Renewal
Assessment Report

Lecanicillium muscarium Ve6

Volume 3MA – B.2 Biological properties

January 2018

Rapporteur Member State: The Netherlands
Co-Rapporteur Member State: France

Version history

When	What
January 2018	Initial RAR

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B Summary of the data and information

B.2 Biological properties of the micro-organism

Note to reader:
Information from the original DAR and/or addenda to the DAR is highlighted grey.

The company Koppert B.V. is submitting a dossier for the re-approval of the microorganism *Lecanicillium muscarium* Ve6 (19-97), further referred to as *Lecanicillium muscarium* Ve6, as an active ingredient under regulation (EC) 1107/2009.

The Microbial Pest Control Agent *Lecanicillium muscarium* Ve6 (formerly *Verticillium lecanii* Ve6) was included in Annex I of Directive 91/414/EEC on 1 May 2009 pursuant to Article 24b of the Regulation (EC) No 2229/2004, (Commission Directive 2008/113/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. *L. muscarium* Ve6 was notified and defended by Koppert Beheer B.V. (Koppert B.V. is a 100% daughter company of Koppert Beheer B.V.). The active ingredient has been evaluated in The Netherlands according to Uniform Principles. The representative formulated product for the initial evaluation was the product MYCOTAL, containing 1.0×10^{10} spores/g.

The microorganism has been previously classified as *Verticillium muscarium.* The strain has been reclassified in 2001 as *Lecanicillium muscarium*, based on molecular analysis as RFLP and ITS sequence analysis by Zare & Gams. The taxonomy change was already considered for the peer review of the pesticide risk assessment of the active substance by EFSA[1].

Here the data is presented that were previously evaluated by RMS The Netherlands in the DAR (June 2007) and DAR addenda (June 2009, October 2009), as well as new data and information based on literature searches and studies. Previously submitted information (consolidated from DAR and addenda) is highlighted in grey, and information on the original DAR Points and the respective EU Points is complemented where necessary.

B.2.1 History of the micro-organism and its uses. Natural occurrence and geographical distribution

B.2.1.1 Historical background

Information from the original DAR

The development of *Verticillium lecanii* as a biological pesticide began at the Glasshouse Crops Research Institute (GCRI), Littlehampton, United Kingdom, in 1972. The first commercial product was

[1] European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Lecanicillium muscarium* strain Ve6, notified as *Verticillium lecanii*. EFSA Journal 2010; 8(1):1446. [45 pp.]. doi:10.2903/j.efsa.2010.1446. Available online: www.efsa.europa.eu

developed for control of aphids on chrysanthemums. The product was called Vertalec and was released in 1981 in the United Kingdom. Consequently, following this research, a different product was developed for control of whitefly, called MYCOTAL. Both products were based upon strains of the entomopathogenic fungus *Verticillium lecanii*, each strain having its specific pathogenicity to its target insects.

The product MYCOTAL is based on the strain that was isolated 1979 (by Dr. R. Hall of the GCRI) from the glasshouse whitefly *Trialeurodes vaporariorum*. The MYCOTAL strain has been identified as *V. lecanii* Ve6 (Holdsworth (1982) in: Appendix 1 of Verhaar, 2000). MYCOTAL was registered in the United Kingdom in 1986 for the control of whitefly in greenhouse crops. In addition, the fungus was also isolated from whitefly-cadavers in a commercial greenhouse cucumber culture, grown on potato dextrose agar (PDA) and identified morphologically as *Verticillium lecanii* (Zimm.) Viégas. by the Commonwealth Mycological Institute and CBS- Baarn (Ekbom, 1979).

New data 2016

No new data is to be submitted under this point.

Previously submitted information is considered to be acceptable to cover current requirements.

B.2.1.2 Origin and natural occurrence

Information from the original DAR

The entomopathogenic fungus *Verticillium lecanii* has a worldwide geographic distribution on many different substrates: as a soil pathogen (on other fungi), as a hyperparasite on rust fungi, and on plant material (Brady, 1979; Hall, 1981a; Rombach and Gillepsie, 1988). *Verticillium lecanii* has also been found as a natural infestation of several green house pests; whitefly on cucumber, chrysanthemum aphids (Ekbom, 1979; Hall, 1975), and has been described to decimate greenhouse populations of aphids and scales (Hall, 1981a).

The notifier submitted literature that describes a broad spectrum of hosts/substrates for the fungus; Hanssler and Hermans (1981) describe the mode of action by which *Verticillium* invades/ attacks the sugar beet cyst-nematode *Heterodera schachtii*, Hill and Lacey (1983) describe the colonisation of ripening grain, Lo and Chapman (1998) describe the occurrence on scales.

The RMS notes that in literature described above no specification is given on a strain level of *Verticillium lecanii*.

New data 2016

No new data is to be submitted under this point.

Previously submitted information is considered to be acceptable to cover current requirements. Please also see point B.2.1.1.

B.2.1.3 Description of target organism(s)

Information from the original DAR

The notifier indicates the use of *Verticillium lecanii* strain Ve6 against whiteflies (*Bemisia tabaci*, *Trialeurodes vaporariorum*) and thrips (*Frankliniella occidentalis*).

New data 2016

White flies (*Trialeurodes vaporariorum*) are most prevalent on cucumbers, tomatoes and pepper. They are about 1.5 mm in size, white powdered, winged insects. These pests damage the plants by direct sucking on plant tissue and multiply by oviposition on the underside of leaves. Hatching larvae develop very fast to grown winged insects and could form up to 10 generations per year in greenhouses.

Thrips are pests of almost all cultures in field and in protected uses. Preferably, they host on onions, leek, white and red cabbage, pea, beans, tomatoes and cucumber and pepper. Most prevalent thrips species are *Thrips tabaci* on tobacco, flower-thrips (*Frankliniella occidentalis*) and pea-thrips (*Kakothrips robustus*). Thrips overwinter in soil in larvae stage and reproduce strongly in early summer. They settle on the plants and damage the plants by sucking on leaves and buds. Symptoms on plants are growth suppression, deformation and death of leaves.

B.2.1.4 Mode of action

Information from the original DAR

Whitefly

Walter et al. (1988) investigated the infection of whitefly with *Verticillium lecanii* Zimm. microscopically (light-and electron microscopically). Different stages of infection were described; spore germination, growth on cuticle, penetration and parasitizing of the interior, and the release of new infection units. Spores of *Verticillium lecanii* germinate on the insects' cuticle within 12 - 48 hours. Strong hyphal growth on the cuticle is observed before penetration of the host. The cuticula is penetrated, and tissue is affected within 48 hours after infection. Once in the host, *V. lecanii* forms blastospores which spread through the haemolymph of the arthropod host and lead to further infection. The insect dies within 7 - 10 days, when a great number of hyphal bodies have been formed inside the body cavity (Walter et al., 1988).

For a better understanding of the development of the fungus on the host, histological studies were performed after different stages of infection. Histological section studies of whitefly killed and fixed after different exposure times indicate fungus penetration and invasion (destruction of the inner organs) of the tissues as the cause of death (Ekbom, 1979).

Thrips

Thrips are probably killed as a result of multiple lesions of the cuticle by enzymatic degradation, as no fungal material was found in the haemolymph of the insect at the time of death (Schreiter et al, 1994). In addition, *Verticillium lecanii* has also been described to secrete lytic enzymes that play a major role in penetrating the cyst wall of *Heterodera schachtii* (Hanssler and Hermanns, 1981)

New data 2016

From an independent literature search on the mode of action of *Lecanicillium* sp., two references were identified, reporting mechanisms of host penetration and repellent effect of plant colonization by *Lecanicillium* sp. It is assumed that *L. muscarium* Ve6 may have similar effects on their hosts.

Lecanicillium spp. use both mechanical forces and hydrolytic enzymes to directly penetrate the insect integument as mode of action against insects (Goettel et al., 2008). Vasantharaj & Selvaraj (2007) confirmed previously information and described the infection process of *Verticillium lecanii* on the whitefly *T. vaporariorum*: "The conidia are aggregated in slimy false heads in groups of 6 - 72. After germination of the conidia, the hyphae grow over the surface of the insect and penetrate through the integument". It is also remarked that humidity is the most limiting factor of successful penetration.

Cited references (abstracts):

Report KMA 2.2.2/01 - Goettel, M.S., Koike, M., Jun Kim, J., Aiuchi, D., Shinya, R., Brodeur, J. (2008), Potential of *Lecanicillium* spp. for Management of insects, nematodes and plant diseases Published report,
J. Invertebr. Pathol., 98: 256-261

Abstract: Fungi in the genus *Lecanicillium* (formerly classified as the single species *Verticillium lecanii*) are important pathogens of insects and some have been developed as commercial biopesticides. Some isolates are also active against phytoparasitic nematodes or fungi. *Lecanicillium* spp. use both mechanical forces and hydrolytic enzymes to directly penetrate the insect integument and the cell wall of the fungal plant pathogen. In addition to mycoparasitism of the plant pathogen, the mode of action is linked to colonization of host plant tissues, triggering an induced systemic resistance. Recently it was demonstrated that development of *Lecanicillium* hybrids through protoplast fusion may result in strains that inherit parental attributes, thereby allowing development of hybrid strains with broader host range and other increased benefits, such as increased viability. Such hybrids have demonstrated increased virulence against aphids, whiteflies and the soybean cyst nematode. Three naturally occurring species of *Lecanicillium*, *L. attenuatum*, *L. longisporum*, and an isolate that could not be linked to any presently described species based on rDNA sequences have been shown to have potential to control aphids as well as suppress the growth and spore production of *Sphaerotheca fuliginea*, the causal agent of cucumber powdery mildew. These results suggest that strains of *Lecanicillium* spp. may have potential for development as a single microbial control agent effective against several plant diseases, pest insects and plant parasitic nematodes due to its antagonistic, parasitic and disease resistance inducing characteristics. However, to our knowledge, no *Lecanicillium* spp. have been developed for control of phytopathogens or phytoparasitic nematodes.

Report KMA 2.2.2/02 - Vasantharaj David, .B., Selvaraj, P. (2007), Entomopathogenic fungi for the control of economically important whiteflies
Published report,
J. Biol. Control, 21: 29-36

Abstract: This paper focuses attention on possible entomopathogenic fungi for the hiocontrol or important whitefly pests, namely, *Bemisia tabaci* (Gennadius), *Singhiella cardamomi* (David and Subramanium). *Trialeurodes vaporariorum* (Westwood). *Aleurolobus barodensis* (Maskell). A few success stories of fungal pathogens of whitefiles have been indicatced. The scope of entomopathogenic fungi, namely, *Verticillium lecanii*, *Aschersonia* spp., *Paecilomyees fumosororoseus*. *Beauveria bassiana* and *Zoophthora* has been brought out. The aspects of different strains, mode of action, available mass multiplication technologies, field application and commercial formulations have been explained. The scope of entomopathogenic fungi and aspects requiring attention are discussed.

B.2.2 Host specificity range and effects on species other than the target harmful organism

Information from the original DAR

Host specificity

The host virulence is strain-dependent. The Vertalec strain (*V. lecanii* Ve2 strain, large size spores) is less virulent to whitefly than the MYCOTAL strain (*V. lecanii* Ve6, small size spores). Hall (1984) describes a relation between the spore size and the epizootic potential to the aphid *Macrosiphoniella sanborni*, affected by the speed of germination. Jackson et al. (1985) describe fast germination, high sporulation rate and high extracellular chitinase activities as the virulence indicators with no relation to spore size. *Verticillium lecanii* also parasitizes fungi that cause rusts on plants (Spencer and Atkey, 1981; Uma and Taylor, 1987). Jun et al. (1991) showed that by analysis on 41 characteristics, that *V. lecanii* could be clustered into 6 phylogenetic groups based on host-specific origin of the strains: aphids, different temperate insects, scale insects and other fungi (rusts).

Effect on species other than the target harmful organism

The notifier submitted open literature available upon the effects of *Verticillium lecanii* on non-target arthropods, which was reviewed independently by Verhaar (2000).

Quinlan and Chaudhry (University of Reading Appendix 1 of Verhaar, 2000), tested 3 strains of *Verticillium lecanii*; among which CBS 456.82 (MYCOTAL -strain) against 5 insect species: *Aedes aegyptae*, *Blatella germanica*, *Pieris brassicae*, *Encarsia formosa*, *Phytoseiulus persimilis*. *Verticillium lecanii* was suspended in tap water at a concentration of 2.5 g/L, and was applied as fine mist to the insects. No infectivity was observed for the first 3 species, and slight infectivity was observed for *Encarsia formosa* and *Phytoseiulus persimilis* only at a relative humidity (RH) of 90%, and not at RH 70%.

In 1986, Flexner et al. published a review on the effects of microbial pesticides on non-target beneficial arthropods, and cited Hall (1982) who showed unaffected *Encarsia formosa* at treatment of whitefly using an aphid *Verticillium lecanii* strain. Kanagaratnam et al. (1982) describe the compatibility of an aphid *Verticillium lecanii* strain with the beneficial insects *Phytoseiulus persim*ilis (against spider mites) and *Encarsia formosa* (against whitefly).

Bethke and Parella (1989) found no negative effects on the leafminer parasite *Dyglyphus beginii*. Only when confining parasites with infected aphids was an effect on longevity found. However, it is very unlikely that these insects (leafminer and aphid) will stay in close contact under practical circumstances.

In a study performed by the notifier (van Doorn, 1998) the use of MYCOTAL did not affect bumblebees.

Steenberg and Humber (1999) showed that a whitefly species was more susceptible to all *Verticillium*

strains/species than *Musca domestica* (the housefly) or *Alphitobius diaperinus* (a meal worm). Sitch and Jackson (1997) tested two aphid-*Verticillium lecanii* against 20 non-target arthropod-species (e.g. insects from the orders of Collembola, Hymenoptera and Coleoptera), and found no signs of infection in any of the 20 non-targets. The treatment mortality was below 10% in all non-target cases, classified as harmless according to the IOBC/WPRS (international organisation for biological control/ West Palaeartic Regional Section) Working Group "Pesticides and Beneficial Organisms". This group has developed testing methods and protocols for testing side-effects of chemical and microbial pesticides on natural enemies, including beneficial fungi (Sterk et al., 1999).

Sterk et al. (1999) report on the effects of *Verticillium lecanii* (used in the form of Micro Germin: containing two strains; Ve2 and VT1 (closely related to Ve6)) on 8 beneficials, and classified *V. lecanii* as harmless (< 30% effected), except the field test with *Typhlodromus pyri* in which it was classified as slightly harmful (25 - 50%).

Verticillium lecanii has not been observed as a pathogen on plants (Gams, 1971) or warm-blooded animals (Hall, 1981a).

New data 2016

The species *L. muscarium* is known to parasitize various hosts. *Lecanicillium* spp. are known for their activity against pest insects, phytoparasitic nematodes (e.g. *Lecanicillium psalliotae*, *Lecanicillium antillanum*, and others), and may be active as against various phytopathogenic fungi as powdery mildew or rust fungi (Goettel et al., 2008). However, host range is in general strain specific. For *L. lecanii* (or *L. muscarium*, respectively), Shinde et al. (2010) reviewed that this species is described to parasitize numerous hosts, but all reported insects parasiztized by *L. lecanii* are mainly sucking insects and pests (belonging to *Homoptera*, *Hemiptera*, *Tysanoptera* and *Acari*) may be infected by *L. lecanii*. This is confirmed by Zare & Gams, (2001, previously submitted) describing that "*Lecanicillium lecanii"* is primarily a pathogen of soft scale insects. Moreover, this species showed a large genetic and morphological variation when isolated from different geographical locations (abiotic conditions), insect-pest hosts (biotic conditions). Thus, the host range may vary strongly between strains, depending on the geographical and host origin.

Aqueel & Leather (2013) studied the virulence of *L. muscarium* Ve6, prepared from MYCOTAL, against the cereal aphids *Rhopalosiphum padi* and *Sitobaon avenae*. *L. muscarium* Ve6 was applied at a dose rate of 0.0, 2.5×10^{10}, 5×10^{10} and 10×10^{10} conidia/L. Seven days after application, the number of dead aphids, showing symptoms of fungal attack, were considered to be killed by *V. lecanii*. Besides the effect on pests, also the effect on the performance of the natural predators *Harmonia axyridis* and *Aphidius colemani* was tested. It was shown that *L. muscarium* was effective against the pests *R. padi* and *S. avenae*. The mortality was increased with the population density of *L. muscarium.* The LC_{50} of *L. muscarium* was determined as 4.8×10^{10} CFU/L and 5.3×10^{10} CFU/L for *R. padi* and *S. avenae*, respectively. It can therefore concluded, that *L. muscarium* Ve6 also affect cereal aphids.

To study the effect on the predation of *H. axyridis*, aphids were treated with *V. muscarium* and infected organisms fed to *H. axyridis* third-instar larvae after 72 h. The number of consumed infected larvae was compared to the consumption of uninfected larvae. Although fewer aphids were consumed by *H. axyridis* when treated with *L. muscarium* Ve6, no significant differences were observed on any stage of *H. axyridis* exept for *H. axyridis* adults feeding on *S. avenae* (consumption was reduced). Besides this, the effect of treatment on the parasitism, emergence and sex ratio of *A. colemani* was tested. It was shown that parasitism was not affected when aphids were exposed to *A. colemani* after treatment. However, the number of emergence of adults from parasitized mummies was lower when aphids were treated.

Report: KMA 2.3/03 –Aqueel, M.A., Leather, S.R. (2013),
Virulence of *Verticillium lecanii* (Z.) against cereal aphids; does timing of infection affect the performance of parasitoids and predators?
Published report,
Pest Management Science, 69: 493-498

Guideline: Not specified

GLP: No

Abstract **Background:**

Entomopathogenic fungi such as *Verticillium lecanii* (Z.) (Mycotal®) are used for pest control as an alternative to chemical control. In this study, the effect of *V. lecanii* on cereal aphids is assessed. In addition, an investigation is carried out to determine whether the use of *V. lecanii* affects the performance of two natural enemies of aphids, the predator *Harmonia axyridis* (P.) and the parasitoid *Aphidius colemani* (V.), in no-choice experiments under laboratory conditions.

Results:

The number of *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.) killed was increased by increasing the concentration of *V. lecanii*. The timing of application of fungus to aphids affected the efficacy of other biocontrol agents, a parasitoid and a predator. Parasitation by *A. colemani* (V.) in both cereal aphids (*S. avenae* and *R. padi*) was not affected by *V. lecanii* when aphids were first treated with *V. lecanii* and then exposed to *A. colemani*. The emergence of adults from parasitised mummies was, however, lower in infected aphids than in uninfected aphids when the aphids were first exposed to the parasitoids and then treated with fungus. The female sex ratio in the emerging adults was lower in *V. lecanii*-treated aphids in both species. When aphids were first treated with *V. lecanii*, 72 h before predation, fewer aphids of

both species were consumed by *H. axyridis* (P.).

Conclusion:

Use of entomopathogenic fungus as a biological control agent could be a complementary strategy in an integrated pest management program against cereal aphids, but it can reduce the efficiency of other biocontrol agents (parasitoids and predators) when applied simultaneously.

Materials and Methods: The active substance *L. muscarium* Ve6 was gained from the formulation MYCOTAL and prepared as a stock solution by mixing 10 g WP in 50 mL deionised water. *L. muscarium* was than applied at a dose rate of 0.0, 2.5×10^{10}, 5×10^{10} and 10×10^{10} conidia/L. The experiment was conducted on plants sown individually in pots in walk-in growth chambers.

To measure the LC_{50}, one adult aphid was placed on one plant and was allowed to reproduce overnight. Before the application (two weeks after reproduction), the number of mixed –instar larvae was counted (31.1 ± 1.51 for S. avenae and 39.7 ± 1.92 for *R. padi*). New produced nymphs were removed daily. Seven days after application, the number of dead aphids, showing symptoms of fungal attack, were considered to be killed by *L. muscarium.*

The effect of *L. muscarium* Ve6 treatment on parasitism, emergence and sex ratio of parasitoids was tested with female parasitoids on treated (72 h before contact) aphids. Parasitoids were removed the following day and the new born nymphs were removed daily. To measure successful parasitism, the numbers of mummies formed were counted and the percentage parasitism was calculated for *L. muscarium*-infected and uninfected aphids. In the second part of this experiment, parasitoids were released first onto the aphids and then the aphids were treated 72 h after parasitoid contact to determine the percentage emergence and female sex ratio of parasitoids. Aphids were checked daily until mummy formation. Sex ratio of parasitoids was determined under binocular and was expressed in the proportion of females and compared to uninfected aphids.

To study the relationship between the ladybird *Harmonia axyridis* and *L. muscarium*, Aphids were treated with *L. muscarium* Ve6 and were fed to 10 third-instar larvae of *H. axyridis* when aphids began to show symptoms of fungus infection (pale green and swollen). 10 additional *H. axyridis* larvae were fed to uninfected aphids. The number of aphids consumed by each larva within a 24 period was rec-

orded. Forth-instar larvae and adults were provided with 90 aphids, and the same procedure was repeated to compare the predation rates.

Findings: It was shown, that *L. muscarium* was effective against the pests *R. padi* and *S. avenae.* The mortality was increased with the population density of *L. muscarium*. The LC_{50} of *L. muscarium* was determined as 4.8×10^{10} CFU/L (4.38 - 5.14 × 10^{10} CFU/L) and 5.3×10^{10} CFU/L (4.82 - 5.81 × 10^{10} CFU/L) for *R. padi* and *S. avenae*, respectively.

When aphids were first treated with *L. muscarium* and then exposed to *A. colemani*, the number of parasitized aphids was not significantly affected by *L: muscarium.*

When aphids were first exposed to the parasitoids and than exposed to the parasitoids, the number of parasitized mummies was lower in infected aphids than in uninfected aphids (see figure below).

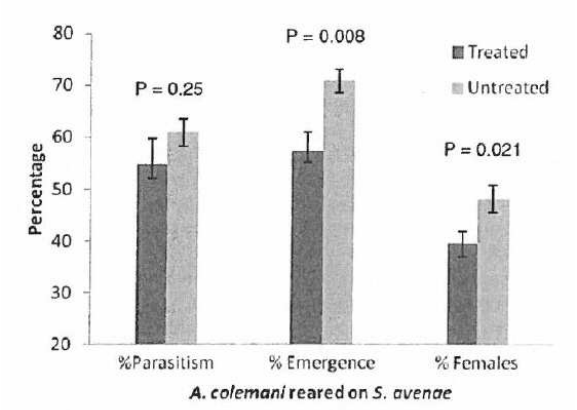

Fig. 2.3-1 Percentage parasitism of *A. colemani* on *V. lecanii*-treated (72 h before parasitism) and untreated *S. avenae*. Percentage emergence and female sex ratio of *A. colemani* when reared on *V. lecanii*-treated (72 h after parasitism) versus untreated *S. avenae* (mean ± SEM, n = 10).

Although fewer aphids were consumed by *H. axyridis* when treated with *L. muscarium* Ve6, no significant differences were observed on any stage of *H. axyridis* exept for *H. axyridis* adults feeding on *S. avenae*.

Conclusions: Application of *L. muscarium* Ve6 effective reduces the aphid pests *R. padi* and *S. avenae* with an LC_{50} of 4.8×10^{10} CFU/ and 5.3×10^{10} CFU/L, respectively.

Application of *L. muscarium* Ve6 did not affect the performance of the natural aphid predator *A. colemani*. However, percentage of newly emerged parasitoids was reduced when aphids were treated after parasitoid contact. It was concluded that *V. muscarium* could be recommended as a biological control agent in an integrated pest management program against cereal aphids.

One article studies the virulence of various *L. muscarium* and *L. longisporum* strains to the white pine weevil, *Pissodis strobi*, a pest of forest trees in Canada and the United States (Kope et al., 2008). Besides indigenous strains, isolated from infected *P. strobi* cadavers and from soil, efficacy of *L. muscarium* Ve6 on the pests was included in the evaluation. Weevils were dipped in conidial sus-

pensions of 1×10^7 conidia/mL for 10 seconds. Weevil mortality was recorded daily for a total of 17 days. Although the commercial isolate *L. muscarium* Ve6 showed a high cumulative mortality under laboratory conditions, other indigenous *Lecanicillium* sp. strains were more effective. This study showed that *L. muscarium* Ve6 may also be used for the control of the weevil pest *P. strobi*. However, results from laboratory trials do not necessarily need to reflect natural conditions.

Report: MA 2.3/04 –Kope, H.H., Alfaro, R.I., Lavallee, R. (2008),
Effects of temperature and water activity of *Lecanicillium* spp. conidia germination and growth, and mycosis of *Pissodes strobi*
Published report,
BioControl, 53: 489-500

Guideline: Not specified

GLP: No

Abstract Selecting entomopathogenic fungal isolates for use as biocontrol agents requires an assessment of their growth and virulence characteristics as affected by environmental conditions. Here we demonstrate a wide temperature and moisture range for colony growth, effective conidial germination and virulence against *Pissodes strobi* Peck (white pine weevil) of several isolates of *Lecanicillium* Gams and Zare, an entomopathogenic fungus distributed worldwide and indigenous to forests on Vancouver Island, British Columbia, Canada. In order to examine the potential *Lecanicillium* as a biological control agent, the pathogenicity of isolates collected from different geographical locations on *P. strobi* cadavers was assessed, and colony growth at different temperatures was evaluated. Colony growth was evident between 5 and 30°C, with optimal growth occurring at 25°C. Various combinations of water activity (0.55, 0.76, 0.85 and 0.99 a w) and temperature (10, 15, 20, and 25°C) were also used to evaluate environmental impacts on conidial germination and cumulative mycosis of adult *P. strobi*. Certain *Lecanicillium* isolates displayed xerophilic (0.85 a w) or psychrophilic (10°C) growth optima. Ultimately, identifying the abiotic limits of this entomopathogenic fungus will be used to determine which isolates have potential for future in situ biocontrol trials.

Materials and Methods: This study was conducted under laboratory conditions. Live weevils were collected from different locations in Vancouver Island, Canada. The adults were maintained in boxes. Cadavers were collected, incubated on moistened filter paper in a sealed Petri plate and observed daily for the development of conidia.

For the application trials with *L. muscarium* Ve6, the formulated product Mycotal® was used. Addi-

tionally, 15 isolates of *Lecanicillium* sp., originating from adult *P. strobi*, and the commercial a.i. from Vertalec® were used.

To measure the colony growth at different temperatures, each colony a freshly grown 5 mm plug was placed in the center of an SDA plate and incubated in darkness at 5, 10, 15, 20, 25, or 30°C for 21 days. Radial growth was measured every 3 days. The experiment was repeated once.

To measure the *Lecanicillium* efficacy to *P. strobi* at different water activities and temperatures, a conidial suspension of 1×10^7 conidia/mL was prepared with Mycotal®, Vertalec® and indigenous *Lecanicillium* sp. strains. For inoculation, eight 4-month old weevils were used and dipped into the conidial suspensions for 10 seconds. Plastic incubation boxes with salt solutions adjusted to a water activity of 0.88-0.85 a_w were used. Each box was sealed and incubated at 10, 15, 20 and 25°C. Every 3 days the number of alive and dead weevils was recorded. The trial was set up in a completely randomized design and repeated once.

Findings: It was shown, that growth of *L. muscarium* Ve6 was increased with temperatures from 5°C (0.22 mm/day) to 25°C (2.03 mm/day), with a maximum growth at 25°C. At 30°C, the growth decreased strongly (0.59°C).

Cummulative mortality of *P. strobi* was highest at 25°C (58.3%), whereas the indigenous strain PFC6 (*L. muscarium*) was much more effective (79.2%) than *L. muscarium* Ve6. For more details, please see the table below.

Table 2.3-1: Cummulative mortality (%) of *P. strobi* infected by *L. longisporum*, *L. muscarium* and *L. pissoidis* and incubated at four temperatures with a low water activity for 15 days.

Temperature (C°)	10	15	20	25
Water activity (a_w)	0.88	0.865	085	0.85
Lecanicillium longisporum				
Vertalec®	0.0	0.0	17.0 a	41.6 d
Lecanicillium muscarium				
Mycotal®	0.0	0.0	12.0 a	58.3 d
PFC 5	0.0	0.0	0.0	12.0 b
PFC 6	8.3 a	17.0 a	33.3 b	79.2 e
PFC 10	0.0	25.0 b	17.0 a	37.5 c
PFC 14	0.0	0.0	0.0	4.2 a
Lecanicillium pissodis				
PFC 19	8.3 a	58.3 c	41.6 b	37.5 c

The mean values for mortality are Abbott's formula calculations which corrected for natural deaths in the control and calculates the proportion of insects killed by the entomopathogen alone. Means in the same column followed by different letters abcde are significantly different ($P < 0.05$, Tukey test)

Conclusions: *L. muscarium* Ve6 was shown to be active against the pest *Pissodes strobi* at 20°C and 25°C under laboratory conditions when inoculated directly for 10 seconds in a conidial suspension, containing 1×10^7 conidia/mL. It is assumed that *L. muscarium* Ve6 might be used for an effective control of the white pine weevil. However, laboratory conditions might not be totally transferrable to

natural conditions.

Cited references (abstracts):

Report MA 2.3/01 - Goettel, M.S., Koike, M., Jun Kim, J., Aiuchi, D., Shinya, R., Brodeur, J.. (2008), Potential of *Lecanicillium* spp. for Management of insects, nematodes and plant diseases
Published report,
J. Invertebr. Pathol., 98: 256-261

Abstract: Fungi in the genus *Lecanicillium* (formerly classified as the single species *Verticillium lecanii*) are important pathogens of insects and some have been developed as commercial biopesticides. Some isolates are also active against phytoparasitic nematodes or fungi. *Lecanicillium* spp. use both mechanical forces and hydrolytic enzymes to directly penetrate the insect integument and the cell wall of the fungal plant pathogen. In addition to mycoparasitism of the plant pathogen, the mode of action is linked to colonization of host plant tissues, triggering an induced systemic resistance. Recently it was demonstrated that development of *Lecanicillium* hybrids through protoplast fusion may result in strains that inherit parental attributes, thereby allowing development of hybrid strains with broader host range and other increased benefits, such as increased viability. Such hybrids have demonstrated increased virulence against aphids, whiteflies and the soybean cyst nematode. Three naturally occurring species of *Lecanicillium*, *L. attenuatum*, *L. longisporum*, and an isolate that could not be linked to any presently described species based on rDNA sequences have been shown to have potential to control aphids as well as suppress the growth and spore production of *Sphaerotheca fuliginea*, the causal agent of cucumber powdery mildew. These results suggest that strains of *Lecanicillium* spp. may have potential for development as a single microbial control agent effective against several plant diseases, pest insects and plant parasitic nematodes due to its antagonistic, parasitic and disease resistance inducing characteristics. However, to our knowledge, no *Lecanicillium* spp. has been developed for control of phytopathogens or phytoparasitic nematodes.

Report MA 2.3/02 - Shinde, S.V., Patel, K.G., Purohit, M.S., Pandya, J.R., Sabalpara, A.N. (2010), *Lecanicillium lecanii* (Zimm.) zare and games an important biocontral Agent for the Management of insect pests - A review
Published report,
Agri. Review, 31: 235-252

Abstract: The Entomopathogenic fungus, *Lecanicillium lecanii* (Zimm.) Zare and Games is one of the potential microbial biocontrol agents which have wide host range. The present review article contains the information related to its taxonomic position, mode of action, toxins and extracellular enzymes produced by the fungus. The various approaches for mass multiplication, carriers for development of commercial formulations and shelf life of formulated products of the fungus are reviewed and discussed. The factors like genetic variability, tritropic interaction, temperature, humidity, formulation base, inoculum level, isolation host, stage of insect host, compatibility with chemical pesticides which affect the performance of the fungus are presented and discussed. Response of *L. lecanii* to different insect pests, under protected conditions and filed conditions, and its effect on non targeted organisms are reviewed and discussed.

B.2.3 Development stages/life cycle of the micro-organism

Information from the original DAR

Verticillium lecanii is an extremely wide-spread spore-forming fungus that produces asexual mitosporic structures (called conidia) directly from the vegetative state. *Verticillium* produces conidia as aggregates in slimy heads (Samson et al., 1984), which are dispersed by contact and hardly get airborne. The spores need a high humidity to germinate, and have a temperature-optimum for growth of 26°C. *V. lecanii* is non-fastidious and will grow on all conventional mycological media; malt extract agar, Sabouraud and potato dextrose agars (Hall, 1981a).

New data 2016

Previously description of the live cycle is confirmed by Vasantharaj & Selvaraj (2007). They described the conidia, aggregated in slimy false heads in groups of 6 - 72. After germination of the conidia, the hyphae grow over the surface of the insect and penetrate through the integument. The authors also confirmed humidity as the most limiting factor for conidial germination and growth.

B.2.4 Infectiveness, dispersal and colonisation ability

Information from the original DAR

Six publications were submitted with regard to the infectiveness, dispersal and colonisation ability.

The MYCOTAL strain of *Verticillium lecanii* (Ve6) was tested by the notifier to obtain the optimum temperature and humidity demands. Spores of *Verticillium lecanii* strain Ve6 germinate and grow radially between 5°C and 30°C: slowest at 5°C, fastest at 21°C. At 37°C germination of some spores was obtained. As no further growth occurred, it was concluded that these spores died after initial sporulation at 37°C. Optimum growth occurred at 21°C, and highest efficacy against *Trialeurodes vaporaroirum* was obtained at high relative humidity (Koppert BV,October 2001).

In a very limited study of Quinlan, effects on fruit ripening and storage were reported (no specific study details were given). Tomato fruits had been dipped in a spore suspension of 2.5 g/L MYCOTAL and stored at 22°C for 13 days. The results implied that MYCOTAL had no effect on tomato fruit ripening or storage and no fungal growth was visible on the tomatoes (Quinlan, date unknown, source unknown,1900a).

In another very limited study of MacQueen & Quinlan, MYCOTAL was applied to fruiting tomato in peat-based growbags. Samples of fruit and leaves were taken at day 0, 1, 2, 3, 4, 11, and 12. Leaves and fruits were washed and dilutions of the wash were plated onto malt extract agar. The numbers of colonies per plate were counted after 5 days. Numbers of colonies on leaves washed from the leaves rose significantly from day 4 up to day 12 (250 fold).

The number of colonies on fruit never exceeded 40% of the original count (MacQueen & Quinlan, date unknown, source unknown 1900a).

Spores of *V. lecanii* KV01 deposited on chrysanthemum or rose leaves remained viable up to 11 (75 - 80%) and 15 days (1 - 2%), respectively. Spores on chrysanthemum leaves were still capable of infecting thrips at that time. A difference in persistence was observed on different leaf surfaces. *Verticillium lecanii* performed significantly better on cucumber, gerbera and tomato than on poinsettia (Beerling et al., 1998). Beerling et al. suggested that several plant characteristics may play a part in the differences in the host plant influence, such as the micro-climate at leaf surface; the chemicals produced by the plant which stimulate or inhibit germination of the fungus. In addition the plant may also influence the infection process indirectly by influencing development rate and susceptibility of the insect for fungal infection. (Beerling et al., 1998).

The mechanism of dispersal is not exactly known. It has been speculated that insects and soil organisms take spores with them from the soil to the leaves, after which other insects can be infected too. Spores are not spread by air, and are not released from conidiophores without water contact. Conidia released in this way, however, have a short life span after drying up, preventing spread of infection by the air (Hall, 1981b referred to in Rombach and Gillespie, 1988). Passive spread can occur by means of splashing, and probably by mechanic transfer by other Arthropods present in the greenhouse (Rombach and Gillespie, 1988).

The study of Hall (1981) submitted by the notifier was not the article Rombach and Gillespie referred to. This article of Hall concerned the compatibility/ incompatibility of other insecticides and fungi-

cides when using Verticillium lecanii and is not relevant for the assessment of infectiveness, dispersal and colonisation ability.

New data 2016

From an independent literature search, one article was identified studying the effect of temperature and water activity on the growth rate of *Lecanicillium* sp. including *L. muscarium* Ve6 (Kope et al., 2008). The effect of temperature on growth rate was measured on SDA plates for 21 days at incubation temperatures of 5, 10, 15, 20, 25, and 30°C. The radial growth rates (mm/day) significantly increased with the temperature up to 25°C (2.03 mm/day), whereas the growth rate declined strongly at 30°C (0.59 mm/day). The influence of the moisture content at different temperatures was expressed as water activity, which is equal to the equilibrium relative humidity (RH %), but expressed as a fraction. It was shown that germination of *L. muscarium* increased with there moisture levels. This confirms previously findings that moisture conditions are essential for the effective use of *L. muscarium* Ve6.

Report: MA 2.5/01 –Kope, H.H., Alfaro, R.I., Lavallee, R. (2008),
Effects of temperature and water activity of *Lecanicillium* spp. conidia germination and growth, and mycosis of *Pissodes strobe*
Published report,
BioControl, 53: 489-500

Guideline: Not specified

GLP: No

Abstract Selecting entomopathogenic fungal isolates for use as biocontrol agents requires an assessment of their growth and virulence characteristics as affected by environmental conditions. Here we demonstrate a wide temperature and moisture range for colony growth, effective conidial germination and virulence against *Pissodes strobi* Peck (white pine weevil) of several isolates of *Lecanicillium* Gams and Zare, an entomopathogenic fungus distributed worldwide and indigenous to forests on Vancouver Island, British Columbia, Canada. In order to examine the potential *Lecanicillium* as a biological control agent, the pathogenicity of isolates collected from different geographical locations on *P. strobi* cadavers was assessed, and colony growth at different temperatures was evaluated. Colony growth was evident between 5 and 30°C, with optimal growth occurring at 25°C. Various combinations of water activity (0.55, 0.76, 0.85 and 0.99 a w) and temperature (10, 15, 20, and 25°C) were also used to evaluate environmental impacts on conidial germination and cumulative mycosis of adult *P. strobi*. Certain *Lecanicillium* isolates displayed xerophilic (0.85 a_w) or psychrophilic (10°C) growth optima. Ultimately, identifying the abiotic limits of this entomopathogenic fungus will be used to

determine which isolates have potential for future in situ biocontrol trials.

Materials and Methods: This study was conducted under laboratory conditions. Live weevils were collected from different locations in Vancouver Island, Canada. The adults were maintained in boxes. Cadavers were collected, incubated on moistened filter paper in a sealed Petri plate and observed daily for the development of conidia.

For the application trials with *L. muscarium* Ve6, the formulated product Mycotal® was used. Additionally, 15 isolates of *Lecanicillium* sp., originating from adult *P. strobi*, and the commercial isolate from Vertalec® were used.

To measure the colony growth at different temperatures, each colony a freshly grown 5 mm plug was placed in the centre of an SDA plate and incubated in darkness at 5, 10, 15, 20, 25, or 30°C for 21 days. Radial growth was measured every 3 days. The experiment was repeated once.

To measure the *Lecanicillium* efficacy to *P. strobi* at different water activities and temperatures, a conidial suspension of 1×10^7 conidia/mL was prepared with Mycotal®, Vertalec® and indigenous *Lecanicillium* sp. strains. For inoculation, eight 4-month old weevils were used and dipped into the conidial suspensions for 10 seconds. Plastic incubation boxes with salt solutions adjusted to a water activity of 0.88-0.85 aw were used. Each box was sealed and incubated at 10, 15, 20 and 25°C. Every 3 days the number of alive and dead weevils was recorded. The trial was set up in a completely randomized design and repeated once.

Findings: It was shown, that growth of *L. muscarium* Ve6 was increased with temperatures from 5°C (0.22 mm/day) to 25°C (2.03 mm/day), with a maximum growth at 25°C. At 30°C, the growth decreased strongly (0.59°C). Please see **Table 2.4-1.**

Table 2.4-1: Effect of temperature (°C) on mean radial growth rates (mm/day) of single spore isolates of *L. longisporum*, *L. muscarium* and *L. pissodis*

Isolate	Temperature (°C)					
	5	10	15	20	25	30
Lecanicillium longisporum						
Vertalec®	0.21 (0.00) b	0.66 (0.010) f	1.10 (0.010) f	1.31(0.010) c	1.86 (0.010) d	0.55 (0.010) d
Lecanicillium muscarium						
Mycotal®	0.22 (0.005) c	0.59 (0.010) c	1.03 (0.010) c	1.59 (0.010) g	2.03 (0.010) h	0.59 (0.010) d
PFC 1	0.16 (0.013) a	0.43 (0.005) b	0.90(0.010) a	1.13 (0. 005) a	1.64 (0.005) c	0.69 (0.010) e
PFC 3	0.15 (0.003) a	0.54 (0.010) c	1.03 (0.004) c	1.35 (0. 010) d	1.86 (0.010) d	0.83 (0.005) f
PFC 4	0.26 (0.006) e	0.63 (0.010) e	1.10 (0.010) e	1.32 (0.005) c	1.86 (0.005) d	0.52 (0.010) d
PFC 5	0.18 (0.003) a	0.58 (0.004) c	0.96 (0.010) b	1.41 (0.005) e	1.93 (0.010) f	0.92 (0.010) h
PFC 6	0.27 (0.003) f	0.59 (0.005) c	1.06 (0.010) d	1.37 (0.005) d	1.83 (0.005) d	0.53 (0.010) d
PFC 9	0.20 (0.006) a	0.58 (0.010) c	1.05 (0.005) d	1.35 (0.005) d	1.80 (0.010) d	0.92 (0.010) h
PFC 10	0.18 (0.009) a	0.63 (0.010) e	1.02 (0.005) c	1.40 (0.005) e	2.07 (0.010) h	0.05 (0.008) a
PFC 11	0.17 (0.006) a	0.58 (0.005) c	1.03 (0.010) c	1.25 (0.010) b	2.05 (0.005) h	0.05 (0.010) a
PFC 12	0.19 (0.009) a	0.64 (0.005) f	1.05 (0.005) d	1.68 (0.010) i	2.01 (0.010) g	0.89 (0.010) g
PFC 13	0.16 (0.005) a	0.38 (0.010) a	0.97 (0.010) b	1.14 (0.010) a	1.10 (0.010) a	0.45 (0.010) c
PFC 14	0.14 (0.005) a	0.83 (0.010) h	1.10 (0.010) f	1.35 (0.010) d	1.24 (0.010) b	0.38 (0.010) b
PFC 16	0.21 (0.010) b	0.55 (0.010) c	1.18 (0.005) h	1.28 (0.005) b	1.93 (0.010) f	0.69 (0.011) e
Lecanicillium pissodis						
PFC 17	0.24 (0.010) d	0.62 (0.010) d	1.00 (0.005) b	1.48 (0.010) f	1.93 (0.005) f	0.79 (0.010) f
PFC 18	0.17 (0.005) a	0.69 (0.010) g	1.14 (0.010) g	1.39 (0.011) e	1.90 (0.005) e	0.59 (0.010) d
PFC 19	0.21 (0.005) b	0.52 (0.010) c	1.00 (0.005) b	1.62 (0.005) h	1.97 (0.010) f	0.83 (0.010) f

Standard errors are in brackets after each mean. Means in the same columns followed by a different letter are significantly different ($P < 0.05$, Tukey test)

Conclusions: *L. muscarium* Ve6 was shown to be active against the pest *Pissodes strobi* at 20°C and 25°C under laboratory conditions when inoculated directly for 10 seconds in a conidial suspension, containing 1×10^7 conidia/mL. It is assumed that *L. muscarium* Ve6 might be used for an effective control of the white pine weevil. However, laboratory conditions might not be totally transferrable to natural conditions.

B.2.5 Relationships to known plant or animal or human pathogens

Information from the original DAR

The genus *Verticillium* has been divided into four sections (Gams, 1971; Gams & van Zaayen, 1982; Bidochka et al., 1999) with *V. lecanii* belonging to the section Prostrata.
Plant pathogenic species (*V. albo-atrum*, *V. nigrescens*, *V. tricorpus* and *V. dahliae* (all causing verticillium wilt)) are included in the section Nigrescentia. *V. fungicola*, causing dry bubble in commercially grown mushrooms is included in the section Albo-erecta.
The enzyme production differs for entomopathogenic and plant pathogenic sections of *Verticillium* species (Bidochka et al., 1999). Entomopathogenic species produce chitinase, whereas the plant-pathogens produce pectinase. Therefore, the RMS agrees with the notifier that it is unlikely that entomopathogenic species like *V. lecanii* will become pathogenic to plants.
No human pathogens are known in the genus *Verticillium.* However, under certain conditions naturally occurring spores of *V. lecanii* may cause hypersensitivity responses. Darke et al. (1976) reported sensitivity of workers harvesting grain to *V. lecanii* spores. Engelhart et al. (2000) reported hypersensitivity pneumonitis of a patient exposed to humidifier water from a humidification, ventilation and air conditioning system, contaminated with *V. lecanii.*

Three publications obtained from public literature report human infections by species from the genus *Verticillium*, which have not been identified upon strain level:
- Das et al. (1997) describe one case in which Verticillium was isolated from a swelling on the arm. This patient had several underlying diseases (after kidney removal the patient received radiotherapy and chemotherapy). The lesion on the left arm responded to antifungal therapy and the swelling disappeared gradually.
- Grandesso et al. (1996) reported on seven patients that suffered from a fungal peritonitis of which in 1 case a *Verticillium* species was identified as the causative agent. All these patients had been treated for bacterial peritonitis and were treated by intraperitoneal antibiotics in the previous two months. Patients were cured by removal of the catheter and antifungal therapy.
- Shin et al. (2002): A 50-year-old man suffered from a case of infectious keratitis caused by a *Verticillium* species, without history of trauma. The patient recovered after antifungal therapy. The author emphasizes that *Verticillium* species are very rare causes of keratitis.

New data 2016

No new data is to be submitted under this point.

Previously submitted information is considered to be acceptable to cover current requirements. Please refer to B.6 for the update of case reports on *Lecanicillium* sp. or *Verticillium lecanii*, respectively.

Lecanicillium muscarium is not known to be a human pathogen or to cause effects on human health.

B.2.6 Genetic stability and factors affecting it

Information from the original DAR

Verticillium lecanii strain Ve6 was stored by the notifier at -85°C and a very low number of generation was used for the production of final product. No alternation in virulence was noted (see Volume 4, annex C). This is considered acceptable.

New data 2016

A comprehensive literature search including genetic stability of fungal biocontrol agents was published in external scientific report for EFSA. The Conclusions of this literature review is provided below. All reports cited in the below paragraph are cited in Mudgal et al., 2013.

"Compared to bacteria, fungi possess low genome plasticity due to the narrower scope of mechanisms available to eukaryotic cells for the incorporation of DNA (Coehlo et al., 2013). In line with this, horizontal gene transfer HGT is still considered to be anecdotal in fungi (Rosewich et al., 2000), although several well-supported events of acquisition of genes from bacteria by conjugation and transformation (Marcet-Houben et al., 2010; Syvanen, 2012) and transfers among fungal species have been reported and are contributing to change this view (Slot et al., 2011; Novo et al., 2009; Khaldi et al., 2008). Nevertheless, those evidences of HGT between fungi did not concern species used as biocontrol agents.

For example, Khaldi et al. (2008) and Slot et al. (2011) respectively described the horizontal transfer of secondary metabolite gene clusters between several fungi - *Magnaporthe grisea*, *Chaetomium globosum*, *Stagonosporanodorum*, *Aspergillus clavatus* and *Aspergillus nidulans* - but none of them are used as fungal biocontrol agents. In the same manner, Novo et al. (2009) provides evidence that gene transfer may occur between *Saccharomyces* and non-*Saccharomyces* species.

Genetic recombination in asexual fungi can occur through the parasexual cycle, during which vegetatively compatible hyphae fuse to form heterokaryonts and exchange genetic material (Castrillo et al., 2004). Parasexuality has been demonstrated in numerous fungi under laboratory conditions, but its occurrence in nature is difficult to detect. While studies on asexual fungi have revealed that nearly all fungi examined exhibit recombining population structures in addition to clonal reproduction, current available tests cannot determine how recombination occurs or how often (Taylor et al., 1999).

Under field conditions in which endemic infections of a pest controlled by a fungal MPCA occur, inundative application of another strain makes co-infections possible or even likely. However, little information is available either on the potential for genetic recombination between strains of fungal MPCAs in agricultural fields and on whether this recombination could result in altered virulence and host range. For example, the frequency of genetic recombination between co-infecting strains of *B. bassiana* strains in a susceptible insect host, the Colorado potato beetle, *Leptinotarsa decemlineata* was assessed in agricultural fields and was evaluated as 5-17% for compatible strains and 0% for non compatible strains (Castrillo et al., 2004). This frequency of recombination of exogenous DNA was higher than the frequency 10^{-3}–10^{-7} estimated by Caten (1981) based on *in vitro* data, but lower than

those reported (90%) in co-inoculation studies with *Paecilomyces fumosoroseus* (Riba et al, 1984). Co-inoculations studies conducted by Leal-Bertioli et al. (2000) and Wang et al. (2002) reported recombination frequency of 10–33% in *M. anisopliae* and 43% in *B. bassiana*, respectively, using wild type strains."

Since fungi possess low genome plasticity and considering that horizontal gene transfer HGT is still considered to be anecdotal in fungi the issue of genetic stability is not considered to be of concern for *Lecanicillium muscarium* Ve6.

Note RMS: Please note that at the moment no conclusion can be drawn if *V. lecanii* complies with the new low risk criteria (see B.2.8). Should the microorganism show multiple resistance to antibiotics than further information on the potential of genetic transfer of resistance genes would be needed. Applicant could then discuss in more depth horizontal gene transfer HGT based on the approach described in EFSA guidance 2017 on food additives (EFSA 2017 Guidance on the characterisation of microorganisms used as feed additives or as production organisms (public consultation version)). In the WG biopesticides this approach was discussed as proposed to be used for micro-organisms intended as plant protection products considering the low risk criteria in the future. Since the data are not strain specific (Verticillium spp.) information on resistance gene transfer to antibiotics or other antimicrobial agents (than Fluconazole and Amphotericin B) should be included in this section.

B.2.7 Information on the production of metabolites (especially toxins)

Information from the original DAR

Several publications were submitted on the production of metabolites by *Verticillium lecanii*. Different strains isolated from different sources were described and metabolite production appeared to be very strain specific, i.e. different strains appeared to produce a different combination of metabolites. Several toxins/metabolites have been reported to be secreted by this fungus:

Kanaoka et al. (1978) and Murakoshi et al. (1978) reported production of Bassianolide, a cyclodepsipeptide, which appeared toxic to silkworms (*Bombyx mori* L.).

Claydon and Grove (1982) reported production of Dipicolinic acid (pyridine-2, 6-dicarboxylic acid) by several strains of *V. lecanii* isolated from the silkworm.

Gindin et al. (1994) extracted phospholipids (that are toxic to *Bemisia tabaci* (sweetpotato whitefly)) from a culture of *V. lecanii* isolated from *Oxycarenus hyalinipennis* in Israel.

Soman et al. (2001) reported extraction of Vertilecanins toxic to *Helicoverpa zea*. At least a part of these toxic substances are however, also synthesized by some non-entomopathogenic and non-entomogenous fungi.

Destruxin (dtx) production by *V. lecanii* has never been documented before and only a limited number of compounds have been described for this fungus. However, the notifier also submitted a more recent,

final report of the RAFBCA project, in which dxt A, B and E were found in some extracts at specific stages of the production process. RAFBCA is funded under the Fifth Framework Programme of the European Commission, Quality of Life and Management of Living Resources Programme (QoL), Key Action 1 - Food, Nutrition and Health. This EC-project's general objective is the identification of metabolites, produced by fungal biological control agents (BCAs) and to establish, whether they enter the food chain and if they pose a risk to human and animal health. This project will generate data that could help address key registration questions (see also http://www.rafbca.com/index.asp). One of the micro-organisms studied in this project is the active ingredient of MYCOTAL, *Verticillium lecanii* strain KV01 (Ve6). As part of this RAFBCA-project, Partner 1 (Butt et al., 2004) describes the identification and quantification of the main metabolites, the persistence of the metabolites and how these metabolites enter the food chain. The last two aspects are addressed in section B.7. The aspect of identification and quantification is summarised below:

Isolation of the fungal metabolites

For isolation and characterization of the metabolites from *Verticillium lecanii* strain KV01 (= MYCOTAL) and strain KV71 (=Vertalec), liquid cultures were grown in liquid medium (under shake and still conditions) and solid state cultures were grown on rice. Liquid culture filtrates were extracted after 15 days of incubation; solid-state culture filtrates after 40 days incubation, both at 26ºC in the dark. Filtrates were extracted with the non-polar solvent dichloromethane or the polar solvent ethyl acetate. Extracts were also made from mycelial material in the liquid – shaken and still – cultures, and extracts from filtrates. Mycelium was crushed using liquid nitrogen, extracted with methanol and partitioned against ethyl acetate. The solvents were evaporated and re-dissolved in proper eluate.

Characterization of the fungal metabolites

Identification was performed using HPLC-analysis in combination with mass-spectroscopy. Dtx were not found in filtrates of liquid or solid cultures nor in the mycelium from strain KV01 (=Ve6, MYCOTAL). Dtxs were also not found in extract from rice cultures. Only extracts from cultures grown under laboratory-scale still liquid conditions of strain KV71 (=Ve2, Vertalec) revealed two groups of peaks (grouped in time) in the HPLC chromatogram. The group eluted first contained the so-called destruxins (dtxs) A, B and E, which are cyclic peptides. This was confirmed by fortifying the extracts with pure isolates of these destruxins. The group eluted secondly contained unidentified compounds. They were characterized as lipophilic compounds with long C-chains and molecular masses of about 500 - 600 g/mol (which excluded aphidicolin).

Interestingly, Partner 3 of the RAFBCA project had identified a number of dtxs in extracts from still liquid cultures from KV01 but metabolite production of KV71 had not been investigated. Considerable variation between different batches as observed for KV71 by partner 1 and higher sensitivity of the

mass spectroscopy (MS) techniques utilised by Partner 3, which had been specifically adapted to the detection of dtxs, might explain this difference.

Fungal metabolite production appeared very dependent on the culture conditions and strain.

Concluding it can be said that dtxs were not produced by KV01 (=Ve6) when produced under conditions as practised in commercial situation (solid state fermentation and in aerated (shaken) liquid fermentation). The destruxins were also not detected in the commercial product MYCOTAL or its non-formulated spores.

New data 2016

Lecanicillium muscarium Ve6 is not known to produce secondary metabolites of toxic relevance. However, production of destruxins is described for related strains formerly described as *Verticillium lecanii.* In a study by Kouvelis et al. (2011), the crude extracts of various biocontrol fungi were tested on their cytotoxic and mutagenic effects by use of the Ames assay and the VITOTOX® test. Besides strains of the genera *Beauveria*, *Gliochladium*, *Trichoderma*, and *Metarhizium*, the strains *L. muscarium* Ve6 and *Lecanicillium longisporum* (Vertalec) were tested for their production of toxic metabolites. Neither the Ames assay, nor the VITOTOX® test showed any toxic effects by the crude extracts the liquid culture supernatants of the tested fungal species. This confirms the absence of the production of toxic metabolites by *L. muscarium* Ve6.

Report: KMA 2.8/01 –Kouvelis, V.N., Wang, C., Skrobek, A., Pappas, K.M., Typas, M.A., Butt, T.M., 2011

Assessing the cytotoxic and mutagenic effects of secondary metabolites produced by several fungal biological control agents with the Ames Assay and the VITOTOX test

Published report,

Mutation Research/Genetic toxicology and Environmental Mutagenesis, 722: 1-6

Guideline: Not specified

GLP: No

Abstract The potential genotoxic effects of several pure secondary metabolites produced by fungi used as biological control agents (BCAs) were studied with the Ames *Salmonella*/microsome mutagenicity assay and the Vitotox test, with and without metabolic activation. A complete set of *Salmonella* tester strains was used to avoid false negative results. To detect possible mutagenic and/or cytotoxic effects of fungal secondary metabolites due to synergistic action, crude extracts and fungal cell extracts of the BCAs were also examined. Although the sensitivity of the methods varied depending on the metabolite used, clearly no genotoxicity was observed in all cases. The results of the two assays

are discussed in the light of being used in a complementary fashion for a convincing risk-assessment evaluation of fungal BCAs and their secondary metabolites.

Materials and Methods:

For the Ames and the vitotox assay various fungal biological control strains were used, including *L. muscarium* Ve6 obtained from the formulated product MYCOTAL. For positive control, *Salmonella typhimurium* tester strains were included in the tests. Crude extracts from *L. muscarium* Ve6 were extracted by enrichment of the cell number in liquid medium.

Ames test was performed by a standard approach and mutagenicity tests were performed by use of a pre-incubation procedure in the presence or absence of S9 mix as an external enzymatic metabolizing system. Tests were obtained with the pure relevant metabolites described for the respective microorganisms (destruxins for *L. muscarium*) and the crude extracts. The test samples consisted of 100 µL bacterial suspension (~ 108 bacteria/mL), 100 mL test solution and 500 µL S9 mix or 500 µL phosphate buffers in experiments without external metabolizing system. All substances were dissolved in methanol or methanol/acetonitrile (1:1, v/v) and used at concentrations of 5, 10, 50, 100 µg/plate for pure metabolites and 50, 100, 250, 500 µg/plate for crude extracts.

The vitotox assays were performed with a VITOTOX® test-kit (Thermo Labsystems, Finland) according to the manufacturer's instruction.

Findings: At tested maximum concentrations, no genotoxicity was detected for either the pure metabolites or the crude extracts of all tested biological control actives in the Ames test. Positive control substances showed clear mutagenic effects.

In the vitotox test neither pure metabolites nor crude extracts from all tested strains had any genotoxic effect on the tester strains. However, different levels of cytotoxicity (S/N < 0.8) were obtained at the higher concentrations of destruxins B, D, and E. According to the manufacturer's instructions for the VITOTOX® test-kit, a product is considered to be cytotoxic when the following criteria are met: a) the S/N ratio decreases below 0.8, b) the Genox/Cytox Reagent ration for the positive controls should be > 1.5; and c) the highest raw data value produced by the positive control with the Genox and Cytox Reagents should be > 3 RLU with the Ascent Luminoskan. Therefore destruxins are considered as cytotoxic.

Conclusions: *L. muscarium* Ve6 didn't show any mutagenic or genotoxic effect in Ames and Vitotox assays. Whereas destruxins B, D, and E showed cytotoxicity in the Vitotox test, but not in Ames tests.

Comment RMS: based on a (submitted not published) study by Skrobek et al. 2005 RMS is of the opinion that destruxin is toxicologically not relevant and no significant residues of toxicologically

relevant toxins/metabolites are expected after the protection action of MYCOTAL (see RMS re-evaluation of the study performed by Skrobek et al. 2005 for the purpose of the renewal in B.7.2.1).

As stated in Vol3. B.6.1.1 different metabolites can be produced RMS cannot exclude the relevance of these metabolites for Lecanicillium muscarium Ve6. Therefore, still the applicant should perform a new literature search including the metabolites only or in combination with toxicology (not only the combination of metabolites and the microorganism).

B.2.8 Antibiotics and other anti-microbial agents

Information from the original DAR

Verticillium lecanii is not known to be resistant to antibiotics or anti-microbial agents used in human or veterinary medicine. Fluconazole (Das et al., 1997; Shin et al., 2002) and Amphotericin B (Shin et al., 2002) have been used successfully to treat human infections with *Verticillium* spp. Although the exact species infecting humans in these studies were not known, it is expected that *V. lecanii* is not resistant to these antibiotics.

New data 2016

No new data is to be submitted under this point. See further new data in B.2.6. Regarding the information on the antibiotic resistance. While the information provided in the original DAR shows that *V. lecanii* can be treated with fluconazole and amphotericin B the information is insufficient to exclude resistance to other antibiotic groups. Therefore, no conclusion can be drawn if *V. lecanii* complies with the new criteria for low risk substance: *"An active substance which is a micro-organism may be considered as being of low-risk unless at strain level it has demonstrated multiple resistance to anti-microbials used in human or veterinary medicine."*

B.2.9 References relied on

Additionally, a literature search according to EFSA Guidance[3] was performed to identify any new information

relevant for production of metabolites, genetic stability, development of resistance of *Lecanicillium muscarium* (Scholze 2016). The search was conducted in June 2016 using the DIMDI database provided by the German Institute of Medical Documentation and comprised searches in four databases MEDLINE, BIOSIS, CAB and SCISEARCH. The search terms used were Lecanicillium OR Verticillium lecanii, Mycotal, genetic transfer OR gene transfer OR gene exchange OR dna uptake OR dna exchange OR genetic stability or resistan? OR metabolite? OR toxin? NOT: breeding OR clone OR cloning OR construction OR engineering OR engineered. Obtained references were first subjected to a rapid assessment based on title and the abstract. Summary records that appeared to be relevant passed to a second step in which a detailed assessment of full text documents was conducted. Only studies that were considered relevant were assessed for reliability (see relevance and reliability criteria). Of the 50 obtained references and based on abstract reading 2 reports were identified to be relevant for biological properties of *Lecanicillium muscarium* Ve6.The study performed by Kouvelis (2011) about the cytotoxic and mutagenic effects of secondary metabolites and the study performed by Ambethgar (2009) about resistance were considered relevant (see B.3.5). For more details, please refer to the literature review report by Scholze (2016).

Relevance criteria:

- Property investigated was relevant for data requirements of Regulation (EC) No 1107/2009
- Identification of the genus referred to as *Lecanicillium muscarium*
- Relevant information on production of metabolites which are either involved in the mode of action or are of toxicological concern
- Relevant information on the development of resistance in the target crops
- Relevant information on the genetic stability of *Lecanicillium muscarium* or related species

Reliability criteria:

Minimum information reported e.g.:

- Test item or related compound
- Test species relevant
- Clear and comprehensive description of material and methods, incl. duration, replicates, test conditions
- Definition of endpoints
- Presentation of result

- Guideline compliance
- Further criteria:
- statistical power
- verification of measurement methods and data
- control of experimental vaiables that could affect measurements
- university of the effects in validated test systems using relevant animal strains and appropriate routes of exposure
- biological plausibility of results
- uniformity among substances with similar attributes and effects

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
Annex II Data and information					
	Scholze, I.	2016	LITERATURE REVIEW ON LECANICILLIUM MUSCARIUM VE6 (19-79): BIOLOGICAL PROPERTIES Koppert, 2191392-MA-02-01 GAB Consulting GmbH, Heidelberg, Germany GLP/GEP: no Published: no	Y New data for active ingredient, not previously submitted nor evaluated	KBS
IIM 1.3.5/01 IIM 2.1/01	Koppert Beheer B.V.	2000	Historical background of *Verticillium lecanii* Koppert Beheer B.V., Department R&D Microbials and Regulatory affairs, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer B.V. - - unpublished statement	Y	KBS
IIM2.2/02	Brady, B.L.K.	1979	Verticillium lecanii. CMI Description of pathogenic fungi and bacteria. Commonwealth Mycological Institute , ferry lane, Kew, Surrey, UK. CMI Description of pathogenic fungi and bacteria, No 610. Published report	N	-
IIM 2.2/13	Lo, P.L., Chapman, R.B.	1998	The role of parasitoids and entomopathogenic fungi in mortality of third-instar and adult *Ceroplastes destructor* and *C. sinensis* (Hemiptera: Coccidae: Ceroplastinae) on citrus in New Zealand. The Horticulture and Food Research Institue of New Zealand, Whangarei, New Zealand, Department of Entomology and Animal Ecology, Lincoln University, PO Box 84, Lincoln, Canterbury, New Zealand.	N	-

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
			- Biocontrol Science and Technology 8, 573-582. - Published report		
IIM 2.2/16	Spencer, D.M., Atkey, P.T.	1981	Parasitic effect of *Verticillium lecanii* on two rust fungi. Glasshouse Crops Research Institute, Littlehampton, W. Sussex, BN16 3PU, Great Britain - Transactions British Mycological Society 77 (3): 535-542 - published report	N	-
IIM 2.2/09	Hanssler, G., Hermanns, M.	1981	*Verticillium lecanii* as a parasite on cysts of *Heterodera schachtii*. Institut für Biologie III (Pflanzenphysiologie), RWTH-Aachen, Worringer Weg, D-5100 Aachen, West Germany - Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, No 88, pp. 678-681 - Published report	N	-
IIM 2.2/10	Hill, R.A., Lacey, J.	1983	The microflora of ripening barley grain and the effects of pre-harvest fungicide applications. Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ, UK - Annals of Applied Biology, 102: 455-465. - Published report	N	-
IIM 2.3.2/03 IIM 2.4/03 IIM	Hall, R.A.	1981a	The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales Glasshouse Crops Research Institute, Littlehampton, England.; Published report: Microbial control of pests and plant diseases 1970-1980 - Burges, H.D. (Ed.) Academic Press, London. Pp. 483-498 - published report	N	-
IIM 2.10/02	Hall, R.A.	1980	Effect of repeated subculturing on agar and passaging through an insect host on pathogenicity, morphology and growth rate of *Verticillium lecanii* Glasshouse Crops research Institute, Worthing Road, Littlehampton, Sussex, UK. - Journal of invertebrate pathology 36, pp. 216-222. - Published statement	N	-
KMA 2.2.2/01	Goettel, M.S., Koike, M., Jun	2008	POTENTIAL OF LECANICILLI-	no	-

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
	Kim, J., Aiuchi, D., Shinya, R., Brodeur, J.		UM SPP. FOR MANAGEMENT OF INSECTS, NEMATODES AND PLANT DISEASES -, not applicable J Invertebr Pathol, 98, 256-261 GLP/GEP: no Published: yes		
-KMA 2.2.2/02	Vasantharaj, D.B., Selvaraj, O.	2007	ENTOMOPATHOGENIC FUNGI FOR THE CONTROL OF ECONOMICALLY IMPORTANT WHITEFLIES -, not applicable GLP/GEP: no Published: no	no	-
KMA 2.3/01	Goettel, M.S., Koike, M., Jun Kim, J., Aiuchi, D., Shinya, R., Brodeur, J.	2008	POTENTIAL OF LECANICILLIUM SPP. FOR MANAGEMENT OF INSECTS, NEMATODES AND PLANT DISEASES -, not applicable J Invertebr Pathol, 98, 256-261 GLP/GEP: no Published: yes **Submitted in: KMA 2.2.2/01**	no	-
KMA 2.3/02	Shinde, S.V., Patel, K.G., Purohit, M.S., Pandya, J.R., Sabalpara, A.N.	2010	LECANICILLIUM LECANII (ZIMM.) ZARE AND GAMES AN IMPORTANT BIOCONTRAL AGENT FOR THE MANAGEMENT OF INSECT PESTS - A REVIEW -, not applicable Agri. Review, 31, 235-252 GLP/GEP: no Published: yes	no	-
KMA 2.3/03	Aqueel, M.A., Leather, S.R.	2013	VIRULENCE OF VERTICILLIUM LECANII (Z.) AGAINST CEREAL APHIDS; DOES TIMING OF INFECTION AFFECT THE PERFORMANCE OF PARASITOIDS AND PREDATORS? -, not applicable Pest Management Science, 69, 493-498 GLP/GEP: no Published: yes	no	-
KMA 2.3/04	Kope, H.H., Alfaro, R.I., Lavallee, R.	2008	EFFECTS OF TEMPERATURE AND WATER ACTIVITY OF LECANICILLIUM SPP. CONIDIA GERMINATION AND GROWTH, AND MYCOSIS OF PISSODES	no	-

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
			STROBI -, not applicable BioControl, 53, 489-500 GLP/GEP: no Published: yes		
KMA 2.4/01	Vasantharaj, D.B., Selvaraj, O.	2007	ENTOMOPATHOGENIC FUNGI FOR THE CONTROL OF ECONOMICALLY IMPORTANT WHITEFLIES -, not applicable GLP/GEP: no Published: no **Submitted in: KMA 2.2.2/02**	no	-
IIM 2.3.2/02 IIM 2.5/02 IIM 2.6/05	Ekbom, B.S.	1979	Investigations on the potential of a parasitic fungus (*Verticillium lecanii*) for biological control of the greenhouse whitefly (*Trialeurodes vaporariorum*). Department of Plant and Forest Protection, Sweden - Swedish Journal of Agricultural Research, No 9, pp. 129-138. - published report	N	-
IIM 2.2/07	Hall, R.A.	1975	Aphid control by a fungus, *Verticillium lecanii*, within an integrated programme for Chrysanthemum pests and diseases. Glasshouse Crops Research Institute, Worthing Road, Littlehampton, Sussex, BN16 3PU, UK. - Proceedings 8th British Insecticide and Fungicide Conference (1975), Pp. 93-99 - Published	N	-
IIM 2.2/15	Rombach, M.C., Gillespie, A.T.	1988	Entomogenous Hyphomycetes for insect and mite control on greenhouse crops. Boyce Thompson Institute for Plant Research at Cornell University, USA, AFRC Institute of Horticultural Research, Littlehampton, West Sussex BN17 6LP, UK - Biocontrol News & Information 9(1), pp. 7-18. - published report	N	-
IIM 2.3.2/05 IIM 2.5/05	Walter, C., Casperson, G., Hirte, W.F.	1988	Light- and electron microscopic investigations on the infection of the whitefly (*Trialeurodes vaporariorum* Westw.) with *Verticillium lecanii* Zimm. WB Bioprozesstechnik, Aussenstelle Kleinmachnow der Humboldt-	N	-

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
			Universität zu Berlin, Max-Reimann-Strasse 16, Kleinmachnow, DDR, Institu für Pflanzenschutzforschung Kleinmachnow der AdL der DDR, Stahnsdorfer Damm 81, Kleinmachnow, DDR - Zentralblatt Mikrobiologie, No. 143, 363-373 - published report		
IIM 2.3.2/04 IIM 2.5/04 IIM 2.6/10	Schreiter, G., Butt, T.M., Beckett, A., Vestergaard, S., Moritz, G	1994	Invasion and development of *Verticillium lecanii* in the western flower thrips, *Frankliniella occidentalis*. Institute of Microbiology, Martin-Luther-University Halle-Wittenberg, Weinbergweg 16a, Halle/S. O-4050, Germany - Mycological Research, No 98 (9), pp. 1025-1034. - published report	N	-
IIM 2.4/02	W. Gams	1971	Cephalosporium-artige Schimmelpilze (Hyphomycetes). Centraalbureau voor Schimmelcultures (Fungal Biodiversity Centre), P.O. Box 85167, 3508 AD Utrecht, The Netherlands Gustav Fischer Verlag, Stuttgart, Germany ISBN: 3-437-30117-9; pp 172-184 not applicable published book	N	-
IIM 2.4/04	Hall, R.A.	1984	Epizootic potential for aphids of different isolates of the fungus, *Verticillium lecanii*. Glasshouse Crops Research Institute, Littlehampton, West Sussex, BN16 3PU, Great Britain - Entomophaga 29(3): 311-321. - published report	N	-
IIM 2.4/05	Jackson, C.W., J.B. Heale, Hall, R.A.	1985	Traits associated with virulence to the aphid *Macrosiphoniella sanborni* in eighteen isolates of *Verticillium lecanii*. Department of Biology, Queen Elizabeth College (University of London), Campden Hill Road, London W8 7AH, Glasshouse Crops Research Institute, Littlehampton, West Sussex BN 17 6LP, United Kingdom. - Annals of Applied Biology, 106: 39-48 - published report	N	-
IIM 2.4/08	Uma, N.U., Taylor, G.S.	1987	Parasitism of leek rust urediniospores by four fungi. Department of Botany, University of Manchester, Manchester M13 9PL, England	N	-

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
			- Transactions British Mycological Society 88(3): 335-340. - published report		
	Kouvelis, V.N., Wang, C., Skrobek, A., Pappas, K.M., Typas, M.A., Butt, T.M.	2011	ASSESSING THE CYTOTOXIC AND MUTAGENIC EFFECTS OF SECONDARY METABOLITES PRODUCED BY SEVERAL FUNGAL BIOLOGICAL CONTROL AGENTS WITH THE AMES ASSAY AND THE VITOTOX TEST -, not applicable Mutat Res, 722, 1-6 GLP/GEP: no Published: yes	N	-
IIM 8.8/01	Verhaar, H.J.M.	2000	The effects of *Verticillium lecanii* on non-target arthropods OpdenKamp Registration & Notification, Koninginnegracht 23, 2514 AB, The Hague, The Netherlands. Koppert Beheer BV F:\oag\Ko\My1-\00001639f-PSD.fm - unpublished statement	Y	KBS
IIM 8.8/02	Koppert Beheer BV	2001c	Side effects of Mycotal on non-target arthropods. Koppert Beheer B.V., Department R&D Microbials and Regulatory affairs, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer BV - - unpublished statement	Y	KBS
IIM 8.8/03	van Doorn, A.	1998	Impact of the fungi *Verticillium* and *Trichoderma* on adult bumblebees and bumblebee brood. Koppert Biological Systems, Department R&D Natupol, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer BV Pr980701 Not GLP unpublished	Y	KBS
IIM 2.7.1/07	W. Gams, A. Van Zaayen	1982	Contribution to the taxonomy and pathogenicity of fungicolous Verticillium species. I. Taxonomy Centraalbureau voor Schimmelcultures (Fungal Biodiversity Centre), Baarn, The Netherlands, Proefstation voor de Champignoncultuur, Horst, The Netherlands. - Netherlands Journal of Plant Pathology 88, 57-78. Not applicable Published report	N	-
IIM 8.8/05	Bidochka, M.J., St Legar, R.J., Stuart, A.,	1999	Nuclear rDNA phylogeny in the fungal genus *Verticillium* and its relationship to insect and plant virulence, extracel-	N	-

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
	Gowanlock, K.		lular protease and carbohydrases Department of Biology, Trent University, Petersboroygh, Ontario, Canada K9J 7B8 - Microbiology 145, pp. 955-963. 1999. - Published report		
IIM 2.7.1/04	C.S. Darke, J. Knowelden, J. Lacey, A. Milford Ward	1976	Respiratory disease of workers harvesting grain. Respiratory Function Unit, Royal Infirmary, the Departments of Community Medicine and Immunology, University of Sheffield, and Rothamsted Experimental Station, Harperden, Herts, UK - Thorax, Vol. 31, pp. 294-302 Not applicable Published report	N	-
IIM 2.12/02	D.K. Das, R.K. Grover, K.L. Chachra, N.C. Bhatt, M. Bibhabati	1997	Fine needle aspiration cytology diagnosis of a fungal lesion of the *Verticillium* species. A case report. Institute of Cytology of Preventive Oncology and the Department of Radiotherapy, Maulana Azad Medical college and Lok Nayak Hospital, and the Department of Microbiology, G.B. Pant Hospital, New Delhi, India - Acta-Cytologica 41(2), pp. 577-582. - Published report	N	-
IIM 2.7.1/08	S. Grandesso, G. Amici, C. Bocci, A. Mottola	1996	Fungal peritonitis in peritoneal dialysis: a critical review of seven cases. Microbiology Laboratory “S. Maria dei Battuti” Regional Hospital, 31100 Treviso, Italy. - Alpe Adria Microbiology Journal 5, 15-21. - Published report	N	-
IIM 2.7.1/09	J.Y. Shin, H.M. Kim, J.W. Hong	2002	Keratitis caused by *Verticillium* species. Department of Ophthalmology, Korea University Hospital, Korea University Medical College, Seoul, Korea - Cornea 21(2), pp.240-242. - Published report	N	-
IIM 4.5.1/03	Zare, R., Gams, W.	2001	A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium* gen.nov. CABI Bioscience, Bakeham Lane, Egham, Surrey TW20 9TY, UK, Centraalbureau voor Schimmelcultures P.O. Box 85167, 3508 AD Utrecht, the Netherlands. - Nova Hedwigia, vol. 73, pp. 1-50. -	N	-

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
			Published		
IIM 2.6/07	Kanaoka, M., Isogai, A., Murakoshi, S., Ichinoe, M., Suzuki, A., Tamura, S.	1978	Bassianolide, a new insecticidal cyclodepsipeptide from *Beauveria bassiana* and *Verticillium lecanii*. Department of Agricultural Chemistry, The Universiy of Tokyo, Bunkyo-ku, Tokyo, Japan - Agricultural and Biological Chemistry, 42(3): 629-635. - published report	N	-
IIM 2.6/08	Murakoshi, S., Ichinoe, M., Suzuki, A., Kanaoka, M., Isogai, A., Tamura, S.	1978	Presence of toxic substance in fungus bodies of the entomopathogenic fungi, *Beauveria bassiana* and *Verticillium lecanii*. Kanagawa Prefecture Sericultural Research Center, Nakashinden, Ebina-shi 243, Japan - Applied Entomology & Zoology 13(2): 97-102 - published report	N	-
IIM 2.7.2/02	Claydon, N., Grove, J.F.	1982	Insecticidal secondary metabolic products from the entomogenous fungus *Verticillium lecanii*. Agricultural Research Council, Unit of Invertebrate Chemistry and Physiology, University of Sussex, Falmer, Brighton, east Sussex BN1 9RQ, England. - Journal of Invertebrate Pathology 40: 413-418 - Published report	N	-
IIM 2.7.2/03	Gindin, G., Barash, I., Harari, N., Raccah, B.	1994	Effect of endotoxic compounds isolated from *Verticillium lecanii* on the Sweetpotato Whitefly, *Bemisia tabaci*. Department of Plant Pathology and Department of Virology, ARO, The Volcanic Center, Bet Dagan 50250, Israel, Department of Botany, Tel-Aviv University, Tel Aviv 69978, Israel. - Phytoparasitica 22(3): 189-196 - Published report	N	-
IIM 2.7.2/06	Soman, A.G., Gloer, J.B., Angawi, R.F., Wicklow, D.T., Dowd, P.F.	2001	Vertilecanins: Ne phenopicolinic acid analogues from *Verticillium lecanii*. Department of Chemistry, University of Iowa, Iowa City, Iowa 52242, and Bioactive Agents Research Unit, Agricultural Research Service, National Center for Agricultural Utilization Research, USDA, Peoria, Illinois 61604 - Journal of Natural Products, 64(2): 189-192 - Published report	N	-

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
IIM 6.1/02	Butt, T.M., Skrobek, A., Wang, C., Shah, F.A., Ben El Hadj, N.	2004	RAFBCA Partner 01, University of Wales, Swansea, UK, Final Report 01.11.01-31.10.04. University of Wales, Swansea, UK, Final Report 01.11.01-31.10.04.; School of Biological Sciences, University of Wales Swansea, Singleton Park, SA2 8PP, UK. - QLK1-2001-01391 - unpublished statement	Y	KBS
IIM 8.8/07	Hall, R.A.	1982	Control of whitefly, *Trialeurodes vaporariorum* and cotton aphid, *Aphis gossypii* in glasshouses by two isolates of the fungus, *Verticillium lecanii*. Glasshouse Crops Research Institute, Littlehampton, West Sussex, BN16 3PU, UK - Annals of applied biology (101), pp. 1-11. - Published	N	-
IIM 8.8/09	Kanagaratnam, P., Hall, R.A., Burges, H.D.	1982	Control of glasshouse whitefly, *Trialeurodes vaporariorum*, by an 'aphid' strain of the fungus *Verticillium lecanii*. Glasshouse Crops Research Institute, Littlehampton, West Sussex, BN16 3PU, UK - Annals of Applied Biology (100), pp. 213-219. - Published	N	-
IIM 8.8/06	Flexner, J.L., Lighthart, B., Croft, B.A.	1986	The effects of microbial pesticides on non-target, beneficial arthropods. Department of Entomology, Oregon State University, Corvallis, Oregon 97331, USA, Environmental Protection Agency, Corvallis Environmental Research Laboratory, Corvallis, Oregon 97333, USA - Agric Ecosystems Environ (16), pp. 203-254. - Published	N	-
IIM 8.8/04	Bethke, J.A., Parrella, M.P.	1989	Compatibility of the aphid fungus *Cephalosporium lecanii* with the leafminer parasite, *Diglyphus beginii* (Hymenoptera: Eulophidae) Department of Entomology, University of California, Riverside, California 92521, USA - Pan-Pacific Entomologist (65), pp. 385-390. - Published	N	-
IIM 2.4/09	A. van Doorn	1998	Impact of the fungi *Verticillium* and *Trichoderma* on adult bumblebees and	Y	KBS

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
			bumblebee brood. Koppert Biological Systems, Department R&D Natupol, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer B.V. Pr980701 Not GLP unpublished report		
IIM 8.8/12	Steenberg, T., Humber, R.A.	1999	Entomopathogenic potential of *Verticillium* and *Acremonium* species (Deuteromycotina: Hyphomycetes). Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Lyngby, Denmark, Section of Zoology, The Royal Veterinary and Agricultural University, Bulowsvej 13, DK-1870 Frederiksberg C, Denmark, USDA-ARS Collection of Entomopathogenic Fungal Cultures, Plant Protection Research Unit, US Plant, Soil and Nutrition Laboratory, Tower Road, Ithaca, New York 14853-2901 - Journal of Invertebrate Pathology (73), pp. 309-314. - Published	N	-
IIM 8.4/13	Sitch, J.C., Jackson, C.W.	1997	Pre-penetration events affecting host specificity of *Verticillium lecanii*. Department of Biology, University of Southhampton, Biomedical Science Building, Bassett Crescent East Southampton SO16 7PX, UK. - Mycological Research (101), pp. 535-541. - Published	N	-
IIM 8.8/13	Sterk, G., Hassan, S.A. *et al.*	1999	Results of the seventh joint pesticide testing programme carried out by the OIBC/WPRS-Working Group 'Pesticides and beneficial Organisms'. Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Obstbau, Schwabenheimers Str. 101, D-69221 Dossenheim, Germany. - Biocontrol (44), pp. 99-117. - Published	N	-
IIM 1.3.3/03	R.A. Samson, E.S. Hoekstra, C.A.N. van Oorschot	1984	Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures (Fungal Biodiversity Centre), P.O. Box 85167, 3508 AD Utrecht, The Netherlands. CBS ISBN: 90-70351-03; pp. 42-46 & 148-149 Not applicable - published book	N	-

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
IIM 2.8/04	Koppert Beheer B.V.	2001	Temperature and humidity demands of Vertalec and Mycotal Koppert Beheer B.V., Department R&D Microbials and Regulatory affairs, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer BV V-46 Not GLP unpublished report	Y	KBS
IIM 2.8/06	R.J. Quinlan	19xx	Effect of MYCOTAL on fruit ripening and storage University of Reading, Reading, Berks, UK Koppert Beheer BV - Not GLP unpublished report	Y	KBS
IIM 2.8/05	M.D. MacQueen, R.J. Quinlan	19xx	Residual *Verticillium lecanii* on the leaves and fruit of a tomato crop treated with Mycotal microbial Insecticide. University of Reading, Reading, Berks, UK Koppert Beheer BV - Not GLP unpublished report	Y	KBS
IIM 2.8/02	E.A.M. Beerling, N.N. Joosten, J. Tolsma, J.J. Fransen	1998	Studies of entomopathogenic fungi for control of thrips and three aphid species on glasshouse ornamentals, final report of participant 04. Research Station for Floriculture and Glasshouse Vegetables (PBG), Linnaeuslaan 2a, 1431 JV Aalsmeer, the Netherlands Koppert Beheer BV AIR3 CT94-1352 Not GLP unpublished report	N	KBS
IIM 2.8/07	M.C. Rombach, A.T. Gillespie	1998	Entomogenous Hyphomycetes for insect and mite control on greenhouse crops. Boyce Thompson Institute for Plant Research at Cornell University, USA, AFRC Institute of Horticultural Research, Littlehampton, West Sussex BN17 6LP, UK - Biocontrol News & Information 9(1), pp. 7-18. - published report	N	-
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Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed* Y/N	Owner**
			Published report		
IIM 2.10/01	Koppert Beheer B.V.	2004f	Genetic stability of *Verticillium lecanii* strain Ve6 and factors affecting it Koppert Beheer B.V., Department R&D Microbials and Regulatory affairs, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer BV - - unpublished statement	Y	KBS
IIM 2.6/07	Kanaoka, M., Isogai, A., Murakoshi, S., Ichinoe, M., Suzuki, A., Tamura, S.	1978	Bassianolide, a new insecticidal cyclodepsipeptide from *Beauveria bassiana* and *Verticillium lecanii*. Department of Agricultural Chemistry, The Universiy of Tokyo, Bunkyo-ku, Tokyo, Japan - Agricultural and Biological Chemistry, 42(3): 629-635. - published report	N	-
IIM 2.6/06	Gindin, G., Barash, I., Harari, N., Raccah, B.	1994	Effect of endotoxic compounds isolated from *Verticillium lecanii* on the Sweetpotato Whitefly, *Bemisia tabaci*. Department of Plant Pathology and Department of Virology, ARO, The Volcanic Center, Bet Dagan 50250, Israel, Department of Botany, Tel-Aviv University, Tel Aviv 69978, Israel. - Phytoparasitica 22(3): 189-196 - published report	N	-
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*: Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted in any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used in any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization, of a plant protection product before the date of submission of the dossier to Rapporteur Member State.

**: Owners' code identifications and names (Code identification: KBS, Name: Koppert Beheer Systems))