is undergoing registration procedures in the EU and US and is commercially available as BIOACT® WG in several countries. To better understand the multitrophic interactions of PL251 in the rhizosphere, dose-response experiments were conducted to evaluate the relationship between the antagonist dose and biocontrol efficacy and fungal persistence. The importance of host- or non-host plants, nematodes, mutualistic fungal endophytes, and mycorrhiza for biological efficacy and unwanted side effects caused by the application of the biocontrol fungus was also investigated. It could be demonstrated that persistence and consequently the biocontrol efficacy of PL251 is not, unlike other nematophagous fungi, linked to the presence of the target nematode nor the host plant. Furthermore, some nematode host plants seem to provide unsuitable conditions in their rhizosphere resulting in rapid decline of fungal density and in some cases reduced efficacy of the antagonist. In contrast to other nematophagous fungi, rhizosphere competence is not a key factor for the biocontrol efficacy of PL251. Multiple applications did increase the persistence of the fungus in soil which was correlated with excellent control of root-knot nematodes under field conditions.


Abstract: The persistence of the nematophagous fungus Paecilomyces lilacinus strain 251 (PL251) and the effect of application rate, substrate type, as well as the presence of the nematode host on its dynamics after application to the soil were investigated under controlled conditions. In all experiments, increase of P. lilacinus colony forming units after application was not found. In contrast, a gradual decline in fungal densities over time was observed. Application rate had no significant effect on the dynamics of the fungal population. Likewise, P. lilacinus density decline in soil was not significantly affected by the presence of the nematode host. Substrate type had a significant effect on P. lilacinus persistence in soil. The fungal agent persisted longer in silty loam and clay soil, with reduced persistence when sand was added to field soil. Conversely, when organic substrate was added to pure sand, persistence was significantly increased. Although persistence of fungal biocontrol agents in soil depends on various biotic and abiotic conditions, baseline data on persistence such as those reported in this study are helpful for biocontrol and environmental risk assessment and merit further study.


Abstract: The facultative egg pathogenic fungus Paecilomyces lilacinus strain 251 (PL251) is one of the most widely tested biocontrol agents for control of plant-parasitic nematodes. Recently, PL251 was included as active substance in Annex I to the directive 91/414/EEC. In the USA, PL251 is registered as bio-nematicide under the trade name MELOCON® WG for use on a variety of crops. So far PL251 has demonstrated efficacy in reducing root-knot, cyst and free living plant-parasitic nematodes on a range of crops. However, to better understand the multitrophic interactions of PL251 with host- or non-host plants, nematodes, mutualistic fungal endophytes, and mycorrhiza, studies were conducted to determine their importance for biological efficacy. In none of the studies conducted, adverse effects on mutualistic fungal endophytes, mycorrhiza, fungal antagonists or entomopathogenic nematodes were observed. Conversely to other nematophagous fungi, rhizosphere competence seems not a key factor for the efficacy of PL251. However, studies
are underway to determine the eggmass colonisation by PL251 using realtime PCR assays which are able to detect 10 CFU per eggmass or less. Monitoring the persistence of PL251 under field conditions using dilution plating techniques and nested PCR revealed a rapid decline of the fungal density in soil over time. Although detection of PL251 in soil was still possible two years after application, the overall suppressiveness of egg pathogenic fungi towards cyst nematodes was not affected.


**Nematol medit 33: 157-162**

**Abstract:** Studies on the management of carnation and gerbera to control root-knot nematode, *Meloidogyne incognita*, in commercial polyhouses using pre-plant (dazomet) and post-plant (chlorpyriphos, carbofuslan and carbofuran) chemicals in a comparison with various combinations of and with bio-agents (*Paeceilomyces lilacinus*, *Pochonia chlamydosporia*) and neem cake were made. Pre-plant treatment of beds with dazomet followed by the application of neem cake (1 kg/m², 15 days later) along with *P. lilacinus* or *P. chlamydosporia* significantly reduced populations of *M. incognita* and the mortality of plants, and suppressed the nematode infection for nearly 2 years. The antagonistic fungi established themselves better in the beds treated with dazomet than in untreated beds. Chlorpyriphos and carbofuran (each applied twice in 6 months) significantly reduced nematode populations in roots and soil. However, there was a build-up of nematode populations in beds treated with these two chemicals after 1 year. On a long term basis, soil management with pre-plant treatment of dazomet followed by the application of oil cakes plus antagonistic fungi, was more effective against *M. incognita* than post-plant treatment with carbofuran, carbofuslan and chlorpyriphos on carnation and gerbera grown in polyhouses.


**Nematol medit 40: 189-196**

**Abstract:** An experiment was conducted to test the effects of two biocontrol agents, *Pseudomonas putida* and *Paecilomyces lilacinus*, on the control of *Meloidogyne incognita* and *Erwinia carotovora* in carrot (*Daucus carota L.*). *Pseudomonas putida* and *P. lilacinus* formulations were enriched in neem cake and evaluated under field conditions, individually and in combination, as seed treatment or as substrate treatment. Twenty grams of this formulation was used for the seed (one kg) treatment and five kg for the enrichment of neem cake (200 kg), which was applied to the beds at the rate of 20 g/m² as a substrate treatment before sowing. Seed treatment with both bioagents and application of neem cake enriched with *P. putida* and *P. lilacinus* proved to be the best of all the treatments, leading to a reduction in the *M. incognita* (J(2)) population (in roots by 69 percent and soil by 47.6 percent) and *E. carotovora* by 66 percent, with a significant increase (27.8 percent) in the yield of carrot. *Pseudomonas putida* and *P. lilacinus* co-existed without affecting root colonization by either.


**Biol Fertil Soils 51: 343-351**

**Abstract:** This study investigated the effects of root-knot nematode biocontrol agent *Paecilomyces lilacinus* (*P. lilacinus*) strain PL1210 on ammonia-oxidizing microorganisms and fungal community composition of tomato rhizosphere. The exchangeableNH4 +--N and NO3 −--N contents were lower in inoculated soils than in the control during 60 days of incubation. Real-time quantitative polymerase chain reaction (qPCR) detected stable colonization of *P. lilacinus* in the tomato rhizosphere and significant inhibition of ammonia-oxidizing bacteria (AOB) and archaea (AOA), which could be responsible for the decrease of NO3 −--N content in soil. PCR-denaturing gradient gel electrophoresis (DGGE) analysis demonstrated no significant difference in soil fungal community composition associated with the application of *P. lilacinus* as shown by Shannon–Wiener diversity index (H′) and Margalef index (D). Cluster analysis showed that the composition
of rhizosphere fungal community was more significantly influenced by time-related differences than by the inoculation of biocontrol agents.

IIM 7.1.2 Water

EU-Dossier: Doc M-IIB, Point 7.1.2

P. lilacinus is a common soil saprophyte with ubiquitous distribution worldwide. Spores of this species may also be found and persist in natural waters, but will be subject to sedimentation and do not find conditions favourable for germination and growth in this compartment. This is supported by a test on brine shrimp, exposed to up to $8.5 \times 10^8$ CFU in 200 mL of water (Q$/c66/ 2000, M-490111-01-1): Brine shrimp were not infected, although spores were recovered from dead shrimp due to ingestion before time of death (see Doc.M, Section 6, Point 8.8, EU dossier Doc.M, Section 6, Point 8.4). Further, spores did not germinate in seawater alone.

Exposure to natural UV-light will to some extent restrict germination and survival of P. lilacinus strain 251 when spread into other environmental compartments, based on the results for strain 251 tested for its UV sensitivity among other P. lilacinus strains (Gunaskera et al., 2000, M-489366-01-1).

New data 2015

Statement on potential interferences with the analytical systems for the control of the quality of drinking water provided for directive 98/83/EC:

According to Council Directive 98/83/EC for drinking water only E. coli, enterococci and Clostridium perfringens are monitored in drinking water. For these bacteria, either highly specific media will be used on which other species do not grow or a highly specific reaction is catalysed by the indicator species allowing a clear identification. These methods were designed to unambiguously determine these pathogens. Methods used were validated for a number of bacteria and fungi. There is no reason to assume that P. lilacinus would be able to grow on these media or will not catalyse the specific indicator reaction and thus interfere with the detection method.

Similarly, non-pathogenic bacteria and fungi naturally occur in drinking water systems and it has never been reported that the presence of these bacteria interferes with the quality control systems. In conclusion, any kind of interference with the analytical systems for drinking water control can be excluded.

A literature search performed in 2015 revealed three publications considered supporting for the dossier. Gunaskera et al. (2010, M-534847-01-1) investigated sewage sludge. P. lilacinum was found among the dominating species in the sludge samples (10% of the total number of strains). These results indicate that P. lilacinum is a commonly occurring fungus in water systems. However, as mentioned above, spores can persist and be translocated in water compartments, but this species will rarely germinate in water. Water potential, light and pH were shown to affect the growth and sporulation of fungi (Uä. Z, 2009; M-534834-01). However these factors may differently affect different strains of the species. For example, for P. lilacinus strain IPC-P optimal water potential for growth was -7.3 MPa and for sporulation -0.3 MPa, while another strain, M-14, grew and sporulated the most efficient at -0.3 MPa. Light inhibited growth of IPC-P, but enhanced the sporulation of M-14. Maximum growth and sporulation occurred on acid media. Gunaskera et al. (2013, M-534747-01-1) investigated fungal biofilms forms on water taps in Germany. P. lilacinus was detected in only one out of 13 studies biofilms, and in extremely low amounts (0.23% of total fungi present). This suggests that tap water is not an optimal habitat for growth of P. lilacinus and thus the risk can be considered negligible.

In addition, it has to be considered that the intended application methods for BioAct WG secure a minimal drift of spores into natural surface waters (see Doc. D1).

Cited references (abstracts):

Mycoscience 50: 321-327

Abstract: To supply essential information for improving mass production and biocontrol efficacy, two-stage cultivation on agar plates was used to evaluate the environmental conditions affecting mycelial growth and sporulation of seven biocontrol fungi. Maximum growth and sporulation occurred on acid media for Paecilomyces (Pa. lilacinus IPC-P, Pochonia (Po.) chlamydospora HSY-12-29), and Lecanicillium lecanii CA-1-G, and on alkaline media for Metarhizium anisopliae isolates. All fungi prefered a certain water potential and temperature for sporulation. Light greatly inhibited the growth of P. lilacinus IPC-P, M. anisopliae SQZ-1-21, and L. lecanii CA-1-G but enhanced the sporulation of L. lecanii M-14, P. chlamydospora HSY-12-14, and L. lecanii CA-16.


Polish J. Environ Stud 19: 635-642

Abstract: Sewage sludge is being used for reclamation of devastated areas and for fertilization of arable soils. However, sludges contain many harmful components, including pathogenic organisms. Many keratinolytic and associated non-keratinolytic fungi are opportunistic pathogens. Our knowledge on fungal occurrence in sludges and sludge-amended soils and on the health risk posed by the fungi is still not sufficient. The present work was part of extensive studies on actidione-resistant fungal pathogens in sludges and sludge-amended soils. Sludges from the Siemianowice-Centrum wastewater treatment plant, Upper Silesia, Poland were examined. Results obtained by means of three methods, i.e. the dilution pour plating method, hair baiting method and most probable number method were compared. The MPN method combines the dilution and hair baiting methods. The dilution pour plating method was found not to be highly informative as to the occurrence of keratinolytic fungi in sludges, while using the method more information was obtained on non-keratinolytic fungi in the sludge environment. Subsequently, the hair baiting method provided the data on fungal growth in the hair spread over the sludge blanket. This qualitative method has often been used for semiquantitative purposes but does not allow for determining fungal quantities. Such quantities were obtained using the MPN method. The method complemented the results obtained using two other methods. The hair baiting and MPN methods use hair and natural media (sterile sludge, sand, and clay) for examination of sludge fungi. The selectiveness of the MPN method was even higher than that of the hair baiting method. Ecological and epidemiological significance of the MPN results was discussed.


Published report

Mycologia 75: 387-397

Abstract: Mass growth of dark fungal biofilms on water taps and associated habitats was observed in various German drinking water distribution systems recently. Customers of affected drinking water systems are anxious about potential and unknown health risks. These environments are known to harbor a fungal flora also comprising a variety of fungal opportunists that are well known to cause superficial mycoses in humans (Exophiala equina, Exophiala lecanii-corni) but are not known to establish dark biofilms so far. To gain profound insight on composition of respective biofilms, a metagenomic approach using Tag-Encoded FLX Amplicon Pyrosequencing (TEFAP) of the ribosomal internal transcribed spacer 2 region in comparison with a classical cultivation approach using Sabouraud agar with chloramphenicol and erythritol-chloramphenicol-agar was performed. E. lecanii-corni was found to be the major component in 10 of 13 biofilms analyzed independently of the method used. Alternaria sp., E. equina, Fusarium spp. and Ochroconis spp. were also relatively abundant. As expected, TEFAP usually revealed a higher diversity than the cultivation approaches. For example, opportunistic species like Candida albicans or Exophiala dermatitidis were detected in very low amounts. In conclusion, TEFAP turned out to be a
promising and powerful tool for the semi-quantitative analysis of fungal biofilms. Referring to relevant literature, potential biological hazards caused by fungi of the dark biofilms can be regarded as low.

IIM 7.1.3 Air

EU-Dossier: Doc M-IIB, Point 7.1.3
Dispersal of spores via aerosols is not anticipated due to the nature of this preparation. Further information on the persistence in air is not required, since the toxicological studies and the temperature growth profile of this strain prove that it is not able to infect humans and imposes no risk for workers, operators or bystanders via the inhalation route or any other route.

IIM 7.2 Other/special studies
No further studies are considered to be necessary.
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<td>A.</td>
<td>2015</td>
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<td>GAB Consulting Agrociencias S.L.U., Valencia, Spain, Bayer CropScience, Report No.: M-542801-01-1, Edition Number: M-542801-01-1, Date: 2015-12-17, GLP/GEP: n.a., unpublished</td>
<td>No</td>
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<td>Interim report - Persistence of Paecilomyces lilacinus in garden soil</td>
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<td>Paecilomyces lilacinus as a biocontrol agent - Chapter 7 to the persistence of Paecilomyces lilacinus after application to soil</td>
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<td>KIIM 7.1.1 /13</td>
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<td>2005</td>
<td>Statement to the DAR reference point: Vol. 3, B 8.1, persistence and multiplication in soil</td>
<td>Bayer CropScience</td>
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<td>2006</td>
<td>Biological control of the root-knot nematode Meloidogyne incognita by Paecilomyces lilacinus strain 251</td>
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