

Renewal Assessment Report

***Lecanicillium muscarium* Ve6**

Volume 3MA – B.1 Identity

January 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: France

Version history

When	What
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B.1 Identity 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 EFSA¹.

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.

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

B.1.1 Applicant

Name	Koppert B.V.
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Contact person	██████████
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B.1.2 Producer

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Contact person	██████████
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B.1.3 Name and species description, strain characterisation

Information from the original DAR

Verticillium is a mitosporic fungus that belongs to the phylum of Deuteromycotina and the order of Hyphomycetes (or Moniliales). Hyphomycetes produce mitosporic asexual structures (called conidia) directly from the vegetative state or hyphae. *Verticillium* produces conidia as aggregates in slimy heads (Samson et al., 1984).

Kingdom: Fungi

Phylum: Deuteromycotina

Order: Hyphomycetes (syn. Moniliales)

Genus: *Verticillium*

Species: *Verticillium lecanii*

Strain: Ve6

Verticillium lecanii Ve6 has recently been reclassified (Zare and Gams, 2001):

Genus: *Lecanicillium*

Species: *Lecanicillium muscarium*

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 are included in the section *Nigrescentia*.

Former *Verticillium* species pathogenic to insects, fungi and nematodes are now included in the genus *Lecanicillium*. The genus *Verticillium* no longer contains entomopathogenic species. Most *Verticillium* spp. in the section *Prostrata* were renamed to *Lecanicillium* spp. Out of the 43 studied *V. lecanii* isolates 10 were renamed to *L. lecanii*, 24 to *L. muscarium*, 5 to *L. longisporum* (formerly known as *Verticillium lecanii* Ve2), 4 to *L. nodulosum* and 4 isolates were renamed to a different genus: *Simplicillium* spp. Besides the *V. lecanii* strains, two strains of *V. fungicola* were renamed to *L. muscarium* too.

New data 2016

Previously submitted information is considered to be acceptable to cover current requirements. No new data will be submitted under this point.

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.

Although Zare & Gams reclassified members of *Verticillium* section Prostata IV to *Lecanicillium* and *Simplicillium* gen. nov., already in 2001, some recent publications in the open literature may still use the old designation. As long as no more information on taxonomy is provided, species identity has to be considered carefully.

B.1.3.1 Accession number in culture collection

Information from the original DAR

CABI Genetic Resource Collection, Surrey, UK (=IMI) 268317.

Centraal bureau Schimmelcultures (CBS), Baarn, The Netherlands CBS 102071.

The Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF) USDA-ARS Plant Protection Research Unit, Ithaca, USA, ARSEF 5128.

New data 2016

Previously submitted information is considered to be acceptable to cover current requirements. No data will be submitted under this point.

It is noted by the RMS that the Centraal bureau Schimmelcultures (CBS) has recently been renamed to Westerdijk Fungal Biodiversity Institute and is currently located in Utrecht, The Netherlands.

B.1.3.2 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism

Information from the original DAR

Indigenous or non-indigenous	Indigenous
Wild type	<i>Lecanicillium muscarium</i> Ve6
Spontaneous or induced mutant*	Not applicable
Genetically modified according to Directive 2001/18/EC*	No

* All known differences between the modified micro-organism and the parent wild strain must be provided

Verticillium lecanii Ve6 has been reclassified in 2001:

Kingdom:	Fungi
Phylum:	Deuteromycotina
Order:	Hyphomycetes (syn. Moniliales)
Genus:	<i>Lecanicillium</i>
Species:	<i>Lecanicillium muscarium</i>
Strain:	Ve6

New data 2016

Previously submitted information is considered to be acceptable to cover current requirements. No data will be submitted under this point.

B.1.3.3 Test procedures and criteria used for identification at strain level

Information from the original DAR

Identity of the active ingredient of MYCOTAL

The information below is no longer presented in Doc J, since information is not considered as confidential.

A positive identification of the active ingredient *Lecanicillium muscarium* Ve6 (*Verticillium lecanii* Ve6) has been carried out on three batches of MYCOTAL by the Centraalbureau voor Schimmelcultures (CBS), the Netherlands.

Verticillium lecanii can be described as follows (Samson et al., 1984; Gams, 1971). Colonies reach a size of 18 –22 mm, white or pale yellow, cotton wool like, hyphae rarely in bundles, after 10 days at 20°C on Malt Extract Agar. The underside of the colony is colourless, yellow or ochraceous. Phialids detached or in few whorls on conidiophores or slightly differentiated hyphae from the aerial mycelium, needle form, high variability in size, 12 - 40 × 0.8 - 3 µm. Conidia one-celled in heads, often parallel to phialide tip, cylindrical with both ends well rounded or ellipse, 2.3 - 10 × 1.0-2.6 µm. Chlamydospores absent. Spore sizes of the MYCOTAL -strain are 4.2 ± 0.9 µm by 1.6 ± 0.2 µm (Determined on Sabouraud Dextrose agar after 6 days at 23°C).

Identification on species level

The *Verticillium lecanii* strain Ve6 can be identified morphologically upon species level. The colonies of *Verticillium* reach 18 - 22 mm in 10 day on malt extract agar (MEA) at 20°C, the colonies are white to pale yellow and cottony. The phialides (size 12 - 40 µm × 0.8 - 3.0 µm) arise from conidiophores or differentiated hyphae in scant whorls or solitarily (Domsch et al., 1980).

The phialides are formed on distinct verticillately branched conidiophores (Samson et al., 1984). Furthermore, Domsch et al. (1980) describe conidia (2.3 - 10 µm × 1.6 - 2.6 µm) in heads or parallel bundles, cylindrical with rounded tips or ellipsoidal. The notifier submitted a German paper that describes a method of identification (Gams, 1971) that was not assessable to the RMS.

Zare and Gams (2001) describe some features specific for the reclassified *Verticillium* species: *Lecanicillium*. *Lecanicillium* species form slender aculeate phialides, with conidia that adhere in fascicles at right angles to the phialide tip.

Colonies of *L. muscarium* reach 14 - 30 mm in diameter after 10 days. They are rather compact, white, with creamcoloured to pale yellow (rarely yellow) or uncoloured reverse. Phialides are produced directly on prostrate hyphae or on secondary branches. Secondary branches are less frequent than in *L. lecanii*. Phialides are generally longer than those of *L. lecanii* and less tapering, measuring (15-)20 - 35 × 1.0 - 1.7 µm. Conidia are produced in globose heads, ellipsoidal to sub cylindrical, more irregular in size and shape, longer and narrower than in *L. lecanii*, measuring 2.5 - 5.5 × 1 - 1.8 µm. Octahedral crystals are commonly present, the temperature optimum is 21 - 27°C, depending on the strain. No growth is observed at 33°C.

L. lecanii can be distinguished from *L. muscarium* on the basis of its shorter, broader and more homogeneous conidia, shorter, broader and more aculeate phialides, more frequent secondary branches, and

deep yellow colour of the colony reverse. The two taxa are distinct in ITS sequences and RFLP patterns. *L. longisporum* is easily distinguished from *L. muscarium* by its longer and broader conidia. Sugimoto et al. (2003) characterised *Verticillium lecanii* based on genetic, morphologic and virulence properties.

Based on the intergenic spacer regions (IGS) of ribosomal DNA (rDNA) size polymorphism 46 isolates were classified into 3 groups: IGS types L (4.0 kb), M (3.0 kb) and S (2.3 kb). Isolates from whitefly and MYCOTAL were typed as IGS-S, isolates from Vertalec were typed as IGS-M. Vertalec is a product (used against aphids) based on the closely related *V. lecanii* Ve2 (renamed to *Lecanicillium longisporum*).

As isolates from aphids were also typed as IGS-S, no classification could be made based on the isolation source. The virulence properties of the different isolates could also not give a clear relation for typing and source of isolation. Isolates from whitefly and MYCOTAL caused a mortality of 12.4 - 47.4% in aphids, whereas isolates from Vertalec and aphids had a mortality of 38.2 - 88.9%. In an additional study of Typas (2002a) MYCOTAL and Vertalec isolates could be distinguished by genetic fingerprinting.

Identification on strain level

See further DAR addendum (June 2009) presented in B.5.1.1.

New data 2016

No new data.

B.1.3.4 Common name or alternative and superseded names and code names used during the development

Information from the original DAR

Code no.	Used by
19-79, GCRI-79 HRI 19-79	R. Hall
Ve6	Tate and Lyle, MRL
KBV 10-88 KBV 88-M01 KV01 3*	Koppert B.V.
<i>Verticillium lecanii</i> Ve6 has been reclassified as <i>Lecanicillium muscarium</i> by Zare and Gams (2001).	

New data 2016

No new data.

B.1.3.5 Relationship to known 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 (with partly prostate conidiphores). Plant pathogenic species are included in the section Nigrescentia. The other sections are not relevant for this report.

The plant pathogens produce high levels of enzymes that degrade pectin (major component of the plant cell wall), whereas the insect pathogens produce high levels of subtilisin-like proteases (active against chymotrypsin substrate). The latter group is distinguished from the plant pathogens as they produce chitinase and are not able to clear pectine (Bidochka *et al.*, 1999).

The notifier did not submit any information that indicates relationship to human pathogens.

New data 2016

No new data.

In B.6.1.1.4 several clinical cases are described. Furthermore, in the literature search covering the last 10 years and focussing on toxicity or pathogenicity of *L. muscarium* on mammals, one article was identified describing the pseudo-outbreak of *Lecanicillium* species in surgery patients.

Note RMS: In the information from the original DAR is stated that the other sections are not relevant for this report. RMS questions whether this is true. Could the applicant explain the situation? As a pseudo-outbreak of *Lecanicillium* species in surgery patients has been identified there could also be a section of human or animal pathogens. Therefore, could applicant check whether there are other relevant articles present in the literature about the plant, human and/or animal pathogenic properties of *Lecanicillium* species.

B.1.4 Specification of the material used for manufacturing of formulated products

B.1.4.1 Content of the micro-organism

Information from the original DAR

The material used for solid medium manufacturing of formulated product contains $\pm 1 \times 10^{11}$ spores per gram technical spore powder (97 - 99%, dried conidiospores, 1 - 3% media remnants).

The material used for liquid medium manufacturing of formulated product contains $\pm 2.5 \times 10^{10}$ spores per gram technical spore powder (49 - 52%, dried blastospores, 48 - 51% additives)

New data 2016

For the renewal of the active substance, a new 5-batch analysis of the formulated product is submitted. The content of *Lecanicillium muscarium* Ve6 ranges from 9.5×10^9 to 1.2×10^{10} spores/g and 5.3×10^9 to 8.9×10^9 CFU/g, respectively. For content of the microorganism in the current 5-batch data, please refer to the summary of the 5-batch data of the formulated product presented in B.1 MP

The nominal content of *L. muscarium* Ve6 is 1×10^{10} spores/g.

B.1.4.2 Identity and content of impurities, additives, contaminating micro-organisms

Information from the original DAR

The technical spore powder from solid production contains 1 - 3% media remnants.

Metabolic products have not been detected in technical spore powder.

No additives are used in the technical spore powder produced by solid fermentation.

The material used for liquid medium manufacturing of formulated product contains $\pm 2 - 5 \times 10^{10}$ spores per gram technical spore with the following additives: emollient, detergent/ oil stabiliser, nutrient/ protectant, anti-oxidant and anti-caking agent.

Composition of technical spore powder of *Verticillium lecanii* strain Ve6: see confidential information DAR, Volume 4 Annex C.

New data 2016

According to But et al., (2005), previously submitted, see for summary B.7.2.1), *L. muscarium* Ve6 does not produce destruxins A, B, or E. No destruxins were detected in spores, mycelium, colonised rice, culture filtrates, or the end-use product.

According to the EFSA conclusion on *L. muscarium* Ve6, the strain does not produce any metabolites of concern.

For the renewal of the active substance, a new 5-batch analysis of the formulated product is submitted. Results on the analysis relevant impurities on current 5 batches are available. Please refer to Volume 4. The batch data complies with the OECD issue paper on microbial contaminants.

B.1.4.3 Analytical profile of batches

Information presented in Volume 4

B. 1.5 References relied on

Annex point / reference number	Author(s)	Year	Title Source (where different from com- pany) Company, Report No GLP or GEP status (where relevant) Published or not	Data Pro- tection Claimed* Y/N	Owner **
Annex II Data and Information					
IIM 1.1.3	CABI Biosci- ence	2005	CABI Genetic Resource Collection CABI Bioscience, Bakeham Lane, Egham, Surrey TW20 9TY UK CABI Bioscience http://www.cabi-bioscience.org/docs/pdf/GRCCatalogue.pdf , CABI nr. 268317 - Public internet database	N	-
IIM 1.1.3	Centraalbu- reau voor Schimmelcul- tures	2005	Data from CBS Fungi database Centraalbureau voor Schimmelcultures (Fungal Biodiversity Centre), P.O. Box 85167, 3508 AD Utrecht, The Nether- lands CBS http://www.cbs.knaw.nl/databases/index.htm , CBS nr. 102071 - Published	N	-
IIM 1.1.3	Humber, R.A., Hansen, K.S.	2004	The Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF) USDA-ARS Plant Protection Research Unit, US Plant, Soil & Nutrition La- boratory, Tower Road, Ithaca, NY 14853-2901 (USA) USDA ARS http://www.ppru.cornell.edu/mycology/insect_mycology.htm , ARSEF nr. 5128 (pp. 13) - published report	N	-
IIM 1.3.5	Gams	1971	Cephalosporium-artige Schimmelpilze (Hyphomycetes). Centraalbureau voor Schimmelcul- tures (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	-

Annex point / reference number	Author(s)	Year	Title Source (where different from com- pany) Company, Report No GLP or GEP status (where relevant) Published or not	Data Pro- tection Claimed* Y/N	Owner **
IIM 1.3.5	Gams, W., Van Zaayen, A.	1982	Contribution to the taxonomy and pathogenicity of fungicolous <i>Verticilli-</i> <i>um</i> species. I. Taxonomy Centraalbureau voor Schimmelcul- tures (Fungal Biodiversity Centre), Baarn, The Netherlands, Proefstation voor de Champignoncultuur, Horst, The Netherlands. - Netherlands Journal of Plant Patholo- gy 88, 57-78. Not applicable Published report	N	-
IIM 1.1.3	Samson, R.A., Hoekstra, E.S., Van Oorschot, C.A.N.	1984	Introduction to food-borne fungi. Centraalbureau voor Schimmelcul- tures (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	-
	Domsch, K.H., Gams, W., Anderson, T.	1980	Compendium of the soil fungi. Institute of soil Biology, Federal Agri- cultural Research Centre, Braun- schweig, Germany, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands - London Ac. Press Vol. I & II, 895 pp. & 405 pp. - Published book	N	-
IIM 1.1.3	Sugimoto, M., Koike, M., Hiyama, N., Nagao, H	2003	Genetic, morphological, and virulence characterization of the entomopatho- genic fungus <i>Verticillium lecanii</i> . Department of Agro-environmental Science, Obihiro University of Agri- culture and Veterinary Medicine, Hok- kaido, Obihiro 080-8555, Japan. - Journal of Invertebrate Pathology, vol. 82, pp. 176-187 Not applicable Published report	N	-
	Zare, R., Gams, W.	2001	A revision of <i>Verticillium</i> section <i>Pro-</i> <i>strata</i> . IV. The genera <i>Lecanicillium</i> and <i>Simplicillium</i> gen.nov. CABI Bioscience, Bakeham Lane, Egham, Surrey TW20 9TY, UK, Cen- traalbureau voor Schimmelcultures P.O. Box 85167, 3508 AD Utrecht, the Netherlands - Nova Hedwigia, vol. 73, pp. 1-50. Not applicable Published report	N	-

Annex point / reference number	Author(s)	Year	Title Source (where different from com- pany) Company, Report No GLP or GEP status (where relevant) Published or not	Data Pro- tection Claimed* Y/N	Owner **
	Koppert Be- heer B.V.	2000	Historical background of <i>Verticillium lecanii</i> Koppert Beheer B.V., Department R&D Microbials and Regulatory af- fairs, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer B.V. - - unpublished statement	Y	KBS
IIM 1.4.3.3/01	Koppert Be- heer B.V.	2005	Identity and content of impurities and contaminating micro-organisms. Koppert Beheer B.V., Department R&D Microbials and Regulatory af- fairs, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer B.V. - - unpublished statement	Y	KBS
	Koppert Be- heer B.V.	2005	Relationships of <i>Verticillium lecanii</i> to known plant or animal or human path- ogens Koppert Beheer B.V., Department R&D Microbials and Regulatory af- fairs, P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands Koppert Beheer B.V. - - unpublished statement	Y	KBS
	Typas, M.A.	2002a	An analysis of Vertalec and Mycotol isolates by genetic fingerprinting. University of Athens, Faculty of Biolo- gy, Department of Genetics and Bio- technology, Panepistemiopolis, Kou- ponia, Athens 15701, Greece. Koppert BV - - Unpublished report	N	KBS
	Typas, M.A.	2002b	An introduction to molecular tech- niques used for the classification of microorganisms, including fungi: an explanataion on characterization of fungal species and strains for the purpose of registration of microbial pesticides. University of Athens, Faculty of Biolo- gy, Department of Genetics and Bio- technology, Panepistemiopolis, Kou- ponia, Athens 15701, Greece. Koppert BV - - Unpublished report	N	KBS

Annex point / reference number	Author(s)	Year	Title Source (where different from com- pany) Company, Report No GLP or GEP status (where relevant) Published or not	Data Pro- tection Claimed* Y/N	Owner **
	Bidochka, M.J., St Legar, R.J., Stuart, A., Gow- anlock, K.	1999	Nuclear rDNA phylogeny in the fungal genus <i>Verticillium</i> and its relationship to insect and plant virulence, extracel- lular protease and carbohydrases Department of Biology, Trent Universi- ty, Petersboroygh, Ontario, Canada K9J 7B8 - Microbiology 145, pp. 955-963. 1999. - Published report	N	-

*: Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used 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)