# European Commission

**VOLUME 3 – Annex B**

**- *Beauveria bassiana* PPRI 5339 -**

**B.1 Identity**

**B.2 Biological, physical, chemical and technical properties**

**B.3 Data on application and further information**

**B.4 Proposal for classification and labelling**

**B.5 Analytical methods**

**Rapporteur Member State: The Netherlands**

**December 2016**

**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**

# Version history page

| Date | Version history |
|---|---|
| December 2016 | Initial DAR |
| | |

**TABLE OF CONTENTS – VOLUME 3 B.1-B.5**

B.1 Identity ..... 6
B.1.1 Identity of the Micro-organism (Annex IIM 1) ..... 6
B.1.1.1 Applicant (Annex IIM 1.1) ..... 6
B.1.1.2 Producer (Annex IIM 1.2) ..... 6
B.1.1.3 Name and species description, strain characterisation (Annex IIM 1.3) ..... 6
B.1.1.4 Specification of the material used for manufacturing of formulated products (Annex IIM 1.4) 10
B.1.2 Identity of the plant protection product (Annex IIIM 1) ..... 11
B.1.2.1 Applicant (Annex IIIM 1.1) ..... 11
B.1.2.2 Manufacturer of the preparation and the micro-organism (Annex IIIB 1.2) ..... 11
B.1.2.3 Trade name or proposed trade name, and manufacturer's development code number of the preparation if appropriate (Annex IIIM 1.3) ..... 11
B.1.2.4 Detailed quantitative and qualitative information on the composition of the preparation (Annex IIIM 1.7) ..... 11
B.1.2.5 Physical state and nature of preparation (Annex IIIM 1.5) ..... 11
B.1.2.6 Function (Annex IIIM 1.6) ..... 11
B.1.3 References relied on ..... 11
B.2 Biological, physical, chemical and technical properties ..... 14
B.2.1 Biological properties of the micro-organism (Annex IIM 2) ..... 14
B.2.1.1 History of the micro-organism and its uses. Natural occurrence and geographical distribution (Annex IIM 2.1 and 2.2) ..... 14
B.2.1.2 Information on target organism(s) (Annex IIM 2.3) ..... 15
B.2.1.3 Host specificity range and effects on species other than the target harmful organism (Annex IIM 2.4) ..... 17
B.2.1.4 Development stages/life cycle of the micro-organism (Annex IIM 2.5) ..... 18
B.2.1.5 Infectiveness, dispersal and colonisation ability (Annex IIM 2.5/2.8) ..... 18
B.2.1.6 Relationships to known plant or animal or human pathogens (Annex IIM 2.7) ..... 22
B.2.1.7 Genetic stability and factors affecting it (Annex IIM 2.10) ..... 23
B.2.1.8 Information on the production of metabolites (especially toxins) (Annex IIM 2.6/2.7.2) 23
B.2.1.9 Antibiotics and other anti-microbial agents (Annex IIM 2.12) ..... 29
B.2.2 Physical, chemical and technical properties of the plant protection product (Annex IIM 2) 29
B.2.2.1 Appearance (colour and odour) (Annex IIIM 2.1) ..... 30
B.2.2.2 Storage stability and shelf-life (Annex IIIM 2.2) ..... 30
B.2.2.3 Explosivity and oxidising properties (Annex IIIM 2.3.1) ..... 35
B.2.2.4 Flash point and other indications of flammability or spontaneous ignition (Annex IIIM 2.3.2) 35
B.2.2.5 Acidity/alkalinity and if necessary pH value (Annex IIIM 2.3.3) ..... 36

B.2.2.6 Viscosity and surface tension (Annex IIIM 2.3.4) ..... 36
B.2.2.7 Technical characteristics of the plant protection product (Annex IIIM 2.4)..... 38
B.2.2.8 Physical, chemical and biological compatibility with other products including plant protection products with which its use is to be authorized (Annex ??) ..... 40
B.2.2.9 Adherence and distribution to seeds (Annex IIIM 2.6) ..... 41
B.2.2.10 Summary and evaluation of data submitted in B.2.2.1 - B.2.2.9 (Annex IIIM 2.7) .... 42
B.2.3 References relied on ..... 42
B.3 Data on application and further information ..... 52
B.3.1 Further information on the micro-organism (Annex IIB 3) ..... 52
B.3.1.1 Function (Annex IIM 3.1) ..... 52
B.3.1.2 Field of use envisaged (Annex IIM 3.3) ..... 52
B.3.1.3 Crops or products protected or treated (Annex IIM 3.3)..... 52
B.3.1.4 Method of production and quality control (Annex IIM 1.4.3)..... 52
B.3.1.5 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s) (Annex IIM 3.6) ..... 52
B.3.1.6 Methods to prevent loss of virulence of seed stock of the micro-organism (Annex IIB 4.1) 54
B.3.1.7 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIM 3.7)..... 54
B.3.1.8 Procedures for destruction or decontamination (Annex IIM 3.8)..... 54
B.3.1.9 Measures in case of an accident (Annex IIM 3.12) ..... 55
B.3.2 Data on application (Annex IIIM 3) ..... 55
B.3.2.1 Field of use envisaged (Annex IIIM 1.6.1)..... 55
B.3.2.2 Mode of action (Annex IIIM 3.1) ..... 55
B.3.2.3 Details of intended use (Annex IIM 3.1) ..... 56
B.3.2.4 Application rate (Annex IIIM 3.3 and 3.4) ..... 59
B.3.2.5 Content of micro-organism in material used (e.g., in the diluted spray, baits or treated seed) (Annex IIIM 3.3)..... 68
B.3.2.6 Method of application (Annex IIIM 3.5)..... 68
B.3.2.7 Number and timing of applications (Annex IIIM 3.6) ..... 68
B.3.2.8 Necessary waiting periods or other precautions to avoid phytopathogenic effects on succeeding crops (Annex IIIM 3.7)..... 69
B.3.2.9 Proposed instructions for use (Annex IIIM 3.8) ..... 69
B.3.3 Further information on the plant protection product (Annex IIIM 4)..... 69
B.3.3.1 Packaging and compatibility of the preparation with proposed packaging materials (Annex IIIM 4.1)..... 69
B.3.3.2 Procedures for cleaning application equipment (Annex IIIM 4.3 and 4.4)..... 69
B.3.3.3 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (Annex IIIM 4.5)..... 70

B.3.3.4 Recommended methods and precautions concerning: handling, storage, transport or fire (Annex IIIM 4.7) ..... 70
B.3.3.5 Measures in the case of an accident (Annex IIIB 4.9) ..... 71
B.3.3.6 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIM 4.10) ..... 71
B.3.4 References relied on ..... 73
B.4 Proposals for classification and labelling ..... 81
B.4.1 Proposals for classification and labelling of the MPCA ..... 81
B.4.2 Proposals for classification and labelling of the MPCP ..... 81
B.4.3 References relied on ..... 81
B.5 Analytical methods (Annex IIM 5) ..... 82
B.5.1 Methods for the analysis of the micro-organism as manufactured and for the analysis of the preparation (Annex IIM 4.3) ..... 82
B.5.1.1 Methods for the analysis of the micro-organism as manufactured ..... 82
B.5.2 Methods for the analysis of the preparation (Annex IIIM 5) ..... 89
B.5.2.1 Methods for the identification and the determination of the content of the micro-organism(s) in the preparation ..... 89
B.5.2.2 Methods to establish regular control of the preparation to show that it does not contain other organisms than the indicated ones and to establish uniform ..... 90
B.5.2.3 Methods to identify any contaminating micro-organisms of the preparation ..... 91
B.5.2.4 Methods used to determine the storage stability and shelf life of the preparation ..... 92
B.5.3 Methods to determine and quantify residues (viable or non-viable) of the micro-organism as manufactured and for the analysis of the preparation (Annex IIM 4.5 and IIIM 5.4) ..... 93
B.5.3.1 Methods to determine and quantify residues (viable or non-viable) of the micro-organism 93
B.5.4 References relied on ..... 93

## B.1 Identity

### B.1.1 Identity of the Micro-organism (Annex IIM 1)

#### B.1.1.1 Applicant (Annex IIM 1.1)

Company: BASF Corporation
North America Regional Headquarters
100 Park Avenue
Florham Park, New Jersey 07932
USA

Contact: [redacted]

Phone: [redacted]
E-Mail: [redacted]

#### B.1.1.2 Producer (Annex IIM 1.2)

This is considered confidential information. Please refer to volume 4 for details on the producer(s).

#### B.1.1.3 Name and species description, strain characterisation (Annex IIM 1.3)

##### B.1.1.3.1 Accession number in culture collection (Annex IIM 1.3.2)

The strain is deposited in the Agricultural Research Culture Collection (NRRL) International Depositary Authority, 1815 N. University Street, Peoria, Illinois 61604, USA under accession number: NRRL 50757

##### B.1.1.3.2 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism (Annex IIM 1.3.1/1.3.4/1.3.6)

| | |
|---|---|
| Species: | *Beauveria bassiana* (Balsamo) Vuillemin |
| Strain: | PPRI 5339 |
| Phylum: | Deuteromycota |
| Class | *Hyphomycetes* |
| Order | Moniliales |

Family: *Moniliaceae*
Genus: Beauveria

Strain PPRI 5339 is a naturally occurring strain and has not been genetically altered in any way. The strain is not originally indigenous to Europe. It was originally isolated by Dr Schalk Schoeman from the larva of a tortoise beetle, *Conchyloctenia punctata* (Coleoptera: Cassidinae) collected in Escombe, Queensburgh, KwaZulu Natal, South Africa. However, *Beauveria bassiana* is now naturally found across Europe (Rehner, 2005).

#### B.1.1.3.3 Test procedures and criteria used for identification (Annex IIM 1.3.3)

*Beauveria* is characterized morphologically by its sympodial to whorled clusters of short-globose to flask-shaped conidiogenous cells, which give rise to a succession of one-celled, hyaline, holoblastic conidia that are borne on a progressively elongating sympodial rachis. Conidia are the principal morphological feature used for species identification in *Beauveria*. In shape conidia may be globose, ellipsoidal, reniform to cylindrical, or commashaped, and range in size from 1.7 to 5.5 mm (Rehner and Buckley 2005).

Although morphologically distinctive as a genus, species identification in *Beauveria* is difficult because of its structural simplicity and the lack of distinctive phenotypic variation. The ongoing difficulties in applying morphologically approaches to species recognition in *Beauveria* have spurred the search for additional sources of taxonomic characters and were summarised by Rehner and Buckley (2005). Alternative character systems to detect genetic variation within *Beauveria* include isoenzymes, chemo taxonomic characters, mitochondrial RFLP, immunological approaches, rRNA sequencing, RFLP, introns in the large subunit rDNA, RFLP and nucleotide sequences of ITS, SSCP analysis of taxon specific markers, RAPD markers and the combined use of morphology and RAPD markers.

Rehner and Buckley (2005) used a gene-genealogical approach to investigate molecular phylogenetic diversity of *Beauveria* and presumptively related *Cordyceps* species with internal transcribed spacer (ITS) and elongation factor 1-alpha (EF1-α) sequences. Based on this approach the authors created six well-supported clades (see Figure B.1.1.3.3-1).

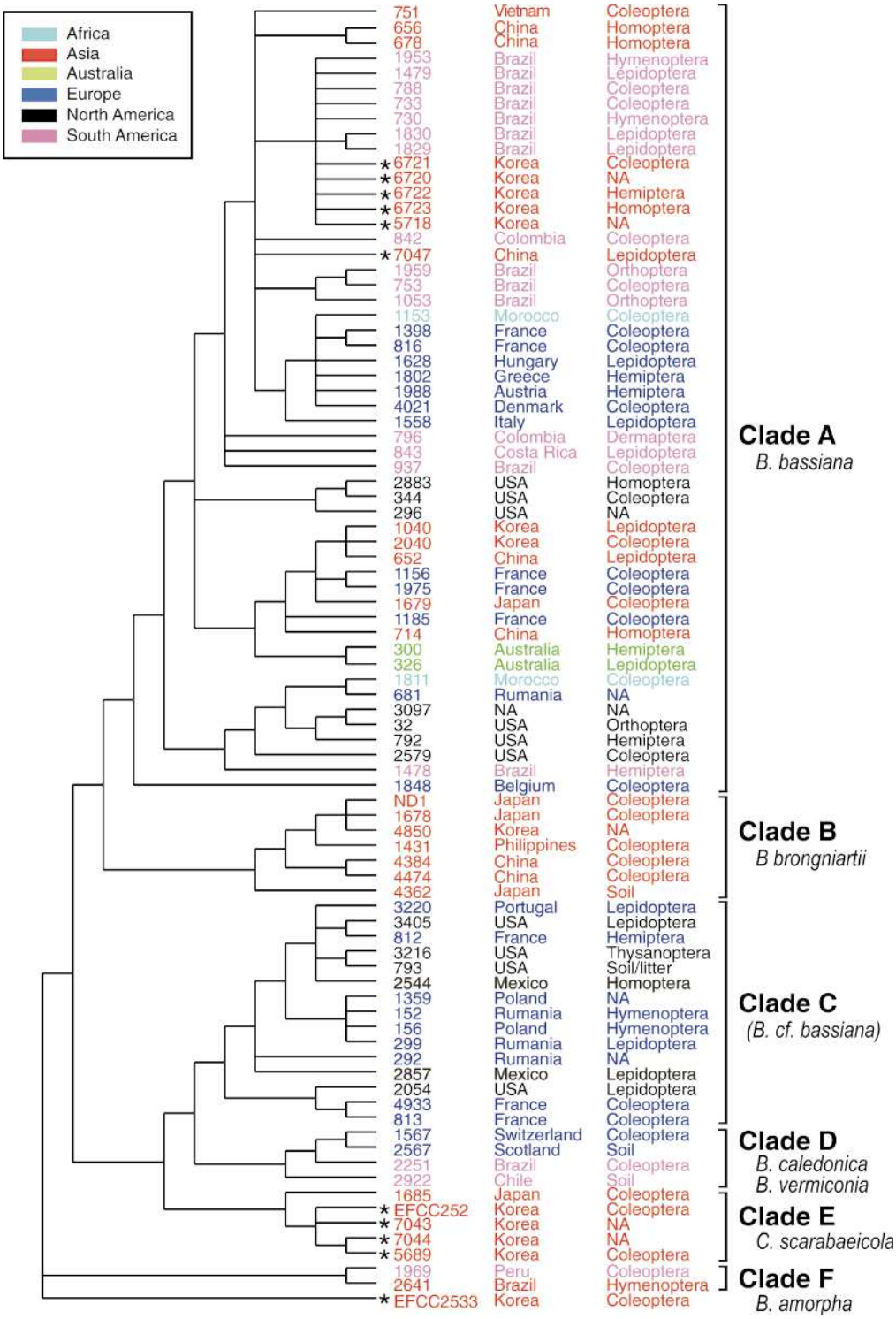



**Figure B.1.1.3.3-1:** Strict (50%) consensus tree of the Bayesian likelihood analysis of the combined TIS and EF1-α data. Terminal branches are labelled according to isolate accession number, continent of origin and the taxonomic class of insect species from which they were isolated. *Cordyceps* teleomorphs are indicated by an asterisk.

Identification at strain level is done by sequencing of the ITS ribosomal region (Berry, 2012, see also B.5.1.1.1). The resulting sequence was BLASTED against sequences deposited in GenBank

(http://www.ncbi.nlm.nih.gov/Blast). Results showed that the isolate PPRI 5339 was most related (99-100% identity) to sequences deposited as *Beauveria bassiana*.

Generating a distance tree of results (http://blast.ncbi.nlm.nih.gov/blast/treeview/treeView.cgi) resulted in a neighbour-joining cladogram depicting relationship between the PPRI 5339 isolate with that of reference sequence in GenBank. Results (Figure B.1.1.3.3-2) showed that the PPRI 5339 isolate (highlighted in yellow) was nestled in between branches of many different *Beauveria bassiana* isolates.

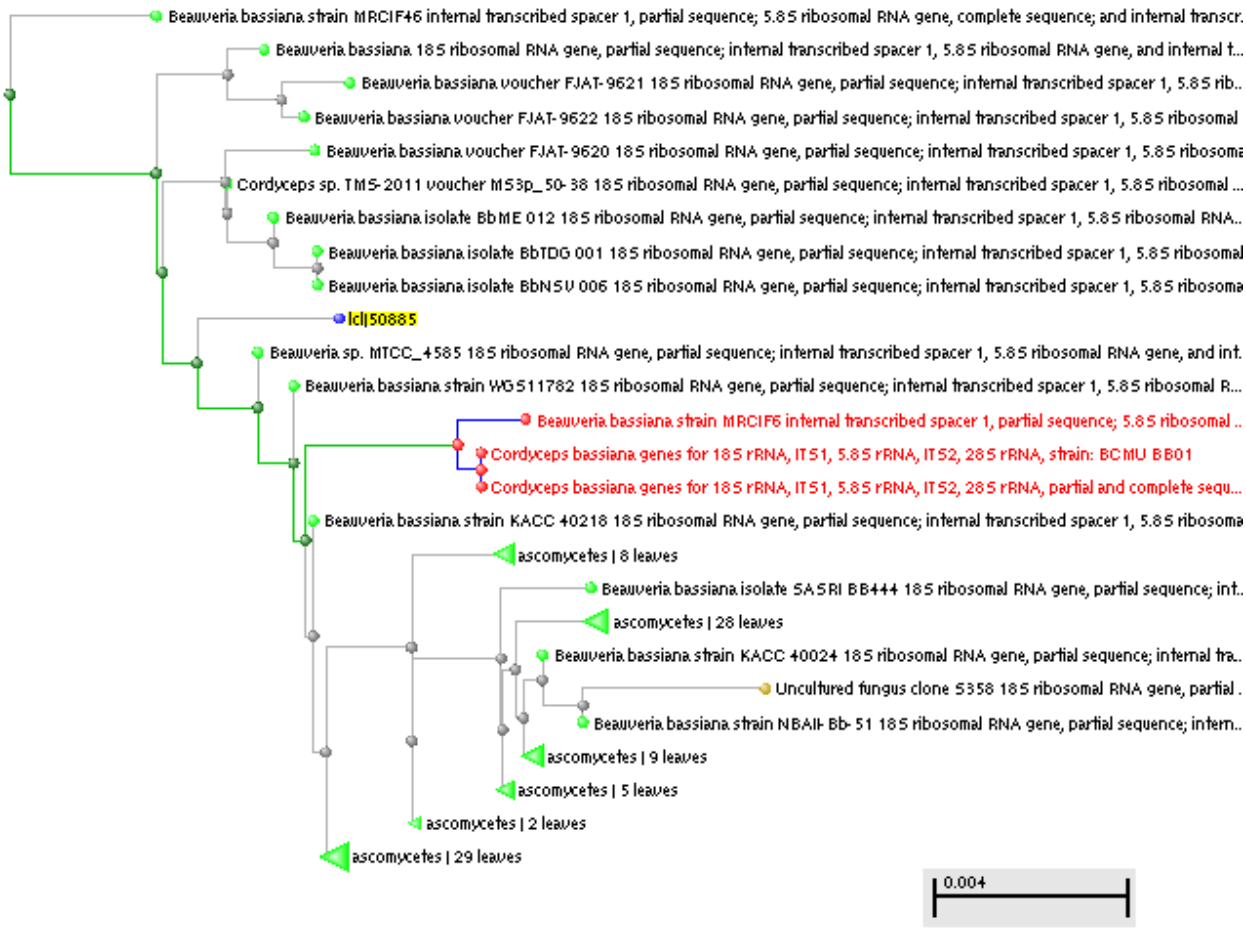



**Figure B.1.1.3.3-2:** Cladogram showing relative position of *Beauveria bassiana* isolate PPRI 5339 (shown in figure as lclj50885) compared to closely related strains.

### B.1.1.3.4 Common name or alternative and superseded names and code names used during the development (Annex IIM 1.3.5)

| | |
|---|---|
| Trade names: | Broadband (South Africa)<br>The active substance is also sold in different formulations under trade names Broadband EC, Bb Plus and Bb Weevil. |
| Common names: | *Beauveria bassiana* strain PPRI 5339 |

Developmental code names: Legacy-Becker Underwood development code number for EUP: BUEXP1778
BASF code number for active ingredient: BAS 480 I
BASF code number for EUP: BAS 480 00 I

#### B.1.1.3.5 Relationship to known pathogens (Annex IIM 2.7)

See B.2.1.6

### B.1.1.4 Specification of the material used for manufacturing of formulated products (Annex IIM 1.4)

#### B.1.1.4.1 Content of the micro-organism (Annex IIM 1.4.1)

Nominal (mean) purity: $1.0 \times 10^{11}$ viable CFU/g in the technical material.

#### B.1.1.4.2 Identity and content of impurities, additives, contaminating micro-organisms (Annex IIM 1.4.2)

As part of the quality control the technical concentrate is analysed for the presence of relevant microbial contaminants (see B.5.1.1.5 for more information on the methods used).

For microbial contaminant the following criteria are applied:

| **Characteristic** | **Standard Test Method number** | **Specification** |
|---|---|---|
| Total aerobic bacterial contamination | SA-STM-1202 | <1 E+5 CFU/g |
| Total fungal contamination | SA-STM-1202 | <1 E+3 CFU/g |
| Total coliforms | SA-STM-1202 | <1 E+3 CFU/g |
| Anaerobic spore formers | SA-STM-1214 | <1 E+5 CFU/g |
| *E. coli* | SA-STM-1214 | Absent in 1g |
| *Staphylococcus aureus* | SA-STM-1214 | Absent in 1g |
| *Pseudomonas aeruginosa* | SA-STM-1214 | Monitoring |
| *Salmonella* | SA-STM-1214 | Absent in 25g |
| *Shigella* | SA-STM-1214 | Absent in 25g |
| *Listeria* | SA-STM-1214 | Absent in 25g |
| *Vibrio* | SA-STM-1214 | Absent in 25g |

In addition, one non-microbial impurity (the metabolite beauvericin) is investigated (see B.5.1.1.5 for more information on the methods used).
See Volume 4 for more details on the batch analysis for these contaminants and the metabolite beauvericin.

#### B.1.1.4.3 Analytical profile of batches (Annex IIM 1.4.4)

For the analytical profile of the batches we refer to Volume 4.

### B.1.2 Identity of the plant protection product (Annex IIIM 1)

#### B.1.2.1 Applicant (Annex IIIM 1.1)

Please refer to B.1.1.

#### B.1.2.2 Manufacturer of the preparation and the micro-organism (Annex IIIB 1.2)

This is considered confidential information. Please refer to volume 4 for details on the producer(s).

#### B.1.2.3 Trade name or proposed trade name, and manufacturer's development code number of the preparation if appropriate (Annex IIIM 1.3)

Development code: BAS 480 00 I

Trade name: Broadband

#### B.1.2.4 Detailed quantitative and qualitative information on the composition of the preparation (Annex IIIM 1.7)

Confidential information, see Volume 4.

#### B.1.2.5 Physical state and nature of preparation (Annex IIIM 1.5)

Formulation type: OD (oil dispersion).

#### B.1.2.6 Function (Annex IIIM 1.6)

Contact insecticide.

### B.1.3 References relied on

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>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 1.3.1/01 | Rehner, SA, Buckley, E | 2005 | A Beauveria phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologica, 97(1), 84-98.<br><br>Not GLP<br>Published | N | N | N/A | Public |
| MA 1.3.3/01 | Berry, S | 2012 | Molecular Identify of the South African *Beauveria bassiana* isolate, strain PPRI 5339. Becker Underwood, Mahogany Ridge, KZN, South Africa | N | Y | Strain specific new laboratory study | BASF |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner |
|---|---|---|---|---|---|---|---|
| | | | and Becker Underwood, NSW 2250, Australia.<br><br>Report Date: 28.11.12<br><br>Not GLP<br><br>Unpublished | | | | |
| MA 1.3.4/01 | Rehner, SA | 2005 | Phylogenetics of the insect pathogenic genus Beauveria. In: Vega, F.E., Blackwell, M. (Eds.), Insect-Fungal Associations: Ecology and Evolution. Oxford University Press, pp. 3–27.<br>Not GLP<br>Published | N | N | N/A | Public |
| MA 1.4.2/01 | Whittaker, M | 2014 | Detection and enumeration of *Beauveria bassiana* and microbial contaminants in five production batches of BROADBAND Microbial Pest Control Product. APIS, Knaresborough Technology Park, Manse Lane, Knaresborough, North Yorkshire HG5 8LF.<br><br>Study Number APIS-BASF-11.<br><br>GLP<br><br>Unpublished | N | Y | Strain specific new GLP laboratory study | BASF |
| MA 1.4.3/01 | Cheng, KM | 2013 | Assay to determine Beauvericin within Beauveria bassiana concentrate with associated validation. Oxford Analytical Ltd, A3, Telford Road, Bicester, Oxfordshire OX26 4LD, UK.<br><br>Study Number OA02266.<br><br>GLP<br><br>Unpublished | N | Y | New strain specific GLP study | BASF |
| MA | Whittaker, | 2014 | Detection and | N | Y | Strain specific | BASF |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner |
|---|---|---|---|---|---|---|---|
| 1.4.3/02 | M | | enumeration of yeasts and moulds in five production batches of PPRI 5339 technical grade active ingredient. APIS, Knaresborough Technology Park, Manse Lane, Knaresborough, North Yorkshire HG5 8LF.<br><br>Study Number: APIS-BASF-009<br><br>Report Date: TBC<br><br>GLP<br><br>Unpublished | | | new GLP laboratory study | |
| **Annex III Data and Information** | | | | | | | |
| | | | No data submitted. | | | | |

## B.2 Biological, physical, chemical and technical properties

### B.2.1 Biological properties of the micro-organism (Annex IIM 2)

#### B.2.1.1 History of the micro-organism and its uses. Natural occurrence and geographical distribution (Annex IIM 2.1 and 2.2)

##### B.2.1.1.1 Historical background (Annex IIM 2.1)

The two entomopathogenic fungal species *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and *Beauveria brongniartii* (Saccardo) Petch were described for the first time about 170 and 110 years ago, respectively (Zimmerman, 2007). There are over 400 reported strains of *Beauveria bassiana* and a few have been commercialised. Most species and strains have some insecticidal properties. *Beauveria bassiana* is used as an insecticide against different species such as *Ostrinia nubilalis* (European corn borer) on corn, *Otiorhynchus sulcatus* (vine weevil) on vine, raspberry and strawberry, *Leptinotarsa decemlineata* (Colorado beetle) on potato, *Cydia pomonella* (Codling moth) on apricot, pear and apple trees, *Hypothenemus hampei* (Coffee berry borer), on coffee, as well as *Trialeurodes vaporariorum* (glasshouse whitefly), grasshoppers, locusts, aphids, thrips and boll worms on various crops.

##### B.2.1.1.2 Origin and natural occurrence (Annex IIM 2.1/2.2)

PPRI 5339 is a naturally occurring, non-modified strain of *Beauveria bassiana*, originally isolated by Dr Schalk Schoeman from the larva of a tortoise beetle, *Conchyloctenia punctata* (*Coleoptera: Cassidinae*) collected in Escombe, Queensburgh, KwaZulu Natal, South Africa.

*Beauveria bassiana*, a hyphomycetous entomopathogenic fungus, is the most widely distributed species of the genus. *Beauveria bassiana* grows naturally in soil and is is generally found throughout a wide range of habitats from alpine soils to heathland, in peat bogs, soils with savannah type vegetation, and in forest and cultivated soils, in sand blows and dunes as well as in desert soils and running water, on all continents of the world (Zimmerman, 2007).

Some studies have investigated the natural occurrence on plants. of *B. bassiana* was assessed in hedgerow vegetation in Denmark (Meyling & Eilenberg, 2006). Populations varied between different plants (*Crataegus monogyna*, *Urtica dioica*, and various members of the *Poaceae*) and between different seasons and were found to be within 0.02 CFU/10 $cm^2$ and 0.75 CFU/10 $cm^2$. In general, lower leaves of *U. dioica* had the highest populations (except for July, when upper leaves had the highest populations), and grasses the lowest. Spores are generally considered to originate from the

soil. Possible ways of dispersion are rain splashes, especially for lower leaves and grasses, but also dispersion by wind, or by infected or uninfected insects. Cadavers of diseased insects in the phyllosphere are another important source of inoculum.

*Beauveria bassiana* has also been isolated from the surface and the interior of plants. It was isolated from bark of elm trees and *Carpinus caroliniana* (Doberski & Tribe 1980; Bills & Polishook, 1991). Wagner & Lewis (2000) and Posada & Vega (2005) described the occurrence of *B. bassiana* as endophyte. In corn approximately 3% of the candida germinated, and less than 1% of these penetrated the leaf surface directly (Wagner & Lewis 2000).
Airaudi & Marchisio (1996) have isolated *B. bassiana* from the air of Turin, Italy. *B. bassiana* was present in 10 out the 12 months measured though with a maximum load of 0.5 CFU/$m^3$ (mean 0.2 CFU/$m^3$.

#### B.2.1.2 Information on target organism(s) (Annex IIM 2.3)

The target organisms are: Whitefly (*Bemisia tabaci, Bemisia argentifolii, Trialeurodes vaporariorum*) and Thrips (*Frankliniella occidentalis, Thrips tabaci*)

##### B.2.1.2.1 Description of target organism(s) (Annex IIM 2.3.1)

Western flower thrips, *Frankliniella occidentalis,* FRANOC and onion thrips, *Thrips tabaci*, THRITB
In the past the most important thrips species in Dutch greenhouses was the onion thrips (*Thrips tabaci*). However, since the mid-eighties western flower thrips *(Frankliniella occidentalis*) are by far the most important one. In contrast to onion thrips, the western flower thrips is an imported species.
The life cycle of thrips consists of six stages; an egg stage, two larval instars, pre-pupa stage, pupa stage and an adult stage. The female lays the eggs in leaves or flower petals. Immediately after egg hatching, the larvae start eating from the bottom side of the leaves. The majority of larvae pupate in the soil. The pre-pupa and pupa stage are mobile but are generally are stationary unless disturbed. The combined pre-pupal, pupal and egg duration equates to more than 50% of the total egg to adult period. Thus, a substantial part of the life cycle is spent as developmental stages which are non-feeding or away from the plant, and consequently are not vulnerable to the active ingredient. The timing of insecticide applications must account for this if control of the pest is to be achieved.
Thrips are causing direct damage because of their feeding behavior. All mobile stages feed on leaf tissue and flowers by puncturing cells and draining the content. Around the feeding spots plant tissue will die, causing pale silvery patches with black dots. This means loss of assimilation and thus less growth. Severe infestation in a young stage can lead to plant deformation.

Tobacco whitefly, *Bemisia tabaci*, BEMITA
*Bemisia tabaci* is a plant sap-sucking insect placed in the family Aleyrodidae in the superfamily Aleyrodoidea (whiteflies). It is broadly polyphagous, feeding on an estimated 600 plant species. Since the early 1980s it has caused escalating problems to both field and protected agricultural crops and

ornamental plants. Heavy infestations of *B. tabaci* may reduce host vigour and growth, cause chlorosis and uneven ripening, and induce physiological disorders. The larvae excrete honeydew on which sooty moulds grow, reducing the photosynthetic capabilities of the plant, resulting in defoliation and stunting. *Bemisia tabaci,* furthermore, is a vector of 111 plant viruses in the genera *Begomovirus* (*Geminiviridae*), *Crinivirus* (*Closteroviridae*) and *Carlavirus* or *Ipomovirus* (*Potyviridae*). Begomoviruses are the most prevalent of the *B. tabaci* transmitted viruses and can cause crop yield losses of between 20% and 100%. *Bemisia tabaci* possibly originated in India and as a result of widespread dispersal, particularly during the last 15 years, is now distributed nearly worldwide.

Eggs, deposited on the underside of leaves, are tiny, oval-shaped, about 0.25mm in diameter and stand vertically on the leaf surface. Newly laid eggs are white then turn brownish. Upon hatching the first instar nymph (0.3mm in length), commonly called the “crawler”, moves about the leaf in search of a place to insert its needle-like mouthparts into the plant to suck up plant phloem. When the crawler finds this site, it molts to the second instar; its legs are pulled up under its body and the rest of the immature stage is sessile. There are three additional nymphal instars (0.4-0.8mm) with the successive stage molting to a slightly larger form. The last nymphal instar develops red eye spots and is commonly called the “red-eyed nymph.” This stage is often incorrectly called the pupal stage; incorrect because insects in this order Hemiptera have incomplete metamorphosis, thus there is no pupal stage. Throughout the nymphal stages, the body of the whitefly is opaque white in colour and is covered by a waxy exoskeleton. As nymphs feed, they excrete large quantities of liquid waste in the form of honeydew. Honeydew is rich in plant carbohydrates and as whiteflies feed and excrete, this waste is distributed onto plant leaves, flowers and fruit and supports the growth of sooty mould fungus, causing the plant to turn black. Adult whiteflies are about 1mm long with two pairs of white wings and light yellow bodies. Their bodies are covered with a waxy powdery material. While whitefly adults can be seen on all plant surfaces, they spend most of their time feeding, mating and ovipositing on the under surfaces of leaves. Males and females are present, typically in even ratios, and mating takes place after an elaborate courtship period. Whiteflies have an interesting reproductive biology (called arrhenotoky) in which females can lay eggs that have not been fertilised and these eggs will result in male offspring. Fertilised eggs will result in female offspring. Each female can produce as many as 200 eggs in her lifetime. It takes 30-40 days to develop from egg to adult, depending on the temperature.

Glasshouse whitefly*, Trialeurodes vaporariorum,* TRIAVA

*Trialeurodes vaporariorum*, commonly known as the glasshouse or greenhouse whitefly inhabits the world’s temperate regions. It is a primary insect pest of many fruit, vegetable and ornamental crops, frequently being found in glasshouses and other protected horticultural environments. Adults are 1-2 mm in length, with yellowish bodies and four wax-coated wings held near parallel to the leaf surface.

Females are capable of mating less than 24 hours after emergence and most frequently lay their eggs on the undersides of leaves. Eggs are pale yellow in colour, before turning grey prior to hatching. Newly hatched larvae, often known as crawlers, are the only mobile immature life-stage. During the first and second larval instars, the appearance is that of a pale yellow/translucent, flat scale which can

be difficult to distinguish with the naked eye. During the fourth and final immature life-stage referred to as the (pupa), compound eyes and other body tissues become visible as the larvae thicken and rise from the leaf-surface. However, this stage cannot be defined as a true pupa stage as Hemipterans do not undergo complete metamorphosis

All life-stages apart from eggs and "pupae" cause crop damage through direct feeding, inserting their stylet into leaf veins and extracting nourishment from the phloem sap. As a by-product of feeding, honeydew is excreted and that alone can be a second, significant source of damage. The third and potentially most harmful characteristic is the ability of adults to transmit several plant viruses. The crops principally affected are vegetables such as cucurbits, potatoes and tomatoes, although a range of other crop and non-crop plants including weed species are susceptible, which can therefore harbor the insect.

#### B.2.1.2.2 Mode of action (Annex IIM 2.3.2)

Like other entomopathogenic fungi, *B. bassiana* attacks its host insects generally percutaneous. 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 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. The penetration is followed by the invasion, which is accompanied by several host immune response activities. During the infection process, *Beauveria* spp. produces proteolytic enzymes and toxins, while the host insect responds with cellular and humoral defence reactions. In the insect body, the fungus multiplies as blastospores, or yeast-like cells, which are distributed passively in the haemolymph. Enzymes begin to destroy the internal structures of the host insect causing morbidity within 36 to 72 hours. Reduced feeding and immobility are rapidly evident, and the insect dies within 4 to 10 days post-infection. The time to death will depend on the insect species, age and conidial dose. After death of the insect, the fungus starts its saprophytic growth: blastospores transform into mycelia, which emerge through the cuticle. Aerial conidia are formed on the surface of the insect cadaver, which build the characteristic white mould. Sporulation occurs only in conditions of high humidity.

### B.2.1.3 Host specificity range and effects on species other than the target harmful organism (Annex IIM 2.4)

*Beauveria bassiana* is not host-specific but an opportunistic entomopathogen capable of attacking insects of a wide range of different taxa (Rehner & Buckley 2005, see MA 1.3.1).

Based on worldwide data, Li (1988) listed 707 insect species as hosts of *B. bassiana*. This list comprised 521 genera and 149 families in 15 orders. In addition, 13 host species of Acarina distributed in 7 genera and 6 families are listed. Nevertheless, despite the prevalence of *B. bassiana*

on a huge number of arthropods, it is known that most isolates have a restricted host range (Goettel *et al.*, 1990, Vestergaard *et al.* 2003). (Doc K-MA 2.2.3/02 and Doc K-MA 2.2.3/03) *Beauveria bassiana* strain PPRI 5339 acts as an insecticide, and the primary target insects are whiteflies (*Aleurodidae*), thrips (*Thysanoptera*).

Based on the wide host range, beneficial insects could be affected by *B. bassiana*. Nevertheless, Hajek and Butler (2000) (Doc K-MA 2.2.3/04) differentiate between the physiological and ecological host range, which means that non-target insects, which are infected under laboratory conditions, may not necessarily be infected in nature (confirmed by Vestergaard *et al.*, 2003) (Doc K-MA 2.2.3/03).

Wang *et al.* (2004) (Doc K-MA 2.2.3/05) monitored the fate of *B. bassiana* strains, which had been inundatively applied against *Dendrolimus punctatus* in Southwest China. They could re-isolate the indigenous and exotic strains during one year. However, the indigenous strains were predominant in the local environment and were not displaced by the exotic ones (Wang *et al.*, 2004) (Doc K-MA 2.2.3/05). An antagonistic effect of *B. bassiana* on plant pathogenic fungi has also been mentioned (Ownley *et al.*, 2004) (Doc K-MA 2.2.3/06).

*B. bassiana* is not known to infect humans (except in extremely rare cases consisting of eye infections after mechanical damage to the corneas, lung diseases and disseminated infections involving profoundly immunocompromised patients) or mammals. In these cases the strain was not characterized (see also Volume 3, B.6 for more details concerning infective potential of *B. bassiana* on humans).

#### B.2.1.4 Development stages/life cycle of the micro-organism (Annex IIM 2.5)

The conidia of *Beauveria bassiana* adhere to the insect cuticle, germinate and penetrate in the insect body, where they replicate as yeast-like cells (blastospores) and destroy the internal structures, causing morbidity within 36 - 72 hours. After death of the insect, the fungus starts its saprophytic growth: blastospores transform into mycelia, which emerge through the cuticle. Aerial conidia are formed on the surface of the insect cadaver, which build the characteristic white growth. *Beauveria bassiana* strain PPRI 5539 requires contact with viable host in order to proliferate.
*Beauveria bassiana* strain PPRI 5339 has no known sexual stage.

#### B.2.1.5 Infectiveness, dispersal and colonisation ability (Annex IIM 2.5/2.8)

Infectiveness:

Despite a 30-year history of research and commercial use, infections in humans in which *Beauveria* species are putatively identified are extremely rare, consisting of eye infections, lung diseases, and from disseminated infections in profoundly immunocompromised patients (see also Volume 3, B.6 for

more details concerning infective potential of *B. bassiana* on humans). In these cases the strain was not characterized. In most cases patients responded well to antimycotic treatments.
No infectiveness to terrestrial vertebrates was seen. Some unclear results were derived for aquatic vertebrates, but no infectiveness was seen for invertebrates as for terrestrial invertebrates (chapter B.9).

Dispersal routes:
*Beauveria bassiana*, a hyphomycetous entomopathogenic fungus, is a naturally occurring ubiquitous fungus. Spores are generally considered to originate from the soil. Possible ways of dispersion are rain splashes, especially for lower leaves and grasses, but also dispersion by wind, or by infected or uninfected insects. For instance Samšiňák (1964) found that the mite *Tyrophagus putrescentiae* is able to transmit spores of *Beauveria bassiana* from fungus-infected larvae of *Galleria mellonella* to healthy ones. *Beauveria bassiana* was also found in high frequency on a great number of the collembolan *Onychiurus subtenuis* present in soil litter (Visser *et al*. 1987). Collembolans can act as vectors of *Beauveria* spp. and thus may play an important role for the dispersal and transmission of these fungi in soil (see B.9.5).

Environmental conditions:

*UV light:*
The conidiospores of *B. bassiana* are sensitive to sunlight radiation. To this end concentrations on the surface of leaves exposed to sunlight were found to decline swiftly (Neethling *et al.*, 2014)

| | |
|---|---|
| **Report:** | Neethling, D, *et al.* (2012), Effect of artificial ultra-violet light on viability of *Beauveria bassiana* strain PPRI 5339. Report Date: 05-06-2014 |
| **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.

**Method**
Technical grade *B. bassiana* strain PPRI 5339 (Batch LE300, 1.43 x 10^11 viable spores/g) powder was placed in a petri dish (Ø90mm). The petri dish was placed on the laboratory worksurface 10 cm from the 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 wetter an isotridecanol-based surfactant agitated to mix and then sonicated for 3 minutes. After sonication, 0.1 ml was plated onto Potato Dextrose Agar plates. The

plates were then incubated at 28ºC 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 MPCA *B. bassiana* strain PPRI 5339 to UV light reduced the germination and viability of the fungal spores (Table B.2.1.5-1). 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%. The drop in viability over time occurred in a logarithmic pattern, typical of a degradation curve with a biological organism (Figure B.2.1.5-1). From the data the $LT_{50}$ can be calculated to be 39.26 minutes (y = -15.99ln(x) + 108.69).

**Table B.2.1.5-1: Effect of exposure to artificial ultra violet under laboratory conditions on the germinations and viability of spores of technical grade *B. bassiana* strain PPRI 5339**

| Time (minutes) | 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 |

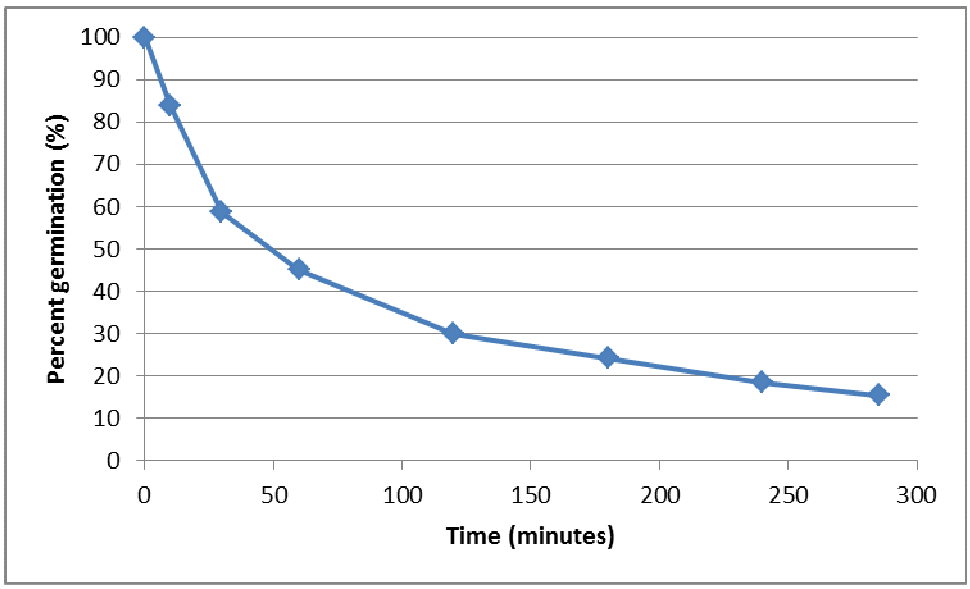



**Figure B.2.1.5-1: Effect of exposure to artificial ultra violet under laboratory conditions on the germination and viability of spores of technical grade *Beauveria bassiana* strain PPRI 5339.**

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 (Table B.2.1.5-1).

**Conclusions**

The study demonstrates that foliar degradation of *B. bassiana* strain PPRI 5339 is fast as has been found for other strains of *Beauveria bassiana*.

*Temperature*

Temperature influences the germination, growth and viability of the fungus. In general, *B. bassiana* grows in a wide temperature range from 5 to 35 °C. Fargues *et al.* (1997) investigated the effect on vegetative growth of 65 isolates of *B. bassiana* and found a maximum thermal threshold at temperatures of >35-37ºC for 50 isolates, >32-35ºC for 12 isolates and >>30-32ºC for 1 isolate. The lower temperature threshold for all isolates tested was below 8 ºC. Optimal temperatures were generally between 25-28ºC with several isolates exhibiting optimal growth at temperatures as low as 20 or as high as 30 ºC.

The effect of temperature on growth of *Beauveria bassiana* strain PPRI 5339 has been investigated and is summarized below.

| | |
|---|---|
| **Report:** | Neethling, D, *et al.* (2012), The effect of temperature on growth of *Beauveria bassiana* strain PPRI 5339. Becker Underwood, Mahogany Ridge, South. Report Date: 21.11.12, Project Number: LE 639 |
| **Guidelines:** | None |
| **GLP:** | No |

**Summary**

The aim of the study was to determine the temperature growth curve of *Beauveria bassiana* strain PPRI 5339. The optimal temperature for growth was determined to be 22 – 28 °C.

**Method**

A spore suspension of *Beauveria bassiana* strain PPRI 5339 (batch BB 5339 sub3 12072010) was prepared by aseptically removing one loop full of spores from a culture slant and added to 9 ml of sterile saline solution and sonicated for two minutes. The suspension was "stab inoculated" onto 21 Potato Dextrose Agar plates at three points. These three points would develop into three colonies. The plates were incubated at 10°C, 18°C, 22°C, 25°C, 28°C, 32°C and 36°C. Three inoculated plates for each fungus were incubated in each of the seven incubators for 8 days, each of these three plates was considered a replicate. The diameter of the colonies were recorded on days 2, 4 and 8.

**Results**

After two days there was no growth of the fungal spores at any test temperature. After four days there was growth at temperatures between 22 and 28 °C (Table B.2.1.5-1, Figure 2.1.5-1). After 8 days

growth had also occurred at lower temperatures of 18 °C. There was no growth at either 10, 32 or 36 °C during the 8 day experiment.

**Table B.2.1.5-1: Effect of temperature on growth of *Beauveria bassiana* strain PPRI 5339 after 4 and 8 days.**

| Temperature (ºC) | Day 4 | | Day 8 | |
|---|---|---|---|---|
| | Area ($mm^2$) | SD | Area ($mm^2$) | SD |
| 10 | 0 | 0.00 | 0 | 0.00 |
| 18 | 0 | 0.00 | 73.391 | 1.83 |
| 22 | 14.74803 | 1.83 | 161.356 | 3.40 |
| 25 | 34.90659 | 0.26 | 245.131 | 3.40 |
| 28 | 22.34021 | 1.83 | 106.901 | 1.05 |
| 32 | 0 | 0.00 | 0 | 0 |
| 36 | 0 | 0.00 | 0 | 0 |

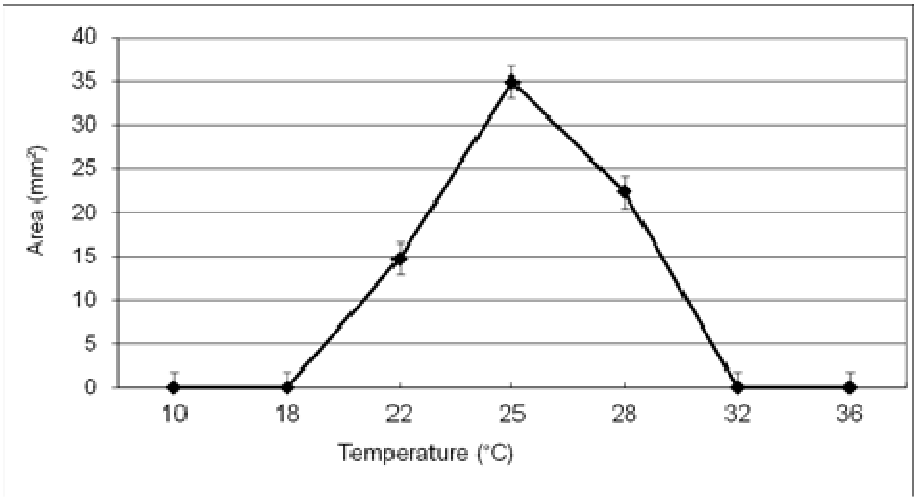



**Figure B.2.1.5-1: Effect of temperature on growth of *Beauveria bassiana* strain PPRI 5339 after 4 days.**

**Conclusions**

Optimal growth of *Beauveria bassiana* strain PPRI 5339 was found to occur at temperatures between 22 and 28 °C.

### B.2.1.6 Relationships to known plant or animal or human pathogens (Annex IIM 2.7)

Endophytic colonisation by *B. bassiana* after spray application was observed in corn, without any signs of pathogenicity (Wagner and Lewis, 2000). Infection mechanisms of *B. bassiana* are highly evolved and specific only to insects.

*Beauveria bassiana* is not linked to known human pathogens. However, like any micro-organism, *B. bassiana* can potentially act as an opportunistic pathogen. A literature search conducted by the notifier

identified reports of human infestations in immunosuppressed patients but all were successfully treated (see also Volume 3, B.6 for more details).

#### B.2.1.7 Genetic stability and factors affecting it (Annex IIM 2.10)

Fungi possess low genome plasticity due to the narrower scope of mechanisms available to eukaryotic cells for the incorporation of DNA (Coehlo *et al.*, 2013). In line with this, Horizontal Gene Transfer (HGT) is still considered to be anecdotal in fungi (Rosewich and Kistler, 2000). Therefore, *Beauveria bassiana* strain PPRI 5339 is not expected to possess the potential for transfer of genetic material.

#### B.2.1.8 Information on the production of metabolites (especially toxins) (Annex IIM 2.6/2.7.2)

Different secondary metabolites isolated from different strains within the genus *Beauveria* have been described in the literature (Strasser *et al.*, 2000). Effects of these metabolites on insects, fungi and bacteria were reported, but also effects on plants, mammals and humans were observed in different experimental systems.

*Beauveria* metabolites can be divided into four different groups:

1. Low molecular weight compounds: oxalic acid.
2. Non-peptide pigments: oosporein, bassianin, and tenellin.
3. Cyclodepsipeptides: beauvericin, bassianolides, beauveriolides, and beauverolides.
4. High molecular weight proteins (with enzymatic activity): bassiacridin, Bclp.

**1. Low molecular weight compounds**

Oxalic acid $(COOH)_2$ is produced by some isolates of *B. bassiana* and is a major pathogenicity factor in the interaction of *B. bassiana* with grasshoppers (*Melanoplus sanguinipes*, Orthoptera; Bidochka & Khachatourians, 1991) and ticks (Ixodidae; Kirkland *et al.*, 2005). Oxalic acid can act as proton donor, electron donor, or as chelating agent to form complexes with divalent cations. Thus, it is able to solubilise insect cuticle components and disrupts cuticles directly (Bidochka & Khachatourians, 1991). Production of oxalic acid by *B. bassiana* isolates depends on composition of the growth media. Activity of *B. bassiana* towards ticks is dependent on acidification and increases drastically below pH 4.5 (Kirkland *et al.*, 2005). In addition, acidification is required for activity of lytic enzymes from *B. bassiana*, which makes oxalic acid a synergistic facilitator of pathogenesis. Oxalic acid is as well produced by plant pathogenic fungi to penetrate plant cell walls, but also by plants used for human consumption like rhubarb, spinach and beets to prevent insects from feeding. In mammals, oxalic acid is an end product of metabolism of natural components of some amino acids as well as glycolate and ascorbic acid. Therefore, oxalic acid is not expected to pose any risk to humans or the environment.

**2. Non-peptide pigments**

Oosporein, bassianin, and tenellin are red, orange, or yellow pigments produced by *Beauveria* strains. They share similar modes of action.

Jeffs & Khachatourians (1997) analysed the effect of tenellin, bassianin and oosporein on ATPase activity. All three metabolites inhibited total erythrocyte membrane ATPase activity in a dose-dependent manner. The effect was more pronounced for $Ca^{2+}$ ATPases than for $Na^{+}/K^{+}$-ATPase activity. The ATPase inhibitory activity for these pigments was not specific but was probably a consequence of membrane disruption, since pigments all caused alterations in erythrocyte morphology and promoted varying degrees of cell lysis.

Eley *et al.* (2007) analyzed biosynthesis of tenellin and produced a mutant lacking genes encoding tenellin biosynthesis enzymes. This strain was as efficient as the wild type strain towards *G. mellonella* larvae, showing that tenellin is not involved in insect pathogenesis.

**Oosporein**

Oosporein (or oosporin) is a red-coloured dibenzoquinone which is produced by some strains of the genus *Beauveria*, most of them belonging to the species *B. brogniartii*. Oosporein acts as an antibiotic against gram-positive bacteria, but has little effect on gram-negative bacteria. Oosporein produced by the cereal post-harvest pathogen *Chaetomium trilaterale* in poultry feed was found to be toxic to chicken (Pegram & Wyatt, 1981). Oosporein is supposed to inhibit activity of enzymes through redox modification of SH groups and to inhibit membrane ATPase activity.

Oosporein together with other metabolites produced by fungi used for biocontrol was analysed for acute toxicity towards the crustaceae *Artemia salina* and *Daphnia magna*. Among all metabolites tested, oosporein showed the lowest toxicity in these systems and $LC_{50}$ data for oosporein in *A. salina* could not be determined. However, sensitivity of *D. magna* to oosporein increased when exposure was extended from 24 h to 36 h (Favilla *et al.*, 2006).

Oosporein has an antifungal effect on the plant pathogen *Phytophthora infestans* (Nagaoka *et al.* 2004). Strasser *et al.* (2000) demonstrated that production of oosporein by a *B. brogniartii* strain varies between 3.2 mg/kg for solid phase fermentation on barley kernels and 300 mg/L for liquid media. Accumulation of oosporein and other metabolites involved in insect pathogenicity only occurs during parasitism of insects (Strasser *et al.*, 2000).

Abendstein *et al.* (2000) applied *Beauveria brogniartii* to seed potatoes in a rate 40-fold higher than the recommended application rate for that specific product. No phytotoxic effect was observed, and *B. brogniartii* did not colonise potato tuber tissue. No oosporein was detected in tubers after harvest of treated plants, nor in tubers spiked with oosporein. This may have resulted from analytical problems in detection or from metabolisation or degradation of oosporein within the tubers. Oosporein was metabolised in potato sap within two hours, indicating that this metabolite is not accumulated by plants.

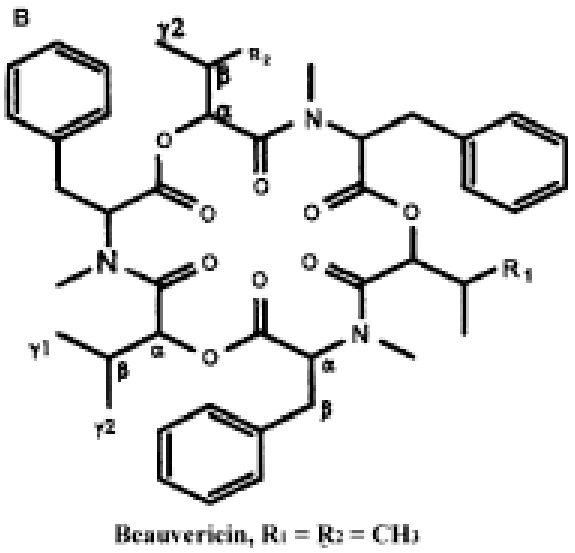
Bassianin, tenellin, and oosporein are produced by some *B. bassiana* strains during interaction with their target hosts or under defined conditions in liquid media. Contents of bassianin, tenellin, or oosporein were not determined for *Beauveria bassiana* strain PPRI 5339. Accumulation of these compounds in natural environments or after application of plant protection products containing *B. bassiana* was not analysed, but does not seem realistic. Accumulation and subsequent effects on vertebrates were only reported for oosporein produced by *Chaetomium trilaterale*, a saprophytic

fungus multiplying in poultry feed. Exposure of humans or non-target organisms to bassianin, tenellin, and oosporein can only occur through consumption of insects infested with *B. bassiana*. However, this exposure already exists as *B. bassiana* is a naturally occurring fungus in Europe.

### 3. Cyclodepsipeptides

This group includes relatively small cyclic peptides as beauvericin, bassianolides, beauveriolides, and beauverolides. They act as ionophore antibiotics and can lead to membrane disruption.

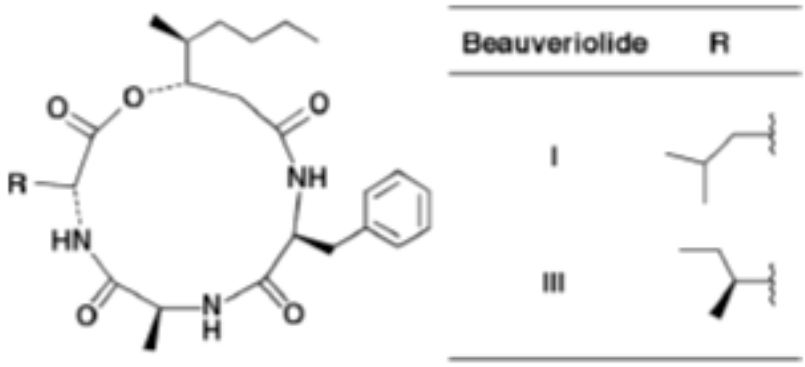
**Beauvericin** is a cyclic hexadepsipeptide which has been isolated from members of the entomopathogenic fungi genera *Beauveria* and *Paecilomyces*, but also from plant pathogenic or saprophytic fungi of the genus *Fusarium* that are common maize and wheat parasites. Beauvericin has antibiotic activity against gram-positive and gram-negative bacteria and moderate insecticidal activity against certain groups of insects (Strasser *et al.*, 2000). It is toxic towards several shrimp species and has even shown toxicity towards murine and human cell lines (Strasser *et al.*, 2000). Beauvericin forms $Na^+$ and $K^+$ complexes leading to increased permeability of membranes. Beauvericin leads to cell death in human acute lymphoblastic leukemia cells through induction of an apoptotic pathway (Jow *et al.*, 2004). Champlin and Grula (1979) could not detect beauvericin in spent media of a *B. bassiana* culture or in the haemolymph of *Heliothis zea* larvae infected with the same strain, which shows high activity against *H. zea* larvae. Likewise, direct injection of beauvericin did not affect *H. zea* larvae whereas bassianolide paralyses *H. zea* larvae. At least for the interaction between this *B. bassiana* strain and *H. zea*, it can be concluded that beauvericin is not a pathogenicity factor. Similar results were reported for *Bombyx mori* larvae. On the other hand, Calo *et al.* (2004) reported cytotoxicity of beauvericin in a *Spodoptera frugiperda* cell culture.
There are some indication of a genotoxic potential available in public literature (see Volume 3 B.6 for full details). Nevertheless, the Ames test provided with *Beauveria bassiana* PPRI 5339 was negative.
Moreover, ten production lots of *Beauveria bassiana* strain PPRI 5339 were analysed for beauvericin contents (Cheng, KM, 2013, see Volume 4 for more detail) and no beauvericin was detected above the limit of quantification of 0.5 mg/kg in any of the lots. For the currently approved *Beauveria bassiana* strain a maximum content of beauvericin was set at 5 mg/kg. It can be concluded the *B. bassiana* PPRI 5339 does not accumulate beauvericin to relevant levels during growth on synthetic media as used during production of BAS 480 I. As summarised above, beauvericin is not necessarily part of insect pathogenesis by *B. bassiana* species.




Chemical structure of beauvericin (Jow *et al.* 2004)

**Bassianolide** is a cyclo-octadepsipeptide which shows insecticidal activities (Champlin & Grula 1979). Overall, little is known on toxicity of bassianolide towards mammals or plants. Production of bassianolide was not analysed for *B. bassiana* strain PPRI 5339.

**Beauveriolides** are cyclotetetradepsipeptides and consist e.g. of L-Phe, L-Ala, D-Leu and (3S,4S)-3-hydroxy-4-methyloctanoic acid (beauveriolide I), and L-Phe, L-Ala, D-allo-Ile, and 3-hydroxy-4-methyloctanoic acid (beauveriolide III). Beauveriolides I and III can be extracted from the culture broth of a *Beauveria sp.* strain originally isolated from soil. Production depends on the supply of specific amino acids. Beauveriolides I and III inhibit cholesteryl ester synthesis through inhibition of activity of acyl-CoA:cholesterol acyltransferase (ACAT). ACAT is the key enzyme catalysing synthesis of cholesteryl esters that are deposited in lipid droplets of macrophages that lead to atherosclerosis in the arterial wall. No cytotoxic effect was observed.




Structure of beauveriolides (Namatame *et al.* 2004)

**Beauverolides** are structurally closely related to beauveriolides. They are as well cyclotetradepsipeptides and consist of three amino acids and a methyl-octanoyl or methyl- decanoyl

moiety. Up to now, 15 different beauverolides have been characterised. Different beauverolides are produced by the same *Beauveria bassiana* strain (Kuzma *et al.* 2001), but also by *Paecilomyces fumosoroseus* species (Jegorov *et al.* 1994). Biological functions of beauverolides are not clear, since they do not possess bactericidal, fungicidal, or direct insecticidal properties. Beauverolide L from *B. brogniartii* does not cause mortality in *Galleria mellonella* larvae, but affects humoral responses. Incubation of isolated plasmatocytes with beauverolide L led to cytoskeleton alterations. A role in insect pathogenicity is possible through exposition of beauverolide on the outer membrane of hyphae penetrating the insect body (Vilcinskas *et al.* 1999). Effects of beauveriolides and beauverolides on plants, insects, or mammals are unknown. The unpolar nature of beauverolides (Vilcinskas *et al.* 1999) makes secretion by the fungus outside the infected host highly improbable. Uptake by plants via leaves or roots seems impossible.

**4. High molecular weight proteins**

Two larger proteins with toxic activity against host insects were described so far, namely Bassiacridin and Bclp.

Bassiacridin was purified from a *Beauveria bassiana* strain originally isolated from the locust *Dociostaurus maroccanus* (Orthoptera; Quesada-Moraga and Vey, 2004) (Doc K-MA 2.7/17). Bassiacridin is a monomer protein with a molecular weight of 60 kDa exhibiting β-glucosidase, β-galactosidase and N-acetylglucosaminidase activities. Melanisation and necrotisation were observed in larvae injected with bassiacridin. Cultured cells from *Spodoptera frugiperda* (Sf- 9) were sensitive and revealed reduced metabolic activity and morphological changes. Cells showed hypertrophy, disruption of organelles and cell membranes, and intense vacuolization. Bassiacridin is the only toxic component in the interaction between this *B. bassiana* strain and locusts, which might either be caused by absence of production of other metabolites or by the capacity of Orthoptera to inactivate cyclic depsipeptides. Upon injection, mortality in nymphs from *Locusta migratoria*, *Dociostaurus maroccanus*, and *Schistocerca gregaria* was observed. Activity is specific for locusts as *Tenebrio molitor* and *Spodoptera littoralis* larvae were not affected. *Galleria mellonella* larvae only showed low toxic effects. Due to the specificity of the effect for Orthoptera hosts, no side-effects on non-target organisms are expected. Production of bassiacridin in other *B. bassiana* strains was not analysed.

Fuguet and Vey (2004) compared mechanisms of mycoses of different *Beauveria* strains (eleven *B. bassiana* strains and five strains from different *Beauveria* species) after injection of spores into *Galleria mellonella* larvae. Symptoms were classified into four groups according to the melanisation of the larvae, showing that pathogenicity mechanisms differ between strains within the species *B. bassiana*, but also among other *Beauveria* species. Mycelium development also differed among strains, with strong mycelium growth associated to the non- melanising type. Two model strains were selected, representing types of pathogenicity linked to melanisation and non-melanisation. Soluble components in the haemolymph of infected larvae were able to cause the same symptoms as living spores. Further characterisation revealed haemolymph components to be thermolabile proteins for the melanizing strain and low-molecular weight components for the non-melanising strain. Fuguet *et al.* (2004) used the same melanising model strain to characterise a chitosanase-like protein (Bclp)

secreted into the medium *in vitro* that upon injection into *Galleria mellonella* larvae caused the same cytotoxic effects and cuticle alterations as those during infection by *B. bassiana*. The non-melanising strain lacked this protein.

**Cephalosporolides**

In addition to those metabolites listed above, Oller-Lopez *et al.* (2005) reported the formation of metabolites cephalosporolides, E and F along with another previously unknown chemical structure, referred to as bassianolone. These metabolites were produced under low nitrogen, stress conditions. More recently, however, Song *et al,* (2014), have shown that the structure of bassianolone is identical to cephalosporolide C (Ces-C), a known naturally occurring compound. Ces C is the natural precursor to cephalosporolides E and F, undergoing acid catalyzed dehydrative rearrangement. Oller-Lopez *et al*. (2005), showed that Ces-E and F have no antimicrobial activity, whereas Ces-C (bassianolone) has been shown to have significant antimicrobial activity.

**Conclusions**

Taken together, *B. bassiana* strains are able to synthesize metabolites with very different chemical and biological properties. Potential effects on humans or the environment also differ between metabolites. Some of these metabolites obviously are key determinants in pathogenicity for *B. bassiana* towards their host insects. Effects of metabolites on target hosts depend on both the *Beauveria* strains that differ in the production of metabolites and the target insects that differ in susceptibility towards metabolites. It has to be pointed out that no detrimental *in vivo* effects of metabolites produced by *B. bassiana* are known so far on humans or non-target organisms. Effects were only described for purified metabolites in cell cultures. Reports on detrimental effects of beauvericin or oosporein on chicken refer to production of this metabolite by *Fusarium spp* or *Chaetomium spp.*, respectively.

This information on *Beauveria* metabolites was summarized for different strains and only reflects biosynthetic capacities within the genus *Beauveria*. Most *Beauveria* strains were only assessed for a single type of secondary metabolites, and no specific information on the capacity to produce different groups of metabolites with potential impact on humans or the environment is available for any strain, including *B. bassiana* strain PPRI 5339. Moreover, conditions under which certain metabolites are produced are widely unknown. For all groups of metabolites, the actual production *in vitro* depends on the media in which isolates are grown and on the age of the culture. Original studies on metabolites were rather focused on chemical structures and biosynthetic capacities than on the ecological role of the respective metabolites. Accumulation of metabolites was observed either in nutrient rich media (and depends on the composition of the media), or locally during parasitation of the target host (Strasser *et al.*, 2000; Fuguet and Vey, 2004). In addition, one or more of several possible pathways may be enhanced or repressed by environmental conditions.

*Beauveria bassiana* does not multiply or show metabolic activity in the absence of host insects, and in consequence no accumulation of potentially harmful metabolites to levels that might affect the

environment is expected. Low amounts of the right metabolites produced and secreted at the right time and place may be sufficient for biocontrol efficacy.

Stability and accumulation of metabolites under conditions of application of *B. bassiana* products seems to be very limited by high metabolic activity of soil micro-organisms. Therefore, accumulation of metabolites with toxic properties towards man or the environment after application of plant protection products containing *B. bassiana* is highly improbable, and exposure of humans or non-target organisms to metabolites from *B. bassiana* can only occur through consumption of insects infested with *B. bassiana*. However, this exposure does not exist for humans and any potential exposure for insectivorous animals to metabolites of *B. bassiana* already existed before the use of this microbial active substance as *B. bassiana* is a naturally occurring fungus in Europe.

Based on these findings, it can be concluded that *Beauveria bassiana* strain PPRI 5339 poses no risk to man or the environment via the production of toxic metabolites.

#### B.2.1.9 Antibiotics and other anti-microbial agents (Annex IIM 2.12)

*Beauveria bassiana* is an opportunistic pathogen that has very rarely caused infections in humans and is not related to any known human pathogen.

Within the open literature, two instances of potential resistance to antifungal agents have been reported. Figuera *et al.* (2012) identified a potential resistance of *Beauveria bassiana* to voriconazole, fluconazole and amphoterin B. It was however very sensitive to posaconazole and to echinocandins. Sonoyama *et al.* (2008) also identified resistance to fluconazole and amphoterin B, but identified voriconazole as the treatment of choice. Spread of resistance (genes) to antimyotic agents between fungal species is not of that concern as for bacteria with regard to antibiotics.

### B.2.2 Physical, chemical and technical properties of the plant protection product (Annex IIM 2)

Data on the oil dispersion (OD) product BAS 480 00 I (Broadband) are summarised in the table below. The product is always applied in a 0.1%v/v dilution.

Two new studies were submitted, which were reported to GLP.

| Guideline and method | Test material purity and specification | Findings | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|
| **B.2.2.1 Appearance (colour and odour) (Annex IIIM 2.1)** | | | | | |
| visual inspection | BAS 480 001<br>Batch No. P1391 | Light brown oily liquid. Faint oily odour. | **Acceptable** | Y | MP 2.1/01 |
| **B.2.2.2 Storage stability and shelf-life (Annex IIIM 2.2)** | | | | | |
| **B.2.2.2.1 Effects of light, temperature and humidity on technical characteristics of the plant protection product** | | | | | |
| | BAS 480 001<br>Batch No. P1391 | No changes in technical characteristics were detected after 7 days storage at low temperature (0 °C).<br>No microbial contamination of BAS 480 00 1 was detected at day 0, so no further testing has been conducted.<br><br>Properties determined before and after storage: active substance content / viability, appearance, pH, foam persistence, dispersion stability, pourability, wet sieve residue, pack weight and performance. | **Acceptable**<br>Low temperature stability testing only requires determination of the dispersion stability and the wet sieve residue. Although not all tests were reported as required by the CIPAC methods, the data shows the product is stable for at least 7 days when stored at 0°C.<br><br>Effect of light and humidity are not | Y | MP 2.1/01 |

| Guideline and method | Test material purity and specification | Findings | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|
| | | | considered relevant. The product is an oil dispersion, which is not hydrophilic and not expected to be affected by humidity. In addition, non-transparent bottles are used to pack the product.<br><br>Long term stability data is currently not available. | | |

**Table 2.2.2.1: Results of low temperature storage stability study and physical properties of test item BAS 480 00 1**

| Test | Notes | Results at analysis point | |
|---|---|---|---|
| | | 0h | 7d |
| Active substance content | | 7.36 x $10^9$ CFU/mL | 6.93 x $10^9$ CFU/mL (94.16% of original) |
| Microbial contaminants[1] | Yeasts and moulds | All absent by test | N/A |
| | Listeria | All absent by test | N/A |
| | S. aureus | All absent by test | N/A |
| | E. coli | All absent by test | N/A |
| | Total coliforms | All absent by test | N/A |
| | Salmonella | All absent by test | N/A |
| | Vibrio | All absent by test | N/A |
| | Shigella | All absent by test | N/A |

| Guideline and method | Test material purity and specification | Findings | | | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|---|---|
| | | | Enterobacter<br>Anerobobes | All absent by test<br>All absent by test | N/A<br>N/A | | |
| | | Appearance | Colour<br>Physical state<br>Odour | Light brown<br>Oily liquid<br>Faint oily odour | Light brown<br>Oily liquid<br>Faint oily odour | | |
| | | Relative density | Neat<br>1:1 dilution | 0.966 g/L<br>0.934 g/L | N/A | | |
| | | pH (neat and 1% dilution) | Neat<br>1% dilution | Mean = 6.1<br>Mean = 6.7 | Mean = 6.7<br>Mean = 7.4 | | |
| | | Persistent foaming | CIPAC A<br><br><br><br><br>CIPAC D | 10 sec = 0 mL<br>1 min = 0 mL<br>3 min = 0 mL<br>12 min = 0 mL<br><br>10 sec = 0 mL<br>1 min = 0 mL<br>3 min = 0 mL<br>12 min = 0 mL | 10 sec = 0 mL<br>1 min = 0 mL<br>3 min = 0 mL<br>12 min = 0 mL<br><br>10 sec = 0 mL<br>1 min = 0 mL<br>3 min = 0 mL<br>12 min = 0 mL | | |
| | | Dispersion stability | 2% in CIPAC A | **0 mins**<br>sediment = 0 mL<br>top cream = 0 mL<br>oil = 0 mL<br><br>**30 mins**<br>sediment = 0.05 mL<br>top cream = 1.15 mL<br><br>**24 hours** | **0 mins**<br>sediment = 0 mL<br>top cream = 0 mL<br>oil = 0 mL<br><br>**30 mins**<br>sediment = 4.5 mL<br>top cream = 1.98 mL<br><br>**24 hours** | | |

| Guideline and method | Test material purity and specification | Findings | | | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|---|---|
| | | | 2% in CIPAC D | sediment = 3 mL, completely dispersed on rotation<br>top cream = 0.8 ml<br><br>**0 mins**<br>sediment = 0 mL<br>top cream = 0 mL<br>oil = 0 mL<br><br>**30 mins**<br>sediment = 0.005 mL<br>top cream = 2.75 mL<br><br>**24 hours**<br>sediment = 3.3 mL, completely dispersed on rotation<br>top cream = 1.4 mL | sediment = 2.75 mL, completely dispersed on rotation<br>top cream = 1.29 ml<br><br>**0 mins**<br>sediment = 0 mL<br>top cream = 0 mL<br>oil = 0 mL<br><br>**30 mins**<br>sediment = 5.5 mL<br>top cream = 2.67 mL<br><br>**24 hours**<br>sediment = 3.44 mL, completely dispersed on rotation<br>top cream = 1.29 mL | | |
| | | Pourability | | Residue = 3.358 g<br>Rinsed residue = 0.921 g | Residue = 2.018 g<br>Rinsed residue = 0.695 g | | |
| | | Wet sieve test | | Residue = 0.445 g | Residue = 0.453 g | | |
| | | Description of packaging | Material<br>Profile and type<br>Colour<br>Capacity<br>Closure type and size | HDPE<br>Round bottle<br>Translucent yellow<br>500 ml<br>yellow screw cap with snap ring | | | |
| | | Pack weight | T175 bottle 12 | 497.77 g | 496.89 g | | |

| Guideline and method | Test material purity and specification | Findings | | | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|---|---|
| | | | T175 bottle 17 | 502.29 g | (99.82 % of original)<br>501.96 g<br>(99.93% of original) | | |
| | | Pack appearance | | **T175 bottle 12** | **T175 bottle 12** | | |
| | | | Sedimentation phase/phase separation | Moderate (1cm sediment) | Moderate (1cm sediment) | | |
| | | | Permanent/temporary deformation | Moderate lateral deformation | Moderate lateral deformation | | |
| | | | Swelling, cracking or crazing of pack walls | Nil | Nil | | |
| | | | Visual contamination | Nil | Nil | | |
| | | | External odour | Nil | Nil | | |
| | | | Colour change of pack walls | Nil | Nil | | |
| | | | Leakage, particularly at seams | Nil | Nil | | |
| | | | Condition of closure | Good | Good | | |
| | | | | **T175 bottle 17** | **T175 bottle 17** | | |
| | | | Sedimentation phase/phase separation | Moderate (1cm sediment) | Moderate (1cm sediment) | | |
| | | | Permanent/temporary deformation | Moderate lateral deformation | Moderate lateral deformation | | |
| | | | Swelling, cracking or crazing of pack walls | Nil | Nil | | |
| | | | Visual contamination | Nil | Nil | | |
| | | | External odour | Nil | Nil | | |
| | | | Colour change of pack walls | Nil | Nil | | |
| | | | Leakage, particularly at seams | Nil | Nil | | |
| | | | Condition of closure | Good | Good | | |

| Guideline and method | Test material purity and specification | Findings | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|
| | | | | | |
| **B.2.2.2.2 Other factors affecting stability** | | | | | |
| | | N/A | | | |
| **B.2.2.3 Explosivity and oxidising properties (Annex IIIM 2.3.1)** | | | | | |
| Theoretical assessment | | The active substance and co-formulants of BAS 480 00 1 are naturally occurring products with no explosive or oxidizing properties. Please refer to Document J for the detailed composition of the product. A study is not required. | **Acceptable**<br>The product contains no constituents classified as explosive or oxidising.<br><br>The product does not need to be classified as an oxidising or explosive mixture based on the criteria of Regulation 1272/2008/EC. | | |
| **B.2.2.4 Flash point and other indications of flammability or spontaneous ignition (Annex IIIM 2.3.2)** | | | | | |
| Theoretical assessment | | The active substance and co-formulants of BAS 480 00 1 are naturally occurring products with no explosive or oxidizing properties. Please refer to Document J for the detailed composition of the product. A study is not required. | **Acceptable**<br>The justification should have addressed the flammability of the | | |

| Guideline and method | Test material purity and specification | Findings | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|
| | | | product, but the product contains no flammable constituents.<br><br>The product does not need to be classified as a flammable mixture based on the criteria of Regulation 1272/2008/EC. | | |
| **B.2.2.5 Acidity/alkalinity and if necessary pH value (Annex IIIM 2.3.3)** | | | | | |
| CIPAC MT 75.3 | BAS 480 001<br>Batch No. P1391 | pH of neat test item: 6.1<br>pH of 1% dilution of test item: 6.7 | **Acceptable**<br>The pH of the undiluted product should be ignored as the product is not an aqueous solution. | Y | MP 2.1/01<br>Whittaker, M., 2014 |
| **B.2.2.6 Viscosity and surface tension (Annex IIIM 2.3.4)** | | | | | |
| OECD 115, ring tensiometer | Broadband Batch 1391 | 33.47 mN/m at 20 °C | **Acceptable** | Y | MP 2.6/01<br>Wiles, J., 2014 |
| OECD 114, rotational | Broadband Batch 1391 | The viscosity of the test item appeared to exhibit what might be very slight shear thinning at both high and low | **Acceptable** | Y | MP 2.6/01<br>Wiles, J., |

| Guideline and method | Test material purity and specification | Findings | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|
| viscometer | | temperatures. | | | 2014 |

**Table 2.2.6/01-1: Results of the determination of the viscosity of Broadband.**

| **Shear Rate** [1/s] | **Viscosity at 20°C** [mPa·s] | **Viscosity at 40°C** [mPa·s] |
|---|---|---|
| 10 | 89.4 | 41.1 |
| 50 | 86.9 | 39.3 |
| 100 | 85.8 | 39.3 |
| 160 | 85.1 | 38.7 |
| 200 | 84.5 | 38.7 |
| 250 | 84.9 | 38.9 |
| 300 | 84.5 | 38.6 |
| | | |
| 290 | 84.4 | 38.6 |
| 250 | 84.9 | 38.4 |
| 200 | 84.9 | 38.1 |
| 160 | 84.6 | 38.4 |
| 100 | 85.0 | 38.1 |
| 50 | 85.5 | 39.3 |
| 10 | 88.5 | 40.3 |

| | | | | | |
|---|---|---|---|---|---|
| CIPAC MT3 | BAS 480 001<br>Batch No. P1391 | Density:<br>Neat: 0.966 g/L<br>1:1 dilution: 0.934 g/L | **Acceptable** | Y | MP 2.1/01<br>Whittaker, M., 2014 |

| Guideline and method | Test material purity and specification | Findings | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|
| **B.2.2.7 Technical characteristics of the plant protection product (Annex IIIM 2.4)** | | | | | |
| **B.2.2.7.1 Wettability (Annex IIIM 2.4.1)** | | | | | |
| | | Not applicable to OD formulations. | | | |
| **B.2.2.7.2 Persistent foaming (Annex IIIM 2.4.2)** | | | | | |
| CIPAC MT47.1 | BAS 480 001<br>Batch No. P1391 | No foaming in CIPAC A or CIPAC D for up to 12 minutes at 0.1%v/v. | **Acceptable** | Y | MP 2.1/01<br>Whittaker, M., 2014 |
| **B.2.2.7.3 Suspensibility and suspension stability (Annex IIIM 2.4.3)** | | | | | |
| | | Not applicable to OD formulations. | | | |
| **B.2.2.7.4 Dry sieve test and wet sieve test (Annex IIIM 2.4.4)** | | | | | |
| CIPAC MT185 | BAS 480 001<br>Batch No. P1391 | Wet sieve test residue: 0.445 g on a 75µm sieve. | **Not acceptable**<br>10mL of test item was used, corresponding to roughly 9.7g, based on the density data reported. Residue was therefore (0.445/9.7)*100% = 4.6%. The 2% maximum is exceeded. | Y | MP 2.1/01<br>Whittaker, M., 2014 |

| Guideline and method | Test material purity and specification | Findings | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|
| | | | Data gap<br>Evidence is required that the product will not cause filter or nozzle blockage of spray equipment. | | |
| **B.2.2.7.5 Particle size distribution (dustable and wettable powders, granules), content of dust/fines (granules), attrition and friability (granules) (Annex IIIM 2.4.5)** | | | | | |
| | | Not applicable to OD formulations. | | | |
| **B.2.2.7.6 Emulsifiability, re-emulsifiablilty and emulsion stability (Annex IIIM 2.4.6)** | | | | | |
| CIPAC MT180 | BAS 480 001<br>Batch No. P1391 | No sediment, cream or oil in CIPAC A or D immediately after rotation at 2%v/v.<br>After 30 minutes:<br>0.05 ml sediment and 1.15 ml cream in CIPAC A<br>0.005 ml sediment and 2.75 ml cream in CIPAC D<br>After 24 hours:<br>Sediment re-dispersed on rotation in CIPAC A and D<br>Cream 0.8 ml in CIPAC A<br>Cream 1.4 ml in CIPAC D | **Not acceptable.**<br>The test is not representative for the intended use (0.1%v/v).<br><br>Data gap<br>Dispersion stability data at 0.1%v/v. | Y | MP 2.1/01<br>Whittaker, M., 2014 |
| **B.2.2.7.7 Flowability, pourability (rinsibility)and dustability (Annex IIIM 2.4.7)** | | | | | |
| CIPAC MT 148 | BAS 480 001 | Pourability: | **Not acceptable** | Y | MP 2.1/01 |

| Guideline and method | Test material purity and specification | Findings | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|
| | Batch No. 1391 | Residue: 3.358 g<br>Rinsed Residue: 0.921 g | The residue should be reported as a percentage of the initial weight of the test item. Considering the initial weight is not reported, the pourability could not be calculated by the RMS.<br><br><u>Data gap</u><br>Pourability data, reported as required by CIPAC MT148. | | Whittaker, M., 2014 |
| **B.2.2.8 Physical, chemical and biological compatibility with other products including plant protection products with which its use is to be authorized** | | | | | |
| | | This product is not intended for use with other products. | | | |
| **B.2.2.8.1 Physical compatibility** | | | | | |
| | | This product is not intended for use with other products. | | | |
| **B.2.2.8.2 Chemical compatibility** | | | | | |
| | | This product is not intended for use with other products. | | | |

| Guideline and method | Test material purity and specification | Findings | Remarks / comments RMS | GLP Y/N | Reference |
|---|---|---|---|---|---|
| **B.2.2.8.3 Biological compatibility** | | | | | |
| | | This product is not intended for use with other products. | | | |
| **B.2.2.9 Adherence and distribution to seeds (Annex IIIM 2.6)** | | | | | |
| | | BASF 480 00 1 is not intended as a seed treatment product. Not required for this submission. | | | |

### B.2.2.10 Summary and evaluation of data submitted in B.2.2.1 - B.2.2.9 (Annex IIIM 2.7)

The representative product BAS 480 00 I (Broadband), is an oil dispersion, with, in aqueous solution, a pH within the neutral range. It does not require classification or labelling based on its physical and chemical properties. BAS 480 00 I does not produce foam when diluted with water.

The technical properties of the product indicate possible issues during application. The wet sieve residue exceeded the limit of 2%. In addition, the pourability was not adequately reported and the dispersion stability was not determined at the proposed in-use concentration of 0.1%v/v.

Long term stability data was not provided, but the product was stable for at least 7 days at 0°C in HDPE.

To complete the product dossier, the following data is required:

1. Long term stability data in the proposed commercial packaging
2. Evidence that the wet sieve residue does not result in filter and/or nozzle blockage during application
3. Pourability data, reported as required by CIPAC MT148
4. Dispersibility (CIPAC MT180) at the proposed in-use concentration of 0.1%v/v

The applicant has submitted an accelerated storage stability study with product BAS 480 00 I (18 weeks at 30°C), Whittaker, M, 2015 (Report No. APIS-BASF-017). The study is not included in the evaluation, however, as it does not comply with the requirements. The dispersion stability was not determined after storage, after 18 weeks only 5.7% of the active substance remained and poorly re-dispersible particles were observed, indicating poor physical stability.

The applicant has developed a new product as well, coded BAS 480 02 I. Data was submitted, but could not be taken into account for evaluation of the substance dossier.

### B.2.3 References relied on

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>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 2.1/01 | Zimmermann, G | 2007 | Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongiartii.* Biocontrol Science | N | N | N/A | Public |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | and Technology 17(5/6), 553-596 Non GLP Published | | | | |
| MA 2.1.2/02 | Meyling, NV, Eilenberg, J | 2006 | Isolation and characterisation of Beauveria bassiana isolates from phylloplanes of hedgerow vegetation. Mycological Research 110:188-195. Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.1.2/05 | Doberski, JW, Tribe, HT | 1980 | Isolation of enntomopathogenous fungi from elm bark and soil with reference to ecology of *Beauveria bassiana* and *Metarhizium anisopliae*. Transactions of the British Mycological Society 74:95-100.<br><br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.1.2/06 | Bills, GF, Polishook, JD | 1991 | Microfungi from *Carpinus caroliniana*. Canadian Journal of Botany 69:1477-1482.<br><br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.1.2/07<br><br>MA 2.5/01 | Wagner, BL, Lewis, LC | 2000 | Colonization of corn, *Zea mays*, by the entomopathogenic fungus *Beauveria bassiana*. Applied Environmental Microbiology 66:3468-3473. Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.1.2/08 | Posada, F, Vega, FE | 2005 | Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*). Mycologia 97:1195-1200. | N | N | N/A | Public |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | Non GLP<br><br>Published | | | | |
| MA 2.1.2/09 | Airaudi, D. Filipello Marchisio, V. | 1996 | Fungal biodiversity in the air of Turin. Mycopathologia 136:95-102.<br><br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.2.1/01 | Cuthbertson, A and Brown, A | 2011 | Efficacy of BUEXP1778 (*Beauveria bassiana* strain PPRI 5339) against two biotypes (Q and B) of *Bemisia tabaci*. FERA, (Food and Environment Research Agency), York, YO41 1LZ, UK, Report R&DFERA0411<br>Non GLP<br>Unpublished | N | Y | Strain specific new laboratory study | BASF |
| MA 2.2.1/02 | Oliveira, M, Henneberry, T, Anderson, P | 2001 | History, current status and collaborative research projects for Bemisia tabaci. Crop Protection, 20, 709-723.<br><br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.2.3/05<br><br>MA 8.6/03 | Wang, C, Fan, M, Li, Z, Butt, TM | 2004 | Molecular monitoring and evaluation of the application of the insect-pathogenic fungus *Beauveria bassiana* in southeast China. Journal of Applied Microbiology 96:861-870.<br><br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.2.3/06<br><br>MA 8.6/10 | Ownley, BH, Pereira, RM, Klingeman, WE, Quigley, NB, Leckie, BM | 2004 | *Beauveria bassiana*, a dual purpose biocontrol organism, with activity against insect pests and plant pathogens. In: Lartey, RT, Caesar, AJ, editors. Emerging concepts in plant health management. India: Research | N | N | N/A | Public |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | Signpost. pp 255-269.<br><br>Non GLP<br><br>Published | | | | |
| MA 2.4/01 | Fargues, J, Goettel, MS, Smits, N, Ouedraogo, A, Rougier, M | 1997 | Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. Mycologia 89:383-392.<br><br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.4/03 | Neethling, D,<br><br>Brown, A | 2012 | The effect of temperature on growth of *Beauveria bassiana* strain PPRI 5339.<br>Non GLP<br>Not Published | N | Y | Strain specific new laboratory study | BASF |
| MA 2.5/01<br><br>MA 2.1.2/07 | Wagner, BL, Lewis, LC | 2000 | Colonization of corn, *Zea mays*, by the entomopathogenic fungus *Beauveria bassiana*. Applied Environmental Microbiology 66:3468-3473.<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.6/01 | Coelho, MA, Gonçalves, C, Sampaio, JP, Gonçalves, P | 2013 | Extensive intra-kingdom horizontal gene transfer converging on a fungal fructose transporter gene. PLOS Genetics 9(6): e1003587 | N | N | N/A | Public |
| MA 2.6/02 | Rosewich, UL, Kistler, HC | 2000 | Role of horizontal gene transfer in the evolution of fungi. Annual Review Phytopathology 38 325–363.<br><br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/01 | Strasser, H, Vey, A, Butt, TM | 2000 | Are there any Risks in using Entomopathogenic Fungi for pest Control, | N | N | N/A | Public |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | with Particular Reference to the Bioactive Metabolites of Metarhizium, Tolypocladium and Beauveria species? Biocontrol Science and Technology (10) , 717- 735<br>Non GLP<br><br>Published | | | | |
| MA 2.7/02 | Bidochka, MJ, Khachatourians, GG | 1991 | The implication of metabolic acids produced by *Beauveria bassiana* in pathogenesis of the migratory grasshopper *Melanoplus sanguinipes*. Journal of Invertebrate Pathology 58, 106-117.<br>Non GLP<br>Published | N | N | N/A | Public |
| MA 2.7/03 | Kirkland, BH, Eisa, A, Keyhani, NO | 2005 | Oxalic acid as a fungal acaricidal virulence factor. Journal of Medical Entomology 42:346-351.<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/04 | Jeffs, LB, Khachatourians, GG | 1997 | Toxic properties of *Beauveria* pigments on erythrocyte membranes. Toxicon 35:1351-1356.<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/05 | Eley, KL, Halo, LM, Song, Z, Powles, H, Cox, RJ, Bailey, A.M, Lazarus, CM, Simpson, TJ | 2007 | Biosynthesis of the 2-pyridone tenellin in the insect patholgenic fungus *Beauveria bassiana* ChemBioChem 8, 289-297<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/06 | Pegram, RA, Wyatt, RD | 1981 | Avian gout caused by oosporein, a mycotoxin produced | N | N | N/A | Public |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | by chaetomium trilaterale Poultry Science 60, 2429-2440<br>Non GLP<br><br>Published | | | | |
| MA 2.7/07 | Favilla, M, Macchia L, Gallo, A, Altomare, C | 2006 | Toxicity assessment of metabolites of fungal biocontrol agents using two different (artemia salina and daphnia magna) invertebrate bioassays . Food Chem Toxicol 44, 1922-1931<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/08 | Nagaoka, T, Nakata, K, Kuono, K, Ando, T | 2004 | Antifungal activity of oosporein from an antagonistic fungus against Phytophtora infestans Z Naturforsch 59c, 302-304<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/09 | Abendstein, D, Pernfuss, B, Strasser, H | 2000 | Evaluation of *Beauveria brongniartii* and its metabolite oosporein regarding phytotoxicity on seed potatoes. Biocontrol Sci Tech 10, 789-796<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/10 | Jow, G-M, Chou CJ, Chen, BF, Tsai JH | 2004 | Beauvericin induces cytotoxic effects in human acute lymphoblastic leukemia cells through cytochrome c release, caspase 3 activation: the causative role of calcium.<br>Cancer Letters, 216, 165-173<br>Non GLP<br>Published | N | N | N/A | Public |
| MA 2.7/11 | Champlin, FR, Grula, EA | 1979 | Noninvolvement of Beauvericin in the | N | N | N/A | Public |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | entomopathogenicity of *Beauveria bassiana*. Appl Environ Microbiol 37, 1122-1125<br>Non GLP<br><br>Published | | | | |
| MA 2.7/12 | Calo, L, Fornelli, F, Nenna, S, Tursi, A, Caiaffa, M.F, Macchia, L | 2004 | Beauvericin cytotoxicity to the invertebrate cell line SF-9. J Appl Genet 44, 515-520<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/13 | Namatame, I, Tomoda, H, Ishibashi, S, Omura, S | 2003 | Antiatherogenic activity of fungal beauveriolides, inhibitors of lipid droplet accumulation in macrophages. Proc Natl Acad Sci USA 101, 737-742<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/14 | Kuzma, M, Jegorov, A, Kacer, P, Havlicek, V | 2001 | Sequencing of new beauverolides by high- performance liquid chromatography and mass spectrometry J Mass Spectr 36, 1108-1115<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/15 | Jegorov, A, Sedmera, P, Matha, V, Simek, P, Zahradinickova, H, Landa, Z, Eyal, J | 1994 | Beauverolides L and La from Beauveria tenella and Paecilomyces fumosoroseus. Phytochemistry 37, 1301-1303<br>Non GLP<br>Published | N | N | N/A | Public |
| MA 2.7/16 | Vilcinskas, A, Jegorov, A, Landa, Z, Gotz, P, Matha, V | 1999 | Effects of beauverolide L and cyclosporin A on humoral and cellular immune response of the greater wax moth, Galleria mellonella Comp Biochem Physiol, Part C 122, | N | N | N/A | Public |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 83-92<br>Non GLP<br><br>Published | | | | |
| MA 2.7/17 | Quesada-Moraga, E, Vey, A | 2004 | Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana.* Mycol Res 108, 441-452<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/18 | Fuguet R, Vey, A | 2004 | Comparative Analysis of the production of insecticidal and melanizing macromolecules by strains of Beauveria spp.:in vivo studies. J Invertebr Pathol 85, 152-167<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/19 | Fuguet, R, Théraud, M, Vey, A | 2004 | Production in vitro of toxic macromolecules by strains of *Beauveria bassiana*, and purification of a chitosanase-like protein secreted by a melanizing isolate. Comp Biochem Physiol, Part C 138, 149-161<br>Non GLP<br><br>Published | N | N | N/A | Public |
| MA 2.7/20 | Oller-López, JL, Iranzo, M, Mormeneo, S, Oliver, E, Cuerva, J, Oltra, JE | 2005 | Bassianolone: an antimicrobial precursor of cephalosporolides E and F from the entomoparasitic funguc *Beauveria bassiana*. Organic Biomolecular Chemistry, 3, 1172-1173.<br>Non GLP<br>Published | N | N | N/A | Public |
| MA | Song, L, Lee, K-H, Lin, Z, Tong, | 2014 | Structural revision of cephalosporolide J | N | N | N/A | Public |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2.7/21 | R | | and bassianolone. The Journal of Organic Chemistry, 79, 1493-1497. Non GLP Published | | | | |
| MA 2.8/01<br>MA 5.1.4/01 | Figueira, L., Pinheiro, D., Moreira, R., Pinto, E., Simões, J., Camisa, E., Torrão, L., Palmares, J., Falcão-Reis, F. | 2012 | *Beauveria bassiana* keratitis in bullous keratopathy: antifungal sensitivity testing and management. Eur J Ophthalmol; 22 (5): 814-818.<br>Not GLP<br>Published | N | N | N/A | Public |
| MA 2.8/02<br>MA 5.1.4/02 | Sonoyama, H., raki-Sasaki, K., Kazama, S., Kawasaki,T., Ideta, H., Sunada, A., Asari, S., Inoue, Y., Hayashi, K. | 2008 | The characteristics of keratomycosis by *Beauveria bassiana* and its successful treatment with antimycotic agents. *Clin Ophthalmol.,* 2(3): 675-678.<br>Not GLP<br>Published | N | N | N/A | Public |
| **Annex III Data and Information** | | | | | | | |
| MP 2.1/01 | Whittaker, M | 2014 | Low temperature storage stability test on the Microbial Pest Control Product BAS 480 00 1. APIS, Knaresborough Technology Park, Manse Lane, Knaresborough, North Yorkshire HG5 8LF. Report No. APIS-BASF-018<br>GLP<br>Unpublished | N | Y | New GLP study on reference product | BASF |
| MP 2.6/01 | Wiles, J | 2014 | Viscosity and surface tension determination of Broadband. CEM Analytical Serves Limited (CEMAS). Imperial House, Oaklands Park, Wokingham, Berkshire RG41 2FD Report No. CEMS-6734<br>GLP: Yes<br>Unpublished | N | Y | New GLP study on reference product | BASF |

| | | | | | | | |
|---|---|---|---|---|---|---|---|

## B.3 Data on application and further information

### B.3.1 Further information on the micro-organism (Annex IIB 3)

#### B.3.1.1 Function (Annex IIM 3.1)

Contact insecticide

#### B.3.1.2 Field of use envisaged (Annex IIM 3.3)

The representative uses are solanaceous and cucurbitaceous fruiting vegetables and ornamentals, all grown under protected conditions.

#### B.3.1.3 Crops or products protected or treated (Annex IIM 3.3)

*Beauveria bassiana* strain PPRI 5339 is intended to be used in glasshouse production of fruits and vegetables and ornamentals at a rate of 100 g a.s./ha for protection against thrips (*Frankliniella occidentalis* and *Thrips tabaci*) and whitefly (*Trialeurodes vaporariorum*, *Bemisia tabaci* and *Bemisia argentifolii*).

#### B.3.1.4 Method of production and quality control (Annex IIM 1.4.3)

Confidential information. See Volume 4.

#### B.3.1.5 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s) (Annex IIM 3.6)

**Potential development of resistance to *Beauveria bassiana* strain PPRI 5339**

To date there have been no published reports of resistance development in field populations to any product containing the active ingredient *Beauveria bassiana*. There have also been no published studies demonstrating the development of resistance to any strain of *Beauveria bassiana* from a laboratory culture of an economically important pest species of protected horticulture as claimed in the *Beauveria bassiana* strain PPRI 5339 GAP. The limited research on laboratory insect populations has shown potential for development of resistance to *Beauveria bassiana* in *Drosophila melanogaster* (Diptera) and *Galleria mellonella* (Lepidoptera), common standard laboratory test organisms. Under laboratory conditions Dubovskiy *et al.* (2013) reported that under constant selective pressure from *B. bassiana*, 25th generation larvae of *Galleria mellonella* exhibited significantly enhanced resistance, which was specific to this pathogen and not to another insect pathogenic fungus *Metarhizium anisopliae*. Dubovskiy *et al.* (2013) hypothesised that the resistance was due to increased prioritisation of pathogen-species-specific defence mechanisms. In this study, systemic immune defences were suppressed in order to allow more resources to be prioritised for enhanced response in the cuticle and epidermis for example the increased expression of the fungal enzyme inhibitor IMPI [insect metalloproteinase inhibitor] and cuticular phenoloxidase activity. Understanding the mode of action of this resistance development demonstrates that, if this development of resistance is also able to occur in wild populations of insects of agricultural importance surrounded by other selection pressures, it will

be possible to manage the resistance within a pest population by rotating with other biological control agents e.g. *Metarhizium anisopliae* (Dubovskiy *et al*. (2013) showed the *B. bassiana* resistance mechanism was specific to this species of fungus) or even potentially different strains of *B. bassiana* which might produce a different range of insect cuticle degrading enzymes not prevented from working by the fungal enzyme inhibitor and cuticular phenoloxidase activity in the resistant population. Dudovskiy et al. (2013) hypothesised that development of resistance to *B. basisana* was a costly trait and rather than being compensated for by a reduction in life-history traits (e.g. fecundity or longevity) the insect would reduce the energy put into other areas of its immune system which would make it more susceptible to other pathogens, which would be to the benefit of an IPM program. Dubovskiy *et al.*, (2013) concluded that, in contrast to insects that develop resistance to synthetic chemical insecticides where a slight change can have a profound effect (e.g. aphid, Silva *et al.*, (2012); whitefly, Alon *et al.* (2008); red flour beetle, Arnaud and Haubruge (2002)) resistance to *B. bassiana* would involve a multiple array of interdependent traits. There are multiple processes involved in infection with *B. bassiana* which reduces the potential for resistance to develop (Gillespie *et al.* (2000)). In addition Dudovskiy *et al.* (2013) concluded that increased resistance to a specific strain of *B. bassiana* did not pose a major threat to the use of pathogenic fungi as biocontrol agents due to three factors 1) There is no cross resistance to other fungal species, 2) There is a cost to the development of resistance with reduced fecundity 3) down regulation of other parts of the immune system will probably make the insect more susceptible to other opportunistic microbial pathogens.

In another study to identify potential development of resistance to *B. bassiana* in *Drosophila melanogaster* Kraaijeveld *et al.* (2012) found no evidence for development of resistance to *B, bassiana* after 15 generations of selection suggesting that development of resistance to *B. bassiana* may be either a mechanism that not all species are capable of and/or a complex evolutionary change that requires many generations to develop. Kraaijeveld and Godfray (2008) discussed the potential for resistance or tolerance to *B. bassiana* in *Drosophila melanogaster* and concluded that "resistance to fungal biopesticides will not evolve easily". The potential development of resistance will be fully evaluated during product registration.

**Potential development of cross resistance between Beauveria bassiana strain PPRI 5339 and synthetic chemical insecticides.**

Because of the mode of action of *B. bassiana* strain PPRI 5339, cross-resistance with chemical insect insecticide active ingredients is unlikely.

**Potential development of cross resistance between Beauveria bassiana strain PPRI 5339 and other microbial plant protection products.**

Following penetration into the insect haemolymph, the insect immune response to both fungal and bacterial microbial pathogens is to produce a range of peptides to kill the infection or encapsulation to prevent it reproducing. However, the immune defence systems of insects have been shown to give different humoral responses upon infection with parasitic fungi such as *Beauveria bassiana* compared to after challenge with bacteria (Wojda *et al.* (2009)) Therefore with different defence pathways, cross-resistance between different kingdoms of microbial pathogens is unlikely.

**International guidelines on management of potential resistance.**

Insecticide Resistance Action Committee (IRAC) is the industry body that monitors, manages and provides recommendations for the management of resistance to insecticides. Currently no biological active ingredients (such as entomopathogenic fungi *B. bassiana*) are included on their classification of resistance management codes therefore it is not possible to provide an IRAC code for *Beauveria bassiana* strain PPRI 5339. Insecticide Resistance Action Committee does however state that management of potential resistance development to any insecticide should "include effective cultural and biological control practices that work in harmony with effective IPM [Integrated Pest Management] programmes".

#### B.3.1.6 Methods to prevent loss of virulence of seed stock of the micro-organism (Annex IIB 4.1)

As part of the production process the strain is passed through an insect (termite) to maintain pathogenicity. Sourcing back from an infected insect rather than sub-culturing at the end of a production batch maintains genetic stability (Refer to confidential information in Volume 4 for further details).

#### B.3.1.7 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIM 3.7)

Handling and storage

No special measures necessary if stored and handled correctly. Ensure thorough ventilation of stores and work areas. When using do not eat, drink or smoke. Hands and/or face should be washed before breaks and at the end of the shift.

Protection against fire and explosion:

No special precautions necessary. The substance/product is non-combustible. Product is not explosive.

Transport

Not classified as dangerous for transport.

#### B.3.1.8 Procedures for destruction or decontamination (Annex IIM 3.8)

Leftover quantities of the product must be disposed of in a commercial incinerator according to local regulations. Incineration must be done under controlled conditions according to the directives 94/67/EC and 2000/76/EC:

- Residence time greater than 2 seconds
- Presence of more than 6 % of oxygen
- Temperature above 850°C

Contaminated packaging has to be thoroughly rinsed with plenty of water, then burned in a commercial incinerator. In field applications the washing solutions have to be added to the spray mixture, otherwise they have to be burned in a commercial incinerator.

#### B.3.1.9 Measures in case of an accident (Annex IIM 3.12)

**Description of first aid measures**

Remove contaminated clothing.

| | |
|---|---|
| If inhaled: | Keep patient calm, remove to fresh air. |
| On skin contact: | Wash thoroughly with soap and water. |
| On contact with eyes: | Wash affected eyes for at least 15 minutes under running water with eyelids held open. |
| On ingestion: | Rinse mouth and then drink plenty of water. |

### B.3.2 Data on application (Annex IIIM 3)

#### B.3.2.1 Field of use envisaged (Annex IIIM 1.6.1)

The representative uses are solanaceous and cucurbitaceous fruiting vegetables and ornamentals, all grown under protected conditions.

#### B.3.2.2 Mode of action (Annex IIIM 3.1)

Like other entomopathogenic fungi, *B. bassiana* attacks its host insects generally percutaneous. 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 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. The penetration is followed by the invasion, which is accompanied by several host immune response activities. During the infection process, *Beauveria* spp. produces proteolytic enzymes and toxins, while the host insect responds with cellular and humoral defence reactions. In the insect body, the fungus multiplies as blastospores, or yeast-like cells, which are distributed passively in the haemolymph. Enzymes begin to destroy the internal structures of the host insect causing morbidity within 36 to 72 hours. Reduced feeding and immobility are rapidly evident, and the insect dies within 4 to 10 days post-infection. The time to death will depend on the insect species, age and conidial dose. After death of the insect, the fungus starts its saprophytic growth: blastospores transform into mycelia, which emerge through the cuticle. Aerial conidia are formed on the surface of the insect cadaver, which build the characteristic white mould. Sporulation occurs only in conditions of high humidity.

### B.3.2.3 Details of intended use (Annex IIM 3.1)

Table B.3.2.3-a provides a summary of the intended uses of the product.

**GAP BAS 480 00 I, new dRR format**

**GAP rev., date: 20/03/2014**

| | | | |
|---|---|---|---|
| **PPP (product name/code)** | **BAS 480 00 I** | **Formulation type:** | **OD** |
| **active substance 1** | **Beauvaria bassiana strain PPRI 5339** | **Conc. of as 1:** | **40g/L or 4 x 10^12 cfu's/L** |
| **active substance 2** | **n.a** | **Conc. of as 2:** | **-** |
| **active substance 3** | **n.a.** | **Conc. of as 3:** | **-** |
| **safener** | **n.a.** | **Conc. of safener:** | **n.a.** |
| **synergist** | **n.a.** | **Conc. of synergist:** | **n.a.** |
| **Applicant:** | **BASF Agricultural Specialities Limited** | **professional use** | ☒ |
| **Zone(s):** | **EU** | **non professional use** | ☐ |
| **Verified by MS:** | **northern/central/southern** | | |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Use-No.** | **Member state(s)** | **Crop and/ or situation (crop destination / purpose of crop)** | **F G or I** | **Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group) | **Application** | | | **Application rate** | | | **PHI** (days) | **Remarks:** |
| | | | | | Method / Kind | Timing / Growth stage of crop & season | Max. number (min. interval between applications) a) per use b) per crop/ season | L product / ha a) max. rate per appl. b) max. total rate per crop/season | g as/ha a) max. rate per appl. b) max. total rate per crop/season | Water L/ha min / max | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | EU (Protected crops) | Solanaceae<br><br>Tomato *Solanum lycopersicum* (LYPES)<br><br>Sweet pepper *Capsicum annuum* (CPSAN)<br><br>Aubergine *Solanum melongena* (SOLME)<br>Solanaceae<br><br>Edible and processed fruits | G | Thrips e.g. *Frankliniella occidentalis* (FRANOC) and *Thrips tabaci* (THRITB)<br><br>Whitefly e.g. *Trialeurodes vaporariorum* (TRIAVA), *Bemisia tabaci* (BEMITA) and *Bemisia argentifolii* (BEMIAR) | Spraying SP | BBCH 11-89 (jan-dec) | a) 122 (3 days)<br>b) 122 | a) 2.5<br><br>b) 305 | a) 100 ($1*10^{13}$ CFU /ha)<br><br>b) 12200 ($1.22*10^{15}$ CFU/ha) | 500-2500 | 0 | Dose rate is 0.1% (0.5L product per 500 L water/2.5L product per 2500 L water) ; 100 mL product per 100 L of water on label.<br><br>Water volume used depends on development stage of crop (low volume – early crops stage, high volume – late crop stage). |
| 2 | EU (Protected crops) | Cucurbitaceae<br><br>Cucumber *Cucumis sativus* (CUMSA)<br><br>Edible and processed fruits | G | Thrips e.g. *Frankliniella occidentalis* (FRANOC) and *Thrips tabaci* (THRITB)<br><br>Whitefly e.g. *Trialeurodes vaporariorum* (TRIAVA), *Bemisia tabaci* (BEMITA) and *Bemisia argentifolii* (BEMIAR) | Spraying SP | BBCH 11-89 (jan-dec) | a) 122 (3 days)<br>b) 122 | a) 2.5<br><br>b) 305 | a) 100($1*10^{13}$ CFU /ha)<br><br>b) 12200 ($1.22*10^{15}$ CFU/ha) | 500-2500 | 0 | Dose rate is 0.1% (0.5L product per 500 L water/2.5L product per 2500 L water) ; 100 mL product per 100 L of water on label.<br><br>Water volume used depends on development stage of crop (low volume – early crops stage, high volume – late crop stage). |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | EU (Protected crops) | Ornamentals<br><br>(e.g. CHYIN, CHYHO, GEBSS roses)<br><br>Non-edible/Non-food crop | G | Thrips e.g. *Frankliniella occidentalis* (FRANOC) and *Thrips tabaci* (THRITB)<br><br>Whitefly e.g. *Trialeurodes vaporariorum* (TRIAVA), *Bemisia tabaci* (BEMITA) and *Bemisia argentifolii* (BEMIAR) | Spraying SP | BBCH 11-99 (jan-dec) | a) 122 (3 days)<br>b) 122 | a) 2.5<br><br>b) 305 | a) 100(1*$10^{13}$ CFU /ha)<br><br>b) 12200 (1.22*$10^{15}$ CFU/ha) | 500-2500 | 0 | Dose rate is 0.1% (0.5L product per 500 L water/2.5L product per 2500 L water) ; 100 mL product per 100 L of water on label.<br><br>Water volume used depends on development stage of crop (low volume – early crops stage, high volume – late crop stage). |

### B.3.2.4 Application rate (Annex IIIM 3.3 and 3.4)

| Crop | Method of application | Rate of application per unit treated (as preparation) | Rate of application per unit treated (as active substance) |
|---|---|---|---|
| Solanacea | Spraying | 2.5 L product/ha in 500 – 2500 L water | 100 g active substance/ha ($1*10^{13}$ CFU /ha) |
| Cucurbitaceae | Spraying | 2.5 L product/ha in 500 – 2500 L water | 100 g active substance/ha ($1*10^{13}$ CFU /ha) |
| Ornamentals | Spraying | 2.5 L product/ha in 500 – 2500 L water | 100 g active substance/ha ($1*10^{13}$ CFU /ha) |

BAS 480 00 I is applied at an application rate of 2.5 L product/ha. A dose rate of 0.1% (100 ml product per hL of water) is proposed. The product should be applied in a water volume ranging from 500 to 2500 L/ha.

**Background information on the application rate used in trials**

*Beauveria bassiana* is a fungus which causes a disease known as the white muscardine disease in insects. Several strains of *Beauveria bassiana,* such as strain GHA and strain ATCC74040, are currently approved under Reg. (EC) No 1107/2009. *Beauveria bassiana* strain PPRI 5339 is a new strain. The representative product is BAS 480 00 I. The product is based on the entomopathogenic fungus *Beauveria bassiana* strain PPRI 5339 and is formulated as an oil dispersion and contains $4.0 \times 10^{12}$ CFU/ L MPCP.

A total of 20 trials conducted with BAS 480 00 I are available. A full efficacy data package to show efficacy against whiteflies and thrips in protected cultivation of fruiting vegetables and ornamentals is not yet available and a more detailed consideration of efficacy will be assessed in the context of subsequent applications for product authorizations.

**Preliminary range finding tests**

Separate preliminary range finding tests were not performed. An indication of the likely recommended dose was derived from two already registered products (Naturalis [*B. bassiana* ATCC 74040] and Botanigard [*B. bassiana* strain GHA]). From practice of these two products it is known that to ensure efficacy under varying conditions a relatively high dose and a high concentration of spores is required. Therefore, BAS 480 00 I is applied at dose rates comparable to that of other similar products.

**Minimum effective dosage**

The minimum effective dose of BAS 480 00 I will be further discussed under *Efficacy* as these two aspects were combined in the trials.

**Efficacy**

Data to support the label claims and which are summarized in this biological dossier were generated in a total of 20 trials carried out under protected conditions in Italy, Spain and the United Kingdom in 2012 and 2013. All trials were carried out by an officially recognized organisation and in accordance with the Principles of Good Experimental Practice (GEP).

**TABLE B. 3.2.4-1 Overview of efficacy trials performed**

| Nr | Test report | Pest | Crop | MS | Year |
|---|---|---|---|---|---|
| 1 | ITA 12156 BA1 | FRANOC[1] | Egg plant | Italy | 2012 |
| 2 | ITA 12156 BA2 | FRANOC | Egg plant | Italy | 2012 |
| 3 | ITA 12156 BA3 | FRANOC | Egg plant | Italy | 2012 |
| 4 | S12-04054-02 | FRANOC | Bell pepper | Spain | 2012 |
| 5 | S12-04054-03 | FRANOC | Bell pepper | Spain | 2013 |
| 6 | S12-04055-02 | FRANOC | Bell pepper | Spain | 2012 |
| 7 | 12-119 | FRANOC | Dwarf French Beans | UK | 2012 |
| 8 | 12-118 | FRANOC | Chrysanthemum | UK | 2012 |
| 9 | 12-216 | FRANOC | Chrysanthemum | UK | 2012 |
| 10 | 12-218 | FRANOC | Gerbera | UK | 2012 |
| 11 | S12-04054-01 | FRANOC | Chrysanthemum | UK | 2013 |
| 12 | S12-04055-01 | FRANOC | Chrysanthemum | UK | 2012 |
| 13 | 12 I CL BU 05 | THRITB[2]<br>BEMITA[3] | Pepper | Spain | 2012 |
| 14 | 12 I CL BU 06 | THRITB | Pepper | Spain | 2013 |
| 15 | 12 I CL BU 07 | THRITB | Pepper | Spain | 2013 |
| 16 | 12 I CL BU 08 | THRITB | Pepper | Spain | 2013 |
| 16 | 13 I CL BU 01 | BEMITA | Bell pepper | Spain | 2013 |
| 13* | 12 I CL BU 05 | BEMITA | Pepper | Spain | 2012 |
| 17 | 13-246 | TRIAVA[4] | Tomato | UK | 2013 |
| 18 | S12-02280-01 | TRIAVA | Tomato | UK | 2012 |
| 19 | S13-03366-01 | TRIAVA | Tomato | UK | 2013 |
| 20 | S13-03366-02 | TRIAVA | Tomato | UK | 2013 |

| | | |
|---|---|---|
| [1]FRANOC | Western flower thrips | *Frankliniella occidentalis* |
| [2]THRITB | Onion thrips | *Thrips tabaci* |
| [3]BEMITA | Silverleaf white fly | *Bemisia tabaci* |
| [4]TRIAVA | Greenhouse white fly | *Trialeurodes vaporariorum* |

**Summary and evaluation of individual trials results – Western flower thrips (*Frankliniella occidentalis*) in fruiting vegetables**

In a total of 6 trials conducted in protected fruiting vegetables, the efficacy of BAS 480 00 I against western flower thrips *(Frankliniella occidentalis)* was tested. In 5 of the trials the efficacy of the proposed dose rate of 0.1% was tested, and the minimum effective dose rate was determined (Test reports: ITA 12156 BA1, ITA 12156 BA2, ITA 12156 BA3, S12-04055-02, S12-04054-03, 12-119).. In 1 trial, 4 different treatments with BAS 480 00 I at a dose rate of 0.05% were compared (S12-04054-02).

*Minimum effective dose*

In a total of five trials, of which three in egg plant (3 trials in Italy) and two in bell pepper (2 trials in Spain) the minimum effective dose was determined. In the trials with bell pepper, no clear differences between any of the treatments including the untreated control were found. Therefore no conclusion on the minimum effective dose can be drawn. In the trials with egg plant, dose rates of 0.005% ($2.0\times10^{10}$ CFUs/hL), 0.05% ($2.0\times10^{11}$ CFUs/hL), 0.1% ($4.0\times10^{11}$ CFUs/hL) and 0.25% ($1.0\times10^{12}$ CFUs/hL) were compared. In general, no clear dose response was observed in the separate trials. In some cases the efficacy of the lowest dose rate of 0.005% tended to be somewhat lower than that of higher dose rates.

*Efficacy*

Eggplant

In Italy 3 trials (test reports: ITA 12156 BA1; ITA 12156 BA2; ITA 12156 BA3) were conducted in eggplant in 2012. Treatments with BAS 480 00 I consisted of 5 applications at an interval of 4 days at the proposed dose rate of 0.1% ($4.0\times10^{11}$ CFUs/hL). Two separate reference treatments were included. BAS 480 00 I was compared with the similarly acting microbial product Naturalis-L and with the chemical product Laser. Applications were performed at crop growth stage 68-69 at a spray volume of 1500 l/ha. The number of nymphs and adults was assessed on 25 leaves per plot.

In the trials pest pressure (adults and nymphs) was high enough to assess efficacy. After the third application the levels of control provided by BAS 480 00 I at 100 ml/hL at 4DAA5, ranged from 29% to 57% for adults and from 19% to 45% for nymphs. Although the levels of control as a result of treatments with BAS 480 00 I were generally low to moderate, the difference with the untreated control was significant in all 3 trials. Moreover, the performance of BAS 480 00 I was similar to that of the microbial product Naturalis-L, showing no significant differences between the products in the 3 trials. The levels of control provided by BAS 480 00 I were generally significantly lower than those provided by the chemical reference product Laser (30-80% for adults; 28-88% for nymphs)).

Bell pepper

In Spain 2 trials (test reports: S12-04055-02; S12-04054-03) were conducted in bell pepper in 2012 and 2013. Treatments with BAS 480 00 I consisted of 5 applications at an interval of 3-4 days at dose

rates of 0.00005% ($2.0\times10^{8}$ CFUs/hL), 0.0005% ($2.0\times10^{9}$ CFUs/hL), 0.005% ($2.0\times10^{10}$ CFUs/hL), 0.05% ($2.0\times10^{11}$ CFUs/hL), 0.1% ($4.0\times10^{11}$ CFUs/hL) and 0.5% ($2.0\times10^{12}$ CFUs/hL). Three separate reference treatments were included. BAS 480 00 I was compared with the similarly acting microbial products Botanigard and Naturalis-L, and with the chemical product Spinosad. Applications were performed at crop growth stage 83-87 at a spray volume of 1000 l/ha. The number of nymphs and adults was assessed on 25 leaves per plot.

In general no significant differences were found between any of the treatments, including the untreated control. Therefore, no conclusions could be drawn from these trials.

**Summary and evaluation of individual trials results – Western flower thrips (*Frankliniella occidentalis)* in dwarf French beans**

Dwarf french bean is not a representative use.

*Minimum effective dose*

In 1 trial conducted in protected dwarf French beans in the UK in 2012, the minimum effective dose was determined. In the trial, dose rates of 0.0028% ($1.1\times10^{10}$ CFUs/hL), 0.01% ($4.0\times10^{10}$ CFUs/hL), and 0.1% (4.0 × 1011 CFUs/hL) were compared. A dose response was not observed in the trial.

*Efficacy*

In 1 trial conducted (test report 12-119) in protected dwarf French beans in the UK in 2012, the efficacy of BAS 480 00 I against western flower thrips *(Frankliniella occidentalis)* was tested. Treatments with BAS 480 00 I consisted of 5 applications at an interval of 5 days at a dose rate of 0.1% ($4.0 \times 10^{11}$ CFUs/hL) and at a dose rate of 0.1% ($4.0\times10^{11}$ CFUs/hL) in tankmix with Codacide oil. Three separate reference treatments were included. BAS 480 00 I was compared with the microbial products Botanigard and Naturalis-L, and with the chemical product Dynamec. Applications were performed at crop growth stage of BBCH 64-65 at a spray volume of 1000 l/ha. The number of nymphs + adults was assessed on 10 stems per plot.

The pest pressure was high enough in this trial. The treatments with BAS 480 00 I resulted in a reduction of the numbers of thrips compared to the untreated control, but the differences were not significant. The levels of control provided by BAS 480 00 I applied solo ranged from 36% to 63% after the third application. The efficacy seemed to be somewhat lower when Codacide was added to the mix, but no significant differences occurred. The levels of control provided by BAS 480 00 I were comparable to those provided by Botanigard and Naturalis-L but generally lower than those provided by Dynamec. However, no significant differences in numbers of thrips per plot were found between the treatments.

**Summary and evaluation of individual trials results –Western flower thrips (*Frankliniella occidentalis)* in ornamentals**

In a total of 5 trials (test reports 12-118; 12-216; 12-218; S12-04054-01; S12-04055-01) conducted in protected ornamentals in the UK, the efficacy of BAS 480 00 I against western flower thrips *(Frankliniella occidentalis)* was tested. In 4 of the trials the efficacy of the proposed dose rate of 0.1% was tested, and the minimum effective dose rate was determined. In 1 trial, 4 different application frequency treatments with BAS 480 00 I at a dose rate of 0.05% were compared (S12-04055-01).

*Minimum effective dose*

In a total of 4 trials in Chrysanthemum (3 trials in the UK) and Gerbera (1 trials in the UK) the minimum effective dose was determined (test reports 12-118; 12-216; 12-218; S12-04054-01). In 2 trials, no clear differences between any of the treatments including the untreated control were found. Therefore no conclusion on the minimum effective dose can be drawn. In 2 trials in Chrysanthemum, dose rates ranging from 0.0028% ($1.1\times10^{10}$ CFUs/hL) to 0.5% ($2.0\times10^{12}$ CFUs/hL) were compared. In general, no clear dose response was observed.

*Efficacy*

In the UK 3 trials were conducted in Chrysanthemum in 2012 (2 trials) and 2013 (1 trial). Treatments with BAS 480 00 I consisted of 5-6 applications at an interval of 4-5 days at the proposed dose rate of 0.1% ($4.0\times10^{11}$ CFUs/hL). Three separate reference treatments were included. BAS 480 00 I was compared with the microbial products Botanigard and Naturalis-L, and with the chemical product Dynamec. Applications were performed at crop growth stage BBCH 65-85 or when the crop covered of 30% of the area at a spray volume of 1000 l/ha. The number of nymphs and adults was assessed on 25 leaves per plot.

In 1 of the trials pest pressure was low and generally no significant differences between any of the treatments were found (12-216). In 2 of the trials pest pressure (adults and nymphs) was high enough. In general a reducing effect on the numbers of thrips as a result of treatment with BAS 480 00 I was seen. Although initially variable levels of control were found in these 2 trials, higher levels ranging from 47% to 72% were found at the last two assessments after the fourth application. However, only in 1 of the trials significant difference in numbers of adults between the untreated control and the plots treated with BAS 480 00 I was seen. The performance of BAS 480 00 I was comparable to that of the microbial products Naturalis-L and Botanigard. Although the numbers of thrips in the plots treated with BAS 480 00 I were generally somewhat higher than those in plots treated with Dynamec at the early assessments, both products performed comparably at later assessments.

In the UK 1 trial was conducted in Gerbera in 2012. Treatments with BAS 480 00 I consisted of 6 applications at an interval of 4 days at the proposed dose rate of 0.1% ($4.0\times10^{11}$ CFUs/hL). Three separate reference treatments were included. BAS 480 00 I was compared with the microbial products Botanigard and Naturalis-L, and with the chemical product Dynamec. Applications were performed at crop growth stage BBCH 65-85 at a spray volume of 1000 l/ha. The number of nymphs and adults was assessed on 25 leaves per plot.

In this trial pest pressure was low and no significant differences were generally found between any of the treatments.

*Minimum application interval*

In the UK, 1 trial (S12-04055-01) was conducted in Chrysanthemum in 2012 to compare different treatments with BAS 480 00 I at a dose rate of 0.05%. The product was applied 10, 6, 5, or 3 times at an interval of respectively 3, 5, 7, or 10 days.

The pest pressure was high enough for evaluation of efficacy. The levels of control as a result of the different treatments with BAS 480 00 I were variable and generally low initially. At 30DAA1 higher levels were found, ranging from 36% to 49% for adults and 61 to 71% for nymphs. At 25DAA1 and 30 DAA1 all treatments gave significant better control of nymphs than the untreated. No clear differences were found between the different treatments.

**Summary and evaluation of individual trials results – Onion thrips (*Thrips tabaci*) in pepper**

In a total of 4 trials (Test reports 12I CL BU 05, 12I CL BU 06, 12I CL BU 07 and 12I CL BU 08) conducted in protected pepper in Spain, the efficacy of BAS 480 00 I against onion thrips (*Thrips tabaci*) was tested. In 2 of the trials the efficacy of the proposed dose rate of 0.1% was tested, and the minimum effective dose rate was determined (Test reports 12I CL BU 07 and 12I CL BU 08). In 2 trials 4 different treatments with BAS 480 00 I at a dose rate of 0.05% were compared (Test report 12I CL BU 05 and 12I CL BU 06).

*Minimum effective dose*

In a total of 2 trials in pepper conducted in Spain the minimum effective dose was determined. In the trials BAS 480 00 I was applied at dose rates of 0.00005% ($2.0\times10^{8}$ CFUs/hL), 0.0005% ($2.0\times10^{9}$ CFUs/hL), 0.005% ($2.0\times10^{10}$ CFUs/hL), 0.05% ($2.0\times10^{11}$ CFUs/hL), 0.1% ($4.0\times10^{11}$ CFUs/hL) and 0.5% ($2.0\times10^{12}$ CFUs/hL). In general, no clear dose response was observed. However, in 1 trial the highest levels of control were seen for BAS 480 00 I at 0.1% and 0.5%.

*Efficacy*

Two trials were conducted in pepper in 2013. Treatments with BAS 480 00 I consisted of 5-6 applications at an interval of 4-5 days at the proposed dose rate of 0.1% ($4.0\times10^{11}$ CFUs/hL). Three separate reference treatments were included. BAS 480 00 I was compared with the microbial products Botanigard and Naturalis-L, and with the chemical product Rufast Avance. Applications were performed at crop growth stage BBCH 65-89 at a spray volume of 1000-1200 l/ha. The number of nymphs and adults was assessed on 25 flowers per plot.

In both trials the pest pressure was high enough for evaluation of efficacy at the majority of assessments. In 1 of the trials levels of control ranging from 31% to 72% were found, but in the other values ranging from -10% to 29% were found for adults and 15% to 54% for nymphs. In general no

significant differences in respect to numbers of thrips were found between the treatment with BAS 480 00 I and the untreated control. The performance of BAS 480 00 I was comparable to that of Botanigard and Naturalis-L. The numbers of thrips found in plots treated with BAS 480 00 I were generally significantly higher than those found in the plots treated with the chemical reference product Rufast Avance.

*Minimum application interval*

In Spain, 2 trials were conducted in pepper in 2012 to compare different treatments with BAS 480 00 I at a dose rate of 0.05%. The product was applied 10, 6, 5, or 3 times at an interval of respectively 3, 5, 7, or 10 days.

The pest pressure was high enough for evaluation of efficacy. The levels of control as a result of the different treatments with BAS 480 00 I were variable and generally low. No clear differences were found between the different treatments.

**Summary and evaluation of individual trials results – Silverleaf white fly (Bemisia tabaci) in pepper**

In a total of 2 trials conducted in protected pepper (Test reports 12 I CL BU 01 and 12 I CL BU 05), the efficacy of BAS 480 00 I against silverleaf white fly (*Bemisia tabaci*) was tested. In 1 of the trials the efficacy of the proposed dose rate of 0.1% was tested, and the minimum effective dose rate was determined (Test reports 12 I CL BU 01). In 1 trial 4 different treatments with BAS 480 00 I at a dose rate of 0.05% were compared (test report 12I CL BU 05).

Minimum effective dose

In 1 trial in bell pepper conducted in Spain the minimum effective dose was determined. In the trial BAS 480 00 I was applied at dose rates of 0.00005% (2.0×108 CFUs/hL), 0.0005% (2.0×109 CFUs/hL), 0.005% (2.0×1010 CFUs/hL), 0.05% (2.0×1011 CFUs/hL), 0.1% (4.0×1011 CFUs/hL) and 0.5% (2.0×1012 CFUs/hL). In general, no clear dose response was observed.

Efficacy

In Spain 1 trial was conducted in bell pepper in 2013 (12I CL BU 01). Treatments with BAS 480 00 I consisted of 5 applications at an interval of 5 days at the proposed dose rate of 0.1% (4.0×1011 CFUs/hL). Three separate reference treatments were included. BAS 480 00 I was compared with the microbial products Botanigard and Naturalis-L, and with the chemical product Confidor. Applications were performed at crop growth stage BBCH 77-83 at a spray volume of 1100-1500 l/ha. The number of nymphs and adults was assessed on 50 leaves per plot.

Pest pressure was high enough for evaluation of efficacy. Initially somewhat lower levels of control as a result of treatment with BAS 480 00 I were found. After the third application BAS 480 00 I provided levels of control ranging from 49% to 80% for adults and from 61% to 72% for nymphs. In general the

difference with the untreated control was significant. The levels of control provided by BAS 480 00 I were comparable to those provided by Botanigard, Naturalis-L and Confidor.

Minimum application interval

In Spain, 1 trial was conducted in pepper in 2012 (12 I CL BU 05) to compare different treatments with BAS 480 00 I at a dose rate of 0.05%. The product was applied 10, 6, 5, or 3 times at an interval of respectively 3, 5, 7, or 10 days.

The pest pressure was high enough for evaluation of efficacy. The levels of control as a result of the different treatments with BAS 480 00 I were initially low and very variable but higher levels (21-86%) were recorded at the last two assessments. However, a statistical analysis of the data could not be performed as Bartlett´s test (on transformed data) did not permit to verify the homogeneity of variances. At the last assessment date a clear effect of the number and interval of the treatments with BAS 480 00 I on numbers of adults was seen. Overall the best performance was seen for 10 treatments with BAS 480 00 I, with 70-86% control at the last two assessments.

**Summary and evaluation of individual trials results – Greenhouse white fly (*Trialeurodes vaporariorum*) in tomato**

In a total of 4 trials conducted in protected tomato, the efficacy of BAS 480 00 I against greenhouse white fly (*Trialeurodes vaporariorum*) was tested (Test reports 13-246, S12-02280-01, S13-03366-01 and S13-03366-02). In these trials the efficacy of the proposed dose rate of 0.1% was tested, and the minimum effective dose rate was determined.

*Minimum effective dose*

In 4 trials in tomato conducted in the UK the minimum effective dose was determined. In the trial BAS 480 00 I was applied at dose rates of 0. 025% ($1.0\times10^{11}$ CFUs/hL), 0.05% ($2.0\times10^{11}$ CFUs/hL), 0.1% ($4.0\times10^{11}$ CFUs/hL) and 0.2% ($8.0\times10^{11}$ CFUs/hL) or at 0.0028% ($1.0\times10^{10}$ CFUs/hL), 0.01% ($4.0\times10^{10}$ CFUs/hL), 0.1% ($4.0\times10^{11}$ CFUs/hL). In 1 trial pest pressure was too low for reliable evaluation of efficacy (Test report S13-03366-01).. In 2 trials, no clear dose response was observed (Test report S13-03366-02, S12-02280-01).

In 1 trial a dose response was observed as a trend ((Test report 13-246).. While the performance of 0.05% and 0.1% BAS 480 00 I was comparable, treatment with these two dose rates resulted in overall higher levels of control than the treatment with 0.025% (respectively 36-67% and 16-44% after the second application). The best results were seen at the treatment with 0.2% BAS 480 00 I (57-77% control after the second application).

*Efficacy*

In the UK 4 trials were conducted in tomato in 2012 (2 trials) and 2013 (2 trials). Treatments with BAS 480 00 I consisted of 5 applications at an interval of generally 5 days at the proposed dose rate of

0.1% ($4.0\times10^{11}$ CFUs/hL). In 1 trial BAS 480 00 I was also applied in tankmix with Codacide oil (Test report S12-02280-01). Three separate reference treatments were included. BAS 480 00 I was compared with the microbial products Botanigard and Naturalis-L, and with a chemical product, either Dynamec or Oberon. Applications were performed at crop growth stage BBCH 67-82 at a spray volume of about 1000 l/ha in 3 trials and at 4000 L/ha in 1 trial. The number of nymphs and adults was assessed on 10-15 leaves per plot.
In 1 trial pest pressure was too low for evaluation of efficacy (Test report S13-03366-01). In 3 trials pest pressure was high enough. Initially very low levels of control as a result of treatment with BAS 480 00 I were found in these trials. After the second application BAS 480 00 I provided moderate levels of control. In general the difference in numbers of thrips with the untreated control was not significant. The levels of control provided by BAS 480 00 I were comparable to those provided by Botanigard, Naturalis-L and lower than those provided by Dynamec and Oberon. Although no significant differences with the other treatment with BAS 480 00 I were found, the addition of Codacide oil appeared to increase the efficacy of BAS 480 00 I against nymphs somewhat.

**Efficacy and dose justification, Summary.**

The aim of the evaluation of an active substance is to determine if the dose rate in the gap is realistic. For microbial products it is often difficult to test the dose rate. There is usually a large variability in efficacy that is caused by factors other than the dose rate. Influence of the actual amounts of CFU in the formulation (which can vary) and external factors, usually cancel out effects of dose rate changes. Effects of dose rate for microbials are usually visible when dose rate is varied on a logarithmic scale, however testing for efficacy and dose rate for product and active substance registration is performed with dose rates with much smaller differences.

In general there was no clear dose response.
The currently submitted efficacy data for the proposed rate shows low to moderate efficacy that is variable both within, and between trials. Variability and lower efficacy is often inherent to microbial products. During product registration, a more complete dossier should be available that allows evaluation of the efficacy. For a determination if the dose rate is realistic usually a smaller dataset will be sufficient, for inherently variable products like microbial products it is often difficult to interpret dose justification effects based on small datasets.

The submitted results show that the product can have sufficient efficacy, and the flat dose response indicates that a higher dose rate is unlikely to have an improved effect.
For the purpose of authorisation of the active substance, the dose rate can be considered to be sufficiently addressed, as it is unlikely that an increase in the dose rate will lead to a large improvement in efficacy.

*Bemisia argentifolii* is not tested in the trials. It does not appear to be relevant to this application but is mentioned in the GAP. The other pests listed in the GAP (*Frankliniella occidentalis, Thrips tabaci, Bemisia tabaci, Trialeurodes vaporariorum) we*re tested in the trials.

The very high number of applications (122 per year with a 3 day interval) is likely to represent a worst case situation. For practical reasons, and because of concerns for resistance development, such high application numbers are unlikely to be reached in a field situation.
A full evaluation of efficacy for the representative uses will be performed at product registration.

### B.3.2.5 Content of micro-organism in material used (e.g., in the diluted spray, baits or treated seed) (Annex IIIM 3.3)

BAS 480 00 I has a nominal concentration of *Beauveria bassiana* strain PPRI 5339 of 40 g/L or 4 x $10^{12}$ cfu/L. Dose rate is 100 mL product per 100 L of water on label, or $4*.10^{11}$ CFU/ hL

### B.3.2.6 Method of application (Annex IIIM 3.5)

BAS 480 00 I should be applied as a foliar spray. It is important to ensure coverage on the bottom side of the leaves where pest might be located.

### B.3.2.7 Number and timing of applications (Annex IIIM 3.6)

| Crop | Method of application | Maximum number of applications | Timing of application |
|---|---|---|---|
| Solanacea | Spraying | 122 | every three days throughout the year |
| Cucurbitacea | Spraying | 122 | every three days throughout the year |
| Ornamentals | Spraying | 122 | every three days throughout the year |

Foliar sprays are started at pest occurrence and are repeated as necessary at an interval of 3-14 days. In practice, at least 3 applications are necessary for control of whitefly and thrips. The maximum number of annual applications is 122.

#### B.3.2.8 Necessary waiting periods or other precautions to avoid phytopathogenic effects on succeeding crops (Annex IIIM 3.7)

No PHI is necessary.

#### B.3.2.9 Proposed instructions for use (Annex IIIM 3.8)

National labels will be made available during product registration.

### B.3.3 Further information on the plant protection product (Annex IIIM 4)

#### B.3.3.1 Packaging and compatibility of the preparation with proposed packaging materials (Annex IIIM 4.1)

BAS 480 00 I formulated end use product (EU) will be stored for sale in UN approved High Density Poly-Ethylene (HDPE) containers of a range of sizes: 0.5, 1.0 and 5.0 L.

#### B.3.3.2 Procedures for cleaning application equipment (Annex IIIM 4.3 and 4.4)

| | |
|---|---|
| **Procedures for cleaning application equipment:** | Cleaning of spray equipment should be performed by triple-rinsing with water (3 times 10% of the tank volume). The equipment can afterwards be used in any crops without damage. No addition of detergents is necessary. |
| **Procedures for cleaning protective clothing:** | Gloves can be washed with a dilute detergent solution and rinsed with water. Any contaminated clothing should be laundered with a dilute detergent solution and thoroughly rinsed with clean water. |

##### B.3.3.2.1 Effectiveness of the cleaning procedures

The product is an insecticide. The effectiveness of cleaning procedures was not studied. Based on the composition of the product, it is expected that the procedure described below will perform adequately to clean equipment and clothing.

**Application equipment**

Cleaning of spray equipment should be performed by triple-rinsing with water (3 times 10% of the tank volume). The equipment can afterwards be used in any crops without damage. No addition of detergents is necessary.

**Protective clothing**

Gloves can be washed with a dilute detergent solution and rinsed with water. Any contaminated clothing should be laundered with a dilute detergent solution and thoroughly rinsed with clean water.

**B.3.3.3 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (Annex IIIM 4.5)**

No re-entry is necessary in treated crops shortly after the application of BAS 480 00 I. BAS 480 00 I is of low toxicity and estimation of worker exposure according to worker exposure models resulted in a good margin of safety. Therefore no re-entry period is required.

**B.3.3.3.1 Pre-harvest intervals, re-entry or withholding periods to minimise residues in crops, plants, plant products, treated areas or spaces**

No PHI is necessary.

**B.3.3.3.2 Information on any specific agricultural, plant health or environmental conditions under which the preparation may or may not be used**

No information.

**B.3.3.4 Recommended methods and precautions concerning: handling, storage, transport or fire (Annex IIIM 4.7)**

**Handling:** PPE is required during mixing, loading or application due to the sensitisation potential of micro-organisms. The following PPE is recommended if there is a risk of skin or eye contact with the product:

| | |
|---|---|
| Hand protection: | Chemical resistant (e.g. nitrile rubber) gloves |
| Eye protection: | Safety glasses/face shield |
| Skin and body protection: | Impermeable clothing |
| Hygiene measures: | Handle in accordance with good industrial hygiene and safety practice. |

**Warehouse storage:** The product should be stored in compliance with GCPF (former GIFAP) Guidelines for Safe Warehousing of crop protection products and GIFAP Guidelines for Safe Handling of Pesticides during their Formulation, Packing, Storage and Transport.

The recommended storage temperature is between 4°C and 25°C.

**User level storage:** The product must be stowed away from food, drink and animal feeding stuffs and without access for children. The product should be stored under conditions which prevent entering any body of water and any misuse by non-authorised persons.

**Transport:** BAS 480 00 I is not classified hazardous under transport regulations. Transport should be in compliance with GIFAP Guidelines for the safe transport of pesticides and Guidelines for Safe Handling of Pesticides during their Formulation, Packing, Storage and Transport.

Suitable extinguishing media: Water spray, foam, dry powder, carbon

| | |
|---|---|
| | dioxide (CO2) |
| Specific hazards during firefighting: | Explosive dust-air mixtures may form |
| Special protective equipment: | No specific equipment is required |
| Additional precautions: | If possible, avoid discharge of fire-extinguishing water into drains or water courses |

**Fire:**

No special precautions necessary. The substance/product is non-combustible. Product is not explosive.

**Waste minimisation**

To minimise surplus product waste, users are recommended to buy not more than is consumed within the shelf life period of the product and to store the product before use according to storage recommendation (cool and dry). Always calculate the amount required and buy the appropriate container sizes.

### B.3.3.5 Measures in the case of an accident (Annex IIIB 4.9)

Personal precautions, protective equipment and emergency procedures

Use personal protective clothing. Avoid contact with the skin, eyes and clothing. Do not breathe vapour/spray.

Environmental precautions

Do not discharge into the subsoil/soil. Do not discharge into drains/surface waters/groundwater.

Methods and material for containment and cleaning up

For small amounts: Pick up with suitable absorbent material (e.g. sand, sawdust, general-purpose binder, kieselguhr).

For large amounts: Dike spillage. Pump off product.

Dispose of absorbed material in accordance with regulations. Collect waste in suitable containers, which can be labeled and sealed. Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations.

### B.3.3.6 Procedures for destruction or decontamination of the plant protection product and its packaging (Annex IIIM 4.10)

**Spill containment**

Prevent entry into drains, waters or soil. Dampen the dust, scoop or sweep up and place into sealable containers. Use a damp cloth to clean floors and other objects after removal of powder and also place in sealable container. Dispose of all waste and contaminated clothing in the same manner as waste chemicals (i.e. via an authorised disposal facility).

Since *Beauveria bassiana* is not considered to have any adverse environmental impact due to its ubiquitous natural occurrence, an accidental release of product indoor or outdoor does not require special treatment.

**Decontamination**

The procedures described above are sufficient for decontamination. Solid surfaces may be further cleaned by washing with detergents.

**Disposal**

Damaged packaging must be rinsed properly, punctured to be made unusable and disposed of as hazardous waste. Absorbents and other contaminated materials must be disposed of as hazardous waste according to local, national or international regulations.

**Protection of emergency workers**

Wear full protective clothing and NIOSH or other regulation-approved self-contained breathing apparatus with full face piece operated in the pressure demand or other positive pressure mode. Keep unnecessary people away. Use as little water as possible. Dike area of fire, to prevent material run-off. Decontaminate emergency personnel with soap and water before leaving the fire area. Avoid breathing dusts, vapours and fumes from burning materials. Control run-off water.

**Protection of bystanders**

Bystanders should not be allowed to remain in the vicinity of accidental release or fire. In case bystanders cannot be removed to a safe location, they should be placed up-wind from the accidental release or fire, and provided with full protective equipment.

**First aid**

| | |
|---|---|
| **Skin contact:** | In case of contact with skin wash off immediately with soap and water. |
| **Eye contact:** | In the case of contact with eyes, rinse immediately with plenty of water for at least 15 minutes. |
| **Ingestion:** | Drink plenty of water. Do not induce vomiting. Seek medical advice. |
| **Inhalation:** | Move to fresh air. |

**Notes to the physician**

| | |
|---|---|
| **Symptoms:** | No specific symptoms are known. |
| **Treatment:** | No specific antidote. Initial treatment should be symptomatic and supportive. |

#### B.3.3.6.1 Controlled incineration

Leftover quantities of the product must be disposed of in a commercial incinerator according to local regulations. Incineration must be done under controlled conditions according to the directives 94/67/EC and 2000/76/EC:

- Residence time greater than 2 seconds
- Presence of more than 6 % of oxygen
- Temperature above 850 °C

Contaminated packaging has to be thoroughly rinsed with plenty of water, then burned in a commercial incinerator. In field applications the washing solutions have to be added to the spray mixture, otherwise they have to be burned in a commercial incinerator.

#### B.3.3.6.2 Others

No methods other than controlled incineration are recommended for disposal.

### B.3.4 References relied on

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>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 3.5.1/03 | Alon, M, Alon, F, Nauen, R, Morin, S | 2008 | Organophosphates' resistance in the B-biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) is associated with a point mutation in an ace 1-type acetylcholinesterase and overexpression of carboxylesterase. Insect biochemistry and molecular biology 38: 940-949<br>Non GLP<br>Published | N | N | N/A | Public |
| MA 3.5.1/04 | Arnaud, L , Harbruge, E | 2012 | Insecticide resistance enhances male reproductive success in a beetle. Evolution 56: 2435-2444<br>Non GLP<br>Published | N | N | N/A | Public |
| MA 3.5.1/01 | Dudovskiy, IM, Whitten, MMA, Yaroslavtseva, ON, Greig, | 2013 | Can insects develop resistance to insect pathogenic fungi? PLoS ONE | N | N | N/A | Public |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner |
|---|---|---|---|---|---|---|---|
| | C, Kryukov, VY, Grzanova, EV, Mukherjee, K, Vilcinskas, A, Glupov, VV, Butt, TM | | 8(4): e60248§<br>Non GLP<br>Published | | | | |
| MA 3.5.1/05 | Gillespie, JP, Bailey, AM, Cobb, B, Vilcinskas, A | 2000 | Fungi as elicitors of insect immune responses. Archives of Insect Biochemistry and Physiology 44:49-68<br>Non GLP<br>Published | N | N | N/A | Public |
| MA 3.5.1/07 | Kraaijeveld, AR, Godfray, HCJ | 2008 | Selection for resistance to a fungal pathogen in *Drosophila melanogaster.* Heredity, 100, 400-406<br>Non GLP<br>Published | N | N | N/A | Public |
| MA 3.5.1/06 | Kraaijeeld, AR, Layen, SJ, Futerman, PH, Godfray, HCJ | 2012 | Lack of phenotypic and evolutionary cross-resistance against parasitoids and pathogens in *drosophila melanogaster*. PLoS ONE 7(12): e53002<br>Non GLP<br>Published | N | N | N/A | Public |
| MA 3.5.1/02 | Silva, AX, Jander, G, Samaniego, H, Ramsey, JS, Figueroa, CC | 2012 | Insect resistance mechanisms in the green peach aphid *myzus persicae* (Hemiptera: Aphididae) I: a transcriptomic survey. PLoS ONE 7: e36366 | N | N | N/A | Public |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner |
|---|---|---|---|---|---|---|---|
| | | | Non GLP<br>Published | | | | |
| MA 3.5.3/01 | Wojda, I, Kowalski, P, Jakbowicz, T | 2009 | Humoral immune response of *Galleria melonella* larvae after infection by *Beauveria bassiana* under optimal and heat-shock conditions. Journal of Insect Physiology, 55, 525-531<br>Non GLP<br>Published | N | N | N/A | Public |
| **Annex III Data on application** | | | | | | | |
| MP 6.1 and MP 6.2/1 | Scrimshaw, O. | 2012 | To determine the efficacy of BUEXP1778 for the control of glasshouse whitefly in protected tomato, 1 trial in the UK, 2012<br>Eurofins Agroscience Services, S12-02280-01<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Scrimshaw, O. | 2012 | Western Flower Thrip - Dose response Eurofins Agroscience Services, S12-04054-01<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Scrimshaw, O. | 2012 | Western Flower Thrip - Dose response Eurofins Agroscience Services, S12-04054-02<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Scrimshaw, O. | 2012 | Western Flower Thrip - Dose response Eurofins Agroscience Services, | N | Y | Y | BASF |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner |
|---|---|---|---|---|---|---|---|
| | | | S12-04054-03<br>GEP, unpublished | | | | |
| MP 6.1 and MP 6.2/1 | Scrimshaw, O. | 2012 | Western Flower Thrip - Dose response, 1 site in the UK<br>Eurofins Agroscience Services, S12-04055-01<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Scrimshaw, O. | 2012 | Western Flower Thrip - Dose response, 1 site in Spain<br>Eurofins Agroscience Services, S12-04055-02<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Dylan G. | 2012 | Western Flower Thrip – Dose Response<br>i2LResearch Ltd 12/216<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Dylan G. | 2012 | Western Flower Thrip – Dose Response<br>i2LResearch Ltd 12/218<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Limon, E. | 2012 | FUNGICIDE EFFICACY AND SELECTIVITY OF BUEXP1778, BUEXP1901 and BUEXP1902 APPLIED AT A RANGE OF APPLICATION FREQUENCIES AGAINST THRIPS (Thrips tabaci) ON PROTECTED PEPPER (GREENHOUSE) IN SPAIN IN 2012<br>PROMO-VERT | N | Y | Y | BASF |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner |
|---|---|---|---|---|---|---|---|
| | | | CROP SCIENCES S.L.<br>12 I CL BU 05<br>GEP, unpublished | | | | |
| MP 6.1 and MP 6.2/1 | Limon, E. | 2012 | FUNGICIDE EFFICACY AND SELECTIVITY OF BUEXP1778, BUEXP1901 and BUEXP1902 APPLIED AT A RANGE OF APPLICATION FREQUENCIES AGAINST THRIPS (Thrips tabaci) ON PROTECTED PEPPER (GREENHOUSE) IN SPAIN IN 2012<br>PROMO-VERT CROP SCIENCES S.L.<br>12 I CL BU 06<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Limon, E. | 2012 | STUDY ON THE EFFICACY AND SELECTIVITY OF BUEXP1778 APPLIED AT A RANGE OF APPLICATION RATES AGAINST THRIPS (Thrips tabaci) ON PROTECTED PEPPER (GREENHOUSE) IN SPAIN IN 2013<br>12 I CL BU 07<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Limon, E. | 2012 | STUDY ON THE EFFICACY AND SELECTIVITY OF BUEXP1778 APPLIED AT A RANGE OF APPLICATION RATES AGAINST | N | Y | Y | BASF |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner |
|---|---|---|---|---|---|---|---|
| | | | THRIPS (Thrips tabaci) ON PROTECTED PEPPER (GREENHOUSE) IN SPAIN IN 2013<br>12 I CL BU 08<br>GEP, unpublished | | | | |
| MP 6.1 and MP 6.2/1 | Pasqualini, E. | 2013 | Evaluate the efficacy of BUEXP1778 against western flower thrips (Frankliniella occidentalis) on eggplant<br>ANADIAG ITALIA SRL, ITA 12156 BA1<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Pasqualini, E. | 2013 | Evaluate the efficacy of BUEXP1778 against western flower thrips (Frankliniella occidentalis) on eggplant<br>ANADIAG ITALIA SRL, ITA 12156 BA2<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Pasqualini, E. | 2013 | Evaluate the efficacy of BUEXP1778 against western flower thrips (Frankliniella occidentalis) on eggplant<br>ANADIAG ITALIA SRL, ITA 12156 BA3<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Scrimshaw, O. | 2013 | Determine the efficacy of BUEXP1778 applied as a curative foliar spray against | N | Y | Y | BASF |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner |
|---|---|---|---|---|---|---|---|
| | | | glasshouse whitefly, 1 site in the UK<br>Eurofins Agroscience Services, S12-03366-01<br>GEP, unpublished | | | | |
| MP 6.1 and MP 6.2/1 | Scrimshaw, O. | 2013 | Determine the efficacy of BUEXP1778 applied as a curative foliar spray against glasshouse whitefly, 1 site in the UK<br>Eurofins Agroscience Services, S12-03366-02<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Dr Whaley C. | 2013 | INITIAL SCREENING OF BUEXP1778 AGAINST WESTERN FLOWER THRIPS ON ORNAMENTALS, UNDER PROTECTED CONDITIONS<br>i2LResearch Ltd 12/118<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Dr Whaley C. | 2013 | INITIAL SCREENING OF BUEXP1778 AGAINST WESTERN FLOWER THRIPS ON ORNAMENTALS, UNDER PROTECTED CONDITIONS<br>i2LResearch Ltd 12/119<br>GEP, unpublished | N | Y | Y | BASF |

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>Published or not | Vertebrate study Y/N | Data protection claimed Y/N | Justification if data protection is claimed | Owner |
|---|---|---|---|---|---|---|---|
| MP 6.1 and MP 6.2/1 | Dr Whaley C. | 2013 | EFFICACY EVALUATION OF BUEXP1778 AGAINST WHITEFLY (Trialeurodes vaporariorum), UNDER PROTECTED CONDITIONS<br>i2LResearch Ltd<br>13/246<br>GEP, unpublished | N | Y | Y | BASF |
| MP 6.1 and MP 6.2/1 | Limon, E. | 2013 | STUDY ON THE EFFICACY AND SELECTIVITY OF BUEXP1778 AND BUEXP1901 AGAINST WHITEFLY (Bemisia tabaci) ON PROTECTED PEPPER (GREENHOUSE) IN SPAIN IN 2013<br>13 I CL BU 01<br>GEP, unpublished | N | Y | Y | BASF |
| | | | | | | | |
| | | | | | | | |

## B.4 Proposals for classification and labelling

### B.4.1 Proposals for classification and labelling of the MPCA

The active substance is a microorganisms and as such is not subject to classification in accordance with Regulation (EC) No 1272/2008 (CLP). The major metabolite beauvericin does have classifications, but exists at concentrations well below applicable concentration limits.

As all microorganisms are considered potential allergens the following warning phrase is proposed:
'Contains *Beauveria bassiana* strain PPRI 5339. Micro-organisms may have the potential to provoke a sensitising reaction'

### B.4.2 Proposals for classification and labelling of the MPCP

No formal classification is required. However, as all microorganisms are considered potential allergens the following warning phrase is proposed:
'Contains *Beauveria bassiana* strain PPRI 5339. Micro-organisms may have the potential to provoke a sensitising reaction'

### B.4.3 References relied on

None

## B.5 Analytical methods (Annex IIM 4)

### B.5.1 Methods for the analysis of the micro-organism as manufactured and for the analysis of the preparation (Annex IIM 4.3)

#### B.5.1.1 Methods for the analysis of the micro-organism as manufactured

Please refer to volume 4 (confidential section).

##### B.5.1.1.1 Methods for the identification of the micro-organism

**Report:** Berry, S (2012), Molecular Identify of the South African *Beauveria bassiana* isolate, strain PPRI 5339. Becker Underwood, Mahogany Ridge, KZN, South Africa and Becker Underwood, NSW 2250, Australia. Report Date: 28.11.12

**Guidelines:** None

**GLP:** Yes

**Summary**

Identification of *Beauveria bassiana* strain PPRI 15339 was confirmed by sequencing of the ITS ribosomal region including the 3' end of the 18S region, the ITS1 region, the 5.8S region and the ITS2 region.

The resulting sequence was BLASTED against sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov/Blast). Results (Table B.5.1.1.1-1) showed that the isolate PPRI 5339 was most related (99-100% identity) to sequences deposited as *Beauveria bassiana*. Of the 40 most similar sequences, 29 sequences were ascribed to *B. bassiana*, 4 sequences were *Cordyceps bassiana*, 4 sequences were *Beauveria* with no species designations, 1 sequence was a *Cordyceps* unknown species, 1 sequence was a strain of *Isaria farinosa* (an entomopathogenic fungus, formerly *Paecilomyces farinose*) strain and 1 sequence was an uncultured fungus clone S358.

**Table B.5.1.1.1-1: List of most similar sequence producing significant alignments to that of *Beauveria bassiana* strain PPRI 5339**

| No. | Accession | Description | Max. Score | Total Score | Query Coverage | Max. Identity |
|---|---|---|---|---|---|---|
| 1 | HQ222971.1 | Beauveria bassiana isolate BbPV 013 | 955 | 955 | 99% | 99% |
| 2 | JQ320361.1 | Beauveria bassiana voucher FJAT-9620 | 953 | 953 | 100% | 99% |
| 3 | HQ222972.1 | Beauveria bassiana isolate BbTDG 001 | 953 | 953 | 98% | 99% |
| 4 | HQ222979.1 | Beauveria bassiana isolate BbNSV 006 | 953 | 953 | 98% | 99% |
| 5 | AB237657.1 | Cordyceps bassiana | 953 | 953 | 100% | 99% |
| 6 | AB044636.1 | Cordyceps sp. 97005 gene | 953 | 953 | 100% | 99% |
| 7 | JX110390.1 | Beauveria bassiana isolate SASRI Woodburn | 952 | 952 | 99% | 99% |
| 8 | JQ320362.1 | Beauveria bassiana voucher FJAT-9621 | 952 | 952 | 99% | 99% |
| 9 | JQ291609.1 | Beauveria bassiana | 952 | 952 | 99% | 99% |
| 10 | EU821491.1 | Beauveria bassiana isolate 2486 | 952 | 952 | 99% | 99% |
| 11 | EU821480.1 | Beauveria bassiana isolate 2475 | 952 | 952 | 99% | 99% |
| 12 | JX122736.1 | Beauveria sp. CRRI-66 18S | 950 | 950 | 99% | 99% |
| 13 | GU189515.1 | Beauveria bassiana isolate B418S | 950 | 950 | 99% | 99% |
| 14 | GQ354257.1 | Beauveria sp. JS-2009a isolate B4B | 950 | 950 | 99% | 99% |
| 15 | GQ354221.1 | Beauveria sp. JS-2009a isolate L11A | 950 | 950 | 99% | 99% |
| 16 | GQ354233.1 | Beauveria sp. JS-2009a isolate L28A | 950 | 950 | 99% | 99% |
| 17 | EU334677.1 | Beauveria bassiana isolate DAOM195005 | 950 | 950 | 99% | 99% |
| 18 | EU334675.1 | Beauveria bassiana isolate INRS-IP | 950 | 950 | 99% | 99% |
| 19 | EU334674.1 | Beauveria bassiana isolate INRS-CFL | 950 | 950 | 99% | 99% |
| 20 | DQ364698.1 | Beauveria bassiana strain EABb 04/01-Tip | 950 | 950 | 99% | 99% |
| 21 | AJ560677.1 | Beauveria bassiana isolate IMI 382295 | 950 | 950 | 99% | 99% |
| 22 | JX110368.1 | Beauveria bassiana isolate SASRI BB444 | 948 | 948 | 99% | 99% |
| 23 | JN713137.1 | Beauveria bassiana strain PAL-B01 | 948 | 948 | 100% | 99% |
| 24 | JN713138.1 | Beauveria bassiana strain PAL-B02 | 948 | 948 | 100% | 99% |
| 25 | JF429895.1 | Isaria farinosa strain STH1 28S | 948 | 948 | 100% | 99% |
| 26 | JF429894.1 | Beauveria bassiana strain STB | 948 | 948 | 100% | 99% |
| 27 | HQ222980.1 | Beauveria bassiana isolate BbME 012 | 948 | 948 | 98% | 99% |
| 28 | AB576868.1 | Beauveria bassiana strain: GHA | 948 | 948 | 100% | 99% |
| 29 | FJ820846.1 | Uncultured fungus clone S358 | 948 | 948 | 100% | 99% |
| 30 | EU573328.1 | Beauveria bassiana strain MRCIF46 | 948 | 948 | 100% | 99% |
| 31 | EU573326.1 | Beauveria bassiana strain MRCIF6 | 948 | 948 | 100% | 99% |
| 32 | EU272502.1 | Beauveria bassiana isolate 2698 | 948 | 948 | 99% | 99% |
| 33 | EF556209.1 | Cordyceps bassiana strain bxq41205 | 948 | 948 | 98% | 99% |
| 34 | EU315003.1 | Cordyceps bassiana isolate FKCB-036 | 948 | 948 | 98% | 99% |
| 35 | AB079126.1 | Cordyceps bassiana | 948 | 948 | 99% | 99% |
| 36 | AB027382.1 | Beauveria bassiana | 948 | 948 | 100% | 99% |
| 37 | JX110387.1 | Beauveria bassiana isolate SASRI 4222 | 946 | 946 | 99% | 99% |
| 38 | JX110371.1 | Beauveria bassiana isolate SASRI C2 | 946 | 946 | 99% | 99% |
| 39 | JX110376.1 | Beauveria bassiana isolate SASRI | 946 | 946 | 99% | 99% |
| 40 | JX110384.1 | Beauveria bassiana isolate SASRI HHGR1 | 946 | 946 | 99% | 99% |

Generating a distance tree of results (http://blast.ncbi.nlm.nih.gov/blast/treeview/treeView.cgi) resulted in a neighbour-joining cladogram depicting relationship between our isolate with that of reference sequence in GenBank. Results (Figure B.5.1.1.1-1) showed that the PPRI 5339 isolate (highlighted in yellow) was nestled in between branches of many different *Beauveria bassiana* isolates.

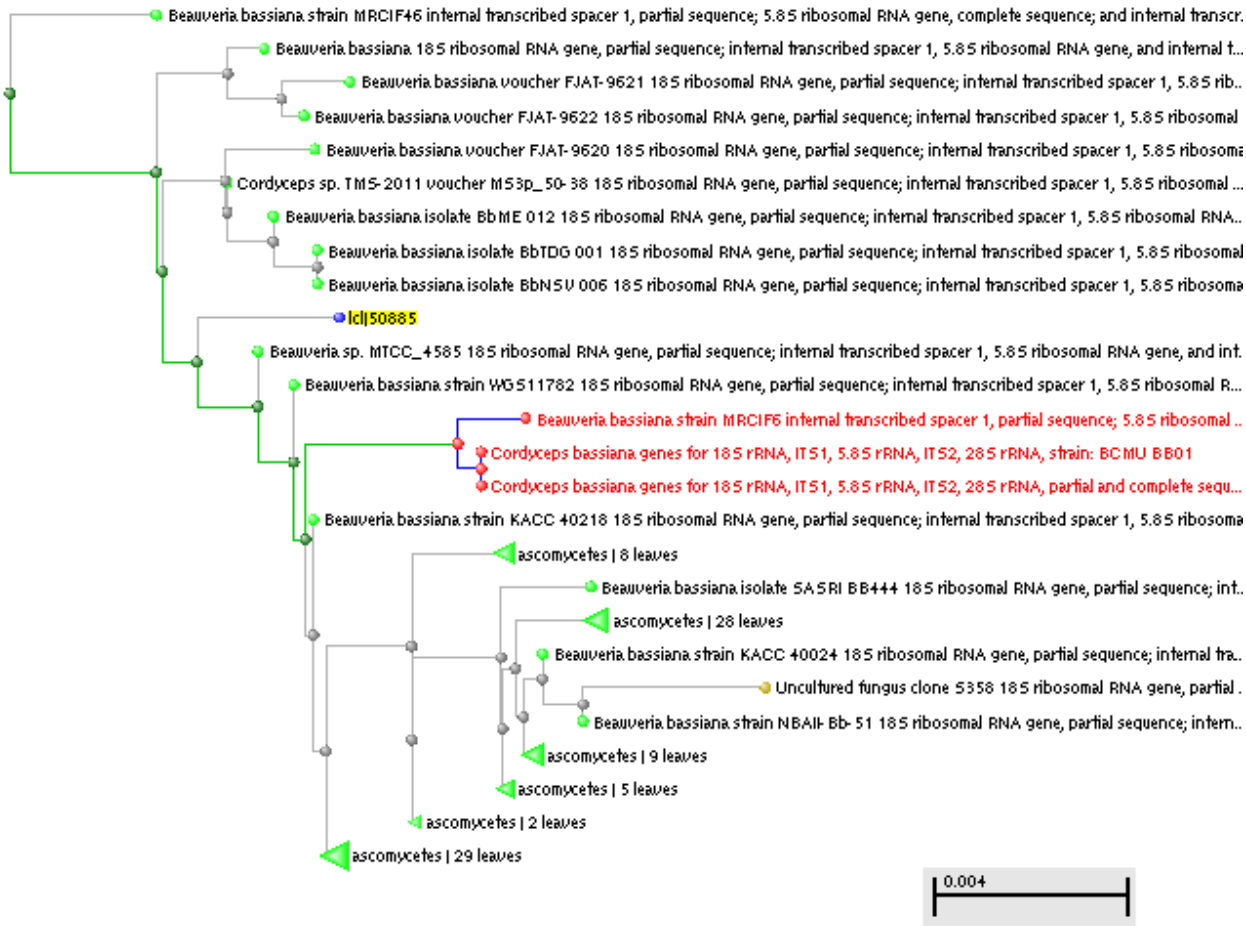



Figure B.5.1.1.1-1: Cladogram showing relative position of *Beauveria bassiana* isolate PPRI 5339 (shown in figure as lclj50885) compared to closely related strains.

The sequences of *Beauveria bassiana* strain PPRI 5339 was then compared with that of two competitor isolates *viz.* Botanigard® (*B. bassiana* isolate GHA, accession number: JN379811.1) and Naturalis® (*B. bassiana* isolate ATCC74040, accession number: FJ972972.1) The sequences of the three isolates were found to be 98-99% similar to each other and can be considered near identical species.

#### B.5.1.1.2 Methods for providing information on possible variability of seed stock/active micro-organism

The molecular method described under B.5.1.1.1 can be used in order to confirm that no spontaneous genetic change has occurred.

#### B.5.1.1.3 Methods to differentiate a mutant of the micro-organism from the parent wild strain

Not relevant, strain PPRI 5339 is a naturally occurring strain and has not been genetically altered in any way.

**B.5.1.1.4 Methods for the establishment of purity of seed stock from which batches are produced and methods to control that purity**

See Volume 3, B.5.1.1.5.

**B.5.1.1.5 Methods to determine the content of the micro-organism in the manufactured material used for the production of formulated products and methods to show that contaminating micro-organisms are controlled to an acceptable level**

The methods to determine the content of the micro-organism and the methods to detect potential contaminating micro-organisms in the MCPA is included below. Results from the 5-batch analysis from the same references (Whittaker 2014a and Whittaker 2014b) are included in Volume 4.

Method to detect content:

For each batch, 0.2 g of test item was weighed out to 1 mg in a weighing boat. A paste was formed by gently stirring Tween 80 into the powder with a glass rod. The paste was washed into a sterile Nalgene pot with 100 mL of sterile PBS and sonicated in an ultrasonic bath for 3 minutes. After swirling, the suspension was sonicated for another 3 minutes to reduce clumps. Serial dilutions were prepared in a logarithmic progression by pipetting 1.0 mL of the suspension into 9.0 mL of sterile PBS. 100 µl from the $1 \times 10^{-7}$, $1 \times 10^{-8}$ and $1 \times 10^{-9}$ dilutions was plated in triplicate on Rose Bengal Agar and incubated upright at 28 ± 2 °C for 96 hours. The concentration of active MPCA in each batch of test item was calculated from plate counts as CFU/g. The triplicate plate count from a dilution producing ~30-300 colonies/plate was averaged for each batch and the mean of this calculation for all batches was used to determine an average count for all 5 batches cumulatively. Standard and relative standard deviations were also calculated.

The results of the enumeration analysis tests shown a mean result of $1.2 \times 10^{12}$ CFU/g MPCA germination with a relative SEM of 17.65%.

Methods to detect contaminating micro-organisms:

26 g of each batch was weighed out accurately (to 1.0 mg) and washed into a sterile flask with 234 mL of sterile phosphate buffered saline (PBS) containing 0.5% Tween 80. This suspension was the stock used for pathogen screening. Positive controls with reference cultures were included to show the suitability of the test system for detection of these contaminants.

1. *Staphylococcus aureus*

1.0 mL of the initial pathogen screening suspension was inoculated onto the surface of each of nine Mannitol Salt agar plates and spread as quickly as possible using a sterile plate spreader. A further three plates were inoculated with a reference culture of *Staphylococcus aureus* to act as a positive control. The plates were allowed to dry with lids on for about 15 minutes at laboratory temperature before being inverted and incubated at 37 ± 2 °C for 24 ± 2 hours. After this time plates were removed

and assessed for growth, with typical colonies being yellow on a yellow background and atypical colonies being pink on a pink background.

2. *Escherichia coli* and other coliforms

The presence of *E. coli* was assessed by plating 1.0 mL of the stock suspension onto each of nine TBX plates, prepared according to the manufacturer's instructions. A further three plates were inoculated with a reference culture of *E. coli* to act as a positive control. The plates were allowed to dry with lids on for about 15 minutes at laboratory temperature before being inverted and incubated at 37 ± 2 °C for 48 hours. After this time plates were removed and assessed for growth, with positive colonies being blue-green.

For other coliforms, 1.0 mL of the stock suspension was plated onto the selective and differential media HiChrome Coliform Agar, prepared according to the manufacturer's instructions. A further three plates were inoculated with a reference culture of *E. coli* to act as a positive control. The plates were allowed to dry with lids on for about 15 minutes at laboratory temperature before being inverted and incubated at 37 ± 2 °C for 48 hours. After this time plates were removed and assessed for growth, with *E. coli* forming dark blue/violet colonies and other coliforms forming salmon/red colonies.

3. *Salmonella*

Two approaches were used to detect *Salmonella* contamination. In the first, 1.0 mL of the initial suspension was inoculated onto the surface of each of nine SSA plates and spread as quickly as possible over the surface using a sterile plate spreader. A further three plates were inoculated with a reference culture of *Salmonella typhimurium* to act as a positive control. The plates were allowed to dry with lids on for about 15 minutes at laboratory temperature before being inverted and incubated at 37 ± 2 °C for 48 hours. After this time plates were removed and assessed for growth, with typical colonies being colourless with black centres.

In the second approach, 1.0 mL of the initial suspension was inoculated onto the surface of each of nine HEA plates and spread as quickly as possible over the surface using a sterile plate spreader. A further three plates were inoculated with a reference culture of *Salmonella typhimurium* to act as a positive control. The plates were allowed to dry with lids on for about 15 minutes at laboratory temperature before being inverted and incubated at 37 ± 2 °C for 24 hours. After this time plates were removed and assessed for growth, with typical colonies being blue-green, sometimes with black centres.

4. *Vibrio*

The flasks of stock solution were incubated at 35 ± 2 °C for 7 ± 1 hours, after which a 3 mm loop from the surface pellicle of the culture was transferred to the surface of nine dried HiChrome Vibrio agar plates and streaked in a manner that would yield isolated colonies. No positive control was included due to inherent health risk to lab personnel. Plates were incubated for 18 - 24 hours at 35 ± 2°C. After this time plates were removed and assessed for growth, with typical colonies being blue-green to purple.

5. *Shigella*

25 g of test item was weighed into 225 mL of *Shigella* broth, to which 0.5 µg/ mL novobiocin had been added following autoclaving. The suspension was held for 10 ± 2 minutes at room temperature and shaken periodically. The supernatant was poured into a sterile 500 mL Erlenmeyer flask and the pH adjusted to 7.0 ± 0.2 with sterile 1 N NaOH.

The flasks were placed in a gas bag containing an AnaeroGen sachet to provide an anaerobic atmosphere and incubated at 44 ± 2 °C for 20 ± 2 hours, after which the enrichment culture suspension was agitated and streaked onto nine SSA and nine HEA plates. No positive control was included due to inherent health risk to lab personnel. Plates were incubated for 20 ± 2 hours at 35 ± 2 °C. After this time plates were removed and assessed for growth, with typical colonies being transparent on both media.

6. *Anaerobic bacteria*

1.0 mL of the initial suspension was inoculated onto the surface of each of nine TSA plates and spread as quickly as possible over the surface using a sterile plate spreader. A further three plates were inoculated with a reference culture of the obligate anaerobe *Bacteroides fragilis* in thioglycollate broth culture to act as a positive control. The plates were immediately transferred to a gas bag containing an AnaeroGen sachet to provide an anaerobic atmosphere and incubated at 28 ± 2 °C for 72 hours.

*8. Yeast and mould*

Approximately 1g of each batch was weighed out accurately and added to 99 ml of sterile (autoclaved) phosphate buffered saline. After vigorous shaking, 1ml of the suspension was plated onto each of nine Yeast and Mould agar plates. Positive controls were prepared by inoculating three plates with a reference culture of *Candida albicans* ATCC 10231 and a further three plates with a reference culture of *Aspergillus brasiliensis* ATCC 16404. Plates were incubated at 26 °C and read after approximately 64 hours.

### B.5.1.1.6 Methods for the determination of relevant impurities in the manufactured material

**Report:** Cheng, KM (2013), Assay to determine Beauvericin within *Beauveria bassiana* concentrate with associated validation. Oxford Analytical Ltd, A3, Telford Road, Bicester, Oxfordshire OX26 4LD. Study Number OA02266.

**Guidelines:** SANCO/3030/99 rev 4.

**GLP:** Yes

**Material and Methods**

Approximately 1000 mg of sample was accurately weighed into a 50 mL volumetric flask, made to volume with acetonitrile and sonicated for 5 minutes. The samples was allowed to cool and then

centrifuged at 3000 rpm for 5 minutes. The sample was then filtered through a Nylon 0.45 µm syringe filter. The resulting solution was analysed by LC/MS. Four ions were monitored using an MSD detector (784.5, 801.5, 806.5, 843.5 m/z). Samples were quantified against a primary standard of Beauvericin (Batch No. BCBG3253V, purity 99.0 %).

The linearity of the method was determined over the range 0.002 to 0.2 µg/mL beauvericin.

The precision of the method was determined by injecting a 0.1 µg/mL beauvericin standard into the LC/MS system six times and the sample precision was determined by injecting a spiked sample six times into the system.

One sample was spiked with 5 mL of a 1 µg/mL beauvericin standard (equivalent to ~ 5 mg/kg beauvericin in the sample) and then prepared according to the procedure above. This sample was analysed by 5 separate injections into the LC/MS system to determine the accuracy of the method.

The low level accuracy of the method was determined by spiking a sample with 0.5 mg/kg beauvericin (equivalent to ~ 0.5 mg/kg beauvericin in the sample).

**Results**

A summary of the validation results for the determination of beauvericin in *Beauveria bassiana* concentrate is shown in Table 4.1/03-1.

**Table 4.1.1/01-1: Summary of validation results for the determination of Beauvericin in *Beauveria bassiana* concentrate**

| **Criteria** | **Beauvericin** |
|---|---|
| Specificity<br>a) retention time match<br>b) interference | <br>RT times match<br>No interferences noted |
| Confirmation of analyte identify<br>a) molecular mass | <br>Matches the reference standard |
| Linearity | R = 0.99998, n = 2 x 5 (5 concentrations in duplicate) |
| IP-Standard | %RSD = 0.45, n = 6 |
| IP-Sample | %RSD = 2.33, n = 6 |
| Accuracy (5 mg/kg) | %RSD = 1.63, n = 5<br>Range 97 - 101 %, Mean = 98.6% |
| Low Accuracy (0.5 mg/kg) | %RSD = 1.94, n = 5<br>Range 104 – 109 %, Mean = 106% |
| Precision (repeatability) | Injection of the Beauvericin standard: %RSD: 0.45 (n=6)<br>Injection of a spiked sample: %RSD: 2.33 (n=6)<br>Injection of (unspiked) Beauveria sample solutions: %RSD: - (not detected) |
| LOD | 0.01 mg/kg |

| | |
|---|---|
| LOQ | 0.5 mg/kg |

**Conclusions**

This method is considered acceptable for the determination of beauvericin in *Beauveria bassiana* concentrate.

**B.5.1.1.7 Methods to control the absence and to quantify (with appropriate limits of determination) the possible presence of any human and mammalian pathogen**

See the methods described in B.5.1.1.5.

**B.5.1.1.8 Methods to determine storage stability, shelf-life of the micro-organism, if appropriate**

See the method described in B.5.1.1.5

**B.5.2 Methods for the analysis of the preparation (Annex IIIM 5)**

**B.5.2.1 Methods for the identification and the determination of the content of the micro-organism(s) in the preparation**

The methods to determine the content of the micro-organism in the MCPP is included below. Results from the 5-batch analysis from the same reference (Whittaker 2014c) is included in Volume 4.

Total spore count:

1.0 mL of each batch was pipetted into a weighing boat and 1.0 mL of G-49 surfactant added, mixing to form a homogeneous paste. The paste was washed into a 100 mL volumetric flask and made up to the mark with deionised water. The spore suspension was sonicated for 5 minutes and then shaken well to ensure the spores were evenly dispersed. A haemocytometer was set up and filled with the spore suspension by capillary action, and then left to stand for 25-30 minutes.

The number of spores were recorded and the average of the total counts calculated. The total spore count per mL of the sample was calculated.

The method was validated by carrying out the procedures above five times sequentially on batch P1373 to demonstrate the suitability of the test method for enumerating the test item.

The results of the validation of the method for total spore counts are shown in Table B.5.2.1-1. There was no significant difference between the enumerations (ANOVA, p = 0.05, ns) and the method was deemed suitable for the detection and enumeration of the test item in the MPCP. The validation of this method demonstrates that the method was appropriate for the analysis of *Beauveria bassiana* in the MPCP, and by extrapolation with the production batches of the MPCA.

**Table B.5.2.1-1: Results of method validation for total spore count of *Beauveria bassiana* in BROADBAND MPCP**

| Square | Haemocytometer counts | | | | |
|---|---|---|---|---|---|
| | **Rep 1** | **Rep 2** | **Rep 3** | **Rep 4** | **Rep 5** |
| A1 | 298 | 316 | 288 | 291 | 352 |
| A2 | 297 | 300 | 293 | 296 | 308 |
| A3 | 312 | 297 | 233 | 280 | 302 |
| A4 | 302 | 341 | 266 | 336 | 314 |
| A5 | 328 | 298 | 304 | 313 | 298 |
| B1 | 315 | 237 | 278 | 333 | 325 |
| B2 | 299 | 218 | 297 | 361 | 308 |
| B3 | 354 | 255 | 362 | 357 | 306 |
| B4 | 316 | 312 | 330 | 344 | 362 |
| B5 | 329 | 326 | 272 | 312 | 308 |
| **Mean** | **315.00** | **290.00** | **292.30** | **322.30** | **318.00** |
| **SEM** | **5.71** | **12.69** | **11.20** | **8.90** | **6.98** |
| **rSEM** | **1.81** | **4.38** | **3.83** | **2.76** | **2.20** |

Germination test:

1.0 mL of each bath was pipetted into 99.0 mL sterile phosphate buffered saline (PBS), sonicated for 3 minutes and allowed to stand for 30-60 minutes. 0.1 v of the diluted sample was plated onto each of three Rose Bengal agar plates. Plates were incubated for 18 hours at 28 °C.

Following incubation, a 2 cm square was cut out of the agar and placed onto a glass microscope slide. A drop of immersion oil was placed directly onto the agar and allowed to settle for 5-10 minutes before colonies were counted at 1000x magnification. Spores were considered germinated when the germ tube equalled the spore diameter. Both germinated and non-germinated spores were counted until the tally of both reached 300. Percentage viability was calculated as follows:

% viability = (G × 100)/ (G+N)

Where: G = germinated spores

N = non-germinated spores

The mean spore count was 7.1 x $10^9$ CFU/ml with a relative SEM of 1.7%.

### B.5.2.2 Methods to establish regular control of the preparation to show that it does not contain other organisms than the indicated ones and to establish uniform

During quality control, a control on the content of the micro-organism is conducted. Microbial contaminants are also checked (see B.5.2.3).

#### B.5.2.3 Methods to identify any contaminating micro-organisms of the preparation

The methods to detect potential contaminating micro-organisms in the MCPP is included below. Results from the 5-batch analysis from the same reference (Whittaker 2014c) is included in Volume 4.

Methods to detect contaminating micro-organisms:

For all contaminants with detection limits stated in 25 mL, a stock suspension was prepared by adding at least 25 mL of each batch of MPCP to 225 mL of sterile PBS. Positive controls with reference cultures were included to show the suitability of the test system for detection of these contaminants.

*1. Yeasts and moulds*

1.0 mL of the stock suspension was inoculated onto each of nine Yeast and Mould Agar (YMA) plates. A further six plates were inoculated with reference cultures of *Candida albicans* (three plates) and *Aspergillus brasiliensis* (three plates). Plates were incubated at approximately 26 °C for approximately 48 hours and then observed for growth.

2. *Listeria*

1.0 mL of the stock suspension was inoculated onto each of nine Listeria Selective Agar (LSA) plates. A further three plates were inoculated with a reference culture of *Listeria monocytogenes*. Plates were incubated at 37 °C for approximately 48 hours and then observed for growth.

3. *Staphylococcus aureus*

1.0 mL of the stock suspension was inoculated onto each of nine CHROMAgar Staph aureus (CASA) plates. A further three plates were inoculated with a reference culture *of Staphylococcus aureus*. Plates were incubated at approximately 37 °C for approximately 24 hours and then observed for growth.

4. *Escherichia coli* and other coliforms

A preliminary screen was conducted with a Colilert-24 test kit using a reference culture of *E. coli* as a positive control. The contents of a Colilert test vial and 1.0 g of test substance were added to 100 mL of deionised water in a sterile non-fluorescing vessel, capped, shaken and then incubated at 35 +/- 1.0°C for 24 hours, after which vessels were examined under UV light. Yellow fluorescence indicates a positive result.

The presence of *E. coli* was assessed by plating 1.0 mL of the stock suspension onto each of nine CHROMAgar E. coli (CAEC) plates. A further three plates were inoculated with a reference culture of *E. coli*. Plates were incubated at approximately 37 °C for approximately 24 hours and then observed for growth.

5. *Salmonella*

1.0 mL of the stock suspension was inoculated onto each of nine CHROMAgar Salmonella Plus (CASPA) plates. A further three plates were inoculated with a reference culture of *Salmonella bongori*.

Plates were incubated at approximately 37 °C for approximately 24 hours and then observed for growth.

6. *Vibrio*

Approximately 25 mL of each batch of test item was weighed into a sterile flask containing 225 mL of alkaline peptone water. The flask was thoroughly mixed for approximately 2 minutes and then incubated at 37 °C for 7 hours. A 3 mm loop from the surface pellicle was streaked onto each of nine CHROMAgar Vibrio (CAVIB) plates. A further three plates were inoculated with a reference culture of *Vibrio alginolyticus*. Plates were incubated at approximately 37 °C for approximately 24 hours and then observed for growth.

7. *Shigella*

25 mL of test item was weighed into 225 mL of *Shigella* broth, prepared according to the manufacturer's instructions, to which 0.5 µg/ mL novobiocin had been added following autoclaving. The suspension was held for 10 +/- 2 minutes at room temperature and shaken periodically. The supernatant was poured into a sterile 500 mL Erlenmeyer flask and the pH adjusted to 7.0 +/- 0.2 with sterile 1 N NaOH.

The flasks were placed in a gas bag containing an AnaeroGen sachet to provide an anaerobic atmosphere and incubated at approximately 44 °C for approximately 18 hours, after which the enrichment culture suspension was agitated and streaked onto nine Hektoen Enteric Agar (HEA) plates. A further three plates were inoculated with a reference culture of *Shigella boydii*. Plates were incubated at approximately 37 °C for approximately 22 hours and then observed for growth.

8. *Enterobacter*

1.0 mL of the stock suspension was inoculated onto each of nine Violet Red Bile Glucose Agar (VRBGA) plates. A further three plates were inoculated with a reference culture of *Enterobacter cloacae*. Plates were incubated at approximately 37 °C for approximately 24 hours and then observed for growth.

*9. Anaerobic plate count*

1.0 mL of the stock suspension was inoculated onto each of nine Anaerobe Basal Agar (ABA) plates. A further three plates were inoculated with a reference culture of *Bacteroides fragilis*. Plates were placed into a gas bag containing an AnaeroGen sachet to maintain a high CO2 environment and then incubated at approximately 37 °C for approximately 24 hours and then observed for growth.

#### B.5.2.4 Methods used to determine the storage stability and shelf life of the preparation

See the method described in B.5.2.1.

### B.5.3 Methods to determine and quantify residues (viable or non-viable) of the micro-organism as manufactured and for the analysis of the preparation (Annex IIM 4.5 and IIIM 5.4)

#### B.5.3.1 Methods to determine and quantify residues (viable or non-viable) of the micro-organism

##### B.5.3.1.1 The active micro-organism(s) on and/or in crop, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant

Not relevant, no method required as no maximum residue level has been set.

##### B.5.3.1.2 Relevant metabolites (especially toxins) on and/or in crop, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant

Not relevant, no method required.

##### B.5.3.1.3 Methods to determine and quantify residues (viable or non-viable) of the micro-organism for the analysis of the preparation

Not relevant, no method required.

### B.5.4 References relied on

| Data point | Author(s) | Year | Title<br>Company Report No.<br>Source (where different from company)<br>GLP or GEP status<br>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 4.1/01<br><br>MA 1.4.2/01 | Whittaker, M | 2014 | Detection and enumeration of *Beauveria bassiana* and microbial contaminants in five production batches of PPRI 5339 technical grade active ingredient. APIS, Knaresborough Technology Park, Manse Lane, Knaresborough, North Yorkshire HG5 8LF. Study Number APIS-BASF-003. | N | Y | New strain specific GLP study | BASF |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | GLP<br><br>Unpublished | | | | |
| MP 4.1/02 | Whittaker, M | 2014 | Detection and enumeration or *Beauveria bassiana* and microbial contaminants in five production batches of BROADBAND microbial pest control product. APIS, Knaresborough Technology Park, Manse Lane, Knaresborough, North Yorkshire HG5 8LF. Study Number APIS-BASF-011.<br><br>Report date: 17.09.2014<br><br>GLP<br><br>Unpublished | N | Y | Strain specific new laboratory study | BASF |
| MA 4.1/03<br><br>MA 1.3/01 | Berry, S | 2012 | Molecular Identify of the South African *Beauveria bassiana* isolate, strain PPRI 5339. Becker Underwood, Mahogany Ridge, KZN, South Africa and Becker Underwood, NSW 2250, Australia.<br><br>Report Date: 28.11.12<br><br>Non GLP<br><br>Unpublished | N | Y | New strain specific GLP study | BASF |
| MA 4.1.1/01<br><br>MA 1.4/01 | Cheng, KM | 2013 | Assay to determine Beauvericin within *Beauveria bassiana* concentrate with associated validation. Oxford Analytical Ltd, A3, Telford Road, Bicester, Oxfordshire OX26 4LD, UK.<br><br>Study Number OA02266.<br><br>GLP<br><br>Unpublished | N | Y | New strain specific GLP study | BASF |
| **Annex III Data and Information** | | | | | | | |
| MP 5.1/01 | Whittaker, M | 2014 | Detection and enumeration or *Beauveria bassiana* and microbial contaminants in five production batches of BROADBAND microbial pest control product. APIS, Knaresborough Technology Park, Manse Lane, Knaresborough, North Yorkshire HG5 8LF. Study Number APIS-BASF- | N | Y | Strain specific new laboratory study | BASF |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | 011.<br><br>Report date: 17.09.2014<br><br>GLP<br><br>Unpublished | | | | |
| | | | | | | | |
| | | | | | | | |