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VOLUME 3 – Annex B

- *Beauveria bassiana* PPRI 5339 -

B.8 Fate and behaviour in the environment

Rapporteur Member State: The Netherlands

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Draft Assessment Report and Proposed decision of the Netherlands prepared in the context of the possible approval of *Beauveria bassiana* PPRI 5339 under Regulation (EC) 1107/2009

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B.8 Fate and behaviour in the environment (Annex IIM 7 and IIIB 9)

Introduction

PPRI 5339 is a naturally occurring, non-modified strain of *Beauveria bassiana*, originally isolated from the larva of a tortoise beetle, *Conchyloctenia punctata* (Coleoptera: Cassidinae) collected in South Africa. Further information on origin can be can be found in B.2.1.1.1.

Currently, two *B. bassiana*, strains ATCC74040 and GHA are evaluated and approved in the EU. Following ribosomal sequencing, strain PPRI 5339 was found to be 98-99% similar to the above strains, see B.5.1.1.1; therefore strain PPRI 5339 can be considered essentially identical to strains ATCC74040 and GHA.

Although species potentially differ in toxicity at the strain level, it is recommended to evaluate persistence at the species level as it was shown by Scheepmaker and Butt (2010) since densities of individual strains often follow a very similar decline.

The body of information in the open literature regarding the fate and behaviour of *B. bassiana* in the environment is extensive. The summation of these data are provided in detail in the review by Zimmermann, 2007 and Scheepmaker and Butt, 2010 and form the basis of the following discussions, supplemented with any other relevant literature that has subsequently been published.

The OECD 2012 guidance regarding the environmental safety evaluation of microbial biocontrol agents ENV/JM/MONO(2012)[1], includes the following specific text for entomopathogenic fungi (EPF) including *B. bassiana* (emphasis underlined) and specifically recommends data in Scheepmaker and Butt, 2010 as being suitable for use to cover the soil persistence data requirements for *B. bassiana*:

"*The review of Scheepmaker and Butt (2010) can be referenced in a waiver/statement to fulfill the persistence in soil data requirement for the three EPF species, B. bassiana, B. brongniartii, M. anisopliae. Since other EPF species are subjected to the same processes in the soil, it is assumed that a similar decrease of inoculum will occur in other EPF species. Therefore, the proposed methodology can be used for other mBCAs as well."*

General information relevant to the assessment

Beauveria bassiana is an opportunistic entomopathogenic fungus. *Beauveria bassiana* attacks its insect hosts percutaneously; the conidia of *B. bassiana* adhere to the insect cuticle by means of hydrophobic interaction between the spore wall and epicuticle lipids. A hydrophobin-type protein and

[1] OECD Guidance to the environmental safety evaluation of microbial biocontrol agents. Series on Pesticides No. 67. ENV/JM/MONO(2012)1

certain enzymes assist in the attachment process. Germination of the conidia and the subsequent successful infection depend on a number of factors, e.g. susceptibility of the host and host stage, and certain environmental factors, such as optimal temperature and humidity. Before penetration, germ tubes may form so-called appressoria and infection pegs. The penetration process is by mechanical means and by the production of several enzymes, including proteases, chitinases and lipases, which degrade the insect cuticle. Penetration is followed by invasion, which is accompanied by several host immune response activities. Further information on mode of action can be can be found in B.3.2.2 and Zimmermann, 2007.

Secondary Metabolites

During the infection process, *Beauveria* spp. produces proteolytic enzymes and toxins (see EFSA supporting publication[2]; also see B.2.1.8 and Zimmermann, 2007) while the host insect responds with cellular and humoral defence reactions.

Beauveria bassiana does not multiply and is metabolically inactive in the absence of host insects, and as a consequence no accumulation of potentially harmful metabolites to levels that might affect the environment is expected. Metabolic inactivity continues as long as the environmental conditions remain unchanged. Furthermore, the effect of fungistasis in non-sterile soils inhibits the germination of conidia. This has been shown by (Watson & Ford 1972) for soil fungi.

In another study by Clerk (1969), it was demonstrated that conidia of *B. bassiana* are subject to fungistatic effects in natural soils.

Study MA 7/04

Report:	Clerk, G.C. (1969) Influence of soil extracts on the germination of conidia of the fungi *Beauveria bassiana* and *Paecilomyces farinosus*. Journal of Invertebrate Pathology 13, 120-14
Guidelines:	none
GLP:	not applicable

The study described effects of untreated and variously modified aqueous extracts of natural soils on the germination of conidia of *Beauveria bassiana.* It is shown that extract of 3 soil zones inhibits the germination. If the extract is filtered, heat sterilised, a dextrose solution is added or silk extract of the cocoon of the silk worm, the germination rose approaching 100%. To detect whether the soils themselves exercised fungistatic effect, conidia were incubated at agar plates covered with soil from

[2] Mudgal, S., De Toni, A., Tostivint, C., Hokkanen, H and Chandler, D. Scientific support, literature review and data collection and analysis for risk assessment on microbial organisms used as active substance in plant protection products –Lot 1 Environmental Risk characterisation. EFSA supporting publication 2013:EN-518 pp 87-93

zone 2. It was demonstrated that while 68.2% germinated in the control agar plate only 7.7% had germinated in the agar that had been covered.

Conclusion

Conidia of *B. bassiana* are subject to fungistatic effects in natural soils.

It can be concluded that secondary metabolites including fungal toxins cannot be produced as long as the germination of conidia is inhibited, e.g. in natural soils. Many of the secondary metabolites produced during the life cycle of *B. bassiana* are produced in response to host defence mechanisms (see section B.2.1.8) and therefore are unlikely to be produced without the presence of a host. Furthermore secondary metabolites are mostly peptides which are considered to be readily biodegradable. In the case of *B. bassiana* and possibly also other entomopathogenic fungi, the highest concentrations of toxins that can occur in nature are expected to be found in the host insects.

Therefore a contamination of environmental compartments might only occur by a plentiful appearance of infected and dead hosts and the risk of soil contamination by secondary metabolites is considered to be negligible.

B.8.1 Persistence and multiplication (Annex IIM 7.1)

B.8.1.1 Soil (Annex IIB 7.1.1)

Beauveria bassiana is an indigenous fungal species, widely distributed in natural and cultivated soils (Quesada-Moraga *et al.,* 2007, Scheepmaker and Butt, 2010 and Zimmermann, 2007).

No specific study on the *B. bassiana* strain PPRI 5339 has been conducted to evaluate its persistence in soil, however extensive literature is available on this topic. Scheepmaker and Butt, 2010 conducted a meta-analysis of the relevant literature available at the time, and provided data concerning natural levels and persistence of *B. bassiana* in soils.

Study MA 7/02

Report:	Quesada-Moraga, E., Navas-Cortes J.A., , Maranhao, E.A. A., Ortiz-Urquiza A., Santiago-Alavarez, C. (2007) Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological research 947-966
Guidelines:	none
GLP:	not applicable

Summary

Quesada-Moraga *et al.,* 2007 investigated the natural occurrence of entomopathogenic fungi on the Iberian peninsula and the Canary and Balearic Archipelagos. 240 soil samples were collected, 127 from cultivated areas and 117 samples from pristine natural habitats. For each soil sample pH (H_2O), organic matter and particle distrubution were determined. Entomopathogenic fungi were isolated from soil using the *Galleria* bait method and identified microscopically based on morphological characteristics using taxonomic keys.

Entomopathogenic fungi were isolated from 175 of the 244 (71.7 %) soil samples, with only two species found, *Beauveria bassiana* and *Metarhizium anisopliae*. Of the 244 soil samples, 104 yielded *B. bassiana* (42.6 %), 18 yielded *M. anisopliae* (7.3 %), and 53 soil samples (21.7 %) harboured both fungi.

Log-linear models indicated no significant effect of habitat on the occurrence of *B. bassiana*, but a strong association between *M. anisopliae* and soils from cultivated habitats, particularly field crops. Also, irrespective of habitat type, *B. bassiana* predominated over *M. anisopliae* in soils with a higher clay content, higher pH, and lower organic matter content. Logistic regression analyses showed that pH and clay content were predictive variables for the occurrence of *B. bassiana*, whereas organic matter content was the predictive variable for *M. anisopliae*. *B. bassiana* that showed a greater occurrence in natural habitats than *M. anisopliae.* within the range of soil factors favouring fungal occurrence, *B. bassiana* is best suited to soils with a higher pH and clay content but lower organic matter content.

Conclusion

B. bassiana was isolated from soils from different habitats both cultivated and natural. The occurrence of *B. bassiana* can be related to soil parameters.

RMS Comments:

The study provides useful information on the occurrence of *B. bassiana* under various environmental and soil conditions.

Study MA 7.1.1/01

Report:	Scheepmaker, J. and Butt, T. (2010)
	Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment and in accordance with EU regulations.
	Biocontrol Science and Technology 20: 503 – 552.
Guidelines:	none
GLP:	not applicable

Summary

An overview is presented of the available data on the natural background levels of some indigenous fungal MBCAs and their persistence. The natural background level of *B. bassiana* was estimated by calculating the 95th percentile of the geometric mean measured CFU/g soil reported in 6 studies. The resulting value of 830 CFU/g soil was considered a reasonable representation of the upper natural background levels of *Beauveria bassiana*, as by using the 95th percentile, very high peaks were excluded.

Data from 10 studies, including laboratory and field studies, concerning the persistence of various strains of *Beauveria bassiana* was evaluated. The data show a decline in conidial levels under different agronomic conditions. Some workers report a rapid decline in levels of introduced *B. bassiana*. Under field conditions a large level of variation was observed. At some sites more inoculum was recovered from control than treated plots and the rate of decline was greater at some sites than others. However the results of the evaluated studies presented an overall trend for gradual decline of *B. bassiana* after inoculation and it was concluded that the upper natural background level was reached after 0.5 – 1.5 years.

Conclusion

Beauveria bassiana exist naturally at a background level of 830 CFU/g soil. After inoculation the population of *B. bassiana* gradually declines until reaching the background level 0.5 – 1.5 years after treatment.

The assessment below is based on the OECD 2012 guidance (ENV/JM/MONO(2012)):

The review by Scheepmaker and Butt showed that applied inoculum of the three EPF species decreases to natural background levels in time and that increases of the inoculum are only temporary and depend on the presence of a population of host insects in the field.

A wide variety of factors explaining the decline of EPF density was described.

Some general situations with a negative impact on the survival and fate of the inoculum:

- the microorganism is subject to competition and parasitism of the autochthon microbial community.
- the microorganism is subject to predator pressure.
- the microorganism does not germinate and/or proliferate/or multiply in the soil due to very specific (micro-) conditions.
- it cannot readily gain energy from hardly degradable substances of limited biodegradability like lignin.

It is not feasible to collect a set of background studies that are similar regarding soil condition, strain, country, crop, etc., for the simple reason that the data in the literature are not uniform and may be very

limited. Moreover, in most cases, studies from the literature are not based on the desired strain for authorization, as these strains often originate from a specific isolate and can therefore only be found in a certain area. For these reasons, it is not feasible to develop standardised methods specifying the minimum number of different conditions, soils, application timings and samplings. This approach is not practicable and too costly.

In general, it is difficult to evaluate the persistence considering only soil, because germination of *B. bassiana* conidia and subsequent multiplication only occurs in the presence of a host. If occurring, the PEC may increase during a short period of time. After this period, a steady decline of the inoculum is expected to occur. From this follows that the length of the period that the applied concentration is higher than the upper background concentration is to be discussed case-by-case. This is clearly the case for *B. brongniartii* and *M. anisopliae*. In general, the persistent mBCA may be present in an inactive state, probably in a patchy distribution confined to small pockets in the soil. The mBCA may be activated under very specific conditions.

A low remaining residual level in soil can be sufficient for restarting of replication on occurrence of a host, so that from its cadaver new spores can reach the soil again. It should be kept in mind that the background levels of *B. bassiana* conidia may vary widely over one field depending on the different appearance of potential host insects and also on the possibly different microclimates. Survival depends on several abiotic and biotic factors. These are specific soil properties, temperature, moisture and water, and agrochemicals, as abiotic factors and soil micro-organisms as well as soil arthropods as biotic factors (Keller & Zimmermann 1989).As described in the study by Clerk (study 7/01) germination of conidia in soil is subject to fungistasis. It might be conceivable to find comparatively high background concentration in one place of the field and almost negligible concentration in another place being only a few meters away.

Predicted environmental concentrations in soil (PEC_S)

BAS 480 00 I is an OD formulation containing 4.0×10^{12} CFU/L, equivalent to 40 g *eauveria bassiana* strain PPRI 5339/L. The BAS 480 00 I formulation is intended for glasshouse application only, details of the proposed use patterns that will be assessed are summarised in Table B.8.1.1-01.

Table B. 8.1.1-01: Proposed uses of BAS 480 00 I

Crop	Application method	Maximum individual application rate (g as/ha)	Number of applications	Maximum total application rate (CFU/ha)	Minimum application interval (days)	Application timing

Tomato Sweet pepper Aubergine Edible and processed fruits Cucumber	Spraying (SP)	100	52 - 122	52 x 10^{13}-1.22 x 10^{15}	3	BBCH 11 - 89
Ornamentals Non-edible / Non-food crop	Spraying (SP)	100	52 - 122	52 x 10^{13}-1.22 x 10^{15}	3	BBCH 11 - 99

A single application of 100 g as/ha is equivalent to 1.0 × 10^{13} CFU/ha.

The impact of formulants is limited to short-term effects such as formation of stable spray dispersions or to facilitate uptake by target organisms, while their influence on long-term processes, such as degradation and distribution of the active substances in the environment is considered negligible. Therefore, for the purposes of this risk assessment it is assumed that the formulants do not influence the fate and behaviour of the active substances in the environment and they are not considered further.

According to the OECD 67 (2012) document (ENV/JM/MONO(2012)1)and also EFSA (2014)[3] on the environmental safety evaluation of microbial biocontrol agents, exposure of soil organisms is not considered relevant and therefore no PECs for glasshouse use are presented.

B.8.1.2 Water (Annex IIB 7.1.2)

The survival of *B. bassiana* strain PPRI 5339 has been studied to evaluate its potential for persistence in water (Doc M-MA 7.1.2/01).

Study MA 7.1.2/01

Report: Whittaker, M (2014)
Hydrophobicity and survival of *Beauveria bassiana* PPRI 5339 in water. APIS, Knaresborough Technology Park, Manse Lane, Knaresborough, North Yorkshire HG5 8LF.
Unpublished report APIS-BASF-010.

[3] EFSA Guidance Document on clustering and ranking of emissions of active substances of plant protection products and transformation products of these active substances from protected crops (greenhouses and crops grown under cover) to relevant environmental compartments. EFSA Journal 2014;12(3):3615, 43 pp., doi:10.2903/j.efsa.2014.3615

Guidelines: None

GLP: Yes

Summary

This study was conducted to determine the hydrophobicity and survival in water of *Beauveria bassiana* PPRI 5339 technical grade active ingredient.

Material and Methods

Test Item

Reference Name:	*Beauveria bassiana* PPRI 5339
Label Identification:	S826
Date received:	08.08.2013
Quantity received:	319.04 g
Physical State:	Off white powder
Storage conditions:	Refrigerated (~ 4 °C)
Nominal purity:	1.0×10^{11} CFU/g
Stability:	Stable at room temperature or under refrigeration

Hydrophobicity test

Approximately 1.0 g of the test item was added to approximately 50 mL of deionised water and photographed. The mixture was agitated gently for approximately 30 seconds, allowed to stand for five minutes, and then photographed again. This procedure was repeated twice more, using vigorous and then very vigorous shaking, with photographs taken after each five minute resting period.

Survival in water test

The test item was handled aseptically. Approximately 1.0 gram of batch S826 was weighed out accurately (to 1.0 mg) and mixed in the weigh boat with Tween 80 to form a smooth paste. Three further weigh boats were set up in the same manner. Each weigh boat was carefully washed into a sterile flask with 98 mL of water. Two flasks were prepared using tap water, and two with tap water that had been sterilised by autoclaving. The flasks were shaken to suspend the spores and then sonicated for 3 minutes in an ultrasonic bath.

One flask of tap water and one of sterile water were placed in an incubator set to 20°C and darkness, with the other flasks being placed on a laboratory bench in full sunlight.

At time 0 and at approximately 24 hour intervals over the following nine days, the flasks were thoroughly agitated to ensure homogeneity, and 1.0 mL withdrawn and diluted in sterile phosphate buffered saline. An aliquot of 100 µL of each dilution was plated in triplicate on Rose Bengal Agar and incubated at 28°C and read after approximately 48 hours.

Findings

Hydrophobicity test

Figures B.8.1.2-01 to B.8.1.2-04 show the hydrophobicity of *Beauveria bassiana* strain PPRI 5339 on application to water and then at 5 minute intervals following periods of increasingly vigorous shaking. The test item was considered extremely hydrophobic, and rapidly returned to a floating layer after shaking.

Figure B.8.1.2-01: *Beauveria bassiana* PPRI 5339 immediately after application to water

Figure B.8.1.2-02: *Beauveria bassiana* PPRI 5339 five minutes after gentle agitation

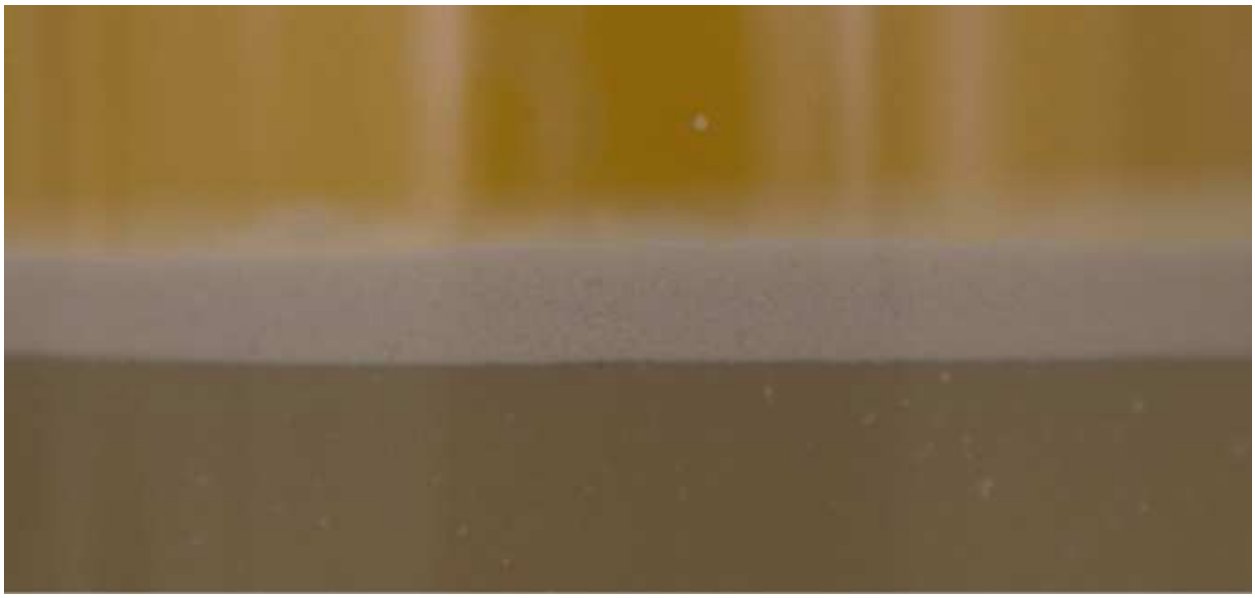


Figure B.8.1.2-03: *Beauveria bassiana* PPRI 5339 five minutes after vigorous agitation

Figure B.8.1.2-04: *Beauveria bassiana* PPRI 5339 five minutes after vigorous agitation

Survival in water

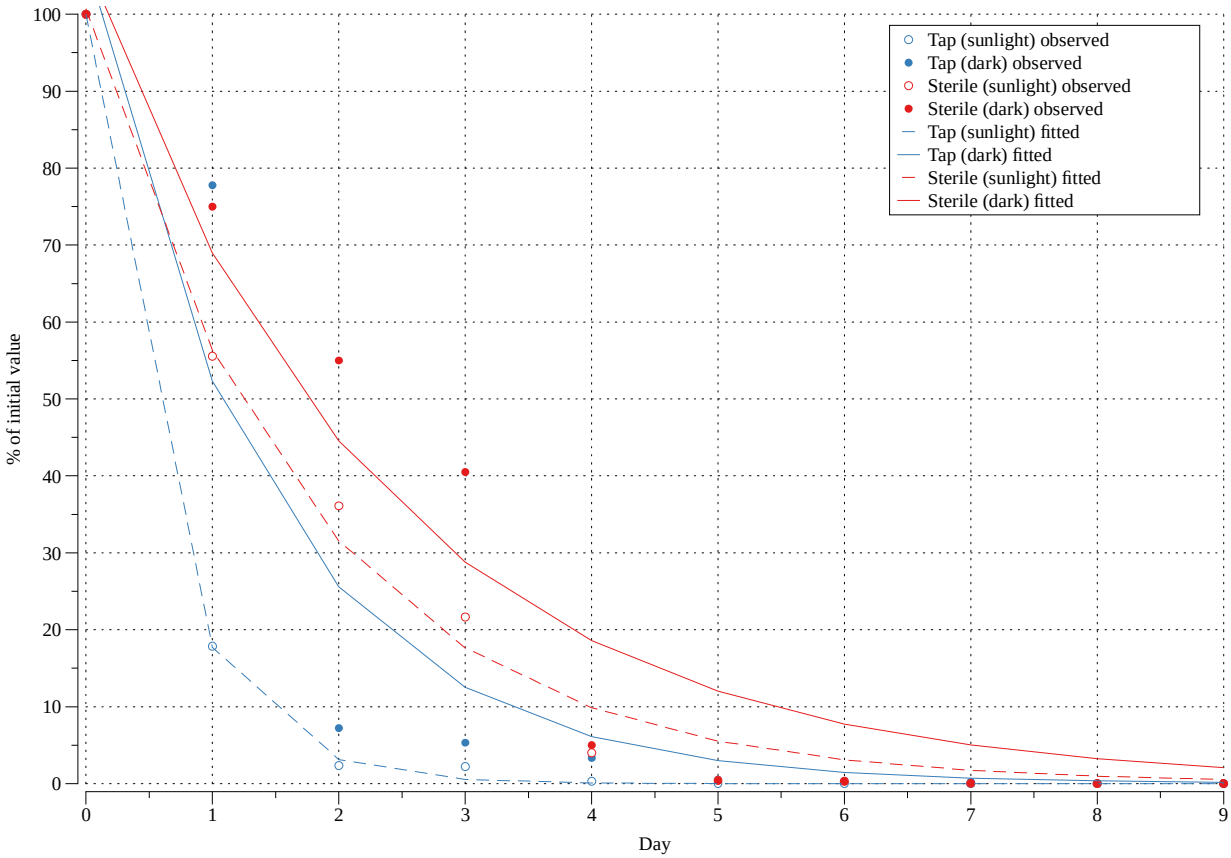
Table B.8.1.2-01 and Figure B.8.1.2-05 show the survival of the test item in water.

Table B.8.1.2-01: *Beauveria bassiana* strain PPRI 5339 spore viability at 0-9 days after addition to water under constant dark and natural sunlight

Day	Mean viability (CFU/mL) at days after treatment				% of initial concentration			
	Tap water		Sterile Water		Tap water		Sterile Water	
	Sunlight	Darkness	Sunlight	Darkness	Sunlight	Darkness	Sunlight	Darkness
0	2.80E+11	1.80E+11	1.80E+11	2.00E+11	100	100	100	100
1	5.00E+10	1.40E+11	1.00E+11	1.50E+11	17.9	77.8	55.6	75.0
2	6.60E+09	1.30E+10	6.50E+10	1.10E+11	2.36	7.22	36.1	55.0

3	6.20E+09	9.60E+09	3.90E+10	8.10E+10	2.21	5.33	21.67	40.5
4	8.70E+08	6.00E+09	7.20E+09	1.00E+10	0.31	3.33	4.00	5.00
5	8.20E+07	9.00E+08	7.70E+08	7.60E+08	0.029	0.500	0.428	0.380
6	6.60E+07	6.70E+08	5.50E+08	6.10E+08	0.024	0.372	0.306	0.305
7	1.00E+07	4.30E+08	7.90E+07	9.50E+07	0.004	0.239	0.044	0.048
8	5.30E+06	5.40E+07	9.60E+06	8.10E+07	0.002	0.030	0.005	0.041
9	5.70E+06	8.90E+07	4.00E+06	4.80E+07	0.002	0.049	0.002	0.024

Figure B.8.1.2-05: Reduction in percentage viable conidia of *Beauveria bassiana* strain PPRI5339 with time in aqueous solutions together with single first order model fit



The results show a slight difference in loss of viability between test solutions maintained in the light and in darkness and a more significant difference between sterile and non-sterile solutions. During the preparation of this dossier, the results were analysed to determine the time to 50% deactivation half-lives assuming single first order kinetic behaviour (see Q7 (p. 539-540) in Scheepmaker and Butt (2010) where the applicability of such kinetics is discussed). These time to 50% deactivate values are presented in Table B.8.1.2-02.

Table B.8.1.2-02: Spore viability in water expressed in SFO Kinetics

Kinetic Parameter	Treatment			
	Tap water		Sterile Water	
	Sunlight	Darkness	Sunlight	Darkness
Rate constant (k)	1.729	0.715	0.580	0.437
Time to 50% deactivation (Days)	0.4	1.0	1.2	1.6
Time to 90% deactivation (Days)	1.3	3.2	4.0	5.3

Conclusions

The test item proved to be extremely hydrophobic, and rapidly formed two phases, even after vigorous mixing. By Day 9, inoculum levels had decreased to below 0.05% of the starting values in all cases, although a high number of viable spores remained.

There was evidence to suggest that the rate of reduction of viable conidia was influenced by biotic and abiotic factors; reduction was ~4 times faster in non-sterile water maintained in sunlight than in sterile water maintained in darkness.

RMS Comments:

The applicant applied a SFO kinetic model to the data to calculate a DT_{50}. In the report this is called DT_{50}. RMS agrees to apply SFO kinetics to the data but does not agree that in this study a DT_{50} (half-life) is derived. The measurements present the reduction in viability of spores during time by testing the culturability. A rate constant for the reduction of culturable spores during time can be derived using SFO kinetics and results in a rate constant. Therefore RMS changed the wording in ‘’time to reach 50% and 90% deactivation” which in this case means the loss of viability/culturability.

Predicted environmental concentrations in surface water (PEC_{SW})

According to the OECD 67 (2012) document (ENV/JM/MONO(2012)1), discharge to surface water should be considered for permanent structures.

The exposure concentrations of *Beauveria bassiana* strain PPRI 5339 in surface water have been estimated for the proposed uses. In order to calculate exposure from greenhouse emission, the standard emission for plant protection products of 0.1% from glasshouses is used for the current assessment as a worst case approach.

The PEC_{sw} was calculated using a standard FOCUS ditch with a rectangular profile 1 m wide and a water depth of 0.3 m. The ditch contains 300 L of water per meter and it was assumed that 0.1% of the dose enters the standard ditch (1 m in length).

In an aquatic environment conidia of *Beauveria bassiana* strain PPRI 5339 will be expected to inactivate rapidly in natural water bodies (see Study MA 7.1.2/01). The worst-case 50% deactivation time was estimated to be 1.6 days in sterile dark conditions at 20 °C.

Table B.8.1.2-03: Surface water initial concentrations (PECsw initial) for *Beauveria bassiana* strain PPRI 5339 in the edge-of-field ditch following application

Use	Rate as/ha (CFU/ha)	Drift rate (%)	Drift rate (CFU/ha)	Loading rate per m^2 ditch (CFU/m^2)	PECsw (CFU/L)
One application	1.0×10^{13}	0.1	1.0×10^{10}	1.0×10^{7}	4760
all uses (n=122)	1.2×10^{15}	0.1	1.2×10^{12}	1.2×10^{8}	5.8×10^{5}

The proposed uses presented in Table B.8.1.1-01 for BAS 480 00 I indicate a minimum interval between applications of 3 days (≈122 applications per year). Based on the inactivation rate noted for *Beauveria bassiana* strain PPRI 5339 there is a theoretical possibility for carry over between applications.

Applicant provided a PECsw calculation based on first order kinetics half-life referenced to Scheepmaker and Butt (2010). RMS does not agree to this concept. The concept of DT_{50} may be described by Scheepmaker and Butt, however the use of the standard equation for chemicals, which is based on first order nonlinear regression at standardised temperature, for time weighted average calculation is not warranted for microbials. In PRAPER M2 it was agreed to calculated the worst case exposure based on the total load without degradation. RMS presented this calculation in Table B.8.1.2-03. Furthermore, RMS calculated the PEC_{sw} using a standard FOCUS ditch taking into account the non-rectangular dimensions of the ditch 1 m wide and a water depth of 0.3 m. That ditch contains 210 L of water per meter and it was assumed that 0.1% of the dose enters the standard ditch (1 m in length).

Potential effect of MPCA on analytical methods for drinking water

Despite the highly restricted persistence of *B. bassiana* strain PPRI 5339 in surface waters (see study MA 7.1.2/01) it may be theoretically possible that single conidia can reach drinking water treatment plants. This, however, is not expected to be relevant for human health as the microorganism is not pathogenic and does not produce metabolites of toxicological concern as in water it is metabolically inert. In addition, there is no evidence that the presence of *B. bassiana* strain PPRI 5339 might affect drinking water monitoring systems. Drinking water quality is monitored by screening for microbial indicator species using highly selective media on which *B. bassiana* strain PPRI 5339 does not grow. This was clearly demonstrated by the outcome of 5-batch analyses of microbial contaminants in the technical material, which included these indicator bacteria.

Potential interference with the analytical systems for the control of the quality of drinking water according to Council Directive 98/83/EC for drinking water coliforms or *E. coli,* enterococci, and *Pseudomonas aeruginosa* are monitored in drinking water.

The methods used are ISO 9308-1, ISO 16266 and ISO 7899-2. In principle, drinking water monitoring requires a concentration step (usually membrane filtration) as drinking water does not contain high densities of micro-organisms. Afterwards, the concentrated samples are subjected to cultivation procedures on media which are highly selective for the above mentioned indicator species. In most cases the media contain specific substrates which are metabolized by the indicator species resulting in a particular colour reaction which is then used as a discrimination criterion. The following media/substrates are used:

- *P. aeruginosa*: acetamide reaction on cetrimid agar (fluorescing colonies) and growth at 42 °C for distinction between *P. aeruginosa* and *P. fluorescence*
- *E. coli* or coliforms: chromogenic agar (tryptone bile salts agar), red or pink colour due to glucuronidase activity
- Enterococci: Slanetz-Bartley medium (triphenyltetrazolium chloride is recued to formazan, red to maroon colorization of enterococci colonies) and/or KAA agar (kanamycin, aesculin, azide), black colorization of enterococci colonies due to esculin-hydrolase activity

Quality assurance for the specific media always includes testing of false positive signals for other indicator species whereby in most case *E. coli, Enterococcus* and *P. aeruginosa* are used which never interfere with the methods used. This indicates that the media are highly specific and it is very unlikely that fungal species will grow on the media and/or exhibits the required enzymatic activities for metabolism of the applied substrates used for identification of the indicator species.

The technical material of *B. bassiana* strain PPRI 5339 was screened for the presence of the above mentioned indicator bacteria using the similar microbiological methods or identification principles. Despite a high density of *B. bassiana* strain PPRI 5339 in the test material, no false positives or any other kind of interference with the applied methods has been observed. It can be therefore concluded, that *B. bassiana* strain PPRI 5339 will not interfere with the microbiological methods used for drinking water monitoring.

Potential Effects on biological methods for sewage treatment

The relevance of sewage treatment plant (STP) assessments for microbial pesticides was discussed in the 2014 Report of the OECD/KEMI/EU Workshop on microbial pesticides: Assessment and management of risks, ENV/JM/MONO(2014)2. It was concluded that this data requirement is not relevant for microbial pesticides and therefore is not discussed further here. This is considered particularly justified for *B. bassiana* strain PPRI 5339 as it has been determined that spores do not germinate in water and are quickly deactivated and similarly vegetative cells, which are un-adapted to aqueous conditions, do not survive. As spores and vegetative cells are unable to proliferate in aqueous conditions they unable to exert a negative competitive effect on sewage microorganisms and furthermore are non-metabolically competent to produce secondary metabolites which may inhibit sewage microorganisms.

RMS Comments:

RMS agrees with the statement that micro-organisms and spores are not capable to survive under aquatic conditions and can have a negative effect on the waste water treatment plant.

B.8.1.3 Air (Annex IIB 7.1.3 and IIIB 9)

The study by Neethling *et al.,* 2014 (see study MA 7.1.3/01, summarised below) provides evidence specific to *B. bassiana* strain PPRI 5339 that *B. bassiana* conidia are highly susceptible to damage from UV light.

Similarly, Edgington *et al*., 2000 (see study MA 7.1.3/02, summarised below) showed that viable *B. bassiana* conidia were almost completely inactivated by exposure to 60 minutes of direct sunlight or 20 seconds of UV light at a wavelength of 302 nm.

Beauveria bassiana conidia are dry, small in size and released in powdery clusters and therefore are readily transported by air (Zimmermann, 2007). Fungi species use aerial spore release as the major dispersion / host location strategy and this is likely the case for *B. bassiana*. Conidia in air will be likely exposed to sunlight.

Sunlight, especially UV-B (290 - 330 nm) and UV-A (330 - 400 nm), has been demonstrated as a detrimental environmental factor affecting the field persistence of fungal insecticides (Zimmermann, 2007). The results presented in the Zimmermann (2007) review reveal that entomopathogens are inactivated within hours or days when exposed to sunlight. The underlying studies from the review that were submitted in the dossier are discussed below. In laboratory experiments under simulated sunlight, 99% of all *B. bassiana* conidia were inactivated at UV-C after nearly 16 min, and at UV-A and UV-B after about 31 min (Krieg *et al*. 1981). The influence of simulated sunlight (295 - 1100 nm) on the survival of conidia of 65 isolates of *B. bassiana* demonstrated that the survival decreased with increasing exposure, i.e. an exposure for 2 h or more was detrimental to all isolates tested (Fargues *et al*. 1996).

Study MA 7.1.3/01

Report: Neethling, D., Neethling, K. and Brown, A (2014).
Effect of artificial ultra-violet light on viability of *Beauveria bassiana* strain PPRI 5339.
Unpublished Report R&D061401

Guidelines: None

GLP: No

Summary

The aim of the study was determine the effect of sunlight on the viability of spores of the Microbial Pest Control Agent (MPCA) *Beauveria bassiana* strain PPRI 5339. Under artificial Ultra Violet light, technical grade *B. bassiana* strain PPRI 5339 demonstrated a degradation curve typical of a *B. bassiana* and was calculated to have an LT_{50} of 39 minutes.

Material and Methods

Assessments on the effect of artificial Ultra Violet (UV) light on survival of *B. bassiana* PPRI 5339 were completed as project LE326 and initiated on 03.02.2005.

Technical grade *B. bassiana* strain PPRI 5339 (Batch LE300, 1.43×10^{11} viable spores/g) powder was placed in a petri dish. The petri dish was placed on the laboratory work surface 10 cm from a germicidal UV lamp producing short-wave UV radiation with a peak at 253.7 nm (UV-C). Samples were removed from the powder after 0, 10, 30, 60, 180, 240 and 285 minutes. After removal, samples were added to 10 mL of G49 wetting agent, agitated to mix and then sonicated for 3 minutes. After sonication, 0.1 mL samples were plated onto Potato Dextrose Agar plates. The plates were then incubated at 28 ºC for 17 hours. Following incubation plates were counted for the number of germinated and un-germinated spores, those that had germinated had the length of the germ tube measured. The experiment was replicated twice.

Results

Exposure of *B. bassiana* strain PPRI 5339 to UV light reduced the germination and viability of the fungal spores. After only 10 minutes the viability had reduced by 17% and at the end of the study at 285 minutes, viability had fallen to 15%. From the data, the LT_{50} was estimated to be *ca* 39 minutes by linear interpolation of log transformed viable spore counts.

Table B.8.1.3-01: Effect of exposure to artificial ultra violet under laboratory conditions on the germination and viability of spores of technical grade *Beauveria bassiana* strain PPRI 5339

Time (mins)	Average percent germination (%)	Average germ tube length (µm)
0	100.00	50.00
10	83.70	47.50
30	58.61	43.75
60	45.05	37.50
120	29.89	36.25
180	24.12	33.75
240	18.47	25.00
285	15.33	33.75

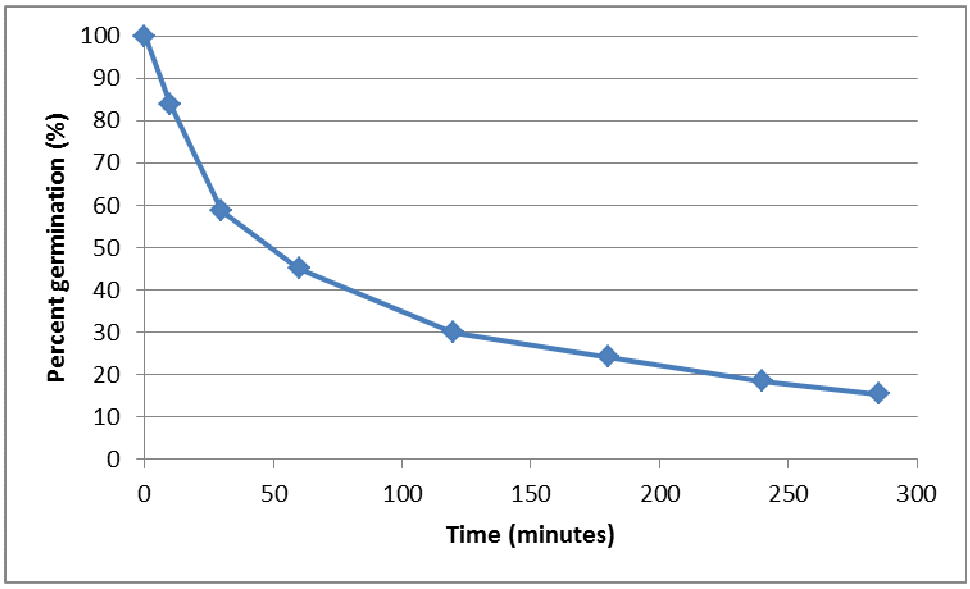


Figure B.8.1.3-01: Effect of exposure to artificial ultra violet under laboratory conditions on the germination and viability of spores of technical grade *Beauveria bassiana* strain PPRI 5339.

It was also shown that, of those spores that did germinate after prolonged exposure to UV light, their germ tubes did not grow as quickly over the 17 hour incubation period as those germinated spores from the untreated control that were measured after zero time exposure to UV light.

Conclusion

Rapid inactivation of *B. bassiana* spores occurred following exposure to UV light.

RMS Comments:

The study demonstrates rapid inactivation of spores of *Beauveria bassiana* strain PPRI 5339 after exposure to UV.

Study MA 7.1.3/02

Report: Edgington, S., Segura, H., De La Rosa, W. and Williams, T (2000).

Photoprotection of *Beauveria bassiana*: testing simple formulations for control of the coffee berry borer.

International Journal of Pest Management, 46(3) 169-176

published

Guidelines: None

GLP: No

Summary

The effect of sunlight and UV light on the viability of *B. bassiana* conidia was assessed in a simple laboratory bioassay. Unprotected *B. bassiana* conidia were almost completely inactivated by exposure to 60 minutes of direct sunlight or 20 seconds of UV light at a wavelength of 302 nm.

Material and Methods

An isolate of *B. bassiana*, "strain 26" was used in the tests. The fungus was grown until sporulation on Sabouraud dextrose agar plus 0.5% yeast extract in sterile Petri dishes at ambient laboratory temperatures, 26 - 30°C. Spores were harvested from the plates and stored in a desiccator at 4°C. Batches of spores showing less than 80% germination in control tests at any time during testing were discarded.

Sunlight test

Spores were suspended to a concentration of approximately 1×10^9 spores/mL in 0.1% Triton X-100. A 3 µL droplet of this suspension was smeared evenly across the length of a clean glass microscope slide. Spores were exposed to direct natural sunlight when sun intensity was at a maximum.

Following exposure, a thin layer of 3% Sabouraud agar (+0.02% aureomycin) was added onto each slide and all were then placed in a dark humid box for 18 h at ambient laboratory temperatures (26 - 30°C). Germination was determined using a phase contrast microscope at ×600 magnification. The mean percent spore germination was calculated for each slide by counting the number of germinated and ungerminated spores in five fields of view. Between three and six replicate slides were used for each treatment.

UV light test

Slides holding spores were prepared as above with the exception that various chemical with potential UV protection were included. Spores were exposed to UV light emitted from a trans illuminator, peak 302 nm. Test slides were located at a distance of 2 cm from the UV filter, and exposed for between 5 and 25 seconds. Spore germination was assessed as previously described.

Findings

Sunlight test

There was a highly significant negative relationship between spore germination and duration of exposure to sunlight ($P < 0.0001$). Unprotected spores were inactivated rapidly; after 60 minutes exposure spore germination had been reduced from an initial mean of 89.4% (range of SE 90.5 - 88.3%) to just 2.8% (range of SE 3.4 - 2.3).

UV light test

Even in the presences of UV protection, spore inactivation was extremely rapid and was linearly correlated to the duration of exposure ($P < 0.0001$). After just 20 seconds of UV irradiation, mean spore germination fell from an original 95.3% (range of SE 94.9 - 95.7%) to 0.74% (range of SE 0.92 - 0.59%).

Conclusion

Rapid inactivation of *B. bassiana* spores occurred following exposure to UV light or natural sunlight.

RMS Comments:

The study demonstrates rapid inactivation of spores of *Beauveria bassiana* “strain 26” after exposure to UV or natural sunlight.

Further information from public literature confirmed the results of the studies above. After irradiation with simulated sunlight (SUV), (Ignoffo and Garcia, 1992) found a half-life of *B. bassiana* conidia of about 2 h. They exposed conidial suspension to SUV for 2, 4, 8, 16, 24 and 32 h, respectively. A non-exposed sample (0 h) was also included which was used to calculate the mean percentage original activity remaining. After exposure the conidia were recovered from each dish and CFU counts performed. After 2 hours exposure about 40% of the total CFU count at 0h exposure remained compared to 87.5% for the dark control.

The survival of conidia exposed to uv-B radiation on leaves and glass was compared in the laboratory. The survival of conidia applied in water onto glass coverslips and on crested wheatgrass was reduced by greater than 95% after 15 min exposure to UV-B radiation. Conidial survival in oil, was more pronounced on glass (74% reduction after 60 min) than on leaves (97% reduction after 60 min) (Inglis *et al*. 1995).

Morley-Davies *et al*., 1995 tested the influence of storage temperatures and also the effect of UV exposure to simulated sunlight on conidia of 14 isolates of the entomopathogenic fungi amongst which 2 *Beauveria bassiana* strains. For the simulated sunlight test samples were prepared as oil formulations. Significant differences in susceptibility to simulated sunlight between the isolates of *B. bassiana* were reported (Morley-Davies *et al*., 1995).

B.8.2 Mobility (Annex IIB 7.1.1 and IIIB 9)

In Soil:

The following study is summarised here to provide indication of where in soil conidia of *B. bassiana* can be found after a spay application. The study provides data regarding the position of viable conidia in soil columns post-irrigation.

Study MA 7.2/01

Report: Storey, G.K. and Gardner W.A. (1988)
Movement of an aqueous spray of *Beauveria bassiana* into the profile of four Georgia soils.
Environmental entomology 17(1): 135 – 139 (1988)

Guidelines: None

GLP: No

Summary

The vertical dispersion of conidia of *B. bassiana* in soil was assessed in a greenhouse and field situation, post irrigation. Conidia were found to be not very mobile in soil and generally remain on the

surface of the soil. The movement of conidia vertically, through the soil profile, is positively correlated with high infiltration rates in soil.

Material and Methods

Four soil series obtained from various locations in Georgia were used in this experiment. They were as follows: The Cecil series (sandy clay loam soil), the Tifton series (sandy loam soil), the Greenville series (sandy clay loam soil) and the Townley series (clay loam soil).

In the study, an intact soil block (20 cm deep) from each of the four Georgian locations was removed with a soil corer. They were then placed in a greenhouse and maintained at 30°C, under a polyethylene tent. Each container was covered with a plastic tray to reduce moisture loss from the blocks. A 100 mL aliquot of an aqueous suspension of commercially formulated *B. bassiana*, containing 9.44×10^7 CFU/mL, was sprayed on the surface of each block.

A soil core from each soil series was removed prior to and 72 h after application. Horizontal sub samples of each of these cores at 0, 4, 8, 12 and 16 cm depth were washed. Dilutions of each wash were then drop-plated on oatmeal-dodine agar and incubated at 25 °C for 7 days. Vertical movement estimates were based on the number of CFUs recovered from each cm^2 of dry soil at each depth of the respective soil cores.

In a field study, field plots measuring 1.8 by 1.8 metres at each of the four Georgian locations were treated with 2 L of an aqueous suspension of a commercial product, containing containing 1.25×10^8 viable conidia per ml. conidia of *B. bassiana*. This was followed by application of 8 L of water to each plot.

Soil cores (15 cm deep) were taken from each of the plots prior to and 2 h after application of the conidia. A 1 g sub sample was removed from three 5 cm vertical sections of each core and processed for CFU enumeration as previously described. Vertical movement was estimated by the number of CFU recovered from each depth of the various soil profiles examined.

Findings

In greenhouse studies, 95% of the total number of CFUs recovered in core samples of the Townley, Greenville and Cecil series soils remained at the surfaces. In the Tifton series soil, 87 % of the total CFU were recovered from the surface with an additional 9 % recovered 4 cm below the surface. Vertical movement of conidia in these soils appears to be greatest in soils with high infiltration rates, such as the Tifton and Greenville series soils. Further details on conidial movement through the four soil series in a greenhouse study are presented in Table B.8.2-01.

Table B.8.2-01: Number of CFUs of *Beauveria bassiana* per cubic centimeter dry soil recovered from selected depths 72 h after application to soil blocks

Depth (cm)	Soil			
	Cecil	Greenville	Townley	Tifton
0	1.13E+08	3.29E+07	2.24E+07	1.44E+07
4	1.20E+05	1.41E+05	3.80E+04	1.50E+06
8	2.80E+04	1.90E+04	7.00E+03	2.70E+05
12	3.70E+04	1.70E+04	4.10E+04	2.86E+05
16	1.07E+05	5.00E+04	1.70E+04	1.60E+04

In a field study, core samples taken 2 h after application indicated that the four soils restricted vertical movement of the conidia. Greater than 94% of the total number of CFU recovered in the cores ranged from the upper 5 cm of the profile of each soil series. At the 5 – 10 cm soil depth, 0.7, 1.8, 3.3 and 4.4 % of the total CFU were recovered from the Cecil, Greenville, Tifton, and Townley series soils, respectively. Retention of the conidia in the upper profile of these soils appears to be caused by mechanical filtration within the soil structure. No significantly correlation between vertical movement and the sand, silt, or clay content was found.

Further details on conidial movement through the four soil series in a field study are presented in Table B.8.2-02.

Table B.8.2-02: Number of CFUs of *Beauveria bassiana* per cubic centimeter dry soil recovered from selected depths 2 h after application to soil blocks

Depth (cm)	Soil			
	Cecil	Greenville	Townley	Tifton
0-5	3.62E+06	4.97E+06	1.85E+06	4.65E+06
5.1 – 10.0	2.40E+04	9.30E+04	8.50E+04	1.61E+05
10.1 – 15.0	4.00E+03	9.10E+04	1.50E+04	4.50E+04

Conclusion

Conidia of *Beauveria bassiana* are not very mobile in soil and generally remain on the surface of the soil. The movement of conidia vertically, through the soil profile, is positively correlated with high infiltration rates in soil.

RMS Comments:

From the study it is clear that conidia of *B.bassiana* show a limited mobility in soil.

Study MA 7.2/02

Report: Kim, J.S. Skinner, M. Gouli, S. Parker, B.L. (2010)
Influence of top-watering on the movement of *Beauveria bassiana*, GHA (Deuteromycota: Hyphomycetes) in potting medium.
Crop Protection 29, 631–634

Guidelines: None

GLP: No

Material and Methods

Beauveria bassiana strain GHA was obtained from the Entomology Research Laboratory Collection at the University of Vermont. A millet grain based solid culture method was used to produce mycotized *B. bassiana* GHA grains, with a concentration of 1.1×10^8 conidia per gram and a germination rate of 98.2% at 24 h and 20 ± 1 °C. The cultures were dried until the moisture content of the conidia powder was less than 5%.

Into 36 plant pots, 135 g of potting medium (sphagnum peat moss, vermiculite, bark, fine bark, starter nutrient charge, dolomitic limestone and wetting agent) was added. Three gravimetric moisture content treatments were tested, 43, 100 and 233% of water saturation, each replicated three times. Into each saturation treatment replicate, 2 g of mycotized millet grains were added and mixed into the upper 3 cm of the medium. Three pots of each treatment received no fungus and served as controls. Treatment and control pots were placed in individual saucers to collect water held at room temperature (25 ± 2 °C). To simulate standard watering practices, every second day after fungal application, pots were top-watered with either 50, 100 or 150 mL over a 18 day period. Control pots received 150 ml of water. Thirty minutes after watering, the amount of released water in each saucer was measured. The number of colony forming units (CFU) of *B. bassiana* was determined by plating the released water on a *B. bassiana* selective medium.

After 18 days the potting medium in each pot was separated into ~ 3 cm layers representing upper, middle and lower layers. From each layer a 20 g subsample was taken and mixed with a 0.02% solution of polysiloxane polyether to make 50 mL soil suspensions with even distribution of *B. bassiana*. Another 5 g subsample from the same layer was taken to measure dry weight.

Findings

No *B. bassiana* CFUs were detected in water from control pots throughout the experiment. Therefore, it is assumed that *B. bassiana* CFUs from pots treated with the mycotized grains were GHA. For the 233% saturation with the 100 mL and 150 mL top watering treatments, a small number of *B. bassiana* CFUs were detected on days 0, 2 and 4 post application in the water released, though differences were not significant between these treatments ($P > 0.05$). A small number of *B. bassiana* CFUs were also detected from water released on days 2 and 4 post-application in the treatments with 43% and 100% saturation and the 150 mL top-watering. On day 6 post-application, a very small amount of *B. bassiana* CFUs were detected from the 43% saturation with the 100 ml top watering treatment, less than 1×10^3 CFUs per pot. No B*. bassiana* CFUs were found after day 6 for all treatments. Overall, very few *B. bassiana* CFUs ($<1 \times 10^6$/pot) were released, when compared with the number of conidia (2.2×10^8 conidia/pot) applied at the start of the study.

Significantly more *B. bassiana* CFUs were detected in the upper than in the middle and lower layers for all moisture content treatments tested. In the upper layer of the 233% saturation treatment, significantly fewer CFUs were found from the 100 and 150 mL watering regime than the 50 mL regime ($P < 0.001$). In addition, when comparing the number of CFUs among percent saturation treatments, significantly fewer were detected in the 233% treatment than the others ($P < 0.001$ in both cases). When data from the number of CFUs detected in the released water after top-watering and the fungal concentration in the three layers of medium in the pots were considered together, the results suggest that by day 6–8 the fungus had inclusively colonized the upper layer of the medium (we observed white mycelium on the potting medium), thereby eliminating any leaching of conidia from water at the bottom of the pot. Minimal fungal growth was detected in the middle and lower levels of the potting medium regardless of the percent saturation or watering regime. Three factors may explain this outcome: (a) fungal colonization of soil or potting media occurs relatively slowly, and 18 d was insufficient time for the fungus to grow throughout the pot, i.e. to the middle and lower layers; (b) fungal colonization is inhibited by abiotic factors such as poor aeration or unsuitable pH conditions; (c) fungal movement is prevented by the filtering effect of the potting medium components such as vermiculite or dolomitic limestone.

Conclusion

In conclusion, a small amount of the *B. bassiana* GHA applied to the upper layer of the potting mix was detected in water at the bottom of the pots, and after 4–6 d, no evidence of fungal leaching was found. After 18 days of regular top-watering, the concentration of *B. bassiana*, based on the number of CFUs, was greatest in the top 3 cm of potting medium, though the percent saturation of the medium and the volume of water it received affected fungal concentration levels. Results of this experiment demonstrate that mycotized millet grains persisted for 18 d post-application, mainly in the top layer. It must be indicated that the potting medium with a consistent saturation of 233% is not common in greenhouse production as it encourages root rot and poor plant growth.

RMS Comments:

From the publication it is understood by RMS that the first measurement (t=0) is thirty minutes after the first watering. Some leaching of conidia was seen during the first 4-6 days of the experiment. The influence of the water saturation rate and the volume of water affected the concentration levels to a large extend. It should be considered that the dimension of the pots is rather small (10x10x12 cm) which is probably one of the causes for this direct influence. It appears from the study that initially a small amount of CFU can move through the soil column (which has a height of 12 cm) after applying a high amount (1.1×10^8 conidia/g).

Predicted environmental concentrations in ground water (PEC_{GW})

Conidia of *Beauveria bassiana* are not mobile in soil and generally remain on the surface of the soil. Therefore contamination of the groundwater can be excluded.

In Air:

In the review by Zimmerman(2007) it is stated that many fungi species use aerial spore release as the major dispersion / host location strategy and this is likely the case for *B. bassiana*. *Beauveria bassiana* conidia are dry, small in size and released in powdery clusters and therefore are readily transported by air. However, for *B. bassiana* it was demonstrated in several studies that rapid inactivation of *B. bassiana* spores occurred following exposure to UV light or natural sunlight (see paragraph B.8.1.3) and therefore transport in air is considered insignificant.

In water:

Conidia are hyaline, globose to broadly ellipsoidal, generally 2 - 3 × 2 - 2.5 µm (Zimmermann, 2007). The globose or ovoid shape of conidia is considered not suitable for efficient transport in water (see also EFSA conclusion on pesticide peer review[4]). Furthermore, it was demonstrated that *B. bassiana* loose viability in water rather quickly (see paragraph B.8.1.2).

B.8.3 References relied on

Literature data

Report:	*Beauveria bassiana* strain PPRI 5339, DOCUMENT M-MA, Section 9, literature search. Biosphere Biopesticide Consulting – September 2014
Guidelines:	European Food Safety Authority; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011.2092
GLP:	no

A literature search was carried out by the notifier following the EFSA guideline “Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009”[5] (M document, section 9).

The search strategy is detailed in the tables below. A broad search was done to look for any references which included the active substance *Beauveria bassiana*, or its major metabolites, in conjunction with any of the key words set out below.

The terms searched for were:

Beauveria bassiana

Tenellin

Vivotoxins

Bassianolide

[4] Conclusion on the peer review of the pesticide risk assessment of the active substances *Beauveria bassiana* strains ATCC-74040 and GHA1. EFSA Journal 2013;11(1):3031

[5] European Food Safety Authority; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p. 1-50). EFSA Journal 2011;9(2):2092. [49 pp.]. doi:10.2903/j.efsa.2011.2092. Available online: www.efsa.europa.eu

Bassianin
Beauvericin

Selection of databases

Search engines used were Thomson Innovation (which covers Web of Science), TOXLINE and Pubmed. A justification for the chosen search engines was provided and were agreed with by the RMS. The search was carried out in June 2014 and covered the 10 year period prior to the search date.

Search strategy

For all databases, a single concept search strategy was adopted, i.e. using terms for *Beauveria bassiana* and its metabolites only (e.g. synonyms, IUPAC name and CAS number).

Criteria for relevance

References were assessed according to the requirements of the EFSA guideline[5] "side-effects on health, the environment and non-target species".

Relevance criteria were created to determine if a literature paper was:

- relevant without restriction;
- relevance is unclear;
- clearly irrelevant.

This was achieved by comparing the paper with discriminating relevance criteria. Two tiers of relevance criteria were applied as follows:

- key qualifying criteria;
- assessment level qualifying criteria.

Key qualifying criteria identify individual fundamental aspects that, if on their own, are deviated from or details of such are omitted, it can be concluded with acceptable certainty that the paper is identified as being "clearly irrelevant". Therefore, identifying a deviation from a key qualifying criteria is sufficient for the assessment to stop.

If all key qualifying criteria were met then the relevance assessment continued to the second tier where the literature was compared to "assessment level qualifying criteria".

Assessment level qualifying criteria are based on a number of scored secondary criteria. These criteria consider the relevance and robustness of the methods used in key areas of the study reported in the literature. Each criterion was scored either "0" meaning unacceptable deviation or "1" meaning that literature detail was acceptable for relevance scoring. The assumption was that if the majority of these secondary criteria were met then the literature can be classified as being "relevant without restriction", if the score was lower, literature was classified as either "relevance is unclear" (e.g. ~50% criteria met) or "clearly irrelevant" (e.g. <50% criteria met). The criteria bands depend on the number

of criteria defined as being assessment level and are shown in the Table B.8.3-01 to Table B.8.3-03. The process is transparent and removes bias.

Table B.8.3-01: Environmental fate relevance criteria – laboratory studies

Key qualifying criteria	
1.	Well-defined test material
2.	Suitable analytical method used and reported or referenced
3.	Relevant substrate type and properties reported
4	Relevant endpoint data
Assessment level qualifying criteria (*each conforming point will score 1, each non-conforming point will receive 0*)	
1.	Exposure/application rates similar to expected use
2.	Experimental conditions similar to guidelines (where appropriate) and of appropriate length
3.	Measured concentrations reported
4.	Suitable replication for robust endpoint
5.	Robust and appropriate model fitting

Table B.8.3-02: Environmental fate relevance criteria – field (dissipation) studies

Key qualifying criteria	
1.	Well-defined test material
2.	Relevant geoclimatic conditions
3.	Suitable analytical method used and reported or referenced
4.	Relevant substrate type and properties reported
5	Relevant endpoint data
Assessment level qualifying criteria (*each conforming point will score 1, each non-conforming point will receive 0*)	
1.	Exposure/application rates similar to expected use
2.	Experimental conditions similar to guidelines (where appropriate) and of appropriate length
3.	Measured concentrations reported
4.	Meteorological information provided
5.	Robust and appropriate model fitting

Table B.8.3-03: Environmental fate relevance criteria – monitoring studies

Key qualifying criteria[a]	
1.	Relevant geoclimatic conditions
2.	Pesticide usage data available for catchment (e.g. crops)
3.	Suitable analytical method used and reported or referenced
4.	Relevant endpoint studied

[a] Due to the nature and wide protocols used in monitoring studies, no assessment level qualifying criteria are assignable. Literature conforming to all key qualifying criteria are considered relevant without restriction.

Results

Table B.8.3-04: An overview of the results of the literature search for *Beauveria bassiana* and its major metabolites

Data requirements	Number of references
Total number of summary records retrieved after all searches of peer-reviewed literature (excluding duplicates)	2495
Number of summary records excluded from search after rapid assessment of relevance	2427
Total number of full-text documents assessed in detail	80
Number of studies excluded from further consideration after detailed assessment of relevance	40
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	40

During the initial rapid assessment, the study titles were scanned to identify whether the studies were relevant to the areas of ecotoxicology, environmental fate or human health. Any studies that were not falling within these areas were excluded. Tables in the report lists the titles of all relevant or unclear studies resulting from this initial rapid assessment ordered by data requirement, and by author according to the requirements of the guidance.

After evaluation of the full text of the papers identified as relevant or of unclear relevance, they were further classified as suitable for inclusion or non-relevant for this dossier. All the papers considered suitable for inclusion (18) are listed in tables, ordered by data requirement and by author. These table are included in the report submitted by the applicant.

RMS Comments:

There is one literature search provided for all aspects which is in line with the requirements of the guidance. There is a general agreement to include the literature research at the beginning of the references relied for each aspect of the DAR. Therefore, RMS selected the relevant records for the section fate and behaviour which are presented in Table B.8.3-05 below, ordered by data requirement.

Table B.8.3-05: Relevant literature records for the fate and behaviour section

Author(s)	Data requirements	Year	Title	Source	Reason(s) for (not) including the study in the dossier
Behie, S.W., Bidochka, M.J.	MA 7.1.1	2014	Ubiquity of Insect-Derived Nitrogen Transfer to Plants by Endophytic Insect-Pathogenic Fungi: an Additional Branch of the Soil Nitrogen Cycle	Applied and Environmental Biology, (2014) 80(5): 1553-1560	Reasons, key qualifying criteria 3 and 4, Table B.8.3-03 not met
Bruck, D.J.	MA 7.1.1	2010	Fungal entomopathogens in the rhizosphere	Biocontrol, (2010) 55(1): 103-112	Reasons, key qualifying criteria 1-4, Table B.8.3-02 not met

Scheepmaker J.W.A., Butt, T.M.	MA 7.1.1	2010	Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment and in accordance with EU regulations	Biocontrol Science and Technology (2010) 20(5): 503-552	Supporting document; summarised under study 7.1.1/01
Quesada-Moraga, E, Navas-Cortes, JA, Maranhao, EAA, Ortiz-Urquiza, A, Santiago-Alvarez, C	MA 7.1.1	2007	Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils	Mycological Ressearch, (2007) 111(8): 947-966	Supporting document; summarised under study 7.1/02
Kim, JS, Skinner, M, Gouli, S, Parker, BL	MA 7.1.1	2010	Influence of top-watering on the movement of *Beauveria bassiana*, GHA (Deuteromycota: Hyphomycetes) in potting medium	Crop Protection, (2010) 29(6): 631-634	Supporting document; summarised under study 7.2/02
Meyling, NV, Eilenberg, J	MA 7.2	2006	Isolation and characterisation of *Beauveria bassiana* isolates from phylloplanes of hedgerow vegetation	Mycological Research, (2006) 110(2): 188-195	Included as supporting document, cited in Zimmermann, 2007

It is stated that the relevance of the resulting hits were checked according to the requirements of the EFSA guideline but no specific relevance criteria are reported. As stated in the EFSA guidance the relevance criteria should be clearly reported in the study report. The notifier is asked to clarify which specific relevance criteria were used in the literature search.

References relied on

Data point	**Author(s)**	**Year**	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
Annex II Data and Information							
MA 7/01 MA 2.1/01 MA	Zimmermann, G.	2007	Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science*	N	N	N/A	Public

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
5/01			*and Technology*, 17 (5/6): 553-596 Not GLP Published				
MA 7/02	Quesada-Moraga E., Navas-Cortes J.A., Maranhao E.A.A., Ortiz-Urquiza A., Santiago-Alvarez C.	2007	Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological Research 111: 947-966. Not GLP Published	N	N	N/A	Public
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MA 7/04	Clerk, G.C.	1969	Influence of soil extracts on the germination of conidia of the fungi Beauveria bassiana and Paecilomyces farinosus. Journal of Invertebrate Pathology 13:120-124. Non GLP Published	N	N	N/A	Public
MA 7.1.1/01	Scheepmaker, J.W.A. and Butt, T.M.	2010	Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation with risk assessment and in accordance with EU	N	N	N/A	Public

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
			regulations. Biocontrol Science and Technology 20, 503-552 Not GLP Published				
MA 7.1.2/01	Whittaker, M.	2014	Hydrophobicity and survival of *Beauveria bassiana* PPRI 5339 in water. APIS, Knaresborough Technology Park, Manse Lane, Knaresborough, North Yorkshire HG5 8LF. Unpublished report APIS-BASF-010. GLP Unpublished	N	Y	Strain specific New GLP laboratory study	BASF
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MA 7.1.3/02	Edgington, S., Segura, H., De La Rosa, W. and Williams, T	2000	Photoprotection of *Beauveria bassiana*: testing simple formulations for control of the coffee berry borer. International Journal of Pest Management, 46(3) 169-176 Not GLP Published	N	N	N/A	Public
MA 7.1.3/03	Krieg, A., Gröner, A., Huber, J., Zimmermann, G.	1981	Inactivation of certain insect pathogens by ultraviolet radiation Journal of plant	N	N	N/A	Public

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
			diseases and production 88(1) 38-48 Not GLP Publised In German including an English translation				
MA 7.1.3/04	Ignotffo, C.M., Garcia, C.	1992	Influence of conidial color ininactivation of several entomogenous fungi (Hyphomycetes) by simulated sunlight. Environmental Entomology, 21(4), 913-917 Non-GLP Published	N	N	N/A	Public
MA 7.1.3/05 MA 6.1/01 MA 6.2.2/01	Fargues, J., Goettel, M.S., Smits, N., Ouedrago, A., Rougier, M.	1997	Effect of temperature on vegetative growth of Beauveria bassiana isolates from different origins. Mycologia, 89(8), 383-392 Non GLP Published	N	N	N/A	Public
MA 7.1.3/06	Inglis G.D., Goettel M.S., Johnson D.L.	1995	Influence of ultraviolet light protectants on persistence of the fungus *Beauveria bassiana*, Biological control 5, 581-590 Not GLP Published	N	N	N/A	Public
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Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
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MA 7.2/01	Storey, G. K. and Gardner W. A.	1988	Movement of an aqueous spray of *Beauveria bassiana* into the profile of four Georgia soils, Entomological society of America 17, 135-139 Not GLP Published	N	N	N/A	Public
MA 7.2/02	Kim, J.S. Skinner, M. Gouli, S. Parker, B.L.,	2010	Influence of top-watering on the movement of *Beauveria bassiana*, GHA (Deuteromycota: Hyphomycetes) in potting medium. Crop Protection 29 631–634 Not GLP Published	N	N	N/A	Public
Annex III Data and Information							
			No data submitted				