European Commission

Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV)

Product data: SPEXIT

Volume 3 – Annex B.3 Data on application and efficacy of the Plant Protection Product

Rapporteur Member State: Spain

April 2020

Version History

When	What
18/09/2018	Completeness check report of the dossier submitted by the notifier
December 2019	DAR submitted to the Notifier for commenting
February 2020	DAR updated with notifier comments
April 2020	DAR updated after EFSA completeness check

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INTRODUCTION

The company Andermatt Biocontrol GmbH submits the current dossier for the approval of the baculovirus (BV) *Spodoptera exigua* multi nucleopolyhedrovirus (SeMNPV) as a new microbial pest control agent (MPCA) and SPEXIT as its reference microbial pest control product (MPCP) to the European Authorities.

BVs used as MPCA in the EU are regulated as microorganism according to Regulation 1107/2009[1]. Data requirements for the registration of BVs as an active substance and their products are laid down in part B of the regulation documents 283/2013 [2]and 284/2013 [3]and the principles for evaluation and authorization of plant protection products contained microorganism according to regulation 546/2011[4].

BV isolates however, represent a unique case in which the wild type isolates are genetically heterogeneous (mixture of different genotypes or pool of isolates). These variations may influence in some biological properties, such as the virulence, but it has no consequences on the safety towards non-target organisms or the environment. Isolation of a single genotype is difficult and even not appropriated, since genetic variation is needed to account for variation in the target organisms and obtain better efficacy in the control of insect populations. Therefore, the BVs were not necessary evaluated at strain level (Sanco/0253/2008). [5]The high similarity between BVs justifies a general assessment at the level of the family *Baculoviridae*, considering species-specific information where necessary. The proposed procedure to include BVs at species level was adopted by the member states and the European Regulatory Authorities already in 2007, when the first BV species was included in Annex I, and for the REBECA proposal 2008[6], for a simplified inclusion of BVs on the species level into Annex I. Most of the formally required data are published and equal for all BVs, already assessed by MS and EU authorities and therefore, some data on the isolate or species level are not mandatory.

The BVs are included on species level in Annex I of directive 1107/2009 and the different pool of isolates were added after they have been evaluated to a separate list, to be maintained in the Review Report and to be amended by taking note in the Standing Committee (Sanco/0253/2008). This approach has been confirmed by a decision in the Standing Committee on May 15, 2007[7] were *S. exigua* NPV was listed at species level in Annex I. The experience that BVs present no risk for the environment have been confirmed by numerous studies during the last fifty years, since their first use as biocontrol agents. With regard to safety considerations, it is important to note that the whole *Baculoviridae* family are naturally present in our environment and are closely associated with their host occurrence. Therefore, their application in pest control would only produce a non-permanent fluctuation of the virus titre in the biotope of the pest insect. Due to their host specificity, BVs do not affect other organisms like vertebrates, arthropods other than their host species, microorganisms, or plants. BVs do not produce any metabolites at all.

For the BV specie *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) a DAR with a reference isolate (Florida isolate SeNPV-F1, the first applied for) was approved in 2006 and the isolate SeNPV-F1 was listed on Annex I.

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[1]*Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union L 309, 1-50.*

[2] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union L 93, 1-84.

[3]*Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. Official Journal of the European Union L 93, 85-152.*

[4]*Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. Official Journal of the European Union L155, 127-175.*

[5]*SANCO/0253/2008 rev. 2, 22 January 2008. Guidance Document on the assessment of new isolates of baculovirus species already included in Annex I of Council Directive 91/414/EEC.*

[6]*Ehlers RU., 2011 Regulation of Biological Control Agents and the EU Policy Support Action REBECA. In: Ehlers RU. (eds) Regulation of Biological Control Agents. Springer, Dordrecht.*

[7]*Review report for the active substance Spodoptera exigua nuclear polyhedrosis virus. Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 15 May 2007 in view of the inclusion of Spodoptera exigua nuclear polyhedrosis virus in Annex I of Directive 91/414/EEC. Spodoptera exigua NPV SANCO/T14/2007 - rev. final1 12 March 2007.*

Two new more isolates were further applied for at Member State level: the SeMNPV-SP2, approved in 2008 and the SeNPV-BV0004, approved in 2010. Conversely, the current dossier was based on the data already assessed by the MS and EU authorities:

- The previous DAR document for the approval of a new active substance SeNPV-F1 submitted by Mitsui Agri Science International S.A and evaluated by The Netherlands in 2007.
- The evaluation report of the new isolate of SeMNPV, BV0004 previously submitted by the company Andermatt Biocontrol GmbH and evaluated by the Netherlans in 2010.

Active substances are approved for maximum period of 10 years under Directive 91/414/EEC[8]. The active substance SeMNPV was under programme of renewal Regulation EU 686/2012 (AIR-III programme[9]). According to draft working document AIR III renewal programme SANCO/2012/11284[10], *Spodoptera exigua* nuclear polyhedrosis virus was included in Batch 9" Active substance *Spodoptera exigua* nuclear polyhedrosis virus No application for renewal of approval has been submitted. Previous expiry date 30/11/2017"

Commission implementing regulation (EU) No 844/2012[11] setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 establishes in its Art 1: "the application for the renewal of an approval of an active substance shall be submitted by a producer of the active substance to the rapporteur Member State, no later than three years before the expiry of the approval"

The application for the renewal of the active substance *Spodoptera exigua* nuclear polyhedrosis virus was not submitted before of three years before the expiry date of the approval of the active substance SeMNPV (30/11/2017).

The applicant then have submitted an application for SeMNPV as a new active substance.

In this RAR, the information submitted regarding *Spodoptera exigua* multicapside nucleopopyhedrovirus (SeMNPV) is evaluated as new active substance, therefore, all information is considered and evaluated as new.

Literature reference included by the applicant comes from a literature search according to EFSA (2011) [12]in order to identify relevant recent published peer reviewed references covering the last 10 years. The RMS has also included relevant studies considered important to support the application for the approval of *Spodoptera exigua* multipolyhedrivirus (SeMNPV) genotype pool BV-0004 and the microbial product SPEXIT.

[8] *Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230 of 19.8.1991.C.*
[9] *Programme of renewal Regulation EU 686/2012 (AIR-III programme).*
[10] *SANCO/2012/11284 –rev. 22, December 2018. Draft working document AIR III renewal programme.*
[11] *Commission implementing regulation (EU) No 844/2012, of 18 September 2012. Setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.*
[12] *Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092.*

PPP (product name/code):	SPEXIT	Formulation type:	SC
Active Substance: (SeMNPV)	*Spodoptera exigua* multicapsid nucleopolyhedrovirus	Conc. of a.s.:	3.75×10^{12} OBs/L
Applicant:	Andermatt Biocontrol GmbH	professional use	☒
Zone(s):	EU	non professional use	☒
Safener:	n.a.	Conc. of safener:	n.a.
Synergist:	n.a.	Conc. of synergist:	n.a.
Verified by RMS:	yes		

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
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**	**OBs/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		**e.g. g safener/synergist per ha**
1	EU	Pepper (CPSAN)	F/G	*S. exigua* (LAPHEG)	Spray	At infestation (preferably on early larva instar: L1 and L2). First treatment just before hatching)	a) 18 (6) b) 18 (6)	a) 0.2 b) 3.6	a) 7.5×10^{11} b) 1.35×10^{13}	200 / 1600	-	- 2 to 3 applications per pest generation, up to 6 generations (i.e. max. of 18 app.). -Interval between applications: min. of 6 sunny days; 2 partially sunny days = 1 sunny day
2	EU	Leafy vegetables (lettuce crops) (3LETC)	F/G	*S. exigua* (LAPHEG)	Spray	At infestation (preferably on early larva instar: L1 and L2). First treatment just before hatching)	a) 18 (6) b) 18 (6)	a) 0.2 b) 3.6	a) 7.5×10^{11} b) 1.35×10^{13}	200 / 1600	-	- 2 to 3 applications per pest generation, up to 6 generations. -Interval between applications: min. of 6 sunny days; 2 partially sunny days = 1 sunny day

Table MP B.4.1. Summary of critical Good Agricultural Praxis for SPEXIT

n.a. Not applicable

B.3 DATA ON APPLICATION AND EFFICACY

With regard to safety considerations, it is important to note that SeMNPV and the whole group of BVs are naturally present in the environment. SeMNPV is a naturally occurring virus worldwide and acts highly specific against larvae of the beet armyworm, *Spodoptera exigua*.

The SeMNPV strain in use was originally isolated in China (Jianfeng, 2005). It is supposed to have no harmful effects on organisms not belonging to the genus *Spodoptera*.

SeMNPV does not produce antibiotics and secondary metabolites of toxicological and/or environmental, or ecotoxicological concern. Neither SeMNPV active ingredient produce nor the end-use product (SPEXIT) contains chemical compounds of critical toxicological, environmental, or ecotoxicological concern. The same would be applied for the semi-synthetic insect diet, which is basedon sugar, wheat germ and dry yeast.

The representative products of three different SeMNPV isolates (SPOD-X in 2006, VIR-EX in 2008 and SPEXIT in 2010, have all been authorised at Member State level for more than six years and have therefore been assessed in line with “Uniform Principles”. Although the different SeMNPV isolates and its representative products have all been authorised at Member State level, according to the article 4(3) of Regulation (EC) No 1107/2009, a PPP shall meet the following requirements (among other requirements): (a) it shall be sufficiently effective, (c) it shall not have any unacceptable effects on plants or plant products.

In the evaluation of the documents it was taken in consideration that t**he representative product SPEXIT and its microbial pest control agent *Spodoptera exigua* nuclueopolyedrovirus isolate BV0004, have been previously authorised at Member State level since 2010 until the end of the approval period in 2017 and have therefore been assessed in line with Uniform Principles”.**

PPP (product name/code):	SPEXIT	Formulation type:	SC
Active Substance: (SeMNPV)	*Spodoptera exigua* multicapsid nucleopolyhedrovirus	Conc. of a.s.:	3.75×10^{12} OBs/L

1	2	3	4	5	6	7	8	9	10	11	12	13
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**	**OBs/ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min / max**		**e.g. g safener/synergist per ha**
1	EU	Pepper (CPSAN)	F/G	*S. exigua* (LAPHEG)	Spray	At infestation (preferably on early larva instar: L1 and L2). First treatment just before hatching)	a) 18 (6) b) 18 (6)	a) 0.2 b) 3.6	a) 7.5×10^{11} b) 1.35×10^{13}	200 / 1600	-	- 2 to 3 applications per pest generation, up to 6 generations (i.e. max. of 18 app.). -Interval between applications: min. of 6 sunny days; 2 partially sunny days = 1 sunny day
2	EU	Leafy vegetables (lettuce crops) (3LETC)	F/G	*S. exigua* (LAPHEG)	Spray	At infestation (preferably on early larva instar: L1 and L2). First treatment just before hatching)	a) 18 (6) b) 18 (6)	a) 0.2 b) 3.6	a) 7.5×10^{11} b) 1.35×10^{13}	200 / 1600	-	- 2 to 3 applications per pest generation, up to 6 generations. -Interval between applications: min. of 6 sunny days; 2 partially sunny days = 1 sunny day

Table MP B.3.1. Summary of critical Good Agricultural Praxis for SPEXIT

Applicant:	Andermatt Biocontrol GmbH	professional use	☒
Zone(s):	EU	non professional use	☒
Safener:	n.a.	Conc. of safener:	n.a.
Synergist:	n.a.	Conc. of synergist:	n.a.
Verified by RMS:	yes		

n.a. Not applicable

B.3.1 FIELD OF USE ENVISAGED

Biological insecticide for the control of the Lepidoptera *S. exigua* in horticulture crops products based on *S. exigua* multipolyhedrovirus **(table MP B.3.1).**

B.3.1.1 Function

S. exigua Nucleopolyhedrovirus (SeMNPV) is used in plant protection products to control the larvae of the armyworm, *S. exigua*. It acts as a highly specific insecticide on vegetables crops.

B.3.1.2 Crops or products protected or treated

SeMNPV is intended to be used in vegetables against larvae of the armyworm, *S. exigua.*

B.3.1.3 Method of production and quality control

See volume 4 Annex C, confidential information.

B.3.1.4 Methods to prevent loss of virulence of seed stock of the microorganism

See volume 4 Annex C confidential document.

B.3.1.5 Recommended methods and precautions concerning handling, storage, transport or fire

- **Handling of SPEXIT** does not require special precautionary measures or protective clothing, as indicated by the submitted toxicological studies (see Volume 3 Annex MP B.2.3- 5). Further, Frommer *et al.*, 1989 relate the different risk classes according to European classification of microorganisms to adequate safety precautions for biotechnology. In this review SeMNPV like other BVs is not listed.

The following general precautionary measures in handling are stated on the Safety Data Sheet (K MP3.9/01):

Recommendations for use

Shake before use!

Tank Mix:

SPEXIT® can be mixed with wettable sulphur and most commonly used fungicides and insecticides. Exempted are strongly acid or alkaline substances (pH <5 and >8.5) and copper products. Always add SPEXIT® last to the tank and do not expose it to concentrated products.

Storage and shelf life:

SPEXIT® can be stored in the refrigerator (< 5°C) for two years. At -18°C, SPEXIT® can be kept for years without any loss of activity. When frozen, SPEXIT stays liquid and is always ready to use.

First aid measures and toxicological information

Keep locked out of the reach of Children

Avoid each unnecessary contact with the product. No typical symptoms and effects known. In all cases of doubt, seek medical attention. Inhalation: This is only possible by exposure to HOT product. Move to fresh air, rest, half upright position, loosen clothing. Oxygen or artificial respiration if there is difficulty in breathing. Seek medical advice after significant exposure. Symptomatic treatment is advised. Skin: Remove contaminated clothing. Seek medical advice if irritation develops. Wash clothes before reuse. After contact with skin, wash immediately with plenty of water. Eye: Rinse thoroughly with plenty of water. Eyelids should be hold away from the eyeball to ensure thorough rinsing. Seek medical advice if irritation develops. Ingestion: No typical symptoms and affects known.

1. To avoid any direct contact with the product.
2. To wash hands after contamination with the product.
3. To keep (remaining) product away from waters.

- Storage conditions of SPEXIT: No specific recommendations.

1. Advises for Storage with other Products: None
2. Other Information on Storage: To maintain quality: Store below 0°C. Avoid elevated temperatures.
3. Store Classification: 11

- Transport conditions of SPEXIT: No restrictions regarding transport on land or sea.

- **Fire precaution of SPEXIT:** In case of fire the following information and instructions are given:

1. Extinguishing media: Water mist, alcohol resistant foam, carbon dioxide, dry powder
2. Measures unsuitable for safety reasons: Water-jet, foam
3. Special hazard properties (products or vapours from thermal combustion): Vapours cause coughing
4. Protective equipment: No specific recommendations
5. Cleaning/disposal: Spray remaining product dispersed in liquid manure. Small amounts can be added to compost.
6. Other advices: Avoid contact with oxidizing agents. Cool closed containers with water.

The standard protection measures for workers are adequately protecting their health in case SPEXIT containing material is released or spilled accidentally at the manufacturing facilities.
The contaminated area may be cleaned by sweeping up spill, and spillage can be safely disposed off in accordance with all applicable federal, state, and local environmental regulations.

B.3.1.8 Procedures for destruction or decontamination

With regard to destruction and decontamination the following accidental release measures and general information and recommendations are given in the Safety Data Sheet (K MP3.9/01):

1. Decomposition/ persistence: Decomposition is achieved by thermal combustion at 600°C without any residues or hazardous products.
2. Special advice for humans and environment: Keep (remaining) product away from waters. Avoid any contact with the product. Non-hazardous to honeybees due to the selectivity of the product. Non-hazardous to relevant populations of beneficials due to selectivity of the product.
3. Disposal/ Product: Waste resulting from the use of the product must be disposed on site or on an approved waste disposal facility. Empty the sprayer out in the field being treated by spraying out on to a relatively pest free part of the field left unsprayed or under-dosed for the purpose. Do not exceed the maximum dose approved for the crop.
4. Disposal/ Packages: Recommendations: add empty packages to local waste disposal or recycling system.
5. Cleaning Agent: no special cleaning necessary

Specific measures in case of an accident are not required, since SpliNPV is a common BV, and this family imposes no risk for environment or health (see Vol.3, Annex MAB6 and MAB9).

B.3.1.9 Measures in case of an accident

First aid measures

1.	General advice:	In all cases of doubt, or if symptoms persist, seek medical attention.
2.	Skin contact:	Remove contaminated clothing. Seek medical advice if irritation develops. Launder clothes before reuse. After contact with skin, wash immediately with plenty of water.
3.	Eye contact:	Rinse thoroughly with plenty of water. Eyelids should be hold away from the eyeball to ensure thorough rinsing. Seek medical advice if irritation develops.
4.	Ingestion: as necessary.	No typical symptoms and affects known. Provide symptomatic/supportive care
5.	Inhalation:	Provide symptomatic/ supportive care as necessary.
6.	Other information:	None
7.	Advice to Physician	Symptomatic treatment is advised.

No specific treatment after contact with SPEXIT contaminated material is required since this strain is not infective for humans. As a general precautionary measure in case of direct contact to this virus the applicant states the below listed first aid instructions in the Safety Data Sheet (K MP3.9/01). In addition, persons who may want to seek medical attention upon accidental contact to SPEXIT, should inform the physician about the identity of the virus on species level, and may show the label of the packaging as supporting information.

B.3.2 MODE OF ACTION

Application of SeMNPV should be timed at hatching of larvae so that early-instar larvae come in contact with the virus. The early instar larval stages of the insect life cycle are the most susceptible to infection with BVs (Evans and Harrap, 1982; Martins *et al*., 2005).
Infection only occurs after ingestion of polyhedra by larvae. Gross pathology is characterised by loss of reactivity of larvae to external stimuli and swelling of the larvae, followed by glossy and moribund appearance 4 to 6 days after infection. From day 6 on, larvae begin to die and subsequently liquefy, releasing further NPV that are able to infect other larvae (OECD, 2002).
After oral intake by the larvae, the OB are dissolved in the alkaline mid gut and occlusion-derived virions (ODV) are released. The nucleocapsids attach to the microvilli and enter the midgut epithelium cells. The nucleocapsids are transported to the nucleus and release their DNA, followed by DNA replication and expression. After virus replication in the midgut cells, budded virus are released through the basal lamina into the hemocoel and subsequently spread to other organs, like the fat body, epidermis, tracheal matrix, muscle, nerve, malphigian tubules, and glandular tissues. Finally, OB are formed, leading to hypertrophy of cells and the swollen appearance of larvae at late infection stages.
Mortality was determined for different isolates and genotypes of SeMNPV and LD50 varied between 9.2 and 49 OB/larva for 2nd stage *S. exigua* larvae (Muñoz et al., 1997; Muñoz et al., 1998).

The product SPEXIT acts highly specific against larvae of the beet armyworm, *S. exigua*, as a biological insecticide. *S. exigua* is a polyphagous pest feeding on over 200 different crops amongst which there are: sugar beet, cabbage, lettuce, soybeans, cotton, maize, tomato, potato, legumes, citrus, strawberry, melon, leek, garlic, onion, rice, flax, and tobacco.
SeMNPV is ingested by feeding larvae. OB dissolve in the alkaline midgut and after infection of the midgut epithelium, other tissues are invaded, e.g. fat body, epidermis, and tracheal matrix as well as the Malpighian tubules. Rapid virus multiplication within the host cells finally results in cell destruction and at the end leads to lysis of the whole organism. The microorganism is not translocated to any part of the plant.

B.3.3 DETAIL OF INTENDED USE

SPEXIT is to be used in a wide range of crops against the beet armyworm. Within this dossier, the use in lettuce and pepper is intended. The details on the intended use of SPEXIT are provided in Table MP B.3.1 above.

.**B.3.4 APPLICATION RATE**

Application of SPEXIT results in an application rate of 1.88 x 10^{11} to 7.5 x 10^{11} OB/ha (200 mL f.p./ha or 12.5 - 100 mL f.p./hL).

The four documents provided by the applicant to support application rate on GAP table were made with the maximum rate per application 0.2L/ha (10cL/hL).

RMS comments:

Has correct the minimum application rate 1.88x10^{11} OB/ha

B3.5 CONTENT OF MICROORGANISM IN MATERIAL USED

SPEXIT (3.75 x 10^{12} OB/L) is applied with 0.05 - 0.2 L product/ha diluted in 200 to 1600 L water/ha. Thus, the concentration of microorganism in the spray solution is between 1.17 x 10^{8} OB/L and 3.75 x 10^{9} OB/L, corresponding to 1.17 x 10^{10} OB/hL and 3.75 x 10^{11} OB/hL.

B.3.6 METHOD OF APPLICATION

The product is applied by tractor drawn spraying equipment, spraying or by knapsack sprayers. The volume of diluent spray varies from 200 L/ha to 1,600 L/ha.

B.3.7 NUMBER AND TIMING OF APPLICATIONS

Spraying should start shortly before first larvae hatch from the eggs or just after hatching on first instar larvae. A second application is recommended after 14 days in the greenhouse or after 8 sunny days (counting 2 partially sunny days as 1 day) in open field. In general, two to three sprayings per generation are sufficient to avoid an infestation of economic importance.

SPEXIT can be mixed with wettable sulphur and conventional fungicides and insecticides, except for strongly acidic or alkaline substances and copper products. The pH has to be between 5 and 8.5.

B.3.8 NECESSARY WAITING PERIODS OR OTHER PRECAUTIONS TO AVOID PHYTOPATHOGENIC EFFECTS ON SUCCEEDING CROPS

Not required. SeMNPV, like all BVs, is highly arthropod-specific and does not harm any plant species.

No limitations exist for the choice of succeeding crops.

No pre-harvest interval is necessary.

B.3.9 PROPOSED INSTRUCTIONS FOR USE

All information is provided in document K MP3.9/01.

Application rate:
SPEXIT® is sprayed with a dosage of 50 to 200 ml per ha, depending on the crop and pest pressure.
Timing: Spray on eggs and 1st instar larvae. Apply with first catches of adult moths in pheromone traps as long as larvae are hatching.
Applications are repeated in intervals of 6-14 days, depending on weather conditions, pest infestation, and overall pest control strategy,
Outdoor: Repeat application of standard dose (200 ml) in intervals of 8 sunny days, two partly sunny days are counted as one sunny day. If you use lower dosage, repeat application in intervals of 6 sunny days.
Greenhouse: Depending on vegetative plant growth and pest pressure, repeat application in intervals of 6 - 14 days. Generally, frequent applications at low rates may be more effective than one or two applications at high rates.
Water volume and application equipment: According to local standard practice. Dissolve product in required amount of water. Use sufficient water to obtain thorough, uniform coverage. Because SPEXIT® acts through ingestion, thorough spray coverage is essential for optimum control of the targeted pest. Using increased water volumes will typically result in better spray coverage. Run-off should be avoided.

B.3.10 EFFICACY DATA

SPEXIT is a highly selective biological insecticide used as spray treatment against larvae of the beet armyworm *S. exigua* since 2007 in Europe. It was authorised in any member state since it was firstly included in 2007. There were three products containing SeMNPV: (1) SPEXIT, contained isolate Bv0004 from China; (2) SPOD-X, contained SeNPV-F1 Florida isolate and (3) VIR-EX, contained isolate SeMNPV-SP2, Spanish isolate. The three of them have been registered in different European countries for the control of armyworm in different crops. A summary of past registrations of the representative use can be found in the following **Table MPB.3.10-01**.

RMS comments: Efficacy data provided by the applicant covers the use in green house for pepper with 3 assays in greenhouse, documents MP3/10.3-01, MP3/10.3-02 and MP3/10.3-03. For open field uses the applicant have included one assay in lettuce, document MP3/10.3-04.

The four efficacy assays for GAP support have been made during last development of the crop period, just before harvest: for paprika, *Capsicum annuum* L at growth stage 7: Development of fruit crop: BBCH 701, when first fruit has reached typical size and form or BBCH 702, 2nd fruit has reached typical size and form (harvest initiation). The BBCH for *Lactuca sativa* L. var. capitata, lettuces, in open field were BBCH 44-45, development of harvestable vegetative plant parts (40% -50% of the expected head size reached).

According to **Table MP B.3.10-01** the product SPEXIT was authorized in EU for greenhouse and open field application in lettuce, pepper, strawberry, herbaceous ornamental, woody ornamental, cucumber and water melon. Other two products contained different SeMNPV isolates were also authorized in EU for in greenhouse and open field application: SPOD-X for ornamental flower and pepper and VIR-EX for pepper.

The provided efficacy assays (3+1) should not be considered enough to fulfil the efficacy data requirement to cover uses according GAP table MP B.3.1.
Compiled results from the four assays showed in table MP B.3.10.2-3 demonstrate consistence and equivalent high effectiveness in the control of the target pest *S. exigua*.
The RMS have also considered previous uses in EU from table MP B.3.10-1 to fully support GAP table and preliminary effectiveness test (Luna-Espino *et al*., 2018; Zamora-Avilés, 2017; Rebolledo *et al*., 2015; Elvira *et al*., 2013; Cabodevilla *et al.,* 2011 and Belda *et al.,* 2000) summarized in table MP B.3.10.1-01. Eventually greenhouse and open field applications of SPEXIT for the control of *S. exigua* in lettuces and peppers are considered robust enough.

Representative use					**Previously authorisations**								
Crop	**Target**	**Situation (e.g. indoor)**	**Formu-lation type and a.s. content**	**Appli-cation method**	**Countr y**	**EU-Zone**	**Since**	**Reg No**	**Produc t**	**Product application rate per treatment (max)**	**Active substance application rate per treatment (max)**	**Number of treatments per season**	**Active substanc e total dose/ha (max)** OBs/ha
Lettuce	*S. exigua*	Field and Greenhouse	SC	Spray	ES	C (S-EU)	27/08/201 5	2559 2	SPEXI T	0.2 L/ha	7.5×10^{11} OBs/ha	3 treatments per pest generation	1.35×10^{13}
Pepper	*S. exigua*	Field and Greenhouse	SC	Spray	ES	C (S-EU)	27/08/201 5	2559 2	SPEXI T	0.2 L/ha	7.5×10^{11} OBs/ha	3 treatments per pest generation	1.35×10^{13}
Strawberry	*S. exigua*	Field and Greenhouse	SC	Spray	ES	C (S-EU)	27/08/201 5	2559 2	SPEXI T	0.2 L/ha	7.5×10^{11} OBs/ha	3 treatments per pest generation	1.35×10^{13}
Herbaceous ornamentals	*S. exigua*	Field and Greenhouse	SC	Spray	ES	C (S-EU)	27/08/201 5	2559 2	SPEXI T	0.2 L/ha	7.5×10^{11} OBs/ha	3 treatments per pest generation	1.35×10^{13}
Woody ornamentals	*S. exigua*	Field and Greenhouse	SC	Spray	ES	C (S-EU)	27/08/201 5	2559 2	SPEXI T	0.2 L/ha	7.5×10^{11} OBs/ha	3 treatments per pest generation	1.35×10^{13}
Cucumber	*S. exigua*	Field and Greenhouse	SC	Spray	ES	C (S-EU)	27/08/201 5	2559 2	SPEXI T	0.2 L/ha	7.5×10^{11} OBs/ha	3 treatments per pest generation	1.35×10^{13}
Watermelon	*S. exigua*	Field and Greenhouse	SC	Spray	ES	C (S-EU)	27/08/201 5	2559 2	SPEXI T	0.2 L/ha	7.5×10^{11} OBs/ha	3 treatments per pest generation	1.35×10^{13}
Ornamental flowers	*S. exigua*	Field and Greenhouse	SC	Spray	ES	C (S-EU)	27/08/201 5	2538 5	SPOD-X	30 L/ha	3×10^{13} OBs/ha	3 applications per campaign	9×10^{13}
Pepper	*S. exigua*	Field and Greenhouse	SC	Spray	ES	C (S-EU)	27/08/201 5	2538 5	SPOD-X	30 L/ha	3×10^{13} OBs/ha	3 applications per campaign	9×10^{13}
Pepper	*S. exigua*	Field and Greenhouse	SL	Spray	ES	C (S-EU)	03/07/201 7	2578 5	VIR-EX	50 L/ha	5×10^{13} OBs/ha	3 applications per campaign	15×10^{13}

Table MP B.3.10-01 Supported representative uses for SeMNPV and their previous authorization status.

B3.10.1 Preliminary tests

RMS comments: There are some information missing on preliminary efficacy test of SeMNPV available in recent open literature summarized in **Table MP B3.10.1-1** that was included and analysed below.

Reference study	Commercial product	Virus strains	Target organism	Crops	efficacy % mortality
Luna-Espino et al., 2018	Spod-X	SeUS2 SeSLP6	*S. exigua*		78% **Table MP B3.10.1-2**
Zamora-Avilés, 2017	SPEXIT, Spod-X VIR-EX	SeUS1 SeUS2 SeSP2 SeMexican	*S. exigua*		50%
Rebolledo et al., 2015	Spod-X	SeUS2	*S. exigua*		82– 93% **Figure MP B3.10.1-1**
Elvira et al., 2013	Vir-ex & SPEXIT		*S. exigua*		25.1% - 31.7% **Table MP B3.10.1-3;** **Figure MP B3.10.1-2**
Cabodevilla et al., 2011		VT-SeA11	*S. exigua*		79.2% **Table MP B3.10.1-4** **Table MP B3.10.1-5**
Belda et al., 2000	Spod-X VIR-EX	SeUS1 SeSP2	*S. exigua*		58.4% -74.7% **Figure MP B3.10.1-3**

Table MP B3.10.1-01 Preliminary studies on SeMNPV efficacy

Reference:	Juan Carlos Luna-Espino, Víctor R. Castrejón-Gómez, Samuel Pineda, José Isaac Figueroa, and Ana Mabel Martínez, 2018. Effect of four multiple nucleopolyhedrovirus isolates on the larval mortality and development of *Spodoptera exigua* (Lepidoptera: Noctuidae): determination of virus production and mean time to death. Florida Entomologist, 101(2):153-159.
Guideline:	N/A
GLP:	No
Summary:	The biological activity of 4 Mexican isolates (SeSIN6, SeSIN8, SeSLP6, and SeSLP8) of the homologous multiple nucleopolyhedrovirus SeMNPV antheir effects on the development of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), were studied. An exotic isolate of SeMNPV from the United States (SeUS2) was used as a reference. Early third-instar larvae were inoculated with 5 × 106 OB per mL, resulting in 78% mortality for SeUS2 and approximately 90% mortality for each of the 4 Mexican isolates at 144 h post-inoculation. At 168 h postinoculation, 100% mortality was obtained in all cases. All of the isolates, including SeUS2, significantly reduced the body weights of *S. exigua* larvae compared with the control larvae; however, at 120 h post-inoculation, the differences among isolates were not significant. All of the isolates reduced the development time of third-instar *S. exigua* larvae (range: 1.2– to 1.6–fold) compared with the control larvae. An independent experiment was performed to determine production of OB and mean time to death. Third-instar *S. exigua* larvae (15 h after molting) were inoculated with each isolate using the same concentration mentioned above. The occlusion body production rate was similar among all isolates. The isolates SeUS2, SeSLP6, and SeSLP8 yielded the fastest mortality (range: 187–191 h). Thus, the biological activities of the Mexican SeMNPV isolates were similar to the activity of the exotic isolate, indicating that these indigenous viruses are promising for the biological control of *S. exigua* in Mexico.

M&M:	Biological activity of four isolates of semnpv and their effects on development. The mortality, average weight, and duration of the third instar for virus-treated larvae were analyzed using one-way ANOVA.
Test substances:	**Antagonist(virus strains):** Four SeMNPV isolates collected from pepper and tomato crops in Mexico (SeSIN6, SeSIN8, SeSLP6, and SeSLP8). The SeUS2 isolate (principal active ingredient of the biological insecticide Spod-X)was used as positive control. **Target***:* Third-instar S. exigua larvae from laboratory using a modified droplet bioassay technique.
Treatments:	Larvae were feed with four different SeNPV isolates (SeSIN6, SeSIN8, SeSLP6, and SeSLP8), a positive control SeUS2, and with no virus. (OBs concentrations 510^6 OB/mL. 12 Groups of molted
Duration:	third-instar larvae. T hird-instar larvae24h, until the larvae consumed the entire plant disc. Days until death (ten days). Larval mortality was determined every 24 h for 7 d (168 h post inoculation)
Results:	- At 120 h post-inoculation, isolates SeSLP8, SeSIN6, and SeSIN8 had caused the highest mortality (range: 77–79%), although the mortality rates were not significantly different from that obtained with the positive control SeUS2 isolate. - The concentration of isolates used in this study (5 10^6 OB/mL for all isolates) resulted in 100% mortality of newly molted third-instar larvae of *S. exigua* at 168 h post-inoculation.

	Larval mortality (%)			
	Hours post-inoculation			
Isolate	72[a]	96[b]	120[c]	144[d]
Control	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
SeUS2	11.1 ± 2.8 c	25.0 ± 8.3 abc	58.3 ± 0.0 bc	77.8 ± 7.34 b
SeSLP6	0.0 ± 0.0 a	8.3 ± 0.0 ab	52.8 ± 11.1 b	88.9 ± 2.8 bc
SeSLP8	0.0 ± 0.0 a	43.7 ± 12.8 c	79.3 ± 8.1 c	94.2 ± 2.9 c
SeSIN6	5.5 ± 2.8 b	44.4 ± 10.0 c	77.8 ± 14.0 c	94.4 ± 2.8 c
SeSIN8	0.0 ± 0.0 a	28.5 ± 7.2 bc	77.3 ± 2.3 bc	94.4 ± 2.8 c

Within the same column, values followed by the same letter are not significantly different from one another (p = 0.05).

[a]*F* = 8.4; df = 5,12; *P* = 0.0001; [b]*F*= 4.54; df = 5,10; *P* = 0.02; [c]*F* = 14.26; df = 5,12; *P* = 0.0001; [d]*F*= 97.38; df = 5,12; *P* = 0.0001.

Table MP B3.10.1-02 Larval mortality (± SE) of third-instar Spodoptera exigua larvae treated with 5 different SeMNPV isolates. (Luna-Espino *et al.,* 2018).

Reference:	Dulce Rebolledo, Rodrigo Lasa, Roger Guevara1, Rosa Murillo, Trevor Williams, 2015. Baculovirus-Induced Climbing Behavior Favors Intraspecific Necrophagy and Efficient Disease Transmission in Spodoptera exigua. PLOS ONE | DOI:10.1371/journal.pone.0136742.
Guideline:	N/A
GLP:	No
Summary:	Shortly prior to death, many species of Lepidoptera infected with nucleopolyhedrovirus climb upwards on the host plant. This results in improved dissemination of viral OB over plant foliage and an increased probability of transmission to healthy conspecific larvae. Following applications of *Spodoptera exigua* multiple nucleopolyhedrovirus for control of Spodoptera exigua on greenhouse-grown sweet pepper crops, necrophagy was observed by healthy S. exigua larvae that fed on virus-killed conspecifics. We examined whether this risky behavior was induced by olfactory or phagostimulant compounds associated with infected cadavers. Laboratory choice tests and olfactometer studies, involving infected and non-infected cadavers placed on spinach leaf discs, revealed no evidence for greater attraction of healthy larvae to virus-killed over non-infected cadavers. Physical contact or feeding on infected cadavers resulted in a very high incidence of transmission (82– 93% lethal disease). Observations on the behavior of *S. exigua* larvae on pepper plants revealed that infected insects died on the uppermost 10% of foliage and closer to the plant stem than healthy conspecifics of the same stage, which we considered clear evidence of baculovirus-induced climbing behavior. Healthy larvae that subsequently foraged on the plant were more frequently observed closer to the infected than the non-infected cadaver. Healthy larvae also encountered and fed on infected cadavers significantly more frequently and more rapidly than larvae that fed on non-infected cadavers. Intraspecific necrophagy on infected cadavers invariably resulted in virus transmission and death of the necrophagous insect. We conclude that, in addition to improving the dissemination of virus particles over plant foliage, baculovirus-induced climbing behavior increases the incidence of intraspecific necrophagy in *S. exigua*, which is the most efficient mechanism of transmission of this lethal pathogen.
M&M:	
Test substances:	Target: Larvae from laboratory colony of *S. exigua,* originally collected from maize fields in Mexico. Plant crop: greenhouse-grown sweet pepper crops
Treatments:	Antagonist (virus strains): SeMNPV OBs from four mexican strain of SeMNPV identical in terms of restriction profiles to SeMNPV-US2 (principal active ingredient of the biological insecticide Spod-X).
Results:	

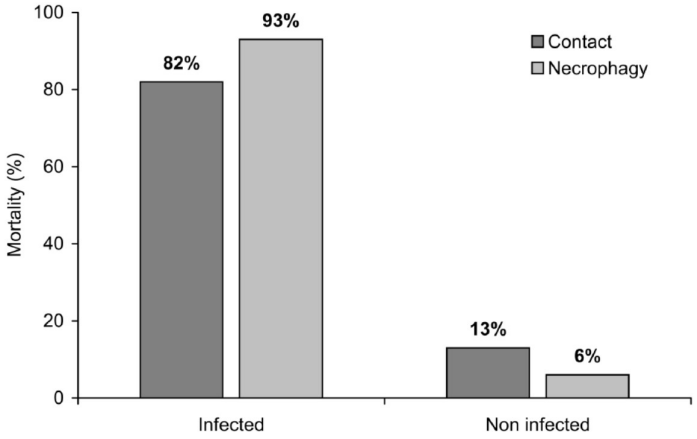


Figure MP B3.10.1-01 Percentage of mortality of S. exigua larvae that fed on (necrophagy) or had

	physical contact with infected and non-infected cadavers (n = 82).

Reference:	Sonia Elvira, M. Angeles Ibargutxi, Noelia Gorria, Delia muñoz, Primitivo caballero, and Trevor Williams, 2013. Insecticidal Characteristics of Two Commercial Spodoptera exigua Nucleopolyhedrovirus Strains Produced on Different Host Colonies. J. Econ. Entomol. 106(1): 50Ð56
Guideline:	N/A
GLP:	No
Summary:	The insecticidal characteristics of two *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) strains produced on two different S. exigua colonies were measured using the same two host colonies. These strains constitute the active ingredients of the biological insecticides Vir-ex and Spexit and were produced on insect colonies from Spain and Switzerland. Demographic characteristics of insects from each colony were examined before infection. Larval developmental time, larval survival, and adult sex ratio did not differ between the colonies, whereas mean pupal weight was significantly higher in the Spanish colony insects. After infection, susceptibility to virus OB (OBs), time to death, larval weight at death, and total production of OBs/larva varied significantly depending on virus strain and the colony used. Vir-ex OBs produced in Spanish colony larvae had improved insecticidal characteristics in terms in lethal dose and speed of kill metrics than other strain-colony combinations. OB production was signifcantly higher in Spanish colony insects infected with Spexit compared with Vir-ex infected insects from the Swiss colony, with intermediate values for the other two strain-colony combinations. Virus strain and host colony origin were highly infuential in determining the insecticidal characteristics of OBs and should be considered as key parameters that require optimization during the production of SeMNPV-based insecticides.
M&M:	- Identification of Viruses Amplified in S. exigua Colonies: OBs amplifed in larvae from each colony were analyzed using BglII. - OB patrhogenecity test and virulence: The median lethal dose (LD50) and mean time to death (MTD) were estimated in second instars from each colony by using the droplet-feeding technique.
Test substances:	**Target (insect colonies)***:* Larvae from 2 laboratory colony of S. exigua, one originally from field collected in Spain Almeria colony) and other from Andermatt Biocontrol AG (Swiss colony) **Plant crop:** greenhouse-grown sweet pepper crops
Treatments:	**Antagonist (virus strains):** SeMNPV OBs from commercial product Vir-ex and SPEXIT Four virus inocula were assayed: the Vir-ex ampliÞed in the Almerian or Swiss colony insects and
Duration:	Spexit ampliÞed in insects from each colony. Experiment was performed 8 times for each colony and results were subject to t-analysis of variance.
Results:	- Biological Comparison of S. exigua Colonies. No differences were observed in larval development time between insect colonies. - OB Production in S. exigua Colonies. Mortality registered in all treatments was between 90 and 95%, with no virus mortality registered in the mock-infected controls. - The weight of larvae postmortem varied significantly between the four treatments. Almerian larvae infected with Spexit were 25.1% and 31.7%, heavier respectively, than Almerian or Swiss larvae treated with Vir-ex. Swiss larvae inoculated with Spexit had intermediate weight values. Total OB production per larva differed significantly between virus colony treatments and also in terms of OBs per milligram larval weight In the Almerian insects infected by Spexit, OB yields, expressed as total OBs per larva and OBs per milligram larval weight, were 2.3- and 1.9-fold higher, respectively, than those of the Swiss insects infected by Vir-ex.

Virus × colony treatment	Host colony	Logit regression[a]				Time to death[b]		
		Intercept (± SE)	LD_{50} (OBs/larvae)	95% CI		MTD (hpi)	95% CI	
				Low	High		Low	High
Vir-ex × Almerian	Almerian	1.47 ± 0.16	7.4a	5.6	9.7	95.1a	89.6	100.9
	Swiss	1.82 ± 0.14	12.8ab	9.7	16.8	103.8ab	97.7	110.3
Vir-ex × Swiss	Almerian	1.79 ± 0.14	15.0b	11.4	19.8	105.5ab	98.6	112.7
	Swiss	1.98 ± 0.14	11.9ab	9.0	15.6	105.8ab	99.8	112.3
Spexit × Almerian	Almerian	2.14 ± 0.14	13.9b	10.9	19.2	115.2b	108.3	122.5
	Swiss	1.98 ± 0.14	17.7b	13.4	23.3	117.9b	110.6	125.7
Spexit × Swiss	Almerian	2.14 ± 0.14	20.4b	15.5	27.0	118.6b	111.6	126.0
	Swiss	1.65 ± 0.13	19.4b	14.7	25.6	121.7b	114.7	129.0

[a] Logit regressions were fitted in GLIM with a common slope of 0.75 ± 0.04 (SE) for all virus inocula. The test of nonparallelism was not significant ($\chi^2 = 5.12$; df = 7; $P = 0.65$). LD_{50} values followed by identical letters did not differ significantly (*t*-test, $P > 0.05$).
[b] Mean time to death (MTD) values labeled with different letters differed significantly (*t*-test, $P < 0.05$, Weibull analysis, GLIM).

Figure MP B3.10.1-03 Logit regression and time-to-death analysis on S. exigua second instars from the Almerian and Swiss colonies after consumption of SeMNPV OBs of two commercial products (Vir-ex and Spexit) produced in fourth instars from each colony.

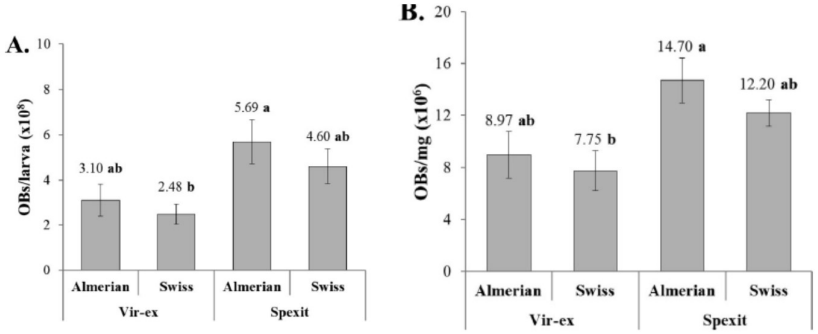


Figure MP B3.10.1-02 Occlusion body production per larva (A) and per milligram of larval body weight (B). Columns headed by different letters differ significantly (ANOVA, Tukey test, P<0.05).

Reference: Oihana Cabodevilla, Eduardo Villar, Cristina Virto, Rosa Murillo, Trevor Williams, and Primitivo Caballero, 2011. Intra- and Intergenerational Persistence of an Insect Nucleopolyhedrovirus: Adverse Effects of Sublethal Disease on Host Development, Reproduction, and Susceptibility to Superinfection. APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2011, p. 2954–2960.

Report No.:

Guideline: N/A

GLP: No

Summary: Sublethal infections by *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) are common in field populations of the beet armyworm (S. exigua, Hu¨bner) in the Almerian horticultural region of Spain. Inoculation of second, third, and fourth instars with OB (OBs) of an isolate (VT-SeAl1) associated with vertically transmitted infections resulted in 15 to 100% of sublethal infection in adult survivors, as determined by reverse transcription-PCR (RT-PCR) detection of viral DNA polymerase transcripts, and quantitative PCR (qPCR) targeted at the DNA polymerase gene. The prevalence of adult sublethal infection was positively related to the inoculum OB concentration consumed during the larval stage. Sublethal infections persisted in OBtreated insects for at least five generations. Viral transcripts were more frequently detected in adult insects than in third instars. qPCR analysis indicated a consistently higher prevalence of sublethal infection than RT-PCR. Sublethal infection was associated with significant reductions in pupal weight, adult emergence, fecundity, and fertility (egg hatch) and significant increases in larval development time and duration of the preoviposition period. Insects taken from a persistently infected experimental population were significantly more susceptible to the OB inoculum than control insects that originated from the same virus-free colony as the persistently infected insects. We conclude that OB treatment results in rapid establishment of sublethal infections that persist between generations and which incur costs in the development and reproductive capacity of the host insect.

M&M: - Effect of instar and OB concentration on the prevalence of covert infection: second, third, and fourth instars were treated with OB concentrations estimated to result in 20 to 80% mortality.

- Susceptibility of healthy and sublethally infected insects to OB inoculum. The susceptibility of sublethally infected second instars to the OB inoculum was compared with that of control insects.

Test substances: **Target***:* from Andermatt Biocontrol AG (Swiss colony).

Plant crop: greenhouse-grown sweet pepper crops

Antagonist (virus strains): SeMNPV isolate VT-SeAl1 a single genotype from green house in Almeria (Spain).

Treatments:

Results:

Instar	OB concn/ml	*n*	% larval mortality (*n*)		Pupation rate (*n*)	% sublethal infection in adults (*n*)	
			NPV	Other causes		RT-PCR	qPCR
L_2	3.8×10^4	120	79.2 (95)	12.5 (15)	8.3 (10)	100 (2)	
	3.8×10^3	120	63.3 (76)	12.5 (15)	24.2 (29)	87.5 (16)	
	3.8×10^2	120	41.7 (50)	15.0 (18)	43.3 (52)	90 (20)	100 (10)
	3.8×10^1	120	17.5 (21)	15.8 (19)	66.7 (80)	40 (20)	
	Control	120	0 (0)	20.0 (24)	80.0 (96)	0 (5)	0 (10)
L_3	3.7×10^4	120	77.5 (93)	12.5 (15)	10.0 (12)	80 (5)	
	3.7×10^3	120	60.8 (73)	16.7 (20)	22.5 (27)	78.6 (14)	
	3.7×10^2	120	40.0 (48)	13.3 (16)	46.7 (56)	45 (20)	60 (10)
	3.7×10^1	120	16.7 (20)	18.3 (22)	65.0 (78)	20 (20)	
	Control	120	0 (0)	17.5 (21)	82.5 (99)	0 (5)	0 (10)
L_4	7.8×10^4	144	78.5 (113)	11.8 (17)	9.7 (14)	80 (5)	
	7.8×10^3	144	61.1 (88)	13.9 (20)	25.0 (36)	84.2 (19)	
	7.8×10^2	144	44.4 (64)	9.0 (13)	46.5 (67)	40 (20)	60 (10)
	7.8×10^1	144	13.2 (19)	13.2 (19)	73.6 (106)	15 (20)	
	Control	144	0 (0)	15.2 (22)	84.7 (122)	0 (5)	0 (10)

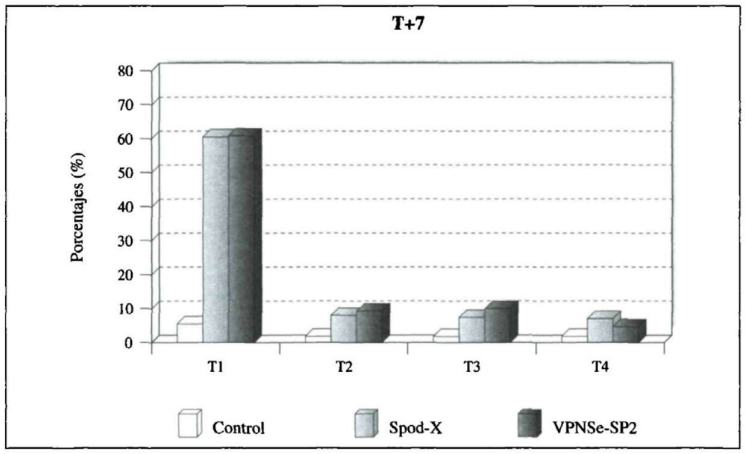
[a] Shown are the percentage of larval mortality due to NPV disease and other causes, pupation rates, and prevalence of sublethal infection (RT-PCR and qPCR) in adults that survived treatment with one of four OB concentrations, or a water control, in the second, third, and fourth instars.

Table MP B3.10.1-04 Percentage of larval mortality due to NPV disease and other causes, pupation rates, and prevalence of sublethal infection in the second, third, and fourth instarsa.

Population	LC_{50} (10^4 OBs/ml)	Slope (mean ± SE)	Intercept (mean ± SE)	Potency	95% fiducial limits for potency
Healthy	2.83	0.82 ± 0.09	−3.67 ± 0.43	1	
Covertly infected	1.49	1.49 ± 0.14	−6.20 ± 0.60	1.90	1.06–3.40

Table MP B3.10.1-05 Probit analysis of occlusion body concentration-mortality response in second-instar *S. exigua* larvae from a healthy or sublethally infected population at the sixth generation postinoculation.

Reference:	J. E. BELDA, E. MIRASOL, A. ESCRIBANO, S. RAPALLO Y P. CABALLERO , 2000. Eficacia de nucleopoliedrovirus (VPNSe) en el control de Spodoptera exigua (Hübner, 1808) (Lepidoptera: Noctuidae) en pimiento de invernadero. Bol. San. Veg. Plagas, 26: 619-628, 2000.
Report No.:	
Guideline:	N/A
GLP:	No
Summary:	Las estrategias de Control Integrado de Plagas pasan por la utilización de insecticidas específicos e inocuos para la fauna auxiliar. El hallazgo en Almería de una cepa autóctona del virus de la poliedrosis nuclear de *Spodoptera exigua* (VPNSe-SP2) y la existencia de un preparado comercial de dicho nucleopoliedrovirus con una cepa de Florida (SPOD-X®) permitieron plantear diversos estudios para comprobar el potencial de estos bioinsecticidas para el control de la plaga en cultivos en invernadero así como las estrategias de utilización. En un primer ensayo se evaluó la eficacia de estos biopreparados aplicados sobre una población alta de S. exigua, pulverizados sobre las plantas cuando el nivel de infestación alcanzó una media superior a 15 larvas por planta. La evaluación de la población a los 6 y 12 días después de la aplicación a una dosis de 10^8 poliedros/m^2, ofreció valores que oscilaron entre el 58,4% y el 74,7% de larvas muertas o enfermas por el patógeno, obteniéndose la máxima mortalidad con el VPNSe-SP2 a los 6 días de la aplicación. Teniendo en cuenta los valores alcanzados por el tratamiento estándar (hexaflumuron 10%), con una mortalidad máxima del 37,4% a los 6 días, y la alta infestación en el cultivo en el momento de la aplicación, se puede considerar una alta eficacia de los bioinsecticidas en el control de la plaga.
M&M:	
Test substances:	**Target***:* Larvae from laboratory colony of *S. exigua,* originally collected from maize fields in Mexico. **Plant crop:** greenhouse-grown sweet pepper crops **Antagonist (virus strains): SeMNPV OBs**
Results:	



	T1	T2	T3	T4
VPNSe-SP2	60,79	9,97	9,98	4,61
Spod-X	60,48	7,89	7,69	7,11
Control	5,18	2,44	2,38	2,38

Figure MP B3.10.1-03 Dispersion assay: percentage of larval mortality due to NPV disease for each treatment and zone after 7 days application.

RMS conclusions:

Similarly, previous studies have found non-significant differences in pathogenicity of between 2 SeMNPV isolates of different genotypes (Murillo et al. 2007) and non-significant differences in activity among 3 genotypic variants of SeMNPV (Muñoz and Caballero 1998). Regarding other species of BVs, Escribano *et al*., 1999 observed that the median lethal concentration (LC_{50}) for second-instar *Spodoptera frugiperda* (Lepidoptera: Noctuidae) did not differ significantly between a *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV) from Nicaragua (Sf-NIC) and a North American virus (Sf-US). Additionally, Rios-Velasco *et al.* 2011, 2012, observed that concentrations of 1.2 to 1.9 × 10^5 OB per mL of several Mexican isolates of SfMNPV obtained from different soil samples caused similar insecticidal effects on second-instar *S. frugiperda*. Small genotypic differences among nucleopolyhedrovirus isolates might have contributed to small differences in biological activity (Smits and Vlak 1988). However, variation in phenotypic traits is associated with genotypic variation in BVs populations (Luna-Espino et al., 2018; Zamora-Avilés, 2017; Rebolledo *et al*., 2015; Elvira *et al*., 2013; Cabodevilla et al., 2011 and Belda *et al.,* 2000).

High levels of genetic and phenotypic variation were observed in natural SeMNPV populations originating from groups of virus-killed larvae collected in the field (Muñoz et al. 1997) and from isolates of greenhouse soils (Murillo et al. 2007). This variation among host populations suggests that heterogeneity is important for virus survival and therefore efficacy (Cooper et al. 2003) and is associated with phenotypic differences (Cory and Myers 2003; Murillo et al. 2007; Serrano et al. 2015).

B.3.10.2 Testing effectiveness. Field trials.

Within this dossier, the efficacy performance of SPEXIT is supported by four field trials, three greenhouse trials on pepper and one on open field lettuce against S. *exigua* (Hübner) (Lepidoptera: Noctuidae carried out during 2006 and 2007 crop seasons in the Mediterranean EPPO climate zone (in Spain). Studies were performed in accordance with EPPO guidelines by GEP Officially Recognised Organisations. The efficacy trials and the efficacy supportive information on **Table MP B.3.10.2-1** and **Table MP B.3.10.2-2** probe SPEXIT efficacy in the control of *S. exigua* larvae.

S. exigua is an herbivorous caterpillar causing feeding damage in a wide variety of crops, including ornamentals and edible crops like sugar beet, cabbage, lettuce, soybeans, cotton, maize, tomato, potato, legumes, citrus, strawberry, melon, leek, garlic, onion, rice, flax, and tobacco. This pest frequently occurs in sub-tropical and tropical regions and in

glass houses of temperate regions. Young beet armyworm larvae feed on the lower surface of leaves where they eat the lamina but often leave the upper epidermis and larger veins intact. Larger larvae make irregular holes in leaves and fully-grown larvae devour foliage completely, leaving only major veins. It attacks the crops at any stage from seedlings to harvested products. In the tropics, breeding can be continuous with four to six generations per year, while in northern regions normally only one or two generations develop. Beet armyworms overwinter in the warmer regions of the Mediterranean countries, North America and Africa and invade the cooler northern regions as temperatures permit. The climate determines the seasonal activity, whereby in warm locations all stages can be found throughout the year. The eggs are laid at night, generally stuck to the lower surface of the lower leaves in clusters of 50 to 150 eggs. After 2 to 4 days, blackish larvae hatch. There are six instars and the maximum size ranges from 37 to 50 mm. Larval development takes 10 to 12 days. In total, the life-cycle lasts about 21 days (Hill, 1983). It is one of the most destructive polyphagous pest species and of world-wide economic importance.

In three efficacy trials SPEXIT was applied to pepper and in one efficacy trial it was applied to lettuce. SPEXIT was tested at 10 mL/hL (1 application), at 20 mL/hL (1 application) and at 10 mL/hL (2 applications). The interval between the two applications was 7 days, also corresponding to the standard product. Intended maximal dose is an interval dose of 200 mL SPEXIT in 200 – 1,600L water, i.e. 12.5 – 100 mL SPEXIT/hL. Therefore, the application rate of 10 mL/hL can be considered below the intended dose range and serves to justify the minimum effective dose. A lower application rate was also tested in order to support the minimum effective dose of SPEXIT. All trials included a treatment with a microbial pest control agents as reference.

Please refer to tables **MP B.3.10.2-1** and **MP B.3.10.2-2** for detailed results and **MP B.3.10.2-3** for an overview of the mean effectiveness percentage.

Greenhouse trials

Product 'SPEXIT' has been tested in three development greenhouse trials which demonstrated efficacious activity and appropriate crop safety in three different *Capsicum annuum* varieties*:* California pepper Var. Melchor (red), hot pepper Var. Furila (red) and California pepper Var. Prometeo (yellow) (**table MP B.3.10.2-1**). The greenhouse trials data supporting effectiveness against *S. exigua* comprise 3 greenhouse trials (MP3.10-01, MP3.10-02, MP3.10-03) conducted in Spain during two consecutives crop seasons 2006 and 2007. The trials were undertaken by "AGRICHEM, S.A." an Officially Recognised Organisation GEP accredited since 1998.

Trials from Spain are representative of the Mediterranean EPPO climatic zone according to EPPO Standard PP1/241 (1). However, since the trials were conducted in protected tomato, the data is representative for the entire EU.

Trial	Document	Treatment	Assessment date[1]	Number of living larvae per plant[3]	Efficacy (%)
1b/06	MP3.10-01	Untreated	0 DBLA	2.38	n.a.
			14 DALA	8.73 c	n.a.
		SPEXIT – 10 mL/hL	0 DBLA	1.75	n.a.
			14 DALA	1.00 a	88.29
		SPEXIT – 20 mL/hL	0 DBLA	2.08	n.a.
			14 DALA	0.83 a	90.15
		SPEXIT – 2 × 10 mL/hL	0 DBLA	2.38	n.a.
			14 DALA	0.38 a	95.48
		MPCA reference – 2 × 10 mL/hL[2]	0 DBLA	2.35	n.a.
			14 DALA	2.23 b	73.32
2b/06	MP3.10-02	Untreated	0 DBLA	3.38	n.a.
			14 DALA	6.85 c	n.a.
		SPEXIT – 10 mL/hL	0 DBLA	3.43	n.a.
			14 DALA	1.18 ab	81
		SPEXIT – 20 mL/hL	0 DBLA	3.43	n.a.
			14 DALA	0.45 a	93
		SPEXIT – 2 × 10 mL/hL	0 DBLA	2.93	n.a.
			14 DALA	0.8 b	88
		MPCA reference – 2 × 10 mL/hL[2]	0 DBLA	2.60	n.a.
			14 DALA	1.70 ab	74
7b/07	MP3.10-03	Untreated	0 DBLA	2.63	n.a.
			14 DALA	15.55 b	n.a.
		SPEXIT – 10 mL/hL	0 DBLA	2.78	n.a.
			14 DALA	0.00 a	100
		SPEXIT – 20 mL/hL	0 DBLA	2.75	n.a.
			14 DALA	0.00 a	100
		SPEXIT – 2 × 10 mL/hL	0 DBLA	3.70	n.a.
			14 DALA	0.33 a	96
		MPCA reference – 2 × 10 mL/hL[2]	0 DBLA	3.35	n.a.
			14 DALA	0.20 a	98

[1] DBLA/DALA: Days Before/After last Application.

[2] Microbial pest control agents: DELFIN, *Bacillus thuringiensis,* var. *kurstaki* (32 % WG), 75 g/hl (2 applications, at 7 days interval)

[3] Data analysis were made using multifactor ANOVA . Data are displayed as the mean of four replicates. When the results of the F-test were significant ($P < 0.05$) in each column, the means were compared using the Tukey´s honestly significant differences (HSD) procedure. With this method, means in each column that are annotated with the same letter are not significantly different ($P < 0.05$) among treatments of the same assessment date and trial.

Table MP B.3.10.2-01 Results of efficacy trials – Protected pepper (CPSAN) / beet armyworm (LAPHEG)

Open field trials

Product 'SPEXIT' has been tested in one open field trial which demonstrated efficacious activity and appropriate crop safety in one *Lactuca sativa* L.: Mini Romana. The open field trial data supporting effectiveness against this target comprise 1 open field assay conducted in Spain during crop season 2007) (**table MP B.3.10-02**). The trial was undertaken by "AGRICHEM, S.A." an Officially Recognised Organisation GEP accredited since 1998. Trial from Spain is representative of the Mediterranean EPPO climatic zone according to EPPO Standard PP1/24.

Trial	Document	Treatment	Assessment date[1]	Number of living larvae per plant[3]	Efficacy (%)
1c/07	**MP3.10-04**	Untreated	0 DBLA	0.25	n.a.
			14 DALA	0.65 c	n.a.
		SPEXIT – 10 mL/hL	0 DBLA	0.23	n.a.
			14 DALA	0.28 bc	41
		SPEXIT – 20 mL/hL	0 DBLA	0.15	n.a.
			14 DALA	0.13 a	80
		SPEXIT – 2 × 10 mL/hL	0 DBLA	0.08	n.a.
			14 DALA	0.13 ab	81
		MPCA reference – 2 × 10 mL/hL[2]	0 DBLA	0.18	n.a.
			14 DALA	0.35 ab	47

[1] DBLA/DALA: Days Before/After Last Application.

[2] Microbial pest control agents: DELFIN, *Bacillus thuringiensis,* var. *kurstaki* (32 % WG), 75 g/hl (2 applications, at 7 days interval)

[3] Data analysis were made using multifactor ANOVA. Data are displayed as the mean of four replicates. When the results of the F-test were significant ($P < 0.05$) in each column, the means were compared using the Tukey´s honestly significant differences (HSD) procedure. With this method, means in each column that are annotated with the same letter are not significantly different ($P < 0.05$) among treatments of the same assessment date and trial.

Table MP B.3.10.2-02 Results of efficacy trials – Open field lettuce (LACSA) / beet armyworm (LAPHEG)

Treatments	Efficacy (%) 14 DALA[1]	
	Green house[3]	**Open field[4]**
SPEXIT – 10 mL/hL	90	41
SPEXIT – 20 mL/hL	94	80
SPEXIT – 2 × 10 mL/hL	93	81
MPCA reference[2] – 2 × 10 mL/hL[2]	82	47

[1] /DALA: Days
Before/After Last Application.

[2] Microbial pest control agents: DELFIN, *Bacillus thuringiensis,* var. *kurstaki* (32 % WG), 75 g/hl (2 applications, at 7 days interval)

3 Protected trials: 1b/06; 2b/06; and 7b/07

[4] Open field trial: 1c/07

Table MP B.3.10.2-03 Overall trials pool data mean efficacy – Protected pepper (CPSAN) / beet armyworm (LAPHEG) and open field lettuce (LACSA) / beet armyworm (LAPHEG)

B.3.11 INFORMATION ON THE OCCURRENCE OF THE DEVELOPMENT OF RESISTANCE OF THE TARGET ORGANISM

SPEXIT is a biological pesticide based on the bv SeMNPV. To date, no resistance development against Bv have been found.

Because of the long existence of the disease-viruses relation, the probability of resistance appearance or development is considered as low. A resistance management strategy is not considered necessary.

The high specificity for the host and the mode of action makes the development of resistance very improbable. Until now, there has been no indication of decreasing efficacy of SPEXIT against *S. exigua* larvae. According to Review report for the active substance *S. exigua* nuclear polyhedrosis virus (SANCO/T14/2007), resistance development will probably not occur quickly and on a large scale. Thus, it is expected that the risk of S. *exigua* developing resistance to SPEXIT is rather low.

Concern about resistance in *S. exigua* against chemical insecticides and against *Bacillus thuringiensis* toxins increased in the last years, but no reports are available on resistance of *S. exigua* towards SPEXIT.

The product is therefore assumed a valuable component in spray programmes of resistance management strategies. The virus kills young instars (L1–L3) and infects older larvae. Furthermore, in contrast to broad-spectrum insecticides, the specifically acting product SPEXIT offers the advantage that natural antagonists of *S.exigua* are not affected. It is well suited for organic and integrated pest management strategies and resistance management programs (Andermatt and Andermatt, 2015).

Even though, the application of BVs SeMNPV as bioinsecticides have been used in Europe for the last ten years and the probability to resistance development must be taken into consideration. BVs are mainly transmitted horizontally from insect to insect, but recent studies on the *S. exigua* host-pathogen system revealed that vertical transmission (from parents to offspring) also occurs. Although the biological mechanisms of the pathway remain mainly unknown, common mutations in the sequence of three genomes of genotypes specialized in vertical transmission were recently identified, including the wild-type genotype SeAL1, known to be capable of being vertically transmitted (Serrano, 2017).

Myers and Cori, 2016 have illustrated the importance of environmental factors in modulating virulence, resistance and the risk of infection of BVs. In particular, the impact of larval food plant in interactions between armyworm, clearly demonstrates that the virulence and pathogenicity of the microorganism can be highly context dependent. The ecology and scale of this system and the sporadic nature of the outbreaks make it a challenging target.

Genetic variation in the SeMNPV population

BVs are often highly variable genetically. The maintenance of this variation in MNPVs is thought to be facilitated, in part, by their unique morphology whereby multiple infective genomes are packaged together in virus particles, which are themselves occluded within the proteinaceous OB (Clem and Passarelli 2013). More interestingly, virulence increases with strain diversity, the combination of two or more BVs strains was more pathogenic than the single isolates (Luna-Espinosa 2018). The mechanism by which this occurs remains to be elucidated, however, it could have major implications for both practical pest management and the evolution of resistance (Cory and Franklin 2012).

Genetic variation in *S. exigua* population

Genetic variation must translate into variation in disease susceptibility for pathogen epizootics to select for resistance or favour specific genotypes. Myers and Cory, 2016 compared the susceptibility of different populations in the peak or prepeak year and then in two subsequent years of population decline. Families showed a large variation in resistance to SeMNPV within each site, and some variation among sites. One population that had not experienced a strong viral epizootic during the previous population decline was more susceptible to infection in the first two years of the study. Larvae from another population that experienced an early epizootic became significantly more resistant in the subsequent year. Thus, it appears that viral infection can select for increased resistance, but this does not stop the epizootic.

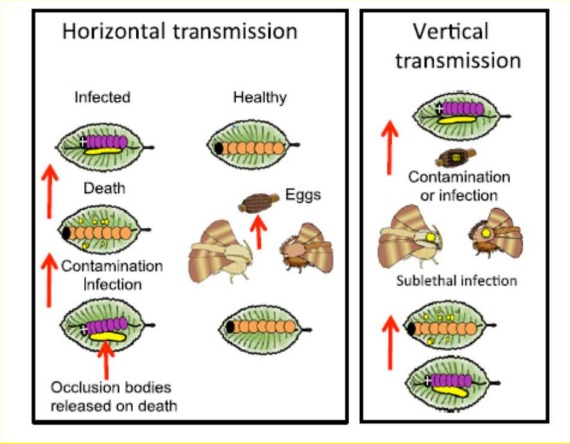


Figure MP B.3.11-01 Transmission of BVs can be horizontal among individuals through environmental contamination, or vertical from parents to offspring on or in the eggs. Upon death of an infected larva, OB are released and other larvae become infected if they ingest the OB on contaminated foliage. Vertical transmission can occur if larvae consume OB (small yellow dots) but pupate before death leading to sublethally infected adults (indicated by larger yellow dot). Adults sublethally infected as larvae might transmit virus to their offspring either on or in the eggs. This can lead to an active infection killing the offspring or potentially a covert infection that is passed on to offspring that will survive. (Myers and Cori, 2016).

Viral persistence

BVs can stablish sublethal infections to transmit the virus from parents to the offspring and persist in the host for long term. However, little is known about the molecular mechanisms that regulate the establishment and persistence of latent and persistent infections. SeMNPV genotypes differed in their ability to induce sublethal infection in the adult stage, indicating that certain genotypes are better adapted to vertical transmission than others (Cabodevilla et al. 2011).

Insect migration and population fluctuations of the host strongly influence the persistence and transmission strategies of pathogens. In the absence of alternative hosts, vertical transmission as either an overt infection or a covert form of virus, or an environmentally persistent life stage, such as a protected occlusion body (is necessary for persistence of pathogens between host outbreaks) **(Fig. MP B.3.11-01)** either through contamination of the outside of the egg (transovum transmission) or within the egg (transovarial transmission). Virus transmission and persistence BVs OB can survive for considerable periods of time outside of their hosts if they are protected from ultraviolet irradiation. Thus, horizontal transmission from this persistent environmental reservoir is likely to be the main route of transmission when infection levels are high. Horizontal transmission is related to host density, and infection levels increase with rising host density above a theoretical threshold (although this can be altered by factors such as behavior).

The presence of such high levels of mortality due to a pathogen like a BV, which primarily relies on horizontal transmission and the persistence of virus OB when the host dies, it is not clear how the virus persists in the system. SeMNPV is host specific, and alternative hosts are therefore not an option. Environmental persistence or direct contact with virus infected cadavers resulting in horizontal transmission (**Figure MP B3.11-01**) will occur within populations at high density. It seems unlikely, however, to explain how the virus survives through the solitary stage and when the insect does not reoccur in the same area for many years.

B.3.12 ADVERSE EFFECTS ON TREATED CROPS

B.3.12.1 Effects on the quality of plants or plant products

SPEXIT is a biofungicide which does not show any phytotoxicity symptoms in any of the four crops evaluated (data supported by documents MP3.10/01, /02, /03 and 04). Therefore, **no particular impact on the quality of plant products (peppers or lettuces) is expected after the use of SPEXIT.**

B.3.12.2 Effects on the transformation process

There is no major processing procedure involving biological reaction such as fermentation with peppers and lettuce crops. Therefore, this sub-part is not considered as relevant in the frame of this biodossier.

B.3.12.3 Effects on the yield of treated plants or plant products

SPEXIT is a biofungicide which does not show any phytotoxicity symptoms.
Therefore, **no particular impact on yield is expected after the use of SPEXIT.**

B.3.13 PHYTOTOXICITY TO TARGET PLANTS (INCLUDING DIFFERENT CULTIVARS), OR TO TARGET PLANT PRODUCTS

Phytotoxicity assessments were evaluated in all efficacy trials according to EPPO 1/135 "Phytotoxicity assessments".

Those trials were carried out by officially recognized organizations in accordance with the Principles of Good Experimental Practice (GEP). All trials were performed under 'worse case' conditions for the MEDITERRANEAN zone (**table MP B.3.13-01**), low light (autumn period) and during development of harvestable vegetative plant parts. In these trials one application was performed with SPEXIT at the N and 2N dose rate (for SPEXIT 10cc/hL and 20cc/hL, respectively) and 2 applications (6 days interval) for SPEXIT 10cc/hL.
Absolutely no symptom of phytotoxicity was observed in all those trials.

Trial	Cultivation system	crop	date	Max Tª (ºC) range	Min Tª (ºC) range	Solar radiation (MJ/m^2day) range
AG 1b/06	greenhouse	pepper	03/10/2006-17/10/2006	22.9-27.3	14.0-17.6	20.9-14.5
AG 2b/06	greenhouse	pepper	27/10/2006-10/11/2006	27.3-20.9	18.7-13.4	16.2-5.7
AG 7b/07	greenhouse	pepper	24/09/2007-08/10/2007	24.5-26.7	16.4-19.3	---
AG 1c/07	Open field	lettuce	01/11/2007-31/11/2007	22.3-14.9	10.3-(-2.9)	---

Table MP B3.13-01: Meteorological data on efficacy assays.

RMS comments:
SPEXIT risk of phytotoxicity is considered as negligible for lettuces and peppers at these doses, number of applications and interval between applications.

B.3.14 OBSERVATIONS ON UNDESIRABLE OR UNINTENDED SIDE-EFFECTS, E.G. ON BENEFICIAL AND OTHER NON-TARGET ORGANISMS, ON SUCCEEDING CROPS, OTHER PLANTS OR PLANTS USED FOR PROPAGATING PURPOSES (E.G. SEEDS, CUTTINGS, RUNNERS)

B.3.14.1 Impact on succeeding crops

SEXIT is a biofungicide and has no herbicidal activity. The effectiveness and selectivity trials have demonstrated that SPEXITL is crop safe on lettuces and pepper crops. Therefore, no adverse effect on crops succeeding the treated one is expected.

B.3.14.2 Impact on other plants, including adjacent crops

SPEXIT is a biofungicide and has no herbicidal activity. The effectiveness and selectivity trials have demonstrated that SPEXIT is crop safe on *solanaceae* family and leaf vegetable crops. Therefore, no adverse effect on crops adjacent to the treated one is expected.

B.3.14.3 Impact on treated plants or plant products to be used for propagation

SPEXITL is a biofungicide that does not present any phytotoxicity. The effectiveness and selectivity trials have demonstrated that SPEXIT is crop safe. Therefore, no adverse effect on parts of plant used for propagating purposes is expected.

B.3.14.4 Effects on beneficial and other non-target organisms

The four GEP studies demonstrated no effect of SeMNPV product SPEXITon any non-target arthropod species.

B.3.15 CONTRIBUTION TO RISK REDUCTION AND INTEGRATED PEST MANAGEMENT STRATEGIES FOR THE TARGETED CROP OR RESOURCE

Due to its nature, SPEXIT can be applied in an Integrated Pest Management program close to harvest and during the period of high risk for pest development.

Considering the use of SPEXIT in IPM, the effect of SPEXIT on other arthropoda organisms was evaluated in a total of three greenhouse studies.

The three greenhouses studies indicate that the total effect of SPEXIT can be classified as harmless.

Therefore, SPEXIT is considered as compatible with integrated pest management strategies.
Considering the data submitted:

- SPEXIT efficacy is considered as variable and partial but, considering this type of product based on microorganisms, this efficacy is considered as acceptable for all claimed uses.
- SPEXIT level of phytotoxicity is considered as negligible.
- The risks of negative impacts on yield, quality, propagation, succeeding and adjacent crops are considered as negligibles.

B.3.16 SUMMARY OF RESULTS

Efficacy

The available trials are enough to support the efficacy of SPEXIT in beet armyworm (LAPHEG) as a new active substance. SPEXIT is human-harmless and environmentally-safe tool for its use in agriculture compatible with other conventional and biological crop protection measures, which fits well within Integrated Pest Management programs and becomes a useful resource for the chemical fungicide resistance management.

Adverse effects

Phytotoxicity has been assessed also in all efficacy trial. There were no phytotoxic effects resulting from the application of SPEXIT in any of the trials. No adverse effects on succeeding crops or adjacent crops are expected since the MPCAs in SPEXIT is not able to enter plant tissues, do not cause injuries to plants and is inactivated by UV light within a few days.

Risk of resistance

SPEXIT acts highly specific against larvae of the beet armyworm, *S. exigua*, as a biological insecticide. SeMNPV only replicates in *S. exigua* cells (Simon et al., 2004), and for this reason SeMNPV is considered among the most specific BVs (Ijkel et al., 1999). This extremely high host-specificity is especially important for assessing the side-effects on beneficial arthropods and other non-target organisms. There is not host alternation in other arthropods, fishes, birds or mammalian, and for this reason is not harmful for other species of non-target organism. Therefore, it is very adequate for using in Integrated Pest Management systems or organic farming.

Additionally, SPEXIT is also a very suitable tool as a component of the strategies for resistance management, because due to its selectivity it does not affect to the natural enemies of *S. exigua*. Vertical transmission may be an interesting feature to improve pest control strategies, and the establishment of covert infections in populations that may eventually trigger fatal disease in larvae causing damages in the crops. SeMNPV transgenerational transmission might reduce the number of applications of baculovirus-based insecticides, improving their effectivity in field and the risk of resistance development.

Resistance towards *S. exigua* based microbial pest control agents has not been documented from reliable sources. Concern about resistance in *S. exigua* against chemical insecticides and also against *Bacillus thuringiensis* toxins increased in the last years, but no reports are available on resistance of *S. exigua* towards SeMNPV. Strategies for resistance management can be found in detail in a revision made Roush (1998).

Despite a considerable time of use of SeMNPV in plant protection products, no indication of decreasing efficacy of SeMNPV against *S. exigua* larvae has been reported until now. Thus, it is expected that the risk of *S. exigua* developing resistance to SPEXIT is rather low. The product is therefore assumed to be a valuable component in spray programmes of resistance management strategies. Furthermore, in contrast to broad spectrum insecticides, the specifically acting product SPEXIT offers the advantage that natural antagonists of *S. exigua* as well as all other species are not affected.

Reference B.3

B.3.17 REFERENCES RELIED ON

ABA – Andermatt Biocontrol AG

References included by the RMS in GREY

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMP 3/01 B.3.10.2	Hill, D.S.	1983	AGRICULTURAL INSECT PESTS OF THE TROPICS AND THEIR CONTROL not available, not available Camebridge University Press, 376 GLP/GEP: no Published: yes	N	N	not protected	-

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMP 3/02 B.3.16	Simon, O., Williams, T., Lopez-Ferber, M., Caballero, P.	2004	Virus entry or the primary infetion cycle are not the principal determinants of host specificity of Spodoptera spp. nucleopolyhedroviruses not available, not applicable Journal of General Virology, 85, 2845-2855 GLP/GEP: no Published: yes	N	N	not protected	-
B.3.1.5	Frommer, W., Ager, B., Archer, G., Collins, C.H., Donikian, R., Frontali, C., Hamp, S., Houwink, E.H., Küenzi, M.T., Krämer, P., Lagast, H., Lund, S., Mahler, J.L., Normand-Plessier, F., Sargeant, K., Tuijnenburg Muijs, G., Vranch, S.P., Werner, R.G	1989	Safety precautions for handling microorganisms of different risk classes not applicable Appl Microbiol Biotechnol, 30, 541-552	N	N	not protected	
B.3.2	Evans, H.F., Harrap, K.A.	1982	Persistence of insect viruses	N	N	not protected	
B.3.2	OECD	2002	Consensus document on information used in the assessment of environmental applications involving baculovirus not applicable ENV/JM/MONO, 1, 1-90 Report-no. not applicable GLP/GEP: no Published: yes	N	N	not protected	

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.3.2	Muñoz, D., Vlak, J.M., Caballero, P.	1998	Naturally Occuring Deletion Mutans are Parasitic Genotypes in a wild-type Nucleopolyhedrovirus Population of S. exigua Appl Environ Microbiol, 64, No. 11, 4372-4377 Report-no. not applicable GLP/GEP: no Published: yes	N	N	not protected	
B.3.2	Martins, T., Montiel, R., Medeiros, J., Oliveira, L., Simones, N.	2005	Occurence and characterization of a nucleopolyhedrovirus from Spodoptera littoralis (Lepidoptera: Noctuidae) isolated in the azores not applicable J Invertebr Pathol, 89, 185-192 Report-no. not applicable GLP/GEP: no Published: yes	N	N	not protected	
B.3.2	Muñoz, D., Vlak, J.M., Caballero, P.	1997	In vivo Recombination between Two Strains of the Genus Nucleopolyhedrovirus in Its Natural Host S. exigua Appl Environ Microbiol, 63, No. 8, 3025-3031 Report-no. not applicable GLP/GEP: no Published: yes	N	N	not protected	
KMP 3.9/01	Anonymous	2018	SPEXIT INSECTICIDE FOR THE BIOLOGICAL CONTROL OF THE BEET ARMYWORM (*S. exigua*) Andermatt Biocontrol AG, CH, not available not available GLP/GEP: no Published: no	N	Y		ABA
B.3.10.1 B.3.11	Luna-Espino, J.C., Castrejón-Gómez, V.R., Pineda, S., Figueroa, J.I., and Martínez, A.M.	2018	Effect of Four Multiple Nucleopolyhedrovirus Isolates on the Larval Mortality and Development of *Spodoptera exigua* (Lepidoptera: Noctuidae): Determination of Virus Production and Mean Time to Death Florida Entomologist, 101(2):153-159 Report-no. not applicable GLP/GEP: no Published: yes	N	N	not protected	

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMP 3.10/01 B.3.10 B.3.12 B.3.13	Mayoral Domínguez, F	2006	SPEXIT efficacy evaluation study 1bl06 GLP/GEP: yes Published: no	N	Y		ABA
KMP 3.10/02 B.3.10 B.3.12	Mayoral Domínguez, F	2006	SPEXIT efficacy evaluation study 2bl06 GLP/GEP: yes Published: no	N	Y		ABA
KMP 3.10/03 B.3.10 B.3.12 B.3.13	Mayoral Domínguez, F	2007	SPEXIT efficacy evaluation study 7bl07 GLP/GEP: yes Published: no	N	Y		ABA
KMP 3.10/04 B.3.10 B.3.12 B.3.13	Mayoral Domínguez, F	2007	SPEXIT efficacy evaluation study 1cl07 GLP/GEP: yes Published: no	N	Y		ABA
B.3.10.1 MA B.1.3.3/9	Zamora-Avilés, N., Murillo, R., Lasa, R., Pineda, S., Figueroa, J.I., Bravo-Patiño, A., Díaz, O., Corrales, J.L. and Martínez, A.M.,	2017	Genetic and biological characterization of four nucleopolyhedrovirus isolates collected in Mexico for the control Report-no. not applicable GLP/GEP: no Published: yes	N	N	not protected	
B.3.10.1	Rebolledo, D., Lasa, R., Roger Guevaral, R., Murillo, R., and Williams,T.	2015	Baculovirus-Induced Climbing Behavior Favors Intraspecific Necrophagy and Efficient Disease Transmission in Spodoptera exigua. PLOS ONE | DOI:10.1371/journal.pone.0136742 GLP/GEP: no Published: yes	N	N	not protected	
B.3.10.1	Elvira, M. S., Ibargutxi, A., Gorria, N., Muñoz, D., Caballero, P., and Williams, T.	2013	Insecticidal Characteristics of Two Commercial Spodoptera exigua Nucleopolyhedrovirus Strains Produced on Different Host Colonies. J. Econ. Entomol. 106(1): 50-56 GLP/GEP: no Published: yes	N	N	not protected	

Data point	Author(s)	Year	Title Owner, Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.3.10.1 B.3.11	Cabodevilla, O., Villar, E., Virto, C., Murillo, R., Williams, T., and Caballero, P.	2011	Intra- and intergenerational persistence of an insect nucleopolyhedrovirus: Adverse effects of sublethal disease on host development, reproduction, and susceptibility to superinfection. Applied and Environmental Microbiology, 77(9), 2954–2960. GLP/GEP: no Published: yes	N	N	not protected	
B.3.10.1	Belda, J. E., Mirasol, E., Escribano, A. Rapallo, S. and P. Caballero ,	2000	Eficacia de nucleopoliedrovirus (VPNSe) en el control de Spodoptera exigua (Hübner, 1808) (Lepidoptera: Noctuidae) en pimiento de invernadero. Bol. San. Veg. Plagas, 26: 619-628, 2000.	N	N	not protected	
B.3.10.1	Murillo, R., Mun˜ oz, D., Ruı´z-Portero, M. C., Alca´ zar, M. D., Belda, J. E., Williams, T. & Caballero, P.	2007	Abundance and genetic structure of nucleopolyhedrovirus populations in greenhouse substrate reservoirs. Biol Control 42, 216–225.	N	N	not protected	
B.3.10.1	Munñoz, D., Castillejo, J. I. and Caballero, P	1998	.Naturally occurring deletion mutants are parasitic genotypes in a wild-type nucleopolyhedrovirus population of Spodoptera exigua. Appl Environ Microbiol 64, 4372–4377.	N	N	not protected	
B.3.10.1	Escribano A, Williams T, Goulson D, Cave RD, Chapman JW, Caballero P.	1999	Selection of a nucleopolyhedrovirus for control of Spodoptera frugiperda (Lepidoptera: Noctuidae): structural, genetic, and biological comparison of four isolates from the Americas. Journal of Economic Entomology 92: 1079–1085.	N	N	not protected	
B.3.10.1	Rios-Velasco C, Gallegos-Morales G, Berlanga-Reyes D, Cambero-Campos J, Romo-Chacón A	2012	Mortality and production of occlusion bodies in Spodoptera frugiperda larvae (Lepidoptera: Noctuidae) treated with nucleopolyhedrovirus. Florida Entomologist 95: 752–757.	N	N	not protected	

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B.3.10.1	Smith PH and Vlak JM.	1988	Biological activity of Spodoptera exigua nuclear polyhedrosis virus against S. exigua larvae. Journal of Invertebrate Pathology 51: 107–114.	N	N	not protected	
B.3.10.1	Cooper D, Cory JS, Myers JH	2003	. Hierarchical spatial structure of genetically variable nucleopolyhedroviruses infecting cyclic populations of western tent caterpillars. Molecular Ecology 12: 881–890.	N	N	not protected	
B.3.10.1	Serrano A, Pijlman PG, Vlak MJ, Muñoz D, Williams T	2015	Identification of *Spodoptera exigua* nucleopolyhedrovirus genes involved in pathogenicity	N	N	not protected	
B.3.11	Andermatt and Andermatt	2015	Product Portfolio. Andermatt Biocontrol AG, Stahlermatten 6, 6146 Grossdietwil, Switzerland.	N	N	not protected	
B.3.11	Serrano , L.	2017	Analysis of genes potentially involved in Vertical Transmission of Spodoptera exigua multiple nucleopolyhedrovirus. Grado en ingeniería agroalimentaria y del medio rural Gradua nekazaritzako elikagaien eta landa ingurunearen ingeniaritzan	N	N	not protected	
B.3.11	Myers, J. H., and Cory, J. S.	2016	Transmission of NPV is largely horizontal although low levels of vertical transmission occur, Ecology and evolution of pathogens in natural populations of Lepidoptera. Evolutionary Applications 231–247.	N	N	not protected	
B.3.11	Clem, R. J., and A. L. Passarelli	2013.	Baculoviruses: sophisticated pathogens of insects. PloS Pathogens 9:e1003729.	N	N	not protected	
B.3.11	Cory, J. S., and M. T. Franklin	2012	Evolution and the microbial control of insects. Evolutionary Applications 5:455–469.	N	N	not protected	

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KMP 3/03 B.3.16	Ijkel, W.F.J., van Strien, E., Heldens, J.G.M., Broer, R., Zuidema, D., Goldbach, R.W., Vlak, J.M.	1999	Sequence and organisation of the Spodoptora exigua multicapsid nucleopolyhedrovirus genome not available, not applicable Journal of General Virology, 80, 3289 - 3304 GLP/GEP: no Published: yes	N	N	not protected	-
KMP 3/04 B.3.16	Roush, R.T.	1998	Strategies for Resistance Management not available, not applicable GLP/GEP: no Published: yes	N	N	not protected	-
KMP 3/05	Vijaykumar, K.B.K., Fakrudin, B.	2003a	Effectiveness of Helicoverpa armigera Nuclear Polyhedrosis Virus Against Insecticide Resistant Strains of Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) not available, not applicable Resistant Pest Managemant Newsletter, 13, 27-28 GLP/GEP: no Published: yes	N	N	not protected	-
KMP 3/06	Vijaykumar, K.B.K., Fakrudin, B.	2003b	Effect of Nuclear Polyhedrosis Virus Infection on the Insecticide Susceptibility of Heliothis armigera Larvae not available, not applicable Resistant Pest Managemant Newsletter, 13, 28-30 GLP/GEP: no Published: yes	N	N	not protected	-
KMP 3.9/01 B.3.1 B.3.9	Anonymous	2018	SPEXIT Insecticide for the biological control of the beet armyworm (Spodoptera exigua) Andermatt Biocontrol AG, CH, not available not available GLP/GEP: no Published: no	N	Y		ABA