European Commission

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

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV)

Active substance data

Volume 3 – Annex B.8 Fate and behavior in the environment

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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B.8 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 in 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 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. 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.

[1]*Regulation EC 1107/2009 – placing on the market of PPPs.*
[2] *Regulation EU 283/2013 - setting data requirements for active substances Communication - list of test methods and guidance documents.*
[3]*Regulation EU 284/2013 – setting data requirements for PPPs Communication - list of test methods and guidance documents*
[4]*Regulation EU 546/2011 - uniform principles for evaluation and authorisation of PPPs.*
[5]*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.*

Active substances are approved for maximum period of 10 years under Directive 91/414/EEC. The active substance SeMNPV was under programme of renewal Regulation EU 686/2012 (AIR-III programme). According to draft working document AIR III renewal programme SANCO/2012/11284, *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 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 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)[6] 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.

B.8. INTRODUCTION FATE AND BEHAVIOR IN THE ENVIRONMENT

SeMNPV is a BV belong to the family Baculoviridae, which are arthropod-specific, enveloped viruses with a circular double-stranded DNA genome. BVs exclusively have been isolated from arthropods, primarily from the three insect orders Lepidoptera, Hymenoptera, and Diptera (OECD, 2002[7]). In general, the host range of most BVs is restricted to one or few species of the genus or family of the host where they were originally isolated.

SeMNPV is a naturally occurring virus worldwide and acts highly specific against larvae of the beet armyworm, *S. exigua*, therefore, the presence of SeMNPV in the environment is linked to the presence of the host, *S. exigua*. Thus, its application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect (Krieg, 1976). The SeMNPV strain BV004 in use was originally isolated in China (Jianfeng, 2005). It is not supposed to have any 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.

Studies submitted to support the application for the approval of *S. exigua* multipolyhedrivirus (SeMNPV) isolate BV0004 and the microbial product SPEXIT are shown in black text; studies provided by the RMS for further support the approval are shown in grey. Literature reference included by the applicant comes from a literature search according to EFSA (2011)[8] in order to identify relevant recent published peer reviewed references covering the last 10 years.

As regards *S. exigua* nuclear polyhedrosis virus (EFSA2013) [9]the European Food Safety Authority, concluded that these substances are not pathogenic to humans and do not require a quantitative consumer risk assessment. In view of that conclusion, the Commission considers that the inclusion of such substances in Annex IV to Regulation (EC) No 396/2005 is appropriate.

[7] Consensus document on information used in the assessment of environmental applications involving baculoviruses

[8] *Guidance of EFSA: 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.*

[9] *EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Journal 2013;11(11):3449, 108 pp. doi:10.2903/j.efsa.2013.3449*

B.8.1 PERSISTENCE

The whole family of Baculoviridae are naturally present in our environment. As BVs are closely associated with their host, occurrence of corresponding BV is linked to the presence of its host. The study conducted by Caballero *et al*. (2009) showed, that the persistence of SeMNPV is high in the soil of all crops, although its incidence is highest in spring and summer. Sublethal infections by SeMNPV are common in field populations of *S. exigua*. Such covert infections were found to persist for at least five generations (Cabodevilla *et al*. 2011). Next to BVs, many *S. exigua* field populations also exhibit covert infections by other virus species (Virto *et al*. 2014). In the field, the prevalence of co-infections in wild population is likely to have an impact on the performance of a SeMNPV insecticide.

The reproduction, dispersal, transmission and / or survival of BVs are enhanced by manipulation of the host behaviour (Han *et al*. 2015, 2017). In the case of SeMNPV, this is evident from the pre-death climbing behaviour (towards elevated positions) of infected larvae (tree-top disease), which makes sense from an evolutionary point of view, since thus the virus may be spread over a larger area of plant foliage, allowing an increased virus transmission to subsequent generations of caterpillars. The underlying processes for this host behavioural manipulation is not understood in detail yet. However, it was found, that the ecdysteroid UDP-glucosyltransferase (*egt*) gene of SeMNPV facilitates the tree-top disease in *S. exigua* larvae by prolonging the larval time to death (Han *et al*. 2015) and further it was observed that light from above in a particular time frame is needed to trigger this behaviour (Han *et al*. 2017). Finally, the tree-top disease allows an increased persistence and multiplication of SeMNPV.

The dynamic of pathogens and the impact on the host population is tricky to examine. The interactions between insects and pathogens are highly context dependent and linked to changes in density, genetic diversity and environmental factors (particularly diet), which can have a strong impact on the persistence and transmission. Seasonal cycles, population cycles and migratory behaviour determine variations in the availability of insect hosts over time. For *S. exigua* outbreak dynamics with multigenerational, long-distance migrations are known and baculovirus occlusion bodies can survive for longer time periods outside of their hosts if they are protected from ultraviolet irradiation. BVs transmission can be horizontal among individuals via environmental contamination, or vertical from parents to offspring. The dynamics in this system influence strongly the persistence possibilities (Myers and Cory 2016).

The extent of persistence and multiplication might be also shaped by vertical transmission. When opportunities for horizontal transmission are unfavourable, vertical transmission represents a common feature of some insect pathogenic viruses, and seems to be essential to virus survival. Virto *et al*. (2013) studied if gender affected transgenerational virus transmission, and found that females, and males are able to transmit the infection to the next generation, although female-mediated transmission resulted in a higher prevalence (twice as efficient as male mediated transmission) of infected offspring. Thereby the main route is the transovarial transmission (process of virus passing to progeny within the eggs), not the transovum (contamination of the egg surface with viral particles that infect neonate larvae when they ingest the chorion) route. Female and male offspring were infected by their parents in similar proportions.

The timing of application is an important determinant of insecticide efficacy, as the phenological state plays a crucial role in the association of the Lepidoptera and the crop. Before or after flowering, plants are less attractive to oviposition butterfly females, since at mature fruit are similarly less optimal for larvae development. Therefore, late applications, during fruit development, have less effectiveness in the control of the insect, due to larvae densities in the crop are usually lower during these stages.

B.8.1.1 Competitiveness under environmental conditions

B.8.1.1.1 UV

The persistence of a virus-based insecticide on the surface of the crop plant strongly influences its efficacy since with greater persistence the likelihood of the pest consuming a lethal OB dose increases. Thereby, solar radiation was found to be the main factor affecting the OB persistence on plant surfaces. For instance, UV radiation is reduced in greenhouses as the walls filters much UV. Arrizubieta *et al*. (2016) surveyed the persistence of a binary co-occluded mixture (HearSP1B:LB6) of *Helicoverpa armigera* single nucleopolyhedrovirus (HearNPV) in greenhouse and field-grown tomato crops. A negative correlation of the persistence with solar radiation in both, greenhouse and field trials, was

observed. However, the persistence was comparable with those of commercial insecticides. Arrizubieta *et al*. (2016) measured at 6 days after treatment 87% remaining viable HearNPV OBs in protected crops, and 7 days after treatment only 7% remaining viable HearNPV OBs in open field.

BVs are highly sensitive to UV degradation. Different studies could show that plant extracts and other oils, as well as the determination of an optimal application regime, optimize the virus efficacy. The study conducted by Shapiro *et al*. (2012) tested a plant extract from kudzu, *Pueraria lobata* (Willd.) Ohwi (Fabaceae) as UV protectant for SeMNPV with and without the addition of an oil / emulsion formulation on collards. The virus activity was determined for 7 days. For unformulated SeMNPV, sunlight exposure of SeMNPV resulted in a huge activity loss, no UV protection was measured in the oil / emulsifier formulated SeMNPV, the addition of kudzu (5 %) provided a significant UV protection, and cottonseed oil / lecithin together with kudzu achieved the greatest UV protection. The study conducted by Salamouny *et al*. (2009) evaluated black tea and lignin as potential UV protectants and measured for both a nearly 100 % UV protection and found no significant visual reduction of the full length viral genomic DNA. Also green tea (decaffeinated and caffeinated) were found to be effective as UV protectants (Shapiro *et al*. 2008).

Reference:	**Report KMA 7.1/01**
Report No.:	Arrizubieta, M., Simón, O., Torres-Vila, L.M., Figueiredo, E., Mendiola, J., Mexia, A., Caballero, P., Williams, T. (2016) Insecticidal efficacy and persistence of a co-occluded binary mixture of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) variants in protected and field-grown tomato crops on the Iberian Peninsula
Guideline:	no
GLP:	no
Summary:	BACKGROUND: A binary co-occluded mixture (HearSP1B:LB6) of *Helicoverpa armigera* single nucleopolyhedrovirus (HearNPV) variants was previously found to be highly pathogenic under laboratory conditions. The insecticidal efficacy and persistence of this mixture were determined in greenhouse and field-grown tomato crops in Spain and Portugal. RESULTS: Concentrations of 10^9-10^{11} occlusion bodies (OBs) L^{-1} of HearSP1B:LB6 resulted in 89-100% mortality of larvae on treated tomato plants in growth chambers. In protected tomato crops, application of 10^{10} OBs L^{-1} of HearSP1B:LB6 was as effective as *Bacillus thuringiensis* (*Bt*) and spinosad in reducing the percentage of damaged fruits, and resulted in higher larval mortality than the *Bt* treatment. In open-field tomato crops, virus treatments were as effective in reducing the percentage of damaged fruit as spinosad, *Bt* and chlorpyrifos treatments. The persistence of the insecticides on tomato plants was negatively correlated with solar radiation in both field and greenhouse settings. Residual insecticidal activity of OBs on protected tomato crops at 6 days post-application was 55 and 35% higher than that of *Bt* and spinosad respectively. On field-grown tomato, OB persistence was significantly lower than with spinosad or chlorpyrifos. CONCLUSION: The efficacy and persistence of HearSP1B:LB6 OBs were comparable with those of commercial insecticides in both field and greenhouse tomato crops. Future studies should focus on reducing application rates to determine insecticidal efficacy at lower OB concentrations.
M&M: **Test substances:**	• **Test Item**: binary co-occluded mixture (HearSP1B:LB6) 10^9-10^{11}OB/mL, of VHearNPV • **Target organism**: *Helicoverpa armigera* second instars. • **Crop**: Tomato.
Test conditions	• **Green house trials:** 2011 experimental green house of 288m^2 in Lisbon, Portugal. • **Open field trials:** 2012 experimental cultural practices according usual procedures in the area, Badajoz, Spain. 26000plant/ha.
Study design & methods	• **Green house trials:** treatments (1) HearSP1B:LB6 (10^{10}OB/mL~10^{13}OB/ha); (2) biopesticide based in *Bacillus thuringiensis*, Turex 50wp (25000IU/mg), 1kg/ha; Spintor 250mL/ha (3) Untreated control, water.

Experimental treatment:	• **Open field trials:** treatments (1) HearSP1B:LB6 (10^{10}OB/mL~10^{13}OB/ha); (2) HearSP1 OBs 10^{10}~10^{13}OB/ha; (3) biopesticide based in *Bacillus thuringiensis*, Turex 50wp (25000IU/mg) 2kg/ha; (4) Spintor 250mL/ha; (5) clorpyrifos 1.25 kg/ha (6) Untreated control, water. • **Green house trials:** 16 plots with 4 replicates/treatment.Replicates of 7.5m^2 contained 28 plants artificially infected with 112 larvae. Experiments repeated twice.
Replicates:	• **Open field trials:** 48 plots (x4m long row (6m^2) 16 plants/plot: 4 application in 4 plots/treatment (24 plots) and 5 applications in 4 plots/treatments (24 plots).
Observations:	• **Green house trials:** 10 day after treatment. • **Open field trials:** every 2 week from early fruit to harvest.
Evaluation:	• **Green house trials:** (1) Number of surviving larvae after 10 day of application; (2) Number of fruit feeding injuries after 10 day of application. Direct counting and results subjected to ANOVA and Tukey´s test $p<0.05$. Correlation between fruit damage and larval mortality by Pearson coefficient. • **Open field trials**: 100 randomly chosen fruits every 3-4 days, grouped by fortnight. Harvest fruit were classified into (1) unmarked green fruits; (2) damage green fruits; (3) unmarked red fruits; (4) scarred red fruits (5) rotten red fruits.
Results:	• Accumulation dose of UV radiation inside greenhouse range is 4,134-16,585 J/m^2, and in open field 12,224-52,517 J/m^2 for the first 10 days after application. • Insecticidal concentration on plant surfaces 1h after application of HearSP1B:B6 was $2.73x10^5 \pm 8.44x10^2$ (in greenhouse) and $1.4x10^6 \pm 1.1x10^3$ (in open field) OB/g leaf material wet weight (100% initial value of insecticidal residue). • Residual activity 3-6 days after application on leaves was similar to the activity just after application in green house and open house applications. Residual activity significant decrease at 9 days post-application (13.3%) (**Figure MA B.8.1-1A**) in green house. In open field, 3 days after application, the residual activity of HearSNPV OBs had decreased to 40.6 and 62.5% of initial activity for HearSP1B:LB6 and HearSP1 OBs respectively (**Figure MA B.8.1-1D-E**) and continued to decrease to 0.1% and 0.05% at 10 days post-application. Residual insecticidal activity on plants decreased significantly over the time and it was negatively correlated with the accumulated dose of UV radiation in green house and open field applications. • In protected crops 87%OBs remained viable after 6 days after application, whereas in open field just 7% OBs remained viable after 7 days.
Conclusions:	• The persistence of HearSNPV OBs on tomato leaves was markedly higher in the greenhouse than in the open field. Consequently, the inactivation of OBs is slower in protected than in open field crops, as the plastic structure are able to filter a large part of incident UV radiation.

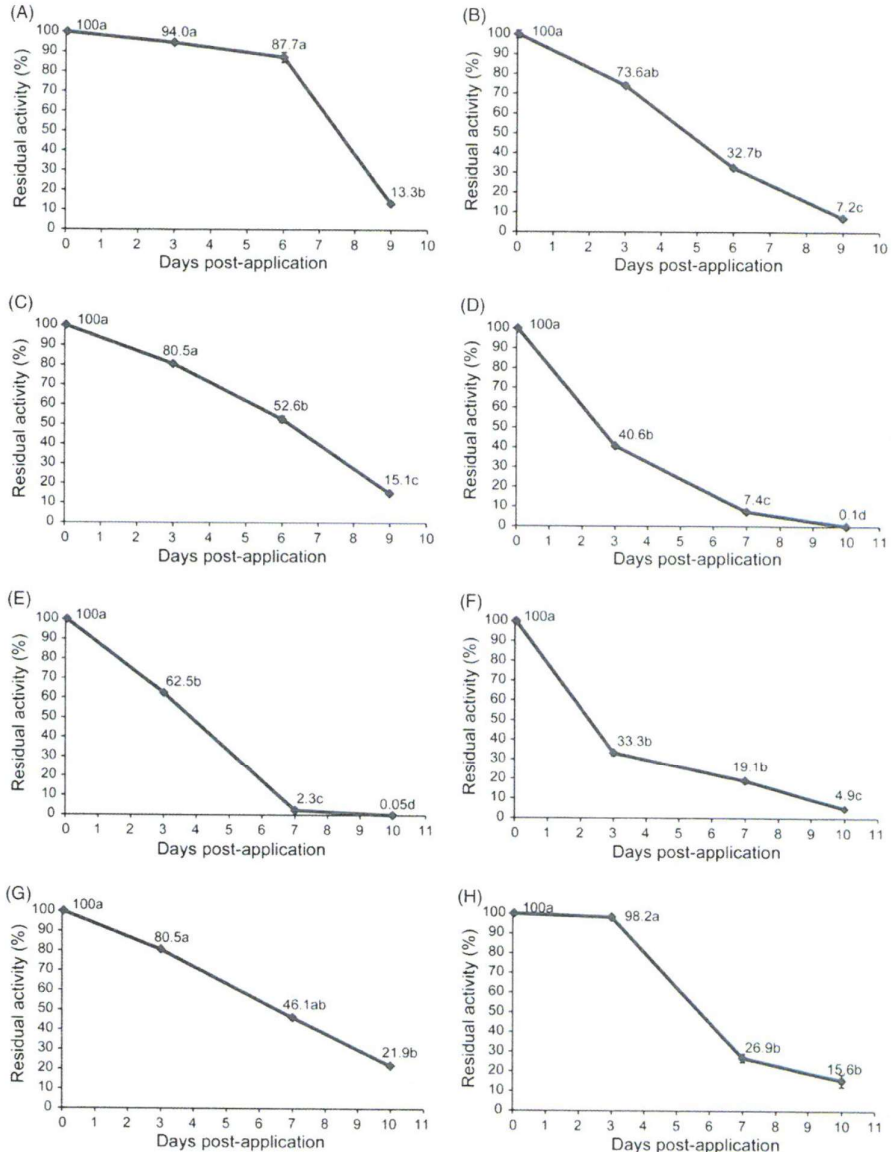

Figure MA B.8.1-1 Percentage of insecticidal residue on tomato leaves at various intervals after application: A) HearSP1 B:LB6 OBs; B) Bt and C) spinosad in protected tomato crops; D) HearSP1 B:LB6 OBs; E) HearSP1 (Wt) OBs; F) Bt; G) spinosad and H) chlorpyrifos in open field tomato crops.

RMS comments: The study confirmed the importance of solar radiation factor affecting the persistence of OBs. The persistence of the OBs on plant surface determine the period during which a lethal dose can be ingested by the larvae. Lasa *et al*, 2007, have been obtained similar level of OBs persistence with SeMNPV on greenhouse grown sweet pepper, in which 61% of SeMNPV OBs maintained efficacy after 6 days post-application.

Reference:	Shapiro, M., El Salamouny, S., Jackson, D. M., Shepard, B. M. (2012) Field Evaluation of a Kudzu/Cottonseed Oil Formulation on the Persistence of the Beet Armyworm nucleopolyhedrovirus
Report No.:	**Report KMA 7.1/08**
Guideline:	no
GLP:	no

Summary:	A plant extract from kudzu, Pueraria lobata (Willd.) Ohwi (Fabaceae), was tested as a UV protectant for the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), nucleopolyhedrovirus (SeMNPV), with and without the addition of an oil/emulsifier (cottonseed oil/lecithin) formulation. Aqueous and oil emulsion formulations of SeMNPV were applied to collards and the residual virus activity was determined for 7 d. Sunlight exposure of SeMNPV resulted in an activity loss of 42%, 85%, and 95% at days 2, 4, and 7, respectively. The addition of the oil/emulsifier to SeMNPV did not provide UV protection. At days 2, 4, and 7, activity losses were 67%, 84%, and 92%, respectively. Whereas the addition of kudzu (5%) to SeMNPV provided significant UV protection during the sunlight exposure period, activity losses of 17%, 62%, and 76% occurred at days 2, 4, and 7, respectively. The greatest UV protection for SeMNPV was achieved when cottonseed oil/lecithin were used in conjunction with kudzu. In this formulation, activity losses were 2%, 40%, and 55% at days 2, 4, and 7, respectively. Although the mode of action is currently unknown, the addition of cottonseed oil to kudzu resulted in an increase of both UVB (280 - 320 nm) and UVA (320 - 400 nm) absorbance.
M&M: **Test substances:** **Test conditions** **Study design & methods** Experimental treatment: Replicates: Observations: Evaluation:	• **Test Item**: SeMNPV product SPOD-X (Certis USA, Columbia, MD) $5x10^7$OB/mL • **Target organism**: *S. exigua* beet armyworm (USADA_ARC, Tifton GA) 3rd instard • **Solar protectants**: Plant extract from *Pueraria lobata (Fabaceae)* and aqueous oil emulsion formulations (cotton seed oil/lecithin). • **Crop**: collard leaves of *Brassica oleaceae* L. • Field test • **Treatments**: 4 virus **(**$5x10^7$OB/mL) aqueous formulations: (1) Control with no solar protectant, sticker-spreader; (2) kudzu 5%; (3) lecithin 1%+ cottonseed oil 8.5%; (4) kudzu 5%+ lecithin 1%+ cottonseed oil 8.5%. • Application of virus formulations to the collar leaves by paintbrush. • 3rd instar larvae were place in cups and feed for 48h with 10 disc of treated leaves • Repetition test 9, 10 larvae per treatments and time. • Virus residue after 0, 1, 2, 4, and 7 days post-application. • Larvae mortality at 5 days and every 2-3 days thereafter until day 14. • OAR% ratio: mortality each day-0 virus treatment divided by mortality of each virus-time.
Results:	• Sun light exposure of SeMNPV reduce virus activity in 42%, 85%, 95% at 2, 4, and 7 days. • Addition of oil emulsion did not provided any UV protection. • Addition of kudzu (5%) to SeMNPV reduce virus activity in 17%, 62%, 76% at 2, 4, and 7 days. • The protection is improved when the kudzu product is applied in conjunction with cottonseed oil emulsion: activity losses were 2%, 40%, 55% at 2, 4, and 7 days.
Conclusions:	• The addition of cottonseed oil to kudszu resulted in an increase of UVB and UVA absorbance.

Reference:	El Salamouny, S., Shapiro, M., Ling, K. S., Shepard, B. M. (2009) Black Tea and Lignin as Ultraviolet Protectants for the Beet Armyworm Nucleopolyhedrovirus
Report No.:	**Report KMA 7.1/09**
Guideline:	no
GLP:	no
Summary:	A major constraint to the use of BVs for biocontrol of insects is their sensitivity to UV degradation. In this study, we evaluated black tea (Lipton®, London, UK) and lignin (Reax 85A™, MeadWestvaco, Charleston, SC) as potential UV protectants for beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) multiple-embedded nucleopolyhedrovirus (SeMNPV). The original activity remaining (OAR%) from SeMNPV upon exposure to various lengths of time (up to 5 h) to a source of

	UVA and UVB was evaluated in bioassays using beet armyworm third-stage larvae under laboratory conditions. Beet armyworm mortality was measured after larvae fed on artificial diet treated with SeMNPV. Mortality of beet armyworm due to SeMNPV, with no UV protectants added, was reduced to 23, 11.3 and 2.1% upon UV exposure for 15, 30 or 60 min, respectively. To investigate the mechanism of reduction in the efficacy of SeMNPV when exposed to UV was due to the degradation of full-length viral genomic DNA, a modified DNA isolation technique was developed to measure levels of the full length viral genomic DNA of SeMNPV through electrophoresis on an agarose gel. The efficacy of SeMNPV on beet armyworm was lost after 2 h of UV exposure, and the full-length genomic DNA also was degraded to levels that were not visible on agarose gel. However, both black tea and lignin provided nearly 100% UV protection for SeMNPV as measured in bioassays even after 5 h of UV irradiation. SeMNPV efficacy against beet armyworm in samples containing black tea or lignin resulted in no significant visual reduction of the full length viral genomic DNA. To investigate the mechanism of UV protection for SeMNPV from black tea and lignin, absorption spectra of both protectants were measured with a spectrophotometer. High rate of absorption in the UV range, especially at the range of UVB (280-320nm), was detected for both materials. The absorption rate was higher with lignin than with black tea. Whereas lignin was a good absorber for both UVB and UVA radiation, black tea was primarily an absorber of UVB. Therefore, both black tea and lignin are potential natural UV protectants in the formulation of baculovirus-based biopesticides.
M&M: **Test substances:** **Test conditions** **Study design & methods** Experimental treatment: Replicates:	• **Test Item**: SeMNPV fron SPOD-X product (Certis USA, Columbia, MD) ($2x10^5$OB/mL) • **Target organism**: Beet armyworm egg from UISADA-ARS, Tifton GA. 3rd larvae instar. • **Solar protectants**: 2% w/w black tea and 2% w/w lignin aqueous solution • 3rd larvae instar were infected by feeding on surface contaminated diet $2x10^4$ OB/30mL • SeMNPV ($2x10^5$OB/mL) irradiated with 15W UVA and UVB during 0 to 300´ • **Bioassay**: for OAR% from SeMNPV upon exposure to various lengths of time (up to 5h) to a UVA/B • **Viral genomic after UV exposure**: DNA from virus was recovered from each sample after UV exposure, extracted, purified and evaluated the difference between genomic DNA concentrations due to the UV breakdown process. • **Solar protectants:** 3 virus **(**$2x10^5$OB/mL) aqueous formulations (1) 2% w/w black tea and (2) 2% w/w lignin; (3) Control with no solar protectant. • 10 larvae per replicate and treatment. Test was repeated at least 3 times.
Results:	• Treating third-stage beet armyworm via surface inoculated diet with $2x10^4$ OBs/cup of unirradiated SeMNPV caused 99% mortality within 10 d. • Decrease in virus-caused mortality of beet armyworm occurred within the 1 -11 h of exposure of SeMNPV to UVA and UVB. • Insect mortality was reduced to 23, 11.3 and 2.1% after 15, 30, and 60 min UV exposure, respectively (Fig. MA B.8.1-1A). Decreased virus activity was accompanied by the gradual degradation of full length. SeMNPV DNA as UV exposure time increased (Fig. MA B.8.1-1A). • Addition of black tea extract (1%) to SeMNPV solution resulted in full protection of virus activity as determined by beet armyworm bioassay (Fig. MA B.8.1-1B). The high viral activity in the black tea-treated samples was also indicated with the presence of full-length virus genomic DNA after various length of exposure to UV (Fig. MA B.8.1-1B). • Infection of *S. exigua* by SeMNPV occurred in lignin-treated samples exposed to UV for up to 5 h (Fig. MA B.8.1-1C).Virus-caused mortality remained at > 99% in samples even after 5 h of UV exposure. Analysis of full-length virus genomic DNA in lignin-treated samples upon

	different period of UV exposure again revealed no major reduction in DNA concentration (Fig. MA B.8.1-1C).
Conclusions:	• Black tea and lignin were effective in protecting SeMNPV from breakdown by UVA/B.

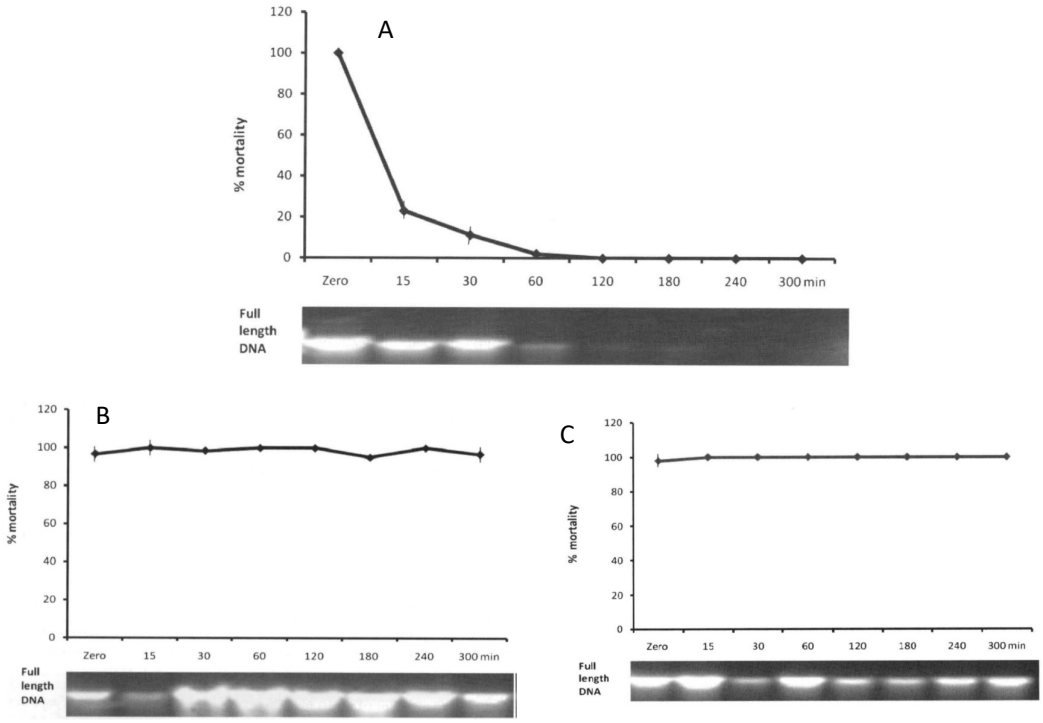

Figure MA B.8.1-1 Mortality of 3rd instar and the presence of full-length viral DNA after UV exposure of SeMNPV (A); and with 1% black tea extract (B) and with 1% lignin (C).

RMS comments: This study, along with above (Shapiro 2012) have demonstrated SeMNPV sensitivity to UV degradation and the importance of UVA/B absorbance in protecting SeMNPV. The studios also have provide information on plant-derived materials as UV protectants for SeMNPV.

Reference:	Shapiro, M., El Salamouny, S., Shepard, B. M. (2008) Green tea extracts as ultraviolet protectants for the beet armyworm, *Spodoptera exigua*, nucleopolyhedrovirus
Report No.:	**Report KMA 7.1/10**
Guideline:	no
GLP:	no
Summary:	The addition of a caffeinated green tea, *Camellia sinensis* L., filtrate (1%) to the nucleopolyhedrovirus (SeMNPV) of the beet armyworm, *Spodoptera exigua* (Hübner), provided almost complete protection following UVB irradiation (30 min) in laboratory tests. There were few differences in UV protection when extracts were prepared at 27 or at 908C. Moreover, few differences in UV protection were demonstrated following infusion times of 5, 15, 30, and 60 min at 908C. At a 1% concentration, decaffeinated and caffeinated green teas were equally effective as UV protectants. At lower concentrations (0.1, 0.01, and 0.001%) caffeinated green tea provided greater UV protection (UVB/UVB 30, 60 min). Virus/tea extracts (caffeinated), under field conditions at 1 and 5%, were ineffective as UV screens. At a 10% concentration, some UV protection was provided and UV protection further increased in a concentration-dependent manner.

M&M:	3 independent assays were made for infusion tea effect on SeMNPV efficacy (1) Effect of green tea concentration expose to UV on *S. exigua* mortality ; (2) effect of different green tea treatments and concentration expose to UV on *S. exigua* mortality (3) effect of caffeinate and decaffeinate green tea expose to UV on *S. exigua* mortality. Field assay (4) for green tea as UV protectant under field conditions
Test substances:	• **Test Item**: OB of SeNPV from Spod-X (Certis USA) • **Target organism**: beet armyworm (BAW) (*S. exigua*, Hübner) 2nd instar • **Infusion tea treatments (solar radiation protectants):** (a) bulk tea standard infusion at 27ºC blending+filtration, concentration 1, 5, 10, 20 and 30% (b) bulk tea standard infusion at 90ºC for 5´,15´, 30´and 60´, blending+filtration (c) caffeinated and decaffeinated green tea infusion • **For field test crop** *Brassica oleaceae L*
Test conditions	• **Radiation source:** SeMNPV suspenssions were exposure for 30´ to UVB (15W)
Study design & methods	**(1)**: Bioassay for irradiation effect: 30´exposure of 10^6 OB/mL (water and green tea filtrate) SeMNPV to UV for 90-95% mortality • Stock filtrate (a) (1%) were diluted 0.001-0.1% • Un-radiated SeMNPV 10^6OB/mL was used as control • Larvae 2nd instar were place in contact with the virus suspensions for 14 days 27ºC
Experimental treatment:	**(2)**: Bioassay for evaluation of green tea as UV protectan: irradiation 4mL of each treatment (a & b) were irradiated expose for 30´ • Infusions teas: (a) and (b) were diluted to 1, 0.1, 0.01, 0.001% • Suspension of 1mL of SeMNPV 10^6OBmL +9mL of each infusion tea treatment • 2nd instar larvae were feed with 0.1mL/30mL • Larvae 2nd instar were place in contact with the virus suspensions for 14 days 27ºC **(3)**: Bioassay for evaluation of caffeine effect in UV protection: of each treatment were irradiated expose for 30´ • Infusions teas: (c) were diluted to 1, 0.1, 0.01, 0.001% • Suspension of 1mL of SeMNPV 10^6OBmL +9mL of each infusion tea treatment • 2nd instar larvae were feed with 0.1mL/30mL • Field assay: Larvae 2nd instar were place in contact with the virus suspensions for 14 days 27ºC
Replicates:	**(4)** Field assay with green tea protectant: Green tea filtrate dilutions (a) 10, 20, and 30% were used for a final concentration of SeMNPV 10^8 OB/mL suspensions (LC=90-95%) (NPV/1% tea; NPV5% tea; NPV/5% tea; 10%NPV tea
Observations:	• Virus suspension applied to collard leaves of *Brassica oleaceae L* and collected after 0, 1, 2, 5 and 7 days after application and place in a cup for feed larvae for 48h
Evaluation:	• 5 replicates with 10 larvae/treatments and replicate for (1) (2) and (3) testes • Three assays with 20 larvae per treatment per time period • Effectiveness of green teas UV protectants: Concentration-mortality regressions. • Mortality at 5days and every 2-4 days thereafter until day 14: LC_{50}. Viral persistence as OAR based on upon 100% mortality at 0days post-treatment.
Results:	**(1)** Irradiation of an aqueous suspension of SeMNPV for 30´ reduced NPV-caused mortality from 95.69% to 5.99% (6.1% OAR). The addition of 1% green tea to SeMNPV, provided complete UV protection. **(2)** There were no differences in percentage OARs among the tea formulated SeMNPV products. **(3)** Caffeinated green tea provided greater UV protection than decaffeinated green tea at 0.1, 0.01, and 0.001%. **(4)** 24h after virus application, virus-caused mortality decreased from 98.3 to 83.3% and at 7 days to 6.7% (i.e. a loss of 96%, **table MA B.8.1-2).** The addition of 10% green tea resulted in a decreased loss in activity on days 5 and 7 compared to the SeMNPV only treatment. The addition of 30% green tea provided the greatest UV protection (i.e. :66% OAR) in comparison to the SeMNPV alone treatment (i.e. :7% OAR) **(table MA B.8.1-2)**

Conclusions:
- There are detrimental effects of UV irradiation in SeMNPV effectiveness.
- These studies demonstrated that UV inactivation of SeMNPV result from the generation of reactive oxygen species and hydrogen peroxide can be partially overcome by antioxidants or oxidative enzymes present in green tea infusions.

Treatment[a]	Days post-treatment	Mortality ±SE[b] (avg%)	OAR[c] (%)
NPV/H_2O			
	0	98.3±3.34	100.0
	1	83.3±7.10	84.7
	2	55.0±5.77	56.0
	5	20.0±2.89	20.3
	7	6.7±1.74	6.8
NPV/GT (10%)			
	0	95.0±3.34	96.6
	1	80.0±8.17	81.4
	2	60.0±5.34	61.0
	5	30.0±3.72	33.3
	7	26.7±3.34	27.2
NPV/GT (20%)			
	0	96.7±3.34	98.4
	1	93.3±6.61	94.9
	2	83.3±7.61	84.7
	5	60.0±5.77	61.0
	7	58.3±3.34	59.3
NPV/GT (30%)			
	0	98.3±3.34	100.0
	1	88.3±8.82	89.8
	2	80.0±8.17	88.9
	5	70.0±6.77	77.8
	7	65.0±5.77	66.1

[a]NPV was used at a final concentration of 1×10^8 OBs/mL. Three replicates; 20 larvae per treatment per replicate; 20 untreated controls per replicate. [b]NPV was applied to collard leaves and treated leaves were collected at different times post-application and were stored at -20°C until usage. When all leaves were collected for a given replicate, leaf disks were cut and larvae were allowed to feed for 48 h. After this time, larvae were transferred to untreated diet and mortality readings were taken. [c]For% original activity remaining (% OAR), all treatments are compared to NPV/H_2O (0 UV), where NPV/H_2O =100% OAR.

Table MA B.8.1-2 Average percent mortality and percentage original activity remaining (OAR): SeMNPV, field test with 10, 20, 30% green tea on brassica collards

Instability upon exposure to sunlight was reported for different BVs, including *Helicoverpa zea* NPV, which is also in the same NPV group II as SeMNPV (Jehle *et al*., 2006). Young (2005) compared the persistence of different BVs and concluded that the half-life under field conditions in sunlight is limited to several hours only. Very little activity remains after a few days because of inactivation by the UV spectrum of sunlight. Half-life values between 2.2 hours under simulated sunlight on glass plates and 53 hours under natural sunlight on soybean leaves were observed. Temperature is not limiting BVs persistence under field conditions (Ignoffo, 1992).

Two different isolates of nucleopolyhedroviruses infecting both *Helicoverpa armigera* and *H. zea*, were compared for their persistence on tomato leaves (Chakraborty *et al*., 1999). These NPVs are closely related to SeMNPV. One isolate (HearNPV) was multiplied in vitro in a cell culture, the other (HzNPV) was multiplied *in vivo* and belongs to the commercial product GemStar. Both were applied to tomato leaves and persistence of activity was determined in a biotest using *H. armigera* larvae. Activity declined rapidly to below 5% within 4 days after application for the HearNPV, and to about 23% after 4 days for the HzNPV.

Shapiro *et al*., 2008, Salamouny *et al*., 2009 and Shapiro *et al*., 2012 have confirmed by *in vivo* and in field assays the sensitivity on SeMNPV to sunlight and the protectant effect of several natural products use as solar protectants. The tree studios were made with SeMNPV from the commercial product SPOD-X.

Shapiro *et al*., 2008 has showed detrimental effects of UV irradiation in SeMNPV effectiveness. In bioassay tests, irradiation of an aqueous suspension of SeMNPV for 30´ reduced NPV-caused mortality from 95.69% to 5.99% (6.1% OAR). The addition of 1% green tea to SeMNPV, provided complete UV protection. In field 24h after virus application, virus-caused mortality decreased from 98.3 to 83.3% and at 7 days to 6.7% (i.e. a loss of 96%, **table MA B.8.1-2).** The addition of 30% green tea provided the greatest UV protection (i.e.:66% OAR) in comparison to the SeMNPV alone treatment (i.e.:7% OAR) **(table MA B.8.1-2).**

Salamouny *et al*., 2009 have confirmed the decrease in virus-caused mortality of beet armyworm occurred within the 1-11 h of exposure of SeMNPV to UVA and UVB. Insect mortality was reduced to 23, 11.3 and 2.1% after 15, 30, and 60 min UV exposure, respectively. In these studios, black tea and lignin were effective in protecting SeMNPV from breakdown by UVA/B.

Similar results were obtained in Shapiro *et al*., 2012 where it have been also evaluated the effect of sun light exposure of SeMNPV reduce virus activity in 42%, 85%, 95% at 2, 4, and 7 days. Addition of kudzu (5%) to SeMNPV reduce virus activity in 17%, 62%, 76% at 2, 4, and 7 days. The protection is improved when the kudzu product is applied in conjunction with cottonseed oil emulsion, with an activity losses of 2%, 40%, 55% at 2, 4, and 7 days.

These studies demonstrated that UV inactivation of SeMNPV result from the generation of reactive oxygen species and hydrogen peroxide can be partially overcome by antioxidants or oxidative enzymes present in natural products as green tea infusions (Shapiro *et al*., 2008), . Decreased virus activity was accompanied by the gradual degradation of full length SeMNPV DNA as UV exposure time increased (Fig. MA B.8.1-1A) (Salamouny *et al*., 2009).

B.8.1.1.2 Phenological stage of the crop and Crop cultivar

Physicochemical characteristic of the crop can influence OB degradation, as exudates of some plants can rapidly inactivate OBs.

Reference:	Caballero, P., Murillo, R., Munoz, D., Williams, T. (2009) El nucleopolyhedrovirus de *Spodoptera exigua* (Lepidoptera: Noctuidae) como bioplaguicida: análisis de avances recientes en España
Report No.:	**Report KMA 7.1/02**
Guideline:	no
GLP:	no
Summary:	The greenhouses of Almeria, Spain, represent the largest extension of covered crops in Europe. Larvae of *Spodoptera exigua* are an important pest in many of these crops and have developed resistance to the majority of insecticides registered in Europe. The nucleopolyhedrovirus (SeMNPV; Baculoviridae) of *S. exigua* is a native pathogen of this insect. The persistence of the virus is high in the soil of all crops, although its incidence is highest in spring and summer. As many as nine genotypic variants of the virus have been identified in this zone. In terms of pathogenicity, virulence and production of progeny virus occlusion bodies (OBs), mixtures of certain genotypes have greater insecticidal potential than pure genotypes. Production of OBs *in vivo* can be up to three-fold greater in larvae treated with juvenile hormone analogues. Compounds derived from stilbene have a synergistic activity with OBs in the laboratory and reduce the lethal dosis in the field. The field efficacy of a simple formulation was greater than that offered by treatments of various synthetic insecticides. The virus is currently being mass-produced in a commercial production facility and the process of registration has begun for its use in sweet pepper crops in Almerian greenhouses. The results of these investigations should facilitate the development of SeMNPV as a biological insecticide in other parts of the world, including Latin America.
Conclusions:	The persistence of the virus is important in the soil of all crops and its incidence is related to the phenological stage of the crop and the season (highest in spring and summer).

RMS comments: The document provides relevance results for persistence and survival of the SeMNPV OBs.

B.8.1.2 Persistence in soil

The inclusion of other BVs results on persistence and mobility in soil is justifiable due to their close family relationship. As a matter of fact, the results of all investigations stated below confirm that the family of Baculoviridae does not reveal deleterious effects to soil. Their application in pest control means only a temporal fluctuation of the virus titre in soil.

BVs can easily reach the soil, either during rain following application or after the death of an infected larva and subsequent release of inclusion bodies, followed by rainfall. Persistence in soil is reported for different BVs (Young, 2005) and is characterised by a rapid adsorption to soil particles, leading to absence of leaching (Evans and Harrap, 1982). In a field study, surface soil was treated with *Trichoplusia ni* NPV. Most viral activity remained in the top 0-2.5 cm of the

undisturbed soil in the 223 weeks of observation (for further references, please refer to Jaques, 1977). The leaching behaviour of unformulated *Mamestra brassicae* MNPV was analysed in two soil types, loamy sand with high humus content and sandy soil with little humus. The greatest proportion of active virus was recovered from the top 4.5 cm in all soil types, but biotest activity was still detected in 18 cm depending on the soil type and irrigation intensity (Krieg, 1983). Lopez-Pila (1988) analyzed the leaching behaviour of a granulovirus and a nucleopolyhedrovirus in comparison to a poliovirus and an f2-bacteriophage as references. Both BVs adsorbed more strongly to the soil than the reference viruses independently from the water used. Leaching was neglectable for the NPV using deionised and buffered water, and more important for groundwater and wastewater. Even if the mechanism of adsorption to soil or sand particles is unclear, a role of the matrix protein polyhedrin is assumed.

Viruses thus persisting in soil following treatments or natural epizootics provide a reservoir for initiation of epizootics in succeeding generations of host insects and is a mechanism by which a long-term effect of virus introductions may be attained (Jaques, 1977). On the other hand, the inactivation rate of viruses not only depends on soil type and pH but also on microbial activity (OECD, 2002).

The survey by Jaques & Harcourt (1971) showed that *Trichoplusia ni* NPV and *T. ni* GV and *Pieris rapae* GV occur naturally in non-treated soil of Ontario crucifer fields. The occurrence of viruses in soil was influenced by the density of the population of the host insect. The viruses appeared to accumulate in soil during the growing season, presumably due to degradation of host larvae killed by the virus. Detectable residues of both viruses were found more frequently in soil sampled late in the season than in soil sampled early in the year. Furthermore, the activity of *T. ni* NPV depends on the soil pH: the lower the pH, the more rapidly the virus was inactivated (Thomas *et al*., 1973).

Jaques (1974 a/b) showed that *T. ni* NPV, *T. ni* GV remained in the soil following application to soil and accumulated in soil following foliar application of the viruses to cabbage plots. However, within one year after application, concentrations in the non-treated plots had increased to equal those in virus-treated plots by accumulation of viruses produced in natural epizootics, demonstrating that natural virus populations depend on the presence of the host. In addition, these results clearly demonstrate that application of BVs in insect control does not lead to an accumulation of virus higher than under natural conditions.

Taken together, BVs are rapidly inactivated when exposed to sunlight. In soil, they rapidly adsorb to soil particles and are not leached to deeper soil layers. Activity is thus maintained in the upper soil and remains accessible to further host generations, leading to a sustainable effect on the host insects.

For more information, see (B2.8.2 Effect of environmental substrates-Soil).

B.8.1.3 Persistence in Water

Most viruses in intact inclusion bodies are reasonably stable in aqueous suspension (Jaques, 1977). *Heliothis* sp. NPV was stable in water at 30°C for one year (Ignoffo, 1992). However, Jaques (1977) continues that it is apparent that the pH and salt concentration of water influences stability. In addition, once introduced into a water body, the viral particles are likely to deposit and are absorbed by sediments. Furthermore, there is evidence of deactivation/destruction by the UV portion of sunlight in aqueous suspensions. Moreover, it is assumed that the protein of the virus will be completely mineralised by bacterial action in water and sediment. There are no risks of pollution of surface or groundwater expected due to the high level of retention of the viral particles by the soil.

B.8.1.4 Persistence in air

A rapid degradation of SeMNPV in air is assumed since inactivation by sunlight is the most important factor causing loss of activity of viruses in the field environment (Krieg *et al*., 1983; Shapiro *et al*., 2008, Salamouny *et al*., 2009 and Shapiro *et al*., 2012). Moreover, SeMNPV has low vapour pressure as it consists of a high molecular weight protein.

B.8.2 MULTIPLICATION AND VIRUSES TRANSMISSION

The intra- and inter-generational transmission of NPVs among individuals in a population of insects is a key factor in understanding the ecology of the virus, and for the efficient use of these pathogens as pest control agents. The highly persistent virus OBs are responsible for horizontal transmission to healthy susceptible larvae that consume OB-contaminated plant material. However, when host population densities are low and conditions for horizontal transmission are unfavourable, vertical transmission, from parents to offspring, plays an important role in the survival of the virus. Vertically-transmitted infections also permit virus dispersal and the colonization of new areas of habitat through the migration of infected adult hosts. For vertical transmission to occur the virus must persist in the adult host as a covert or sublethal infection which does not prevent adult reproduction. Sublethal BVs infections have been reported in a number of lepidopteran species (Cabodevilla *et al*., 2011). Vertical transmission has been reported in the *S. exigua*-SeMNPV pathosystem (Bianchi *et al*., 2001; Smits and Vlak,1988), but only recently PCR-based quantification has been employed to estimate the importance of this transmission route in host populations (Cabodevilla *et al*., 2011; Murillo *et al*.,2007). Moreover, a recent study on *S. exigua* demonstrated transovarial transmission of SeMNPV, and the role of the parental female in the persistence of the virus population from one generation to the next (Virto *et al*., 2013).

B.8.2.1 Vertical and horizontal transmission

Reference:	Cabodevilla, O., Villar, E., Virto, C., Murillo, R., Williams, T., Caballero, P. (2011) Intra- and Intergenerational Persistence of an Insect Nucleopolyhedrovirus: Adverse Effects of Sublethal Disease on host Development, Reproduction, and Susceptibility to Superinfection
Report No.:	**KMA 2.2.2/06; KMA 7.1/03**
Guideline:	no
GLP:	no
Summary:	Sublethal infections by *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) are common in field populations of the beet armyworm (*S. exigua*, Hübner) in the Almerian horticultural region of Spain. Inoculation of second, third, and fourth instars with occlusion bodies (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 OB treated 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: **Test substances:**	• **Test Item**: OB of SeMNPV Spanish isolate (VT-SeAl1) with a single genotype (from green houses in Almeria 2007). Considered to have been vertically transmission from parents to offspring. • **Target organism**: beet armyworm (*S. exigua*, Hübner) second, third and fourth instars from Andermatt Biocontrol AG.
Test conditions	• **Bioassay:** OB production: Second, third and fourth instar larvae (L2, L3, L4) were treated with OB concentration resulted in 20-80% mortality. OB from each instar were offered one of four OB concentration in 10-fold increments. Larvae ingested OB suspension within 10 minutes transferred to 24-well tissue culture plates with semisynthetic diet. 25±1ºC 50%±5 RH.

Study design & methods	• **Detection of viral DNA polymerase transcripts by RT-PCR** specific viral gene were target. Amplicon products were confirmed by sequencing. For the detection of viral genome DNA, total DNA was extracted from adult untreated control and adult survivor of OB treatments. • **Quantitative analysis by PCR (qPCR)** for the estimation of latent infections with SYBR fluorescence.
Experimental treatment:	1) Effect of larvae instar stage and OB inoculum concentration on larval mortality and adult emergence. 2) Detection of sublethal SeMNPV infections. 3) Transgenerational persistence of sublethal infections. 4) Cost of survival OB treatments on host development and reproduction. 5) Susceptibility of healthy and subhealthy infected insects to OB inoculum.
Replicates:	• Four suspension concentrations in 10-fold increments of OB from each instar (L2, L3 and L4).
Observations:	• Control larvae were fed with OB-free suspension. • 24 larvae ingested OB suspension four OB concentration for each of the three larvae instar.
Evaluation:	• Bioassay were performed 5 times for L2 and L3 and six for L4. • Daily observation of larvae until death or pupation.
	• 1) Fitting generalized linear models. • 2) Polymerase transcripts by RT-PCR. • 3) Quantitative analysis by PCR (qPCR).
Results:	• Prevalence of adult sublethal infection is positively related to the inoculum OB concentration consumed during the larval stage. • Sublethal infections persisted in OB treated insects for at least five generations. • Viral transcripts were more frequently detected in adult insects than in third instars. • 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.
Conclusions:	OB treatment results in rapid establishment of sublethal infections that persist between generations.

Reference:	Virto, C., Navarro, D., Tellez, M.M., Herrero, S., Williams, T., Murillo, R., Caballero, P. (2014) Natural populations of *Spodoptera exigua* are infected by multiple viruses that are transmitted to their offspring
Report No.:	**Report KMA 7.1/04**
Guideline:	no
GLP:	no
Summary:	Sublethal infections by BVs (Baculoviridae) are believed to be common in Lepidoptera, including *Spodoptera exigua*. In addition, novel RNA viruses of the family *Iflaviridae* have been recently identified in a laboratory population of *S. exigua* (*S. exigua* iflavirus-1: SeIV-1; *S. exigua* iflavirus-2: SeIV-2) that showed no overt signs of disease. We determined the prevalence of these viruses in wild populations and the prevalence of co-infection by the different viruses in shared hosts. Infection by *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) and iflaviruses in *S. exigua* adults (N = 130) from horticultural greenhouses in southern Spain was determined using qPCR and RT-PCR based techniques respectively. The offspring of these insects (N = 200) was reared under laboratory conditions and analyzed to determine virus transmission. Overall, 54% of field-caught adults were infected by SeMNPV, 13.1% were infected by SeIV-1 and 7.7% were infected by SeIV-2. Multiple infections were also detected, with 8.4% of individuals harboring SeMNPV and one of the iflaviruses,

	whereas 2.3% of adults were infected by all three viruses. All the viruses were transmitted to offspring independently of whether the parental female harbored covert infections or not. Analysis of laboratory-reared insects in the adult stage revealed that SeIV-1 was significantly more prevalent than SeMNPV or SeIV-2, suggesting high transmissibility of SeIV-1. Mixed infection involving three viruses was identified in 6.5% of laboratory-reared offspring. We conclude that interspecific interactions between these viruses in co-infected individuals are to be likely frequent, both in the field, following applications of SeMNPV-based insecticides, or in laboratory colonies used for SeMNPV mass production.
M&M: **Test substances:** **Study design & methods** Evaluation:	• **Test Item**: Natural populations of S. *exigua* adults from horticultural area of Almería (Spain). • **Target organism**: SeMNPV and SeIVs • **Detection of viral covert infections** by purification of total DNA and RNA from field-caught and F1 adults. The presence of SeMNPV and and SeIVs *S. exigua* field-caught adults, abdomens of 130 moths were analyzed by qPCR and RT-PCR. • **Detection of transgenerational transmission:** Virus transmission to offspring was investigated by detection of SeMNPV in offspring (F1) adults. Ten field-caught females, either infected or non-infected by SeMNPV were selected at random from those that had produced offspring.
Results:	• 53.8% insects were positive for the DNA polymerase SeMNPV gene. • There were no observe covert infection by SeMNPV in any of the offspring. • Field-collected adults were found to harbor SeIV and SeMNPV, alone and in mixed infections, in reproductively active moths. A high prevalence of sublethal infection was detected; overall 62% of moths had one or more of the viruses, the majority of which were individuals infected by SeMNPV (54%). We examined whether the presence of SeMNPV might influence the transmission of the iflaviruses (or vice versa) from field-collected insects to their laboratory-reared offspring. Unexpectedly, the prevalence of iflavirus infection increased dramatically in F1 insects, as high percentages of the offspring of iflavirus-negative females were found to be positive for SeIV-1 (39%) or SeIV-2 (19%) infection. A combination of highly efficient vertical and horizontal transmission could explain these results, since under laboratory conditions the transition from apparently healthy *S. exigua* colonies to 100% infection by the SeIV-1 was achieved in a single host generation Pre- vious studies on *S. exigua* indicated that covert infections by SeMNPV affect host fitness by increasing their susceptibility to superinfection (Cabodevilla *et al*., 2011b). In line with this result, SeMNPV pathogenicity differed when bioassayed in covertly infected insects in comparison with virus-free insect lines (Cabodevilla *et al*., 2011a). Increased susceptibility to alphabaculo- virus infections may be desirable in pest populations targeted for virus-based biological control, but ongoing laboratory bioassays will reveal whether covert infections by SeIV modify insect responses following consumption of lethal or sublethal doses of SeMNPV OBs.
Conclusions:	• Infections by SeMNPV were detected in *S. exigua* field-collected adults and in laboratory reared offspring.

RMS comments: The study has evaluated the prevalence of BVs infections in a field population of *S. exigua* present in the horticultural greenhouse agroecosystem of Almeria, and confirmed the ability for vertical transmission. It is also relevant for the analysis of the potential influence of certain RNA viruses in *S. exigua* population dynamic.

B.8.2.2 Effect of host infection in Persistence. Climbing behavior

Reference:	Han, Y., van Houte, S., Dress, G. F., van Oers, M. M., Ros, V. I. D. (2015) Parasitic Manipulation of Host Behaviour: BV SeMNPV EGT Facilitates Tree-Top Disease in *Spodoptera exigua* Larvae by Extending the Tine to Death.

Report No.:	**Report KMA 7.1/05**
Guideline:	no
GLP:	no
Summary:	Many parasites enhance their dispersal and transmission by manipulating host behaviour. One intriguing example concerns BVs that induce hyperactivity and tree-top disease (*i.e.*, climbing to elevated positions prior to death) in their caterpillar hosts. Little is known about the underlying mechanisms of such parasite-induced behavioural changes. Here, we studied the role of the ecdysteroid UDP-glucosyltransferase (*egt*) gene of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) in tree-top disease in *S. exigua* larvae. Larvae infected with a mutant virus lacking the *egt* gene exhibited a shorter time to death and died before the induction of tree-top disease. Moreover, deletion of either the open reading frame or the ATG start codon of the *egt* gene prevented tree-top disease, indicating that the EGT protein is involved in this process. We hypothesize that SeMNPV EGT facilitates tree-top disease in *S. exigua* larvae by prolonging the larval time to death. Additionally, we discuss the role of *egt* in BV-induced tree-top disease.
M&M:	Study whether deletion of the viral egt gen or egt start codon from SeMNPV affect viral infectivity, the time to death and the role in SeMNPV-induced tree-top disease.
Test substances:	• **Test Item**: SeMNPV Wt (G25) naturally occurring strain and SeMNPV US1 • **Target organism**: beet armyworm (*S. exigua*, Hübner)
Study design & methods	• **Infection assay:** infectivity for each virus • **Mortality assay:** Effect of virus on the time death • **Behaviour assay:** egt gene role in tree top disease
Experimental treatment:	• **Infection assay:** 3rd instar *S. exigua* infected with 5 SeMNPV viruses at 5 concentration (6-fold dilutions: $10x10^3$ to $1.3x10^6$OB/ml). Control virus-free sucrose solution • **Mortality assay:** 3rd instar *S. exigua* infected with 10^6OB/mL • **Behaviour assay:** 3rd instar *S. exigua* infected with 10^6OB/mL
Replicates:	• **Infection assay:** three replicates; 24-36 larvae/replicate • **Mortality assay:** three replicates; 36 larvae • **Behaviour assay:** Twice x 2 replicates, 30-40 larvae
Observations:	• **Infection assay:** scored for mortality until or larvae had died • **Mortality assay:** larvae evaluation twice/day until died • **Behaviour assay:** Vertical position of the larvae twice/day until died
Evaluation:	• **Infection assay:** LC_{50} by logistic regression analysis • **Mortality assay:** MTD mean time to death • **Behaviour assay:** position at death by linear regression model
Results:	• **Mortality assay:** Larvae infected with mutant virus lacking egt gene presented a shorter time to death and die before the onset of pre-death climbing behaviour. • **Behaviour assay:** The absent of the gen prevented tree top-disease, indicating that the EGT protein is necessary for the occurrence of tree-top disease. EGT protein facilitated the pre-death climbing behaviour by prolonging the larvae time to death.
Conclusions:	In SeMNPV-infected *S. exigua* larvae, EGT facilitates tree-top disease via prolonging the larval time to death

Reference:	Han, Y., van Houte, S., van Oers, M. M., Ros, V. I. D. (2017) Timely trigger of caterpillar zombie behaviour: temporal requirements for light in BV induced tree-top disease
Report No.:	**Report KMA 7.1/06**
Guideline:	no
GLP:	no
Summary:	Host behavioural manipulation is a common strategy used by parasites to enhance their survival and/or transmission. BVs induce hyperactivity and tree-top disease (pre-death climbing behaviour) in their caterpillar hosts. However, little is known about the underlying mechanisms of this behavioural manipulation. A previous study showed that the BV *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) induced tree-top disease at 3 days post infection in third instar *S. exigua* larvae and that light plays a key role in triggering this behaviour. Here we investigated the temporal requirements for the presence of light to trigger this behaviour and found that light from above was needed between 43 and 50 h post infection to induce tree-top disease. Infected larvae that were not exposed to light from above in this period finally died at low positions. Exposure to light prior to this period did not affect the final positions where larvae died. Overall, we conclude that light in a particular time frame is needed to trigger SeMNPV-induced tree-top disease in *S. exigua* larvae.
M&M:	
Test substances:	• **Test Item**: OB of SeMNPV G25, naturally occurring Wt • **Target organism**: beet armyworm (*S. exigua*, Hübner)
Test conditions	• Infection larvae with 10^6OB/mL • Jar contained a cube of artificial diet at the bottom and lined with mesh wire to facilitate larvae climbing
Study design & methods	• 27ºC 50%Hr
Experimental treatment:	• **Behaviour assay 1:** light from above from light to dark conditions • **Behaviour assay 2:** Light from below from light to dark conditions • **Behaviour assay 3:** light from above, from dark to light conditions
Replicates:	• 3 Behaviour assays repeated twice with 30 larvae/treatment. Control larvae fed with free-virus solution
Observations:	• Vertical position where larvae died is recorded 5 days after infection
Evaluation:	• Time point which light is needed to trigger positive phototaxis • Determination of the importance of direction of light • Determination of the importance of light at the beginning of the infection
Results:	• Light was needed between 43 and 50h post infection to induced tree-top disease • Light from above is needed between 43 and 50 hpi to induce tree-top disease, but is not needed when the actual climbing takes place • Positive phototaxis is trigger between 43 and 50hpi • When the light is provided from the below, larvae stay at the bottom until die
Conclusions:	SeMNPV induce hyperactivity and tree-top disease (pre-death climbing behaviour) in *S. exigua*. Light in a particular period is needed to trigger SeMNPV-induced tree-top disease.

B.8.2.3 The role of pathogens in insect population

Reference:	Myers, J. H., Cory, J. S. (2016) Ecology and evolution of pathogens in natural populations of Lepidoptera
Report No.:	**Report KMA 7.1/07**
Guideline:	no
GLP:	no
Summary:	Pathogens are ubiquitous in insect populations and yet few studies examine their dynamics and impacts on host populations. We discuss four lepidopteran systems and explore their contributions to disease ecology and evolution. More specifically, we elucidate the role of pathogens in insect population dynamics. For three species, western tent caterpillars, African armyworm and introduced populations of gypsy moth, infection by nucleopolyhedrovirus (NPV) clearly regulates host populations or reduces their outbreaks. Transmission of NPV is largely horizontal although low levels of vertical transmission occur, and high levels of covert infection in some cases suggest that the virus can persist in a nonsymptomatic form. The prevalence of a mostly vertically transmitted protozoan parasite, *Ophryocystis elektroscirrha*, in monarch butterflies is intimately related to their migratory behaviour that culls highly infected individuals. Virulence and transmission are positively related among genotypes of this parasite. These systems clearly demonstrate that the interactions between insects and pathogens are highly context dependent. Not only is the outcome a consequence of changes in density and genetic diversity: environmental factors, particularly diet, can have strong impacts on virulence, transmission and host resistance or tolerance. What maintains the high level of host and pathogen diversity in these systems, however, remains a question.
Conclusions:	Transmission of NPV is largely horizontal and low levels of vertical transmission occurs. High levels of covert infection suggested that the virus could persist in a non-symptomatic form.

Reference:	Virto C., Zárate, C. A., López-Ferber, M., Murillo, R., Caballero, P., Williams, T. (2013) Gender-Mediated Differences in Vertical Transmission of a Nucleopolyhedrovirus
Report No.:	**Report KMA 7.1/11**
Guideline:	no
GLP:	no
Summary:	With the development of sensitive molecular techniques for detection of low levels of asymptomatic pathogens, it becoming clear that vertical transmission is a common feature of some insect pathogenic viruses, and likely to be essential to virus survival when opportunities for horizontal transmission are unfavorable. Vertical transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) is common in natural populations of *S. exigua*. To assess whether gender affected transgenerational virus transmission, four mating group treatments were performed using healthy and sublethally infected insects: i) healthy males (H♂) × healthy females (H♀); ii) infected males (I♂) × healthy females (H♀); iii) healthy males (H♂) × infected females (I♀) and iv) infected males (I♂) × infected females (I♀). Experimental adults and their offspring were analyzed by qPCR to determine the prevalence of infection. Both males and females were able to transmit the infection to the next generation, although female-mediated transmission resulted in a higher prevalence of infected offspring. Malemediated venereal transmission was half as efficient as maternally-mediated transmission. Egg surface decontamination studies indicated that the main route of transmission is likely transovarial rather than transovum. Both male and female offspring were infected by their parents in similar proportions. Incorporating vertically-transmitted genotypes into virus-based insecticides could provide moderate levels of transgenerational pest control, thereby extending the periods between bioinsecticide applications.
Results:	65-85% of sublethal infection was detected in adult survivors of an inoculum thjat killed 57.6% of the insects.
Conclusions:	Vertical transmission in SeMNPV was demonstrated when male or female parents harbored a sub lethal infection. The maternally mediated transmission is double that of parentally mediate transmission.

RMS comments: The document provided valuable information on vertical transmission of the insect SeMNPV virus and the role of this strategy in the survival of these pathogens in naturel populations. It was confirmed SeMNPV can establish sublethal infections in larvae that survive after having consumed OBs and these infections can be dose-dependent.

B.8.3 MOBILITY

Krieg *et al*. (1983) investigated the content of active viruses (*Mamestra brassicae* nuclear polyhedrosis virus (MbNPV)) in different layers of the soil column and but not investigate the leachate itself. No information is provided about the amount of eluate and of viruses in the eluate. Information about the temperature during the experiment is missing. The leaching trial was conducted with loamy sand (pH 5.6, 2.5 % o.c., 34.5 % sand, 4.9 % clay) and sand (pH 7.0, 0.7 % o.c., 61.5 % sand, 5 % clay). The soil columns were 30 cm high and 5 cm in diameter. The virus suspension contained was applied with 2.8×10^{10} cleared polyhedra of MbNPV in 3 mL water per column. Two irrigation variants applied (A) 786 mL water over a period of 4 days and (B) 393 mL water over a period of 2 days. The accumulated virus-related mortality was measured for 9 samples per column on larvae of *Mamestra brassicae*. Virus activity was still detectable in the bioassay down to a depth of 15 cm for the loamy sand and down to 30 cm in the sand. However, an exponential decrease of activity was documented.

B.8.3.1 Mobility in soil

The study by Lopez-Pila (1988) showed the ability of granuloviruses to leach through a column of soil, but also the low risk of reaching the ground water. The good retention of these viruses by soil is probably attributed to the particular protein envelope of the virus particles consisting of granulin. The column leaching investigation used a sand and organic contaminated soil as substrate. The amount of irrigation was 2400 mL. The authors used different types of irrigation water including deionised water, ground water, buffer and waste water. They applied four different bacteria and viruses including granuloviruses. The column fillings were 48 mm diameter and 200 mm length. The authors leave several experimental conditions unmentioned, e.g. the amount of viruses applied to the columns. Duration of the experiment and the method of virus detection in the eluate. For granuloviruses the study shows that a low percentage of up to 4 % reach the eluate for all types of waters in the sand experiments. For the organic contaminated soil experiment up to 24 % of the granuloviruses for the deionised water and the buffer and up to 4 % for the ground water and the waste water reaches the eluate. The author mentions a field lysimeter experiment which was conducted 1987 in Marienfelde for 7 month. After application of the granuloviruses the leachate was collected at 14 - day intervals and quantified in pooled samples. The lysimeter was not actively irrigated but provided with natural rainfall. No information is given about the amount of granuloviruses applied, the type of soil, the amount of rainfall, the amount of leachate, and the number of investigated samples. During the 7 month investigation period none of the samples contained viruses.

In summary, the studies demonstrated that BVs and granuloviruses respectively are able to leach through a column of soil. Viral activity has been found in a depth of 15 cm for the loamy sand and down to 30 cm in the sand (Krieg, 1983). Lopez-Pila (1988) demonstrated that 4 % of the applied amount of granuloviruses were still detectable in the eluate of 20 cm column of sand and 24 % in a 20 cm long column of organic contaminated soil. The exponential decrease of activity in the study conducted by Krieg (1983) and the results of the field lysimeter experiment conducted 1987 in Marienfelde (Germany) (Lopez-Pila, 1988) indicate a low risk of reaching deeper soil layers and therefore the groundwater. The good retention of BVs by soil is probably attributed to the particular protein envelope of the virus particles consisting of granulin (Lopez-Pila, 1988).

Stability of BVs would not appear to be directly influence by wind, but it has not been study. Virus can be translocated by wind, as the substrate such as soil, insect debris, or foliage is moved (Young, 2005).

The phenomenon of tree-top disease is described by different researchers (van Houte *et al*. 2014a/ 2015, Dobson *et al*. 2015, Rebolledo *et al*. 2015). This BVs induced host behavioral manipulation contributes to the SeMNPV mobility. Infected *S. exigua* larvae migrate to the top of the plant prior to death. It is assumed that this behavior is adaptive for the virus, since it ensures the optimal dissemination of progeny virus onto lower foliage and enhances the visibility (of the

dying larvae) for birds, spreading the virus over longer distance (van Houte *et al*. 2014a / 2015). Thereby, von Houte *et al*. (2014a / 2015) suspected that positive phototaxis prior to death is specifically triggered during virus infection and makes the larvae climbing upwards. However, other researchers (Dubson *et al*. 2017) criticised the study design and interpretation basis and determined this phenomenon to result rather from optimally timed larval killing. Rebolledo *et al*. (2015), concluded that baculovirus-induced climbing behaviour increases the incidence of intraspecific necrophagy in *S. exigua*, which is considered to be the most efficient mechanism of virus transmission. The mobility may be promoted by another observed BV induced alteration of the host behaviour: Van Houte *et al*. (2014b) found that *Autographa caliofornia* nuclear polyhedrovirus (AcMNPV) induces hyperactivity, consequently increased mobility, in infected *S. exigua* larvae and that the protein tyrosine phosphatase (*ptp*) gene is involved in this process.

Reference:	van Houte, S., van Oers, M.M., Han, Y., Vlak, J.M., Ros, V.I.D. (2014a) Baculovirus infection triggers a positive phototactic response in caterpillars to induce 'tree-top' disease
Report No.:	**KMA 7.2/03** , **KMA 2.2.2/11**
Guideline:	NO
GLP:	NO
Summary:	Many parasites manipulate host behaviour to enhance parasite transmission and survival. A fascinating example is BVs, which often induce death in caterpillar hosts at elevated positions (‘tree-top’ disease). To date, little is known about the underlying processes leading to this adaptive host manipulation. Here, we show that the BV Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) triggers a positive phototactic response in *S. exigua* larvae prior to death and causes the caterpillars to die at elevated positions. This light-dependent climbing behaviour is specific for infected larvae, as movement of uninfected caterpillars during larval development was light-independent. We hypothesize that upon infection, SeMNPV captures a host pathway involved in phototaxis and/or light perception to induce this remarkable behavioural change.
Results:	• Hypothesis that ‘tree-top’ disease results from a positive phototactic response, i.e. attraction to light.
Conclusions:	• SeMNPV triggers a positive phototactic response in *S. exigua* larvae prior to death and causes the caterpillars to die at elevated positions.

RMS conclusions: Infection (SeMNPV) causes *S. exigua* larvae to die in an elevated position. This result would improve dissemination of viral occlusion bodies over plant foliage and would increase probability of transmission to healthy conspecific larvae. The study does provide information of host behavior in the environment (effect on light expose), but does not indicate any relationship between climbing behavior and infected insects. It is not relevant for the intended used of SeMNPV as a bioinsecticide.

Reference:	van Houte, S., van Oers, M.M., Han, Y., Vlak, J.M., Ros, V.I.D. (2015) Baculovirus infection triggers a positive phototactic response in caterpillars: a response to Dobson *et al.* (2015)
Report No.:	**KMA 7.2/04** ; **KMA 2.2.2/13**
Guideline:	
GLP:	YES
Summary:	The research group recently reported that BV *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) triggers positive phototaxis in *Spodoptera exigua* larvae, leading to death at elevated positions. However, the experimental set up and the conclusions from the results were criticized by another research team. In this paper van Houte's team is explaining more detailed their experiments and in summary conclude, that the other research team comments would not convince them does not invalidate their main conclusion that light is the cue for "tree-top" disease.

RMS conclusions: No relevant results. There were no statistical data analysis performed.

Reference:	Dobson, A.D.M., Auld, S.K.J.R., Tinsley, M.C. (2015) Insufficient evidence of infection-induced phototactic behaviour in *Spodoptera exigua*: a comment on van Houte *et al*. (2014)
Report No.:	**KMA 7.2/05; KMA 2.2.2/12**
Guideline:	NO

GLP:	NO
Summary:	A recent paper by van Houte *et al*. (2014) claims to demonstrate that (i) infection with the BV *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) causes *S. exigua* larvae to die in an elevated position; and (ii) this is achieved by the virus triggering a positive phototactic response in its larval host. Their study is grounded in knowledge that BVs manipulate climbing behaviour in some lepidopteran species. Dobson *et al*. (2015) argue van Houte *et al*.'s study would have significant limitations: the experimental design cannot test the authors' hypotheses, and the data presented are open to other interpretations that do not support the authors' conclusions.

RMS conclusions: No relevant results.

Reference:	Rebolledo, D. Lasa, R., Guevara, R., Murillo, R., Williams, T. (2015) Baculovirus-Induced Climbing Behaviour Favors Intraspecific Necrophagy and Efficient Disease Transmission in *Spodoptera exigua*
Report No.:	**KMA 7.2/06** ; **KMA 2.2.2/14**
Guideline:	NO
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 occlusion bodies 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 BV-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, BV-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:**	• **Test Item**: insect infected cadavers of *S. exigua* infected with SeMNPV-SU2 • OB of SeMNPV isolate US2 • **Target organism**: beet armyworm (*S. exigua*, Hübner) from maize fields (from Monterey, Mexico)
Study design & methods **Experimental treatment:** **Evaluation:**	• **Crop:** insects were fed with Spinach leafs. • **Petri dish bioassay** contained spinach leaf, infected cadaver and larvae. • Assessment of larvae behaviour to infected cadavers(97 larvae replicates). • Assessment of feeding stimulant effect of infected cadavers (45 larvae replicates). • Response to volatile components of infected cadavers (55 larvae replicates). • Assessment of BVs climbing and foraging -induced behaviour of larvae on plants (30 larvae replicates).

Results:	• Laboratory studies in Petri dish arenas indicated no differences in the frequencies of selection, contact or necrophagous feeding on infected and non-infected cadavers. • Physical contact and feeding on infected cadavers resulted in a high prevalence of lethal virus infection in experimental insects. • Low levels of virus infection were observed in insects that had no direct contact with the infected cadaver. • No evidence for differential responses to volatiles emitted by infected and non-infected cadavers placed on leaf discs. • Insect pathogenic viruses have not been reported to produce volatile compounds that favor their transmission. • Greenhouse observations indicating that SeMNPV-infected cadavers were attractive to healthy conspecifics, laboratory tests and olfactometer studies provided no evidence for the existence of virus-associated olfactory or phago-stimulant factors that might induce intraspecific necrophagy in *S. exigua* larvae. • Baculovirus-induced climbing behavior resulted in infected insects dying in the upper 10% of the plant, which was significantly higher up the plant than the site at which non-infected conspecifics were located at the moment of death of the diseased insect.
Conclusions:	• Climbing in BVs infected insects has been shown to be a pathogen induced behavior that increases the dispersal of viral OBs on the host plant as the insect cadaver disintegrates and OBs fall, or are washed by rainfall, over inferior plant foliage. • baculovirus-induced climbing behavior, involving an increase in the height of infected larvae on the plant and their movement close to the central plant stem, increases the frequency of encounters between virus infected cadavers and healthy larvae foraging for young foliage. This resulted in a very high incidence of intraspecific necrophagy; a behavior that resulted in transmission of SeMNPV.

RMS conclusions: The study is interesting in term of virus transmission and dispersion.

Reference:	van Houte, S., Ros, V.I.D., van Oers, M.M. (2014b) Hyperactivity and tree-top disease induced by the baculovirus AcMNPV in *Spodoptera exigua* larvae are governed by independent mechanisms.
Report No.:	**KMA 7.2/07**
Guideline:	NO
GLP:	NO
Summary:	Although many parasites are known to manipulate the behavior of their hosts, the mechanisms underlying such manipulations are largely unknown. BVs manipulate the behavior of caterpillar hosts by inducing hyperactivity and by inducing climbing behavior leading to death at elevated positions (tree-top disease or Wipfelkrankheit). Whether hyperactivity and tree-top disease are independent manipulative strategies of the virus is unclear. Recently, we demonstrated the involvement of the protein tyrosine phosphatase (ptp) gene of the BV *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) in the induction of hyperactivity in *Spodoptera exigua* larvae. Here we show that AcMNPV ptp is not required for tree-top disease, indicating that in *S. exigua* baculovirus induced hyperactivity and tree-top disease are independently induced behaviors that are governed by distinct mechanisms.
Results:	• To determine whether the AcMNPV ptp gene plays a role in tree-top disease in *S. exigua* larvae, a climbing studies with mock-, AcMNPV WT- and AcMNPV Δptp-infected *S. exigua* larvae were performed. • No differences were observed in the climbing behavior of WT- and Δptp-infected larvae. Similar to WT-infected larvae that died as fourth instars, Δptp-infected larvae that molted to the fourth instar died at high positions.

Conclusions:	• Hyperactivity and tree-top disease are two independent behavioral manipulations that are induced by distinct mechanisms. This implies that BVs have evolved multiple strategies to alter host behavior, probably by manipulating distinct host signalling pathways. • It can be exclude a role for ptp and egt in tree-top disease in AcMNPV-infected *S. exigua* hosts, it is as yet unknown whether a specific viral gene is responsible for tree-top disease.

B.8.3.2 Effects of the microorganism on drinking water analysis

Most viruses in intact inclusion bodies are reasonably stable in aqueous suspension (Jaques, 1977). Heliothis sp. NPV was stable in water at 30°C for one year (Ignoffo, 1992). However, Jaques (1977) continues that it is apparent that the pH and salt concentration of water influences stability. In addition, once introduced into a water body, the viral particles are likely to deposit and are absorbed by sediments. Furthermore, there is evidence of deactivation/destruction by the UV portion of sunlight in aqueous suspensions. Moreover, it is assumed that the protein of the virus will be completely mineralised by bacterial action in water and sediment. There are no risks of pollution of surface or groundwater expected due to the high level of retention of the viral particles by the soil.

S comments: Even all the evidence of the potential inactivation of the BV, most viruses in intact inclusion bodies are reasonably stable in aqueous suspension. No information has been provided in relation to potential interference of PepMV with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC7.

Drinking water quality is monitored by screening for microbial indicator species. Potential interference with the analytical systems for the control of the quality of drinking water according to Council Directive 98/83/EC needs to be addressed. For drinking water coliforms or *E. coli, enterococci, and Pseudomonas aeruginosa* need to be monitored. The lack of close relationship with the microorganisms listed under Directive 98/83/EC, or the absences of information on interaction with bacteria DO NOT CONFIRM the NEGLIGIBLE risk of interference.

No information has been provided in relation to potential interferences with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC. **A data gap is therefore identified.**

B.8.4 REFERENCES RELIED ON

A literature search according to EFSA guidance (2011)[10] was conducted in January 2018 covering the last 10 years. The literature research was carried out using the search-engine ProQuest Dialog™. After rapid assessment based on title and abstract; 39 references were submitted to a detailed assessment of full text documents. From a total of 39 references, 17 references, potentially relevant for fate and behaviour of SeMNPV in the environment, were subjected to full text analysis. Of those, 1 report was identified as non-relevant, 16 reports were identified as relevant for the information on environmental fate and behaviour of SeMNPV in the environment. For more details please refer to Gueli Alletti (2018, provided in KMA 8/01).

Report KMA 7/01 – Gueli Alletti, G. (2018), Literature review on *S. exigua* multiple nucleopolyhedrovirus and its metabolites: Fate and behaviour in the environment

RMS comments:

- RMS has considered all document as new information on the current Draft Assessment Report for the new microbial pest control agent SeMNPV.
- In the opinion of the RMS, the literature research made by the applicant according to EFSA 2011 guidance covered the most relevant news for SeMNPV. The RMS has also included some new references considered important for the evaluation.

ABA – Andermatt Biocontrol AG

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
KMA 7/01	Gueli Alletti, G.	2018	Literature review on Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV: Fate and behaviour in the environment Andermatt Biocontrol AG, CH, 356159-MA-07-01 GAB Consulting GmbH, Heidelberg, Germany GLP/GEP: no Published: no	N	Y		ABA
KMA 7.1/01 B.8.1.1/01	Arrizubieta, M., Simon, O., Torres-Vila, L.M., Figueiredo, E., Mendiola, J., Mexia, A., Caballero, P., Williams, T.	2016	Insecticidal efficacy and persistence of a co-occluded binary mixture of Helicoverpa armigera nucleopolyhedrovirus (HearNPV) variants in protected and field-grown tomato crops on the Iberian Peninsula not available, not applicable Pest Management Science, 72, 660-670 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1/02 MA B.8.1/01	Caballero, P., Murillo, R., Munoz, D., Williams, T.	2009	El nucleopoliedrovirus de Spodoptera exigua (Lepidoptera: Noctuidae) como bioplaguicida: análisis de avances recientes en España not available, not applicable REvista Colombiana de Entomología, 35(2), 105-115 GLP/GEP: no Published: yes	N	N	not protected	-

[10] Guidance of EFSA: 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

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
KMA 7.1/03 MA B.8.1/02 MA B.8.2/01	Cabodevilla, O., Villar, E., Virto, C., Murillo, R., Williams, T., Caballero, P.	2011	Intra- and Intergenerational Persistence of an Insect Nucleopolyhedrovirus: Adverse Effects of Sublethal Disease on Host Development, Reproduction, and Susceptibility to Superinfection not available, not applicable Applied and Environmental Microbiology, 77(9), 2954-2960 GLP/GEP: no Published: yes	N	N	not protected	-
MA B.8.2/02	Bianchi, F. J., J. M. Vlak, R. Rabbinge, andW. Van der Werf.	2002	Biological control of beet armyworm, *Spodoptera exigua*, with baculoviruses in greenhouses: development of a coMArehensive process-based model. Biol. Control 23: 35–46.	N	N	not protected	
MA B.8.2/04	Murillo, R., D. Muñoz, C. Ruız-Portero, D. M. Alcazar, E. J. Belda, T. Williams, and P. Caballero.	2007	Abundance and genetic structure of nucleopolyhedrovirus populations in greenhouse substrate reservoirs. Biol. Control 42: 216–225.				
KMA 7.1/04 MA B.8.1/03	Virto, C., Navarro, D., Tellez, M.M., Herrero, S., Williams, T., Murillo, R., Caballero, P.	2014	Natural populations of Spodoptera exigua are infected by multiple viruses that are transmitted to their offspring not available, not applicable Journal of Invertebrate Pathology, 122, 22-27 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1/05 MA B.8.1/04 MA B.8.2.2/01	Han, Y., van Houte, S., Drees, G.F., van Oers, M.M., Ros, V.I.D.	2015	Parasitic Manipulation of Host Behaviour: Baculovirus SeMNPV EGT Facilitates Tree-Top Disease in Spodoptera exigua Larvae by Extending the Time to Death not available, not applicable insects, 6, 716-731 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1/06 MA B.8.1/05 MA B.8.2.2/02	Han, Y., van Houte, S., van Oers, M.M., Ros, V.I.D	2017	Timely trigger of caterpillar zombie behaviour: temporal requirements for light in baculovirus-induced tree-top disease not available, not applicable Parasitology, 1-6 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1/07 MA B.8.1/06 MA B.8.2.2/03	Myers, J.H., Cory, J.S.	2016	Ecology and evolution of pathogens in natural populations of Lepidoptera not available, not available Evolutionary Applications, 9(1), 231-247 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
KMA 7.1/08 MA B.8.1.1/02 MA B.8.1.4/04 MA B.8.2.2/03	Shapiro, M., El Salamouny, S., Jackson, D. M., Shepard, B. M.	2012	Field Evaluation of a Kudzu/Cottonseed Oil Formulation on the Persistence of the Beet Armyworm nucleopolyhedrovirus not available, not available Journal of Entomological Science, 47(3), 197-207 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1/09 MA B.8.1.1/03 MA B.8.1.4/03	Salamouny, S., Shapiro, M., Ling, K. S., Shepard, B. M.	2009	Black Tea and Lignin as Ultraviolet Protectants for the Beet Armyworm Nucleopolyhedrovirus not available, not available Journal of Entomological Science, 44(1), 50-58 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1/10 MA B.8.1.1/04 MA B.8.1.4/02	Shapiro, M., El Salamouny, S., Shepard, B. M.	2008	Green tea extracts as ultraviolet protectants for the beet armyworm, Spodoptera exigua, nucleopolyhedrovirus not available, not available Biocontrol Science and Technology, 18(6), 591-603 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1/11 MA B.8.1/07 MA B.8.2/04	Virto C., Zárate, C. A., López-Ferber, M., Murillo, R., Caballero, P., Williams, T.	2013	Gender-Mediated Differences in Vertical Transmission of a Nucleopolyhedrovirus not available, not available PloS ONE, 8, e70932 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/01 MA B.8.1.1/05	Jehle, J.A., Lange, M., Wang, H., Hu, Z., Wang, Y., Hauschild, R.	2006	Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera not available, not applicable Virology, 346, 180-193 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/02 MA B.8.1.1/06 MA B.8.1.2/01 MA B.8.3.1/03	Young, S.	2005	Persistence of viruses in the environment not available, not applicable http://www.lsuagcenter.com/s265/young.htm GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/03 MA B.8.1.1/07 MA B.8.1.3/02 MA B.8.3.2/02	Ignoffo, C.M.	1992	Environmental factors affecting persistence of entomopathogens not available, not applicable Fla Entomol, 75, 516-525 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/04 MA B.8.1.1/08	Chakraborty, S., Monsour, C., Teakle, R., Reid, S.	1999	Yield, biological activity, and field performance of a wild-type Helicoverpa nucleopolyhedrovirus produced in *H. zea* cell cultures not available, not applicable Journal of invertebrate Pathology, 73, 199-205 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
KMA 7.1.1/05 MA B.8.1.2/02	Evans, H.F., Harrap, K.A.	1982	Persistence of insect viruses not available, not applicable Virus Persistence, Publisher: Cambridge University Press, 58-96 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/06 MA B.8.1.2/03 MA B.8.1.3/01 MA B.8.3.2/01	Jaques, R.A.	1977	Stability of entomopathogenic viruses not available, not applicable Misc Publ Entomological Soc America, 10(3), 99-119 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/07 MA B.8.1.2/04 MA B.8.1.4/01 MA B.8.3.1/02	Krieg, A.	1983	Testing of a nuclear polyhedrosis preparation MbNPV (unformulated) for leaching behaviour (German Original) Andermatt Biocontrol GmbH, A55490 BBA, Darmstadt, Germany GLP/GEP: no Published: no	N	N	not protected	ABA
KMA 7.1.1/08 MA B.8.1.2/05 MA B.8.3.1/01	Lopez-Pila, J.M.	1988	Effect of Baculoviruses on Groundwater and Drinking water (German Original) not available, not applicable In: Mitteilung aus der Biol. Bundesanstalt, 246, p 178-203 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/09 MA B.8.1.2/06	OECD	2002	Consensus document on information used in the assessment of environmental applications involving baculoviruses not available, not applicable OECD Organisation for Economic Co-operation and Development, 2002 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/10 MA B.8.1.2/07	Jaques, R.P., Harcourt, D.G.	1971	Viruses of Trichoplusia ni (lepidoptera: Noctuidae) and pieris rapae (lepidoptera: pieridae) in soil in fields of crucifers in southern Ontario not available, not applicable The Canadian Entomologist, Journal, 103, 1285-1290 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/11 MA B.8.1.2/08	Thomas, E.D., Reichelderfer, C.F., Heimpel, A.M.	1973	The effect of soil pH on the persistence of cabbage looper nuclear polyhedrosis virus in soil not available, not applicable Journal of invertebrate Pathology, 21, 21-25 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.1.1/12 MA B.8.1.2/09	Jaques, R.P.	1974a	Occurrence and accumulation of viruses of Trichoplusia ni in treated field plots not available, not applicable Journal of invertebrate Pathology, 23, 140-152 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**
KMA 7.1.1/13 MA B.8.1.2/10	Jaques, R.P.	1974b	Occurrence and accumulation of the granulosis virus of Pieris rapae in treated field plots not available, not applicable Journal of invertebrate Pathology, 23, 351-359 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.2/03 MA B.8.3.1/04	van Houte, S., van Oers, M.M., Han, Y., Vlak, J.M., Ros, V.I.D.	2014a	Baculovirus infection triggers a positive phototactic response in caterpillars to induce tree-top disease not available, not applicable Biology Letters, 10, 1-4 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.2/04 MA B.8.3.1/05	van Houte, S., van Oers, M.M., Han, Y., Vlak, J.M., Ros, V.I.D.	2015	Baculovirus infection triggers a positive phototactic response in caterpillars: a response to Dobson et al. (2015) not available, not applicable Biology Letters, 11, 1-4 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.2/05 MA B.8.3.1/06	Dobson, A.D.M., Auld, S.K.J.R., Tinsley, M.C.	2015	Insufficient evidence of infection-induced phototactic behaviour in Spodoptera exigua: a comment on van Houte et al. (2014) not available, not applicable Biology Letters, 11, 1-3 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.2/06 MA B.8.3.1/07	Rebolledo, D. Lasa, R., Guevara, R., Murillo, R., Williams, T.	2015	Baculovirus-Induced Climbing Behavior Favors Intraspecific Necrophagy and Efficient Disease Transmission in Spodoptera exigua not available, not applicable PloS ONE, 1-16 GLP/GEP: no Published: yes	N	N	not protected	-
KMA 7.2/07 MA B.8.3.1/08	van Houte, S., Ros, V. I. D., van Oers, M. M.	2014b	Hyperactivity and tree-top disease induced by the baculovirus AcMNPV in Spodoptera exigua larvae are governed by independent mechanisms not available, not available Naturwissenschaften, 101, 347-350 GLP/GEP: no Published: yes	N	N	not protected	-