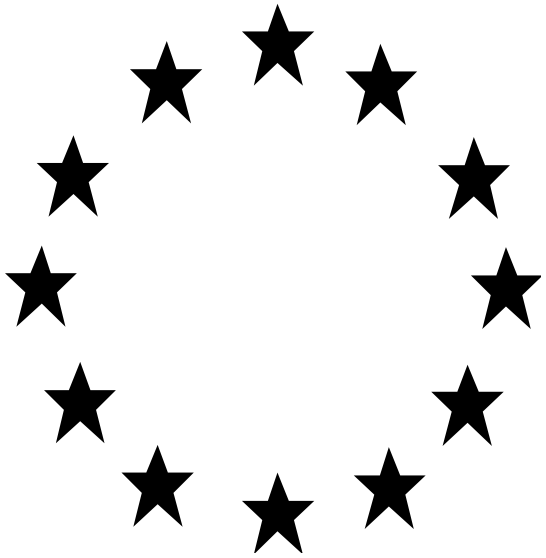

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Germany for the existing active substance

BEAUVERIA BASSIANA GHA

of the fourth stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, part 4, B.8

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Annex B

Beauveria bassiana GHA

B-8: Environmental fate and behaviour

B.8 Fate and behaviour in the environment (OECD IIM 7, IIIM 9)

General information about the exposure with secondary metabolites of *Beauveria bassiana*

Beauveria bassiana is known to produce a variety of toxins. In order to assess the risk coming from these toxins, the time of their production in relation to the life cycle of fungi and therefore the exposure should be assessed.

It is important to be aware that almost all toxins produced by fungi belong to the group of secondary metabolites. Secondary metabolites are produced during the changeover from the logarithmic growth stage (log stage) of a micro-organism to the steady-state stage, when micro-organisms stop growing because of several reasons, e.g. the lack of nutrients and oxygen, abiotic stress, limited space and so on. With beginning growth (corresponding with the lag stage) and the log stage, micro-organisms mainly produce primary metabolites that are non-toxic and that are produced by every living organism. Secondary metabolites are synthesised on the basis of primary metabolites declining growth speed and reduction of primary metabolism. (Madigan 2001, page 430-432).

Environmental conditions inhibiting the growth of conidia and the building of mycelium, therefore, prevent the synthesis of secondary metabolites, since during the dormancy of conidia, metabolism is inhibited in general.

Dormancy continues as long as the environmental conditions remain unchanged.

In the case of the entomopathogenic fungus *B. bassiana* the germination of conidia starts with the contact between conidia and a suitable host, provided that the temperature range is between 0 °C and 38 °C (Müller-Kögler 1965; Roberts & Campbell 1977 cited in Zimmermann 2007) and especially the microhumity at the surface of the host is sufficiently high (100 – 92 %) (Prior et al. 1988; Bateman et al. 1993; Vidal et al. 2003 cited in Zimmermann 2007).

The effect of fungistasis in non-sterile soils inhibits the germination of conidia. This has been shown by Watson & Ford (1972 cited in Zimmermann 2007). A widespread fungistasis in soils was found and postulated about 50 years ago (Dobbs & Hinson 1953 cited in Zimmermann 2007). According to Clerk (1969 cited in Zimmermann 2007), several authors have reported that conidia of *B. bassiana* are subject to fungistatic effects in natural soils. However, the nature of the inhibitor(s) responsible for soil fungistasis is still unknown, although several authors consider that inhibitory substances released by soil micro-organisms play a major role.

From this, 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.

Conidia of *B. bassiana* start germinating when in contact with the cuticle of a suitable host insect. They penetrate the cuticle and proliferate within the host by formation of hyphal bodies or blastospores. These steps correspond with the lag-stage in the beginning and the log-stage, when the fungus grows through the body of the insect. The fungal growth enters the steady-state stage, when all nutrients have been consumed and the fungus has been grown out of the dead insect and produces new conidia. At this point of time, secondary metabolites (fungal toxins) are mainly produced.

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 occure by a plentiful appearance of infected and dead hosts.

B.8.1 Persistence and multiplication (Annex IIM 7.1)

B.8.1.1 Soil (Annex IIM 7.1.1)

The studies cited in the following were carried out using the strain ATCC 74040 and the appropriate formulation Fermone Naturalis-L 225, respectively (please refer to Draft Assessment Report W4L-6155 *B. bassiana* strain ATCC 7404, Reference No. IIM 7.1/01, IIM 7.1/02 and IIIM 10.4/02). It is assumed that both strains behave similar with respect to fate and behaviour in soil.

Reference:	IIM 7.1 Cited from DAR W4L-6155 *B. bassiana* strain ATCC 74040
Author:	Krygsman, A
Title:	Effects of Naturalis-L Bio-insecticide on beneficial insects during the 1992 experimental use permit season
Date:	May 20, 1993
Doc ID:	Unpublished report no.: FERM.ENVFA EUP-1 BVL No.: BOD2006-251
Guideline:	Not documented
GLP:	Not documented
Acceptability:	The study is considered to be acceptable.

Material and methods:

Test material:	Naturalis L *Beauveria bassiana strain ATCC 74040*
Test concentration:	937.2 CFU/cm^2 (leaf surface), 10.1 x 10^3 CFU/ ? (soil) In order to investigate survivability further, numerous trials were conducted with various researchers in a number of States. Naturalis L was applied at 0.283 - 0.425 kg per acre (0.699 – 1.05 kg/ha).
Test system:	Application in a greenhouse to cotton. 16 cotton plants were treated.
Temperature:	Not given
Sampling time points:	0, 1 ,2 ,3 ,4 ,5 days
Method of analysis:	CFU in each sample
Soil characteristics:	Not given

Findings:

Six hours after treatment, an 80 % reduction in *B. bassiana* was observed. Thirty hours post-inoculation, less than 1 % of the original viable population existed. Further details on survivability of *B. bassiana* in soil and leaves are presented in Table B.8.1-1.

Table B.8.1-1: Survivability of *B. bassiana* (ATCC 74040) in greenhouse cotton soil

Days	CFU/?*
0	10.1×10^3
1	5.6×10^3
2	1.2×10^3
3	0
4	0
5	0

* No information is given what the given CFU refer to (?).

Conclusion:

The results are presented as "CFU". It is not clear to which amount of soil the CFU refer to. Nevertheless it was shown, that *Beauveria bassiana* strain ATCC 74040 rapidly degrades within 48 hours after application, and, therefore, is unlikely to persist in the terrestrial environment. The study results are plausible.

Reference:	IIM 7.1 Cited from DAR W4L-6155 *B. bassiana* strain ATCC 47040
Author:	Wright, J.
Title:	Determination of *Beauveria bassiana* ATCC 74040 in soil and on foliage
Date:	1993
Doc ID:	Unpublished report No.: FCI.93 EUP MONIT.1, BVL-No.: BOD2006-252
Guideline:	Standardized Protocol for the Quantification of *Beauveria bassiana* Guidelines were not available at the time test was performed.
GLP:	Not documented
Acceptability:	The study is considered to be acceptable.

Material and methods:

Test material:	*Beauveria bassiana strain ATCC 74040*
Test system:	Soil and foliage samples were collected from five sites in the states of Texas, Florida, Louisiana, Mississippi and California in the United States of America. Three replicates of the upper 5 cm of the soil layer were taken at least 24 hours pre-treatment and again at 2 – 24 hours post-treatment with *Beauveria bassiana* strain ATCC 74040. Soil (20 g) samples were rinsed with a solution of sterile distilled water and Tween 80. Soil samples were then sonicated for 30 minutes to 3 hours. Soil samples were then inoculated on oatmeal-dodine agar and incubated in the dark in an environmental chamber at 25 °C for up to six days.
Temperature:	25 °C
Sampling time points:	Soil samples were sonicated for 30 minutes to 3 hours and inoculated on oatmeal-dodine agar and incubated in the dark in an environmental chamber for up to six days.

Findings:

Details of background levels of *B. bassiana* are presented in Table B. 8.1-2. Although soil samples indicated that *Beauveria bassiana* was not detectable in many areas, it is readily found in specific locations in Florida and Texas. Background levels of *B. bassiana* ranged from 0 to 7.0×10^5 CFU/mL in soil; post-treatment levels of the organism ranged from 0 to 4.4×10^8 CFU/mL. Further details of post-treatment data are presented in Table B. 8.1-3.
In Prosper, Texas, despite background levels of *Beauveria bassiana* in soil of 7.0×10^5 CFU/mL were measured on July 8, on July 15, i.e. 24 hours post application, a 99.87 % decrease in levels of *Beauveria bassiana* was recorded. This decrease does not refer to the level immediately after application. In Raymond, Mississippi, *Beauveria bassiana* was not detected in any samples, neither before application on July 3 nor on July 14, 24 hours after application.
In Mexia, Texas, levels of *Beauveria bassiana* were higher in post-application samples than before application, both samples were determined on July 8.

Table B. 8.1-2: **Background soil populations of *Beauveria bassiana* at various locations in five states**

Date	Location	Crop	Soil population (CFU/mL)
June 25	Orlando, Florida	Tomatoes	5.2×10^3
June 23	Orlando, Florida	Cantaloupe	6.7×10^3
June 21	St. Joseph, Louisiana	Cotton	0
July 6	M.S.U., Mississippi	Cotton	0
Juli 3	**Raymond, Mississippi**	**Cotton**	**0**
July 8	**Prosper, Texas**	**Cotton**	**7.0×10^5**
July 8	**Mexia, Texas**	**Cotton**	**2.2×10^4**
Aug 9	Edinburg, Texas	Cotton	0
Sept 9	Brawley, California	Cotton	0

Table B. 8.1-3: Post-treatment soil populations of *Beauveria bassiana* at various locations in five states at 2-24 hours after treatment

Date	Location	Crop	Soil population (CFU/mL)
July 14	**Raymond, Mississippi[1]**	**Cotton**	**0**
July 15	**Prosper, Texas[1]**	**Cotton**	**9.1×10^2**
July 23	Ennis, Texas	Cotton	1.3×10^4
July 8	**Mexia, Texas[2]**	**Cotton**	**4.4×10^8**
Sept 2	Commerce, Texas[1]	Cotton	0

[1]= 24 hours post application, [2]= at the day of application

The counts give no uniform information about degradation after application.

Conclusion:

Beauveria bassiana strain ATCC 74040 can be present naturally in the environment. Background levels of this organism vary from 0 (below detection limits) to 7.0×10^5 CFU/mL. Post-treatment levels vary from 0 to 4.4×10^8 CFU/mL. There are only 3 locations, where pre- and post-treatment levels were determined. Post-treatment levels were found to be higher (Mexia, Texas), lower (Prosper, Texas) or equal (Raymond, Mississippi) compared to pre-treatment levels. According to this data, no estimation about degradation is possible, because one single measurement after application is not sufficient to follow the decline with time.
According to the initial greenhouse study *B. bassiana* is effective for approximately 3 days. Validation of the study is not possible, but the study results are acceptable.

Reference:	IIIM 10.4 Cited from DAR W4L-6155 *B. bassiana* strain ATCC 74040
Author:	Krygsman, A
Title:	Fermone Naturalis-L 225 monitoring studies during the 1993 experimental use permit season
Date:	April 17, 1994
Doc ID:	Troy Biosciences Inc. Unpublished report No. Fereup93mon2 BVL-No.: ANA2006-143
Guideline:	US EPA, Experimental Use Permit Recommended studies, EPA correspondence of 3/12/93
GLP:	No
Acceptability:	The study is considered to be acceptable.

Material and methods:

Test material:	*Beauveria bassiana* strain ATCC 74040, Fermone Naturalis-L 225
Test concentration:	2.8×10^4 CFU
Test system:	Cotton plots were conducted in Arizona, Texas, Louisiana and Mississippi. Greenhouse plots in Texas and tomato plots in Florida were also sampled.
Temperature:	not specified
Sampling time points:	1 h, 24 h, 48 h

Method of analysis: For the analysis of soil residues, the upper 5 cm of soil was taken 24 hours before treatment and 2 - 24 hours post treatment. The samples were then stored at 4 °C until analysis. Twenty gram samples were rinsed and sonicated for 30 minutes – 3 hours in a solution of sterile distilled water and Tween 80, plated on oatmeal-dodine sugar agar and held in an environmental chamber at 25 ° C for up to 6 days

Soil characteristics: Not specified

Findings

Soil samples were collected from different locations. At Nemec Farms in Texas cropped with cotton, levels of *B. bassiana* in the soil pre-spray were determined to be 2.8 x 10^4 CFU/g soil. The indicated levels of the fungus declined over the sampling time. One hour after treatment, 1 x 10^5 CFU/g soil were recorded in soil samples, which slowly declined to 7 x 10^4 CFU/g soil at 24 hours and 2.8 x 10^4 CFU/g soil at 48 hours after treatment.

Although samples were taken from cotton trials in Arizona and Texas (Schuster Farms) before and after treatment, it was difficult to ascertain the breakdown of the fungal organism over time. Initial pre-spray counts failed to detect the organism as well as determinations 24 hours after treatment.

At Galloway Farms, Monte Alto, Texas, soil samples were collected before and after spraying at three different times during the growing season (Table B. 8.1-4).

Table B. 8.1-4: Residue levels of *B. bassiana* ATCC 74040 in Soil Galloway Farms

Sample time	Sample interval	CFU/g soil
Early	Pre spray	0
	1 hour post	1 x 10^4
	24 hours post	6.5 x 10^3
	48 hours post	5.0 x 10^3
Mid-season	Pre spray	1.5 x 10^4
	1 hour post	5.9 x 10^4
	24 hours post	4.5 x 10^4
	48 hours post	1.7 x 10^4
Pre-harvest	Pre spray	5.9 x 10^4
	1 hour post	4.4 x 10^4
	24 hours post	5.5 x 10^4
	48 hours post	5.7 x 10^4

Although the soil levels initially follow the breakdown trend, levels at the later sampling times indicate high residual levels in the soil. Levels for the mid-season sampling time also illustrate the reduction trend.

Data from the pre-harvest fail to give this result. Further observation of this data also reveals high levels of *Beauveria bassiana* in the pre-spray samples. In addition, a review of the replicate samples indicates a wide degree of variability. It was assumed that contamination occurred at some time during sample preparation and evaluation rather than the establishment of a *Beauveria bassiana* colony in the soil at this location. Unfortunately, sampling was not carried out after the 48 hour period.

Conclusion:

Data from Nemec indicate the breakdown of the fungus over time in soils over the 48-hour testing period: A decrease of 72 % was reached. Data from the trials at Galloway Farms indicate breakdown patterns only in the early (decrease to 50 %) and mid-season (decrease of 81 %) sampling periods. Especially data from pre-harvest fail to indicate breakdown pattern.
It was assumed, that contamination occurred at some time during sample preparation and evaluation rather than the establishment of a *Beauveria* colony in the soil at this location.
Validation of the study is not possible, but the study results are plausible.

Overall conclusion soil:

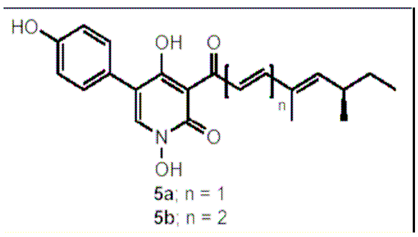
According to the greenhouse study and the monitoring studies at Nemec Farms and Galloway Farms in the early and mid-season, there is more than 50 % decline of CFU of *Beauveria bassiana* after 48 hours. This speaks for a rapid decrease, though a complete decrease is not proven.
There were also investigations where conidia of *Beauveria bassiana* could not be detected even after application, or investigations, where higher levels were determined after application than before treatment, but further measurements were lacking to confirm the results. This especially applies for tests with more than one application within a year.

In general, it is difficult to evaluate the persistence considering only soil, because multiplication does take place in the hosts. 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. On the other hand, in 5 of 9 monitoring studies, no background levels of *B. bassiana* were detected, although *B. bassiana* is naturally present in the environment. However, it should be kept in mind that the backround level of *Beauveria bassiana* conidia may be strongly different in various parts of 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 cited in Zimmermann 2007). So that it might be conceivable to find comparatively high backround concentration in one place of the field and almost negligible concentration in another place being only few meters away.
Persistence up to several years (e.g. Inglis et al., 1997; Studdert, 1990 cited in Zimmermann 2007), but also reduction of introduced stains is reported (e.g. Fargues & Robert, 1985 cited in Zimmermann 2007).
Germination and multiplication of entomopathogenic fungi in natural, non-sterile soils can be excluded as long as no potential hosts are present, since microorganisms living in soil must have the ability to degrade heavily degradable and unsolvable substances like lignin and humus (Campbell 1981, page 117-118). Furthermore the effect of fungistasis inhibits germination of conidia in non-sterile soils (Watson & Ford 1972 cited in Zimmermann).
In this respect, there is no indication of accumulation.

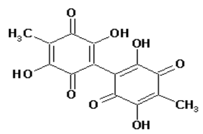
Assessment of a possible soil contamination with secondary metabolites:

The only way of soil contamination is via a plentiful appearance of infected and dying insects. Concentrations of metabolites in natural environment and their stability are not known. However, most of them are peptides which are usually well degradable. Also oxalic acid, a common product in metabolism, is biodegradable. Bassianin, tenellin and oosporein are non-peptide pigments. The chemical name of oosporein is [Bi-1,4-cyclohexadien-1-yl]-3,3',6,6'-tetrone. Tenellin and bassianin are the 3-[(*E*,*E*)-4,6-dimethylocta-2,4-dienoyl] and 3-[(*E*,*E*,*E*)-6,8-dimethyldeca-2,4,6-trienoyl] derivatives of 1,4-dihydroxy-5-(*p*-hydroxyphenyl)-2(1*H*)-pyridone (see Figure B.8.1-1). Studies conducted for the RAFBCA project have shown that oosporein degrades quickly under moderate alkaline conditions (DT_{50} = 12 days at 23°C, pH 8) und is more stable under moderate acidic conditions (DT_{50} = 74 days at 23 °C, pH 6). Elevation of the temperature reduces the stability of oosporein remarkably (DT_{50} = 0,3 day at 53 °C and pH 8). Due to the structure, biodegradability is assumed for all three substances. So many bacteria and fungi are able to cleave aromatic cycles in the manly presence of oxygen, but also without oxygen. Multiple substituted aromatic cycles as the non-peptide secondary metabolites are degraded to Protocatechuat. This is further metabolised to succinate and acetyl ~ CoA (see Figure B.8.1-1). Hydroxy-groups are inserted into the cycle in any case, since a 1,2-dihydroxybenzene structure is needed for the cleavage of the cycle (Schlegel 1992). In the case of these secondary metabolites hydroxy-groups already exist as substituents in the aromatic cycle. These hydroxyl- and the carbonyl-groups being further constituents should accelerate the pathway of biodegradation. Therefore the risk of soil contamination by secondary metabolites is considered to be negligible.

Figure B.8.1-1: Non-peptidic metabolites of *Beauveria bassiana*

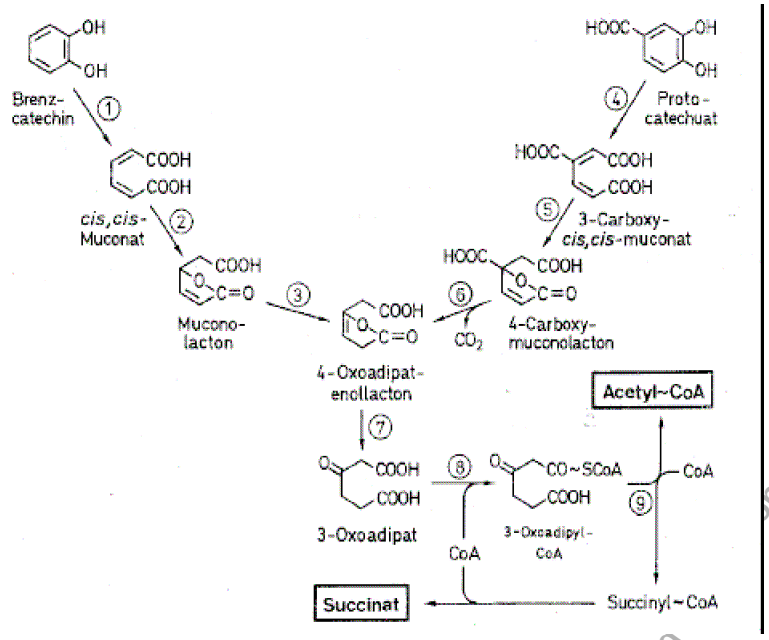


5a: Tenellin
5b: Bassianin



Oosporein

Figure B.8.1-2: Ortho-cleavage of aromatic cycle and the 3-oxoadipat-pathway (Schlegel 1992)



B.8.1.2 Water (Annex IIM 7.1.2)

Reference:	IIM 7.1.2
Author:	Jaronski, S.T. and Britton, J.A.
Title:	Effect of pH and metal ions on *Beauveria bassiana* strain GHA in aqueous suspension.
Date:	04. November 1993
Doc ID:	Unpublished report no. 93-025, BVL-No.: WAS2006-143
Guideline:	151A-16 for Technical grade active ingredient stability data requirements
GLP:	No
Acceptability:	The study is considered to be acceptable.

Material and methods:

Test material:	*Beauveria bassiana* strain GHA (Mycoide GH TGAI)
Test concentration:	5 x 10^8 *B. bassiana*/mL represents the use rate of 1 x 10^{13} conidia per acre (4 x 10^{12} CFU/ha), applied in spray volume of 20 L per acre
Test system:	25 mL of 0.1 M aqueous buffers at pH 5, 7 or 9 and in the following metal ion solutions: NaCl, $MgCl_2$, $CaCl_2$, $FeSO_4$, $CuSO_4$
Temperature:	22 – 25 °C
Sampling time points:	0, 24, 48 h
Method of analysis:	Observation of conidial germination; relative number of bacteria in each sample; viability of conidia
Water characteristics:	Control: unbuffered glass-distilled water

0.15 g of *Beauveria bassiana* strain GHA conidia, at a nominal concentration of 5 x 10^8 CFU *B. bassiana*/mL, was suspended in 25 mL of 0.01 M aqueous buffers at pH 5, 7 or 9 and in the following metal ion solutions: NaCl, $MgCl_2$, $CaCl_2$, $FeSO_4$, $CuSO_4$. Initial samples were removed and placed on Sabouraud dextrose agar with yeast plus antibiotic and incubated overnight to determine initial spore viability.
Subsequent samples were incubated at 22 - 26 °C. Three replicate sub samples of each buffer and metal ion solution were removed at 24 and 48 h. Observation of conidial germination and relative number of bacteria in each sample were made using a 400x magnification and phase contrast illumination. Conidial viability was evaluated as previously described.

Findings:

Viability of conidia of *B. bassiana* incubated in water decreased from 97.5 % after 0 hours to 39.0 %, after 48 h. Initial conidial viability at pH 5, 7 and 9 was 94.7 %, 91.9 % and 93.3 %, respectively. Decreases were observed in spore viability after 48 h incubation at pH 5, 7 and 9, respectively. Details of conidial survival in water and pH 5, 7 and 9 buffers are presented in Table B.8.1-5.
Decreases in conidial viability of *B. bassiana* were observed in sodium, magnesium, calcium, iron and copper ion solutions after 48 h incubation, respectively. Further details on conidial survival in metal ion solutions are presented in Table B.8.1-6.
Bacterial levels in all test systems greatly increased in the 24 and 48 h incubation periods with the exception of the copper solution. By 24 hrs fermentation gases were already noticeable. Further details on bacterial levels in the various test systems are presented in Table B.8.1-7.

Table B.8.1-5: Mean percent viability of *Beauveria bassiana* during incubation in pH 5, 7 and 9 buffers

Test system	Incubation		
	0 h	24 h	48 h
Water	97.5	60.5	39.0
pH 5	94.7	62.6	47.8
pH 7	91.9	87.5	59.3
pH 9	93.3	89.3	62.9

Table B.8.1-6: Mean percent viability of *Beauveria bassiana* conidia incubated in various metal ion solutions

Test system	Incubation		
	0 h	24 h	48 h
Water	97.5	60.5	39.0
Sodium	96.7	73.8	23.3
Magnesium	96.1	82.6	41.5
Calcium	95.8	83.2	28.0
Iron	89.5	41.1	24.4
Copper	76.1	0.1	0.0

Table B.8.1-7: Relative bacterial levels in the test systems*

Test system	Incubation		
	0 h	24 h	48 h
Water	0	1	2
pH 5	0	1	2
pH 7	0	1	2
pH 9	0	1.67	2
Sodium	0	2	2
Magnesium	0	0	0
Calcium	0	0	2
Iron	0	1.67	2
Copper	0	1	2

* = Average of three samples (0 = <10 bacteria per 400 x field, 1 = 10-100 per field, 2 = > 100 per field)

Conclusion:

On the basis of the information presented under this annex point, it is only possible to notice a loss in conidial viability of *Beauveria bassiana* strain GHA in distilled water and aqueous suspensions at pH 5, 7 and 9 or in metal ion solutions in the presence of bacteria. The rapid growth of bacteria may be caused by the loss in conidial viability. There was no system with pond water. After 48 hours of incubation there are 39 % viable conidia in distilled water, 59 % at pH 7, and 63 % at pH 9, which was the slowest loss. The conidia do not germinate in the different tested aqueous systems.

No other information is given about persistence and multiplication in water. However survival of spores in sterile water is well-known (e.g. Castellani, 1939; Boesewinkel, 1976; Müller-Kögler & Zimmermann, 1980 cited in Zimmermann 2007). On the other hand germination of conidia and therefore multiplication in water is not expected, since *Beauveria bassiana* is no aquatic fungus and is therefore not adapted to the conditions of the aqueous environment

(Campbell 1981). It may be concluded that conidia of *Beauveria bassiana* in water will be degraded by bacteria and protozoa in natural non-sterile waterbodies, rapidly.

B.8.1.3 Air (Annex IIM 7.1.3)

Reference:	**IIM 7.1**
Author:	Inglis, G.D., Goettel, M.S. and Johnson, D.L.
Title:	Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, *Beauveria bassiana*
Date:	1995
Doc ID:	Report no.: 01094 R; Publication: Biological Control 5, 581 – 590 (1995), BVL No.: BOD2006-319
Guideline:	None
GLP:	None
Acceptability:	laboratory experiment: not applicable for assessing sunlight effects field experiment: acceptable

Material and methods (laboratory experiment):

Test material:	*Beauveria bassiana* strain GHA (supplied by Mycotech Corp., Butte, MT)
Test concentration:	2×10^5 conidia/µL 1 µL pipetted on to sterile round coverslips and onto the surface of leaf pieces of field-collected wheatgrass in various UV protectants
Test system:	Radiation from 260 nm to 400 nm 10 cm below a UV-B fluorescent bulb 601 to 675 µW/cm²
Temperature:	25 ± 1 °C
Sampling time points:	0, 15, 30, 45, or 60 min
Method of analysis:	Wash solution was further diluted; placed on semi-selective oatmeal dodine-agar medium; cultures were incubated at 25 °C for 6 – 7 days; number of colony forming units (CFU) were enumerated at 30 – 300 CFU per dish

Material and methods (field experiment):

Test material:	*Beauveria bassiana* strain GHA (supplied by Mycotech Corp., Butte, MT)
Test concentration:	3×10^{13} conidia/ha in various water-compatible or oil-compatible UV protectants
Test system:	field experiment in Lethbridge, Canada 3.72 105 kJ/m^2 in trial one 2.79 105 kJ/m^2 in trial two
Temperature:	in trial one (mean temperature 16.2 °C) in trial two (mean temperature 15.2 °C)
Sampling time points:	10 leaves from the top canopy randomly collected 0, 1, 2, 4, 6, 8, 12, and 16 days

Method of analysis: wash solution was further diluted;
placed on semi-selective oatmeal dodine-agar medium;
cultures were incubated at 25 °C for 6 – 7 days; number of colony forming units (CFU) were enumerated at 30 – 300 CFU per cm²

The investigation is divided into a lab experiment and a field experiment part. In both experiments conidia of the fungus *Beauveria bassiana* were applied in water or in an oil emulsion, both containing one of several UV-protectants.

The lab experiment was conducted at 25 °C and radiation from 260 nm to 400 nm. The light intensity was (10 cm below a UV-B fluorescent bulb) 601 to 675 µW/cm². The survival of conidia applied in water onto glass coverslips or crested wheatgrass leaves was reduced by greater than 95 % after 15 min exposure to UV-B radiation. Substitution of oil for water increased the survival of conidia on both substrates. However, conidial survival in oil was more pronounced on glass (74 % mortality after 60 min) than on leaves (97 % mortality after 60 min). The water compatible or the oil-compatible formulations enhanced survival of conidia after 3 h exposure to UV-B radiation only in a few cases.

The field experiment was conducted in Canada (Leathbridge) from July 28 (trial one) and August 12 (trial two) in 1993. The target concentration was 3×10^{13} conidia/ha applied in 4 water (at a rate of 100 L/ha) and 4 oil (at a rate of 5 L/ha) compatible formulations. After spraying ten leaves from the top canopy were randomly sampled at time 0, 1, 2, 4, 6, 8, 12, and 16 days. The CFU/cm² was determined. All relevant weather and climate parameters were measured during the experiment.

A detailed statistically analysis was performed by the authors. For the water compatible formulations they found: “Although a strong relationship was observed between cumulative solar radiation and conidial persistence, light was generally a less effective predictor of conidial survival than was time.” and “in both trials, time and formulation were significant […]”. Only for the clay formulation in trial two “a stronger relationship was observed between conidial persistence and light than with time.” For the oil compatible formulations the authors found similar results with “a strong relationship […] between cumulative light and conidial persistence”. However, the influence of time was nearly in 6 out of 8 stronger, because “light was almost as good a predictor of conidial survival as was time”. “For oil compatible formulations, time but not formulation influenced conidial persistence.”

Results

For the lab experiment the authors assume a strong effect of UV-B radiation on *Beauveria bassiana* survival. However, they state “but the mechanism causing death is unknown” (page 588). Additionally, no effort was made by the authors to transfer the lab conditions to a typical European radiation or temperature situation in the field. Consequently, the lab experiment part of this study is not considered applicable for accessing the effect of ultraviolet radiation on *Beauveria bassiana* under field-relevant conditions in Europe.
In contrast, the field experiment from Canada could be used to evaluate the persistence of *Beauveria bassiana* under field-relevant conditions in Europe. However, again no effort was made by the authors to transfer the weather and climate condition into an European situation. Besides that, the study demonstrates that not the effect of radiation but the effect of time is the strongest factor on persistence of *Beauveria bassiana*. This explains why most sunscreen formulants had no increasing effect on the survival rate. The fact that there is also a good

relationship between degradation and light may be explained by a strong correlation between time and cumulative light.

Conclusion:

It may be concluded that the degradation of *Beauveria bassiana* on leaves under field condition is fast. This is also supported by a study performed by Gardner WA, Sutton RM, Noblet R. 1977. This effect is may not only caused by sunlight radiation, but other unknown factors could play an important role. It is possible to increase the survival rate by adding a formulation. Consequently, this should be considered by evaluating the final plant protection product. However, *Beauveria bassiana* is not expected to persist in air as the viability of conidia of *Beauveria bassiana* are greatly reduced following exposure to sunlight during a period longer than 24 hours. Furthermore, in the absence of a specific host insect, conidia of *Beauveria bassiana* will not persist in air for more than 2 days.

Reference:	IIM 7.1.3
Author:	Jaronski, S.T.
Title:	TGAI physical stability study of *Beauveria bassiana* strain GHA: sunlight effects.
Date:	18. October 1993
Doc ID:	Doc No 93-001, BVL-No.: CHE2006-765
Guideline:	151A-16
GLP:	No
Acceptability:	The study is considered to be acceptable.

Material and methods:

Test material:	*Beauveria bassiana* strain GHA
Test concentration:	2.6×10^9 viable conidia/mL
Test system:	Sprayed on cover slips dried for about 0.5 hour indoors at room temperature (19 °C) and in the dark
Temperature:	Irrigated: 17.3 – 26.2 °C; Shaded control: 17.2 – 24.7 °C
Sampling time points:	At study initiation and at 1, 2, 4, 6 and 8 hours after the start of exposure
Method of analysis:	Sample was plated on Sabouraud Dextrose agar prior to incubation at 22 - 26 °C overnight

Findings:

Conidia of *Beauveria bassiana* strain GHA were rapidly inactivated in the irrigated samples. No results or information are given for the shaded control. The half-life of *Beauveria bassiana* strain GHA conidia in the irrigated samples was calculated 2.58 hours by using only the 1, 2, and 4 hour samples neglecting the 0, 6, and 8 hour sample.

Conclusion:

It remains unclear if natural sunlight or just temperature or time is responsible for the rapid inactivation, since no comparison with the shaded control is given. Inglis et al. (1995) found time to be the most relevant factor and not light.

Overall conclusion air:

Beauveria bassiana is dry, of small size and it is produced in powdery clusters. Therefore, these types of conidia can be easily transported through the air. Also transmission of fungal spores through the air by insects is possible.
It may be concluded, that the degradation of *Beauveria bassiana* on leaves under field condition is fast. This effect is assumedly not only caused by sunlight radiation, but also by other unknown factors It is possible to increase the survival rate by adding a formulation. Consequently, this should be considered by evaluating the final plant protection product. However, *Beauveria bassiana* is not expected to persist in air as the viability of conidia of *Beauveria bassiana* are greatly reduced following exposure to sunlight for a period greater than 24 hours. Furthermore, in the absence of a specific host insect, conidia of *Beauveria bassiana* will not persist in air for more than 2 days.

B.8.2 Mobility (Annex IIM 7.1 and IIIM 9)

Soil:

Reference:	IIM 7.1.1
Author:	Storey, G.K. and Gardner W.A.
Title:	Movement of an aqueous spray of *Beauveria bassiana* into the profile of four Georgia soils.
Date:	1988
Doc ID:	Publication: Environmental entomology 17(1): 135 – 139 (1988), BVL No.: BOD2006-320
Guideline:	None
GLP:	None
Acceptability:	The study is considered to be not applicable

Material and methods (laboratory experiment):

Test material:	*Beauveria bassiana* (ABC 6112, Abbott Lab., North Chicago, Ill.)
Test concentration:	9.44×10^7 conidia per mL
Test system:	100 mL sprayed on soil block of 40 x 20 x 20 cm depth in greenhouse under polyethylene tent covered with plastic tray
Temperature:	30 °C
Sampling time points:	Prior to and 72 h after application at 0, 4, 8, 12, and 16 cm depths
Method of analysis:	CFU per g of dry soil at each depth

Material and methods (field experiment):

Test material:	*Beauveria bassiana* (commercial product: ABC 6178)
Test concentration:	1.25×10^7 conidia per mL
Test system:	2 L sprayed on plot of 1.8 x 1.8 metres
Temperature:	Field study at Georgia, USA

Sampling time points: Prior to and 2 h after application 5 cm vertical sections
Method of analysis: Percentage of CFU compared to total number initially applied

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). These soil type characteristics are described in Table B.8.2-1.
In a greenhouse study, an intact soil block (20 cm deep) from each of the four Georgian locations was removed. 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 viable conidia/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 cm² 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 conidia of *B. bassiana*. This was followed by application of 8 L of water to each plot.
Soil cores (15 cm deep) were removed 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 CFUs recovered from each depth of the various soil profiles examined.

Table B.8.2-1: Characteristics of the A horizons of four Georgia soil series

Soil characteristics (%)	Cecil	Greenville	Townley	Tifton
Clay	22.4	27.6	32.2	5.4
Sand	58.7	64.5	28.7	86.6
Silt	18.9	7.9	39.1	8.1
Organic matter	1.8	0.8	1.2	1.1
Infiltration	0.06	0.18	0.08	1.80

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 CFUs 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.8.2-2 .

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 CFUs 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 CFUs 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.8.2-3.

Table B.8.2-2: Number (x 10^4) of CFUs of *Beauveria bassiana* per cubic centimetre dry soil recovered from selected depths 72 h after application to soil blocks

Depth (cm)	Cecil	Greenville	Townley	Tifton
0	1,1310.0	3294.0	2242.0	1440.9
4	12.0	14.1	3.8	149.9
8	2.8	1.9	0.7	27.0
12	3.7	1.7	4.1	28.6
16	10.7	5.0	1.7	1.6

Table B.8.2-3: Number (x 10^3) of CFUs of *Beauveria bassiana* per cubic centimeter dry soil recovered from selected depths 2 h after application to soil blocks

Depth (cm)	Cecil	Greenville	Townley	Tifton
0 – 5	362.0	497.1	184.6	464.7
5.1 – 10.0	2.4	9.3	8.5	16.1
10.1 – 15.0	0.4	9.1	1.5	4.5

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.
Besides leaching, movement in the soil in horizontal and vertical direction is possible by soil arthopods, especially collembola (Dromph 2003; see chapter 6.3 cited in Zimmermann 2007).

Water:
The globose or ovoid shape of conidia is not suitable for efficient transport in water.

Air:
In the air *Beauveria bassiana* can be easily transported, as it is dry, of small size and it is produced in powdery clusters. Also transmission of fungal spores through the air by insects is possible. However, all studies principally indicate that *Beauveria bassiana* is not expected to persist in air as the viability of conidia of *Beauveria bassiana* is greatly reduced for a period greater than 24 hours. Furthermore, in the absence of a specific host insect, conidia of *Beauveria bassiana* will not persist in air for more than 2 days.

B.8.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 7 (OECD)	Campbell, R.	1981	Mikrobielle Ökologie Verlag Chemie GmbH, Weinheim pages 117,118,190 GLP: N, published: Y	N	LIT
KIIM 7	Zimmermann,G.	2007	Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii* Biocontrol Science and Technology, Volume 17, Issue 6: 553-598 GLP: N, published: Y 1673365	N	LIT
KIIM 7.1 (OECD)	Gardner, W.A., Sutton, R.M. and Noblet, R.	1977	Persistence of *Beauveria bassiana*, *Nomuraea rileyi*, and *Nosema necatrix* on soybean foliage. Environmental Entomology 6:6161-618.	N	LIT
KIIM 7.1 KIIIM1 9 (OECD)	Inglis, G. D., Goettel, M. S. and Johnson, D. L.	1995	Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, *Beauveria bassiana* Biological control 5, 581-590 01094 R GLP: N, published: Y 1300992 / BOD2006-319	N	MEU
KIIM 7.1 (OECD)	Krygsman, A.	1993	Effects of Naturalis-L bio-insecticide on beneficial insects during the 1992 experimental use permit season FERM.ENVFA EUP-1 GLP: N, published: N 1639820 / BOD2006-251	N	ACE
KIIM 7.1 (OECD)	Wright, J.	1993	Determination of *Beauveria bassiana* ATCC 74040 in soil and on foliage GLP: N, published: N 1639821 / BOD2006-252	N	ACE
KIIM 7.1 (OECD) KIIIM1 10.4	Krygsman, A.	1994	Fermone Naturalis-L 225 monitoring studies during the 1993 experimental use permit season KIIIM 10.4/02 GLP: N, published: N 1689943 / ANA2006-143	N	ACE
KIIM 7.1.1 (OECD) KIIIM1 9 (OECD)	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 GLP: N, published: Y 1300993 / BOD2006-320	N	MEU

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 7.1.2 (OECD)	Jaronski, S. T. and Britton, J. A.	1993	Effect of pH and metal ions on *Beauveria bassiana* strain GHA in aqueous suspension 93-025 GLP: N, published: N 1300994 / WAS2006-143	Y	MEU
KIIM 7.1.3 (OECD)	Jaronski S.T.	1993	TGAI Physical stability study of *Beauveria bassiana* strain GHA: sunlight effects. 93-001 GLP: Y, published: N 1689949 / CHE2006-765 /CHE-2006-755	Y	LAM

Codes of owner

LAM	Laverlam International Corporation
LIT	Literature
MEU	Mycotech Europe Ltd.