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| REGISTRATION REPORT Part B  Section 7: Efficacy Data and Information  Detailed summary |
| Product code:  FORAY® 76B (ABG-6431)  Active Substance:  *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351  206.5 g/L |
| Central Zone  **(zRMS: Poland)** |
| CORE ASSESSMENT |
| Applicant: XXXX  Submission Date: January 2024  Evaluation date: May 2024  MS Finalisation date: September 2024 |

Version history

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| **When** | **What** |
| January 2024 | Initial version submitted by the applicant for Art. 43 on request of zRMS Poland |
| May 2024 | Version evaluated by zRMS |
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**Summary**

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| Comments  of zRMS: | This registration report concerns the extending of authorization of the plant protection product Foray 76B (product code ABG-6431), containing the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 (206,5 g/l) in the EU Central Zone, under Article 43 of Regulation (EC) No. 1107/2009. The zRMS is Poland and cMS are DE, HU and RO.  *B. thuringiensis* subsp. *kurstaki* strain ABTS-351 was first assessed for use as PPP in the EU in 2008 and was included in Annex I of Directive 91/414/EC as a new active substance on 01 May 2009. Application for renewal of this active substance was submitted in 2016 under Regulation (EC) No. 1107/2009 and EFSA on the peer review did not indicate any critical areas of concern.  Foray 76B is currently authorized across the EU for the use as an insecticide to control lepidopteran defoliating caterpillars on deciduous and coniferous forests, pine trees, ornamental trees, and shrubs or amenity areas (parks, gardens).  In Central zone, according to the GAP table, Foray 76B is intended to be used for the control of Lepidoptera caterpillars (L1 to L4) as follows:  – in Poland for control of *Lymantria monacha* (LYMAMO) and *Dendrolimus pini* (DENDPI) in pine trees, *Operophtera brumata* (CHEIBR) *Tortrix viridana* (TORTVI) and *Euproctis chrysorrhoea* (EUPRCH) in deciduous forest,  – in Romania for control of *Choristoneura* spp. (CHONSP), *Lymantria monacha* (LYMAMO), *Thaumetopoea pityocampa* (THAUPI) and *Dendrolimus pini* (DENDPI) in coniferous forest and *Hyphantria cunea* (HYPHCU), *Malacosoma neustria* (MALANE), *Stilpnotia salicis* (LEUOSA), *Euproctis chrysorrhoea* (EUPRCH), *Lymantria dispar* (LYMADI), *Thaumetopoea processionea* (THAUPR), *Tortrix viridana* (TORTVI) and *Operophtera brumata* (CHEIBR) in deciduous forest.  **–** in Hungary for control of *Lymantria dispar* (LYMADI), *Hyphantria cunea* (HYPHCU), *Euproctis chrysorrhoea* (EUPRCH), *Aporia crataegi* (APORCR), *Thaumetopoea processionea* (THAUPR), *Tortrix viridana* (TORTVI) *Geometridae* (1GEOMF), *Tortricidae* (1TORTF), *Gracillariidae* (1GRACF) in deciduous forest species (also on public areas) and *Dendrolimus pini* (DENDPI), *Rhyacionia buoliana* (EVETBU) *Gracillariidae* (1GRACF) in pine species (also on public areas) and also *Lymantria dispar* (LYMADI), *Hyphantria cunea* (HYPHCU), *Euproctis chrysorrhoea* (EUPRCH) *Aporia crataegi* (APORCR), *Thaumetopoea processionea* (THAUPR), *Tortrix viridana* (TORTVI), *Geometridae* (1GEOMF), *Tortricidae*  (1TORTF), *Gracillariidae* (1GRACF), *Dendrolimus pini* (DENDPI). *Rhyacionia buoliana* (EVETBU) in ornamental trees, bushes (also on public areas)  **–** in Germany for control of *Lepidoptera* caterpillars L1 to L3 (when caterpillars are visible following egg hatch & foliage growth sufficient for deposition) in coniferous forest, deciduous forest and ornamental trees.  The applicant did not provide any data on the efficacy of Foray 76B for controlling fungal diseases, giving the note that the information provided by the applicant to support the existing authorization of Foray 76B is still valid as the range of its current registrations is exactly the same as the intended uses presented in the GAP table in Section 2.3.  **Possible development of resistance or cross-resistance**  The resistance of pests to pesticides is caused by, among others, using the same active substances too often during the year and in the following years. This applies, in particular to insecticides, because controlling pests on many plant species requires multiple insecticide treatments. A good example is beetle control on potatoes because, in case of an intense attack of this pest, several treatments are necessary.  Some authors indicate that some species have developed any significant resistance to B. thuringiensis-based products under field use conditions. *Plutella xylostella* was the first insect with field populations resistant to B. thuringiensis. The data on resistance *P. xylostella* or other pests to *B. thuringiensis* add to overall knowledge about the variation of resistance in pest populations.  **Conclusion**. Taking into account the lack of new data, zRMS concludes that the extending of Foray 76B registration in all interested countries (cMS) is justified at the range of current registrations.  Due to the possible development of resistance, appropriate provisions regarding the resistance management strategy should be included in the product label. The proper resistance management programs can be used to mitigate against development of resistance. B. thuringiensis-based products like any other insecticide should be used in IRM (Insecticide Resistance Management) or IPM (Integrated Pest Management) programs. Prolonged use as the only insecticide of choice should also be avoided. |

IIIM 6 Efficacy Data and Information (including Value Data) on the Plant Protection Product

This registration report is submitted to the Ministry of Agriculture and Rural Development (Poland) as zonal Rapporteur Member State (zRMS) and cMS (DE, HU, RO) in August 2023 to support the authorisation of the plant protection product (PPP) Foray® 76B (product code ABG-6431) in the EU Central Zone under Article 43 of Regulation (EC) No. 1107/2009. The formulation Foray® 76B is an aqueous suspension concentrate (SC) containing 206.5 g/L the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351. The content of *B. thuringiensis* subsp*. kurstaki* strain ABTS-351 in Foray® 76B range between 1.17 x 1013 CFU/L and 1.69 x 1013 CFU/L (nominal concentration of 1.51 x 1013 CFU/L). It is currently authorised across the EU for use as an insecticide to control lepidopteran defoliating caterpillars on deciduous and coniferous forest, pine trees, ornamental trees and shrubs or amenity areas (parks, gardens).

*B. thuringiensis* subsp. *kurstaki* strain ABTS-351 was first assessed for approval for use as PPP in the EU in 2008 by Denmark as Rapporteur Member State (RMS). It was included in Annex I of Directive 91/414/EC as a new active substance on 01 May 2009. Application for renewal of the active substance was submitted to Denmark (RMS) and the Netherlands (co-RMS) in 2016 under Regulation (EC) No. 1107/2009, replacing Directive 91/414/EC. EFSA Conclusion on the peer review of risk assessment of *B. thuringiensis* subsp. *kurstaki* strain ABTS-351 was published on 22 October 2021 (EFSA Journal 2021;19(10):6879). No critical areas of concern were identified in the EFSA Conclusion. Renewal of approval of *B. thuringiensis* subsp. *kurstaki* strain ABTS-351 was granted on 23 May 2023 (entry into force 1 July 2023); Commission Implementing Regulation (EU) 2023/999.

When the AIR 4 dossier was submitted for EU renewal of *B. thuringiensis* subsp*. kurstaki* strainABTS-351, an application to demonstrate technical equivalence of *B. thuringiensis* subsp*. kurstaki* strainABTS-351produced at a new manufacturing site for XXXX, was also submitted to Denmark. Technical equivalence was granted in January 2018.

DiPel® DF (product code ABG-6404) is the representative formulation used to support the application for renewal of approval of *B. thuringiensis* subsp. *kurstaki* strain ABTS-351, thus have been evaluated during the approval process. The representative uses are outdoor vegetables (cabbage) and indoor vegetables (tomato).

Efficacy data and information has not changed since the last evaluation.

IIIM 6.1 Efficacy data

IIIM 6.1.1 Preliminary range-finding tests

No changes since last evaluation.

IIIM 6.1.2 Minimum effective dose tests

No changes since last evaluation.

IIIM 6.1.2 Minimum effective dose tests

No changes since last evaluation.

IIIM 6.1.3 Efficacy tests

No changes since last evaluation.

IIIM 6.1.4 Effects on yield and quality

IIIM 6.1.4.1 Impact on the quality of plants and plant products

No changes since last evaluation.

IIIM 6.1.4.2 Effects on the processing procedure.

No changes since last evaluation.

IIIM 6.1.4.3 Effects on the yield of treated plants and plant products

No changes since last evaluation.

IIIM 6.2 Adverse effects

IIIM 6.2.1 Phytotoxicity to host crop

No changes since last evaluation.

IIIM 6.2.2 Adverse effects on beneficial organisms (other than bees)

No changes since last evaluation.

IIIM 6.2.3 Adverse effects on parts of plant used for propagating purposes

No changes since last evaluation.

IIIM 6.2.4 Impact on succeeding crops

No changes since last evaluation.

IIIM 6.2.5 Impact on other plants including adjacent crops

No changes since last evaluation.

IIIM 6.2.6 Possible development of resistance or cross-resistance

Limited information is available in literature to address development of resistance specifically to *B. thuringiensis* subsp. *kurstaki* strain ABTS-351. Therefore, this section is addressed with general information available regarding development of resistance to *B. thuringiensis strains*.

As with any insecticide, too frequent use of one type of *B*t strain or one type of *Bt* delta-endotoxin can result in resistance of the insect to the active ingredient. Only one species (*Plutella xylostella*) has developed any significant resistance to *B. thuringiensis*-based products under field use conditions (Shelton *et al*., 2000). *P. xylostella* developed resistance to *B. thuringiensis* subsp. *kurstaki* (*Btk*). However, most of these studies dealt with finding isolated field populations of diamondback moth in areas with heavy *B. thuringiensis* use (Shelton *et al*. 2000; Tabashnik *et al*., 1994). The *Btk* resistance was adequately addressed in these regions in early 1990’s with rotations of either chemical insecticides or *Bt* products containing other delta-endotoxins such as present in *Bt* subspecies *aizawai*.

Under laboratory conditions, some insect species can develop resistance to *B. thuringiensis* strains. Resistance to different *B. thuringiensis* has been demonstrated following artificial selection of laboratory populations of insect larvae to sub-lethal doses. In total, thirteen insect species have been reported to develop some level of resistance. However, eleven of these species have not developed any known resistance to various strains of *B. thuringiensis* toxin in the field. These include, *Ostrinia nubilalis* (the European corn borer; Huang *et al.*, 1999), *Heliothis virescens* (the tobacco budworm; Gould *et al*., 1997), *Pectinophora gossypiella* (the pink bollworm moth; Liu *et al*., 1999), *Culex quinquefasciatus* (the Southern house mosquito; Wirth *et al*., 1997), *Cadra cautella* (the almond moth; McGaughey and Beeman, 1988), *Chrysomela scripta* (the cottonwood leaf beetle; Frederici and Bauer, 1998), *Spodoptera exigua* (the beet armyworm; Moar *et al*., 1995), *Spodoptera littoralis* (the Egyptian cotton leafworm; Salama and Matter, 1991), *Trichoplusia ni* (the tiger moth; Janmaat and Myers, 2003), *Leptinotarsa decemlineata* (the Colorado potato beetle; Rahardja and Whalon 1995), and *Aedes aegypti* (the yellow fever mosquito; Loke *et al*., 2010).

There are different ways to monitor development of resistance to insecticides. Susceptibility of caterpillar species to insecticides can be monitored by leaf dip bioassay or bioassay using a semi-synthetic diet if available for the specific caterpillar species. An example of the latter is given by Mascarenhas & Boethel (1997) on Soybean looper (*Pseudoplusia includens*). Insecticide resistance monitoring for spinosad, indoxacarb and emamectin benzoate in field populations of *Plutella xylostella* using cabbage leaf dip assays has been well established, the same technique can be and has been used for *Btk* (Shelton *et al*., 2000; Zhao *et al*., 2002; Zhao *et al*., 2006). Other test methods are available such as a droplet feeding method or force-feeding method as developed by Van Frankenhuyzen *et al*. (1995) who examined the geographic variation in susceptibility of *Choristoneura fumiferana* (Spruce budworm) populations across Canada to *Btk*.

*Bts* can be alternated with other environmentally friendly insecticides, such as spinosad, since there is no cross resistance to the different modes of action. The inherently unique nature of *Bt* products provides for a combination of control methods. Use of only a single Cry protein from *Bts* may result in resistance in mosquitos within only a few generations. However, *Bts* have multiple toxins and when at least two or more proteins are used in combination, resistance is unlikely (Georghiou and Wirth, 1997; da Silva Carvalho *et al.,* 2018). This is further supported by Zhu *et al.* (2015) and Fayad *et al.* (2019), who noted that *Btk* strain HD1 and *Bti* AM65-52 for example, contain plasmids carrying genes for several crystal proteins (cry1Aa, cry1Ab, cry1Ac, cry1Ia, cry2Aa, and cry2Ab in the case of strain HD1; Cry4Aa, Cry4Ba, Cry10Aa, Cry11Aa, Cyt1Aa and Cyt2Ba in the case of AM65-52) and that each protein shows individual toxicity. However, their combination and synergistic interactions delays resistance evolution in the field and allows a more pronounced efficacy and an enhanced toxicity of the individual toxins. In addition, the spore is active, germinating within the insect and causing septicemia.

Resistance management programs may also involve the existence of a nearby location of a susceptible population. Individuals from the susceptible population are expected to diffuse into the treated population to mate, thereby maintaining a higher frequency of susceptible genes. It has been shown that resistance can be resolved through a rotation with a different *Bt* subspecies (example *Btk* and *Bta*) when introduced into a rotation during a season-long control program. Any program would need to be based upon the IRAC guidelines for rotating substances throughout the season, with reference to the *Btk* IRAC classification as Main group 11, sub-group 11A. Overall, resistance development from field use of DiPel® DF is not expected. Moreover, proper resistance management programs can be used to mitigate against development of resistance. *B. thuringiensis*-based products like any other insecticide should be used in IRM (Insecticide Resistance Management) or IPM (Integrated Pest Management) programs. Prolonged use as the only insecticide of choice should also be avoided.

For context, abstracts of published literature referred to are provided below:

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| Data point addressed | IIIM 6.2.2/01 |
| Author(s) (year) | Shelton, A.M., Sances, F.V., Hawley, J., Tang, J.D., Boune, M., Jungers, D., Collins, H.L., and Farias, J. (2000) |
| Title | Assessment of Insecticide Resistance After the Outbreak of Diamondback Moth (Lepidoptera: Plutellidae) in California in 1997 |
| Report number | Journal of Economic Entomology, June 2000, Vol. 93, Issue 3, p. 931-936 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

During an outbreak of the diamondback moth, *Plutella xylostella* (L.), in California in 1997, nine populations were collected from the major broccoli areas throughout the state. Populations were assayed for their susceptibility to currently used materials (*Bacillus thuringiensis* subsp. *kurstaki*, permethrin, and methomyl) and to newer materials that had not yet been commercially used in California (spinosad, emamectin benzoate, and chlorfenapyr). For the currently used insecticides, elevated levels of resistance were seen only with permethrin and seven of the nine populations had tolerance ratios (TR) of ˃100. With the newer chemistries, TR values were all ˂15. To compare potential cross-tolerance, TR values of the currently used insecticides were compared with TR values of the newer insecticides. There were significant relationships found between: methomyl and emamectin benzoate, methomyl and spinosad, and permethrin and spinosad. Further biochemical studies are needed to confirm the actual mechanisms that lead to these relationships and field tests are needed to determine what impact, if any, such TR levels would have on control in the field. These data indicate that resistance to at least one of the commonly used insecticides (permethrin) may have played a role in the outbreak during 1997. However, other factors may have been at least equally important. The winter of 1996 - 1997 was warmer than normal, and during the period from February through August of 1997 the amount of rainfall was, 50% of normal. Hot and dry conditions are known to be conducive to outbreaks of *P. xylostella*. These data add to an overall knowledge about the geographic variation of resistance in *P. xylostella* populations within the United States. They also serve as a baseline for monitoring changes in susceptibility to these newer insecticides and can also help explain the occurrence of outbreaks caused by factors other than insecticide resistance.

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| Data point addressed | IIIM 6.2.2/02 |
| Author(s) (year) | Tabashnik, B.E., Finson, N., Groeters, F.R., Moar, W.J., Johnson, M.W., Luo, K., and Adang M.J. (1994) |
| Title | Reversal of resistance to Bacillus thuringiensis in *Plutella xylostella* |
| Report number | Proceedings of the National Academy of Sciences (PNAS), May 1994, Vol. 91, Issue 10, p. 4120-4124 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Continued success of the most widely used biopesticide, *Bacillus thuringiensis*, is threatened by development of resistance in pests. Experiments with *Plutella xylostella* (diamondback moth), the first insect with field populations resistant to *B. thuringiensis*, revealed factors that promote reversal of resistance. In strains of *P. xylostella* with 25- to 2800-fold resistance to *B. thuringiensis* compared with unselected strains, reversal of resistance occurred when exposure to *B. thuringiensis* was stopped for many generations. Reversal of resistance was associated with restoration of binding of *B. thuringiensis* toxin CryIA(c) to brush-border membrane vesicles and with increased biotic fitness. Compared with susceptible colonies, revertant colonies had a higher proportion of extremely resistant individuals. Revertant colonies responded rapidly to reselection for resistance. Understanding reversal of resistance will help to design strategies for extending the usefulness of this environmentally benign insecticide.

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| Data point addressed | IIIM 6.2.2/03 |
| Author(s) (year) | Huang, F., Buschman, L., Higgins, R.A., and McGaughey, W.H. (1999) |
| Title | Inheritance of Resistance to *Bacillus thuringiensis* Toxin (Dipel ES) in the European Corn Borer |
| Report number | Science, May 1999, Vol. 284, Issue 5416, p. 965-967 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Resistance in the European corn borer, *Ostrinia nubilalis* (Hübner), to a commercial formulation of *Bacillus thuringiensis* (*Bt*) Berliner toxin, Dipel ES, appears to be inherited as an incompletely dominant autosomal gene. This contrasts with the inheritance of resistance to *Bt* in other insects, where it has usually been characterized as a recessive trait. The proposed high-dose/refuge strategy for resistance management in *Bt* maize depends on resistance being recessive or partially recessive. If field resistance turns out to be similar to this laboratory resistance, the usefulness of the high-dose/refuge strategy for resistance management in *Bt* maize may be diminished.

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| Data point addressed | IIIM 6.2.2/04 |
| Author(s) (year) | Gould, F., Anderson, A., Jones, A., Sumerford, D., Heckel, D.G., Lopez, J., Micinski, S., Leonard, R., and Laster, M. (1997) |
| Title | Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens* |
| Report number | Proceedings of the National Academy of Sciences, April 1997, Vol. 94, Issue 8, p. 3519-3523 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

The risk of rapid pest adaptation to an insecticide is highly dependent on the initial frequency of resistance alleles in field populations. Because we have lacked empirical estimates of these frequencies, population–genetic models of resistance evolution have relied on a wide range of theoretical estimates. The recent commercialization of genetically engineered cotton that constitutively produces an insecticidal protein derived from the biocontrol agent, *Bacillus thuringiensis* (*Bt*) has raised concern that we lack data needed to quantify the risk of insect pests such as *Heliothis virescens* rapidly adapting to this ecologically valuable class of toxins. By individually mating over 2,000 male *H. virescens* moths collected in four states to females of a *Bt* toxin-resistant laboratory strain, and screening F1 and F2 offspring for tolerance of the toxic protein, we were able to directly estimate the field frequency of alleles for resistance as 1.5 × 10−3. This high initial frequency underscores the need for caution in deploying transgenic cotton to control insect pests. Our single-pair mating technique greatly increases the efficiency of detecting recessive resistance alleles. Because alleles that decrease target site sensitivity to *Bt* toxins and other insecticides are often recessive, this technique could be useful in estimating resistance allele frequencies in other insects exposed to transgenic insecticidal crops or conventional insecticides.

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| Data point addressed | IIIM 6.2.2/05 |
| Author(s) (year) | Liu, Y.B., Tabashnik, B.E., Dennehy, T.J., Patin, A.L., and Bartlett, A.C. (1999) |
| Title | Development time and resistance to *Bt* crops |
| Report number | Nature, August 1999, Vol. 400, Issue 6744, p. 519 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Crop plants genetically engineered to produce insecticidal toxins derived from the bacterium *Bacillus thuringiensis* (*Bt*) are being grown on millions of hectares, but their success will be short-lived if pests adapt to them quickly. The primary strategy for delaying insect resistance to transgenic *Bt* plants is to provide refuges of host plants that do not produce *Bt* toxins. This potentially delays the development of insect resistance to *Bt* crops by providing susceptible insects for mating with resistant insects. But our laboratory results with a worldwide pest of cotton, pink bollworm moths (*Pectinophora gossypiella*), contradict an important assumption of the refuge strategy. We find that a resistant strain of larvae on *Bt* cotton takes longer to develop than susceptible larvae on non-*Bt* cotton. This developmental asynchrony favours non-random mating that could reduce the expected benefits of the refuge strategy.

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| Data point addressed | IIIM 6.2.2/06 |
| Author(s) (year) | Wirth, M.C., Georghiou, G.P., and Federici, B.A. (1997) |
| Title | CytA enables CryIV endotoxins of *Bacillus thuringiensis* to overcome high levels of CryIV resistance in the mosquito, *Culex quinquefasciatus* |
| Report number | Proceedings of the National Academy of Sciences (PNAS), September 1997, Vol. 94, Issue 20, p. 10536-10540 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Cry proteins produced by *Bacillus thuringiensis* are selective biodegradable insecticides used increasingly in bacterial insecticides and transgenic plants as alternatives to synthetic chemical insecticides. However, the potential for development of resistance and cross-resistance in target insect populations to Cry proteins used alone or in combination threatens the more widespread use of this novel pest control technology. Here we show that high levels of resistance to CryIV proteins in larvae of the mosquito, *Culex quinquefasciatus*, can be suppressed or reduced markedly by combining these proteins with sublethal quantities of CytA, a cytolytic endotoxin of *B. thuringiensis*. Resistance at the LC95 level of 127-fold for a combination of three CryIV toxins (CryIVA, B, and D), resulting from 60 generations of continuous selection, was completely suppressed by combining sporulated powders of CytA in a 1:3 ratio with sporulated powders of a CryIVA, CryIVB, and CryIVD strain. Combining the CytA strain with a CryIVA and CryIVB strain also completely suppressed mosquito resistance of 217-fold to the latter toxins at the LC95 level, whereas combination of CytA with CryIVD reduced resistance in a CryIVD-selected mosquito strain from greater than 1,000-fold to less than 8-fold. The CytA/CryIV model provides a potential molecular genetic strategy for engineering resistance management for Cry proteins directly into bacterial insecticides and transgenic plants.

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| Data point addressed | IIIM 6.2.2/07 |
| Author(s) (year) | McGaughey, W.H., and Beeman, R.W. (1988) |
| Title | Resistance to *Bacillus thuringiensis* in Colonies of Indianmeal Moth and Almond Moth (Lepidoptera: Pyralidae) |
| Report number | Journal of Economic Entomology, February 1988, Vol. 81, Issue 1, p. 28-33 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Colonies of Indianmeal moth, *Plodia interpunctella* (Hübner), and almond moth, *Cadra cautella* (Walker), reared in the laboratory on diet treated with *Bacillus thuringiensis*, became resistant to *B. thuringiensis*. However, resistance did not progress at the same rate or to the same extent in all of the colonies. Resistance in five Indianmeal moth colonies increased from 2- to 29-fold within three generations, and from 15- to 100-fold in ca. 40 generations under relatively low selection pressure. With higher selection pressure, resistance in one colony increased >250-fold. Resistance in an almond moth colony increased only ca. 7-fold in 21 generations of intensive selection. Resistance was stable when selection was discontinued after the resistance levels reached a plateau but declined if selection was discontinued earlier. The resistance was partially recessive in the five Indianmeal moth colonies, but not to an equal extent. The resistance assorted independently of the recessive genetic markers *copper, golden*, and *white-eye*.

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| Data point addressed | IIIM 6.2.2/08 |
| Author(s) (year) | Federici, B.A., and Bauer, L.S. (1998) |
| Title | Cyt1Aa Protein of *Bacillus thuringiensis* Is Toxic to the Cottonwood Leaf Beetle, *Chrysomela scripta*, and Suppresses High Levels of Resistance to Cry3Aa |
| Report number | Applied and Environmental Microbiology, November 1998, Vol. 64, Issue 11, p. 4368-4371 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

The insecticidal activity of *Bacillus thuringiensis* is due primarily to Cry and Cyt proteins. Cry proteins are typically toxic to lepidopterous, coleopterous, or dipterous insects, whereas the known toxicity of Cyt proteins is limited to dipterans. We report here that a Cyt protein, Cyt1Aa, is also highly toxic to the cottonwood leaf beetle, *Chrysomela scripta*, with a median lethal concentration of 2.5 ng/mm2 of leaf surface for second-instar larvae. Additionally, we show that Cyt1Aa suppresses resistance to Cry3Aa greater than 5,000-fold in *C. scripta*, a level only partially overcome by Cry1Ba due to cross-resistance. Studies of the histopathology of C. scripta larvae treated with Cyt1Aa revealed disruption and sloughing of midgut epithelial cells, indicating that its mechanism of action against *C. scripta* is similar to that observed in mosquito and blackfly larvae. These novel properties suggest that Cyt proteins may have an even broader spectrum of activity against insects and, owing to their different mechanism of action in comparison to Cry proteins, might be useful in managing resistance to Cry3 and possibly other Cry toxins used in microbial insecticides and transgenic plants.

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| Data point addressed | IIIM 6.2.2/09 |
| Author(s) (year) | Moar, W.J., Pustztai-Carey, M., Van Faassen, H., Bosch, D., Frutos, R., Rang, C., Luo, K., and Adang, M.J. (1995) |
| Title | Development of *Bacillus thuringiensis* CryIC Resistance by Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) |
| Report number | Applied and Environmental Microbiology, June 1995, Vol. 61, Issue 6, p. 2086-2092 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Selection of resistance in *Spodoptera exigua* (Hübner) to an HD-1 spore-crystal mixture, CryIC (HD-133) inclusion bodies, and trypsinized toxin from *Bacillus thuringiensis* subsp. *aizawai* and *B. thuringiensis* subsp. *entomocidus* was attempted by using laboratory bioassays. No resistance to the HD-1 spore-crystal mixture could be achieved after 20 generations of selection. Significant levels of resistance (11-fold) to CryIC inclusion bodies expressed in *Escherichia coli* were observed after seven generations. Subsequent selection of the CryIC-resistant population with trypsinized CryIC toxin resulted, after 21 generations of CryIC selection, in a population of *S. exigua* that exhibited only 8% mortality at the highest toxin concentration tested (320 mg/g), whereas the 50% lethal concentration was 4.30 mg/g for the susceptible colony. Insects resistant to CryIC toxin from HD-133 also were resistant to trypsinized CryIA(b), CryIC from *B. thuringiensis* subsp. *entomocidus*, CryIE-CryIC fusion protein (G27), CryIH, and CryIIA. In vitro binding experiments with brush border membrane vesicles showed a twofold decrease in maximum CryIC binding, a fivefold difference in Kd, and no difference in the concentration of binding sites for the CryIC-resistant insects compared with those for the susceptible insects. Resistance to CryIC was significantly reduced by the addition of HD-1 spores. Resistance to the CryIC toxin was still observed 12 generations after CryIC selection was removed. These results suggest that, in *S. exigua*, resistance to a single protein is more likely to occur than resistance to spore-crystal mixtures and that once resistance occurs, insects will be resistant to many other Cry proteins. These results have important implications for devising *S. exigua* resistance management strategies in the field.

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| Data point addressed | IIIM 6.2.2/10 |
| Author(s) (year) | Salama, H.S., and Matter, M (1991) |
| Title | Tolerance level to *Bacillus thuringiensis* Berliner in the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lep., Noctuidae) |
| Report number | Journal of Applied Entomology, December 1991, Vol. 111, Issue 1-5, p. 225-230 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

A standard laboratory strain of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) was treated with *Bacillus thuringiensis* var. *kurstaki*-HD1 (Dipel 2X) for eight successive generations. Selection with *B. thuringiensis* did not establish true resistance in the population. On the contrary, a latent toxicity was demonstrated. Slight delay in response to the toxic effect of the pathogen was developed after continuous selection for four successive generations. This may be attributed to the increase in vigour tolerance due to intrinsic or extrinsic factors rather than development of true resistance.

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| Data point addressed | IIIM 6.2.2/11 |
| Author(s) (year) | Janmaat, A.F., and Myers, J. (2003) |
| Title | Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni* |
| Report number | Proceedings of the Royal Society B, November 2003, Vol. 270, Issue 1530, p. 2263-2270 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

The microbial insecticide *Bacillus thuringiensis* (*Bt*) has become the mainstay of non–chemical control of Lepidopteran pests, either as sprays or through the incorporation of *Bt* toxins into transgenic crops. Given the wide use of *Bt*, it is striking that currently only one pest species, *Plutella xylostella*, has been reported to have developed significant resistance to *Bt* outside the laboratory. By contrast, we report here the frequent and rapid development of resistance to *B. thuringiensis kurstaki* (Dipel, Abbott) in populations of cabbage loopers, *Trichoplusia ni*, in commercial greenhouses. Resistance to *Bt* appears to be costly and there is a rapid decline of resistance in populations collected from greenhouses and maintained in the laboratory without selection. Management of pests resistant to *Bt* in vegetable greenhouses will require sporadic use of *Bt*–based sprays or alternatively use of sprays containing other *Bt* toxins.

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| Data point addressed | IIIM 6.2.2/12 |
| Author(s) (year) | Rahardja, U., and Whalon, M.E. (1995) |
| Title | Inheritance of Resistance to *Bacillus thuringiensis* subsp. *tenebrionis* CryIIIA δ-Endotoxin in Colorado Potato Beetle (Coleoptera: Chrysomelidae) |
| Report number | Journal of Economic Entomology, February 1995, Vol. 88, Issue 1 p. 21-26 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

We investigated the genetic inheritance of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), resistance to *Bacillus thuringiensis* CryIIIA 8-endotoxin. Standard reciprocal crosses and backcrosses between susceptible (S) and resistant (R) strains were used to determine the characteristics of resistance. Analysis of probit lines from the F1 reciprocal crosses indicated that *B. thuringiensis* δ-endotoxin resistance was inherited autosomaly without maternal effects. We estimated the degree of dominance to be 0.77 and 0.76 for the (R × S) and (S × R) F1 generations, respectively, indicating that *B. thuringiensis* CryIIIA δ-endotoxin resistance is conferred by incompletely dominant genes. Chi-square analysis of mortality responses of backcrossed offspring suggested that resistance might be caused by more than one locus. The stability of resistance was also studied by testing seventeen generations of resistant beetles after the selection pressure was removed. When the selection pressure was removed, the resistance level of the selected colony decreased after five generations. The resistance level did not decrease further when the selection was removed for > 12 generations.

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| Data point addressed | IIIM 6.2.2/13 |
| Author(s) (year) | Loke, S.R., Andy-Tan, W.A., Benjamin, S., Lee, H.L., and Sofian-Azirun, M. (2010) |
| Title | Susceptibility of Field-Collected Aedes aegypti (L.) (Diptera: Culicidae) to Bacillus thuringiensis israelensis and temephos |
| Report number | Tropical Biomedicine, July 2010, Vol. 27, Issue 3, p. 493-503 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

The susceptibility status of field-collected *Aedes aegypti* (L.) from a dengue endemic area to *Bacillus thuringiensis israelensis* (*Bti*) and temephos was determined. Since August 2007, biweekly ovitrap surveillance (OS) was conducted for 12 months in 2 sites, A & B, in Shah Alam, Selangor. Site A was treated with a *Bti* formulation, VectoBac® WG at 500 g/ha, from December 2007 – June 2008 while Site B was subjected to routine dengue vector control activities conducted by the local municipality. *Aedes aegypti* larvae collected from OS in both sites were bred until F3 and evaluated for their susceptibility. The larvae were pooled according to 3 time periods, which corresponded to *Bti* treatment phases in site A: August – November 2007 (*Bti* pre-treatment phase); December 2007 – June 2008 (*Bti* treatment phase); and July – September 2008 (*Bti* post-treatment phase). Larvae were bioassayed against *Bti* or temephos in accordance with WHO standard methods. Larvae collected from Site A was resistant to temephos, while incipient temephos resistant was detected in Site B throughout the study using WHO diagnostic dosage of 0.02 mg/L. The LC50 of temephos ranged between 0.007040 – 0.03799 mg/L throughout the year in both sites. Resistance ratios (LC50) indicated that temephos resistance increased with time, from 1.2 – 6.7 folds. The LC50 of *Aedes aegypti* larvae to *Bti* ranged between 0.08890 – 0.1814 mg/L throughout the year in both sites, showing uniform susceptibility of field larvae to *Bti*, in spite of Site A receiving 18 *Bti* treatments over a period of 7 months. No cross-resistance of *Aedes aegypti* larvae from temephos to *Bti* was detected.

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| Data point addressed | IIIM 6.2.2/14 |
| Author(s) (year) | Mascarenhas, R.N. and Boethel, D.J. (1997) |
| Title | Responses of field-collected strains of soybean looper (Lepidoptera: Noctuidae) to selected insecticides using an artificial diet overlay bioassay |
| Report number | Journal of Economic Entomology, October 1997, Vol. 90, Issue 5, p. 1117-1124 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Third instar F1, progeny of several field-collected strains of soybean looper, *Pseudoplusia includens* (Walker), were exposed to artificial diet that was surface-treated with several concentrations of selected insecticides (permethrin [Ambush], *Bacillus thuringiensis* variety *kurstaki* [Condor OF), thiodicarb [Larvin], chlorfenapyr [Pirate], emamectin benzoate [Proclaim], or spinosad [Tracer]). LC50 s (72 h) for field strains were compared with a susceptible USDA reference strain to evaluate possible tolerance to these insecticides. Significant differences were found among LC50 s of all field strains and the susceptible USDA reference strain in the permethrin bioassays and among several field strains and the USDA strain in the *B. thuringiensis*, thiodicarb, and emamectin benzoate bioassays. In the chlorfenapyr bioassays, only 1 field strain from Winnsboro, LA, had a significantly greater LC50 than that of the USDA strain. In the spinosad bioassays, the only field strain with a significantly different LC50 than that of the USDA strain was the strain collected from Hamburg, LA, and this strain had a lower LC50 than that of the USDA strain. These data will serve as a historical database for monitoring soybean looper resistance to these compounds and should prove useful in the development of an insecticide resistance management program for this pest.

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| Data point addressed | IIIM 6.2.2/15 |
| Author(s) (year) | Shelton, A.M., Sances, F.V., Hawley, J., Tang, J.D., Boune, M., Jungers, D., Collins, H.L., and Farias, J. (2000) |
| Title | Assessment of Insecticide Resistance After the Outbreak of Diamondback Moth (Lepidoptera: Plutellidae) in California in 1997 |
| Report number | Journal of Economic Entomology, Jun 2000, Vol. 93, Issue 3, p. 931-936 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

During an outbreak of the diamondback moth, *Plutella xylostella* (L.), in California in 1997, nine populations were collected from the major broccoli areas throughout the state. Populations were assayed for their susceptibility to currently used materials (*Bacillus thuringiensis* subsp. *kurstaki*, permethrin, and methomyl) and to newer materials that had not yet been commercially used in California (spinosad, emamectin benzoate, and chlorfenapyr). For the currently used insecticides, elevated levels of resistance were seen only with permethrin and seven of the nine populations had tolerance ratios (TR) of > 100. With the newer chemistries, TR values were all < 15. To compare potential cross-tolerance, TR values of the currently used insecticides were compared with TR values of the newer insecticides. There were significant relationships found between: methomyl and emamectin benzoate, methomyl and spinosad, and permethrin and spinosad. Further biochemical studies are needed to confirm the actual mechanisms that lead to these relationships and field tests are needed to determine what impact, if any, such TR levels would have on control in the field. These data indicate that resistance to at least one of the commonly used insecticides (permethrin) may have played a role in the outbreak during 1997. However, other factors may have been at least equally important. The winter of 1996-1997 was warmer than normal, and during the period from February through August of 1997 the amount of rainfall was < 50% of normal. Hot and dry conditions are known to be conducive to outbreaks of *P. xylostella*. These data add to an overall knowledge about the geographic variation of resistance in *P. xylostella* populations within the United States. They also serve as a baseline for monitoring changes in susceptibility to these newer insecticides and can also help explain the occurrence of outbreaks caused by factors other than insecticide resistance.

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| Data point addressed | IIIM 6.2.2/16 |
| Author(s) (year) | Zhao, J.Z., Collins, H.L., Li, Y.X., Mau, R.F.L, Thompson, G.D., Hertlein, M., Andaloro, J.T., Boykin, R., and Shelton, A.M. (2006) |
| Title | Monitoring of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, indoxacarb, and emamectin benzoate |
| Report number | Journal of Economic Entomology, Feb 2006, Vol. 99, Issue 1, p. 176-181 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Six to nine populations of the diamondback moth, *Plutella xylostella* (L.), were collected annually from fields of crucifer vegetables in the United States and Mexico from 2001 to 2004 for baseline susceptibility tests and resistance monitoring to spinosad, indoxacarb, and emamectin benzoate. A discriminating concentration for resistance monitoring to indoxacarb and emamectin benzoate was determined based on baseline data in 2001 and was used in the diagnostic assay for each population in 2002-2004 together with a discriminating concentration for spinosad determined previously. Most populations were susceptible to all three insecticides, but a population from Hawaii in 2003 showed high levels of resistance to indoxacarb. Instances of resistance to spinosad occurred in Hawaii (2000), Georgia (2001), and California (2002) as a consequence of a few years of extensive applications in each region. The collaborative monitoring program between university and industry scientists we discuss in this article has provided useful information to both parties as well as growers who use the products. These studies provide a baseline for developing a more effective resistance management program for diamondback moth.

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| Data point addressed | IIIM 6.2.2/17 |
| Author(s) (year) | Zhao, J.Z., Li, Y.X., Collins, H.L., Gusukuma-Minuto, L., Mau, R.F.L., Thompson, G.D., and Shelton, A.M. (2002) |
| Title | Monitoring and characterization of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad |
| Report number | Journal of Economic Entomology, April 2002, Vol. 95, Issue 2, p. 430-436 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Fourteen populations of the diamondback moth, *Plutella xylostella* (L.), were collected from fields of crucifer vegetables in the United States, Mexico, and Thailand in 1999 and 2000 for susceptibility tests with Spinosad. Most populations were susceptible to Spinosad and similar to earlier baseline values, but populations from Thailand and Hawaii showed high levels of tolerance. A statewide survey in Hawaii in 2000 and 2001 indicated resistance problems on several islands. One colony collected in October 2000 from Pearl City, HI, was subjected to further selection pressure, using spinosad in the laboratory, and then was used as the resistant strain (Pearl-Sel) for other tests. Spray tests using the recommended field rates of Spinosad on potted broccoli plants in the greenhouse confirmed that field control failures due to resistance were possible in the areas of these collections. Analysis of probit lines from F1 reciprocal crosses between the Pearl-Sel and S strain indicated that resistance to Spinosad was inherited autosomally and was incompletely recessive. A direct test of monogenic inheritance based on the F1 x Pearl-Sel backcrosses suggested that resistance to Spinosad was probably controlled by one locus. The synergists S,S,S-tributyl phosphorotrithioate and piperonyl butoxide did not enhance the toxicity of Spinosad to the resistant colony, indicating metabolic mediated detoxification was probably not responsible for the Spinosad resistance. Two field colonies in Hawaii that were resistant to Spinosad were not cross-resistant to emamectin benzoate or indoxacarb. Resistance developed in Hawaii due to the continuous cultivation of crucifers in which as many as 50 applications of Spinosad per year may have been made to a common population of *P. xylostella* in sequential plantings, although each grower might have used the labelled restrictions for resistance management. Resistance management strategies will need to address such cropping and pest management practices.

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| Data point addressed | IIIM 6.2.2/18 |
| Author(s) (year) | van Frankenhuyzen, K., Nystrom, C.W., and Tabashnik, B.E. (1995) |
| Title | Variation in tolerance to Bacillus thuringiensis among and within populations of the spruce budworm (Lepidoptera: Tortricidae) in Ontario |
| Report number | Journal of Economic Entomology, February 1995, Vol. 88, Issue 1, p.97-105 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

Variation in tolerance to *Bacillus thuringiensis* Berliner subsp. *kurstaki* (strain HD-1-S-1980) among and within populations of the spruce budworm, *Choristoneura fumiferana* (Clemens), was assessed in the laboratory. Force-feeding assays using offspring of females collected as pupae from nine locations throughout Ontario and from a laboratory colony (DCF) demonstrated limited variation in tolerance among populations. Variation among populations was comparable with the variation observed among repeated assays with different batches of larvae from the DCF colony. Population LC50s were not significantly associated with age of the outbreak, host-plant species, incidence of the microsporidian *Nosema fumiferanae* (Thomson), or size of the female parent. Upper limits for genetic variation in tolerance were estimated by examining variation among full-sibling families within same populations. Mortality of individual families ranged from 6.5 to 70.9% within five field populations and from 2.7 to 93.3% within two laboratory colonies in response to a dose that caused a mean mortality of 40%. Familial factors accounted for 32.8% of the phenotypic variation in response across field populations, as compared with 3% for population factors. These data suggest that the phenotypic variation in tolerance to *B. thuringiensis* has a substantial genetic component and may provide a basis for evolution of resistance given sufficient selection pressure.

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| Data point addressed | IIIM 6.2.2/19 |
| Author(s) (year) | Georghiou, G.P., and Wirth, M.C. (1997) |
| Title | Influence of exposure to single versus multiple toxins of *Bacillus thuringiensis* subsp. *israelensis* on development of resistance in the mosquito *Culex* *quinquefasciatus* (Diptera: Culicidae) |
| Report number | Applied and Environmental Microbiology, March 1997, p1095-1101, Vol. 63, Issue 3, p. 1095-1101 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

The impending widespread use of transgenic crop plants encoding a single insecticidal toxin protein of Bacillus thuringiensis has focused attention on the perceived risk of rapid selection of resistance in target insects. We have used B*. thuringiensis* subsp. *israelensis* toxins as a model system and determined the speed and magnitude of evolution of resistance in colonies of the mosquito *Culex quinquefasciatus* during selection for 28 consecutive generations with single or multiple toxins. The parental strain was synthesized by combining approximately 500 larvae from each of 19 field collections obtained from the states of California, Oregon, Louisiana, and Tennessee. At least 10,000 larvae were selected in each generation of each line at an average mortality level of 84%. The susceptibilities of the parental and selected lines were compared in parallel tests in every third generation by using fresh suspensions of toxin powders. The normal toxin complement of *B. thuringiensis* subsp. *israelensis* consists of four toxins, CryIVA, CryIVB, CryIVD, and CytA. Resistance became evident first in the line that was selected with a single toxin (CryIVD), attaining the highest level (resistance ratio [RR], >913 at 95% lethal concentration) by generation F(inf28) when the study was completed. Resistance evolved more slowly and to a lower level (RR, >122 by F(inf25)) in the line selected with two toxins (CryIVA+CryIVB) and lower still (RR, 91 by F(inf28)) in the line selected with three toxins (CryIVA+CryIVB+ CryIVD). Resistance was remarkably low (RR, 3.2) in the line selected with all four toxins. The results reveal the importance of the full complement of toxins found in natural populations of *B. thuringiensis* subsp. *israelensis* as an effective approach to resistance management.

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| Data point addressed | IIIM 6.2.2/20 |
| Author(s) (year) | Fayad, N., Patiño-Navarrete, R., Kambris, Z., Antoun, M., Osta, M., Chopineau, J., Mahillon, J., El Chamy, L., Sanchis, V., and Awad, M.K. (2019) |
| Title | Characterization and Whole Genome Sequencing of AR23, a Highly Toxic *Bacillus thuringiensis* Strain Isolated from Lebanese Soil |
| Report number | Current Microbiology, September 2019, 76(12), p. 1503-1511 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

The demand for sustainable and eco-friendly control methods of pests and insects is increasing worldwide. From this came the interest in *Bacillus thuringiensis*, an entomopathogenic bacterium capable of replacing chemical pesticides. However, the possibility of pests developing resistance to a particular strain may impair its use, and there is a need to identify novel strains of this species as potential commercial biopesticides. *B. thuringiensis* sv. *israelensis* is one of the most successful serovars, widely commercialized for its activity against black fly and mosquito larvae. In this study, we isolated, characterized, and sequenced a new Lebanese *B. thuringiensis* sv. *israelensis* isolate, strain AR23. Compared to the commercialized reference strain AM65-52 (Vectobac®, XXXX), AR23 showed an increased activity against several mosquito species. The genomic analysis revealed that this strain, compared to AM65-52, possesses a simplified plasmid content and an additional functional *cry4Ba* coding gene that most likely accounts for the increased effectiveness of this strain in mosquito larvae killing.

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| Data point addressed | IIIM 6.2.2/21 |
| Author(s) (year) | Zhu, L., Peng, D., Wang, Y., Ye, W., Zheng, J., Zhao, C., Han, D., Geng, C., Ruan, L., He, J., Yu, Z., and Sun, M. (2015) |
| Title | Genomic and transcriptomic insights into the efficient entomopathogenicity of *Bacillus thuringiensis* |
| Report number | Scientific Reports, September 2015, 5(14129), p. 1-14 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

*Bacillus thuringiensis* has been globally used as a microbial pesticide for over 70 years. However, information regarding its various adaptions and virulence factors and their roles in the entomopathogenic process remains limited. In this work, we present the complete genomes of two industrially patented *Bacillus thuringiensis* strains (HD-1 and YBT-1520). A comparative genomic analysis showed a larger and more complicated genome constitution that included novel insecticidal toxicity-related genes (ITRGs). All of the putative ITRGs were summarized according to the steps of infection. A comparative genomic analysis showed that highly toxic strains contained significantly more ITRGs, thereby providing additional strategies for infection, immune evasion and cadaver utilization. Furthermore, a comparative transcriptomic analysis suggested that a high expression of these ITRGs was a key factor in efficient entomopathogenicity. We identified an active extra urease synthesis system in the highly toxic strains that may aid *B. thuringiensis* survival in insects (similar to previous results with well-known pathogens). Taken together, these results explain the efficient entomopathogenicity of *B. thuringiensis*. It provides novel insights into the strategies used by *B. thuringiensis* to resist and overcome host immune defences and helps identify novel toxicity factors.

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| Data point addressed | IIIM 6.2.2/22 |
| Author(s) (year) | Da Silva Carvalho, K., Crespo, M.M., Araújo A.P., Santana da Silva, R., de Melo-Santos, V.M.A., Fontes de Oliveira, C.M, and Silva-Filha, M.H.N.L. (2018) |
| Title | Long-term exposure of Aedes aegypti to *Bacillus thuringiensis* svar. *israelensis* did not involve altered susceptibility to this microbial larvicide or to other control agents |
| Report number | Parasites & Vectors, December 2018, Vol. 11, Issue 1, p. 1-11 |
| Test facility | Not applicable |
| Published | Yes |
| Test guideline | Not applicable |
| Deviations | Not applicable |
| GLP | No |

**Abstract**

**Background:** *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) is an effective and safe biolarvicide to control *Aedes aegypti*. Its mode of action based on four protoxins disfavours resistance; however, control in endemic areas that display high mosquito infestation throughout the year requires continuous larvicide applications, which imposes a strong selection pressure. Therefore, this study aimed to investigate the effects of an intensive *Bti* exposure on an *A. aegypti* strain (RecBti), regarding its susceptibility to *Bti* and two of its protoxins tested individually, to other control agents temephos and diflubenzuron, and its profile of detoxifying enzymes.

**Methods:** The RecBti strain was established using a large egg sample (10,000) from Recife city (Brazil) and more than 290,000 larvae were subjected to *Bti* throughout 30 generations. Larvae susceptibility to larvicides and the activity of detoxifying enzymes were determined by bioassays and catalytic assays, respectively. The Rockefeller strain was the reference used for these evaluations.

**Results:** *Bti* exposure yielded an average of 74% mortality at each generation. Larvae assessed in seven time points throughout the 30 generations were susceptible to *Bti* crystal (resistance ratio RR ≤ 2.8) and to its individual toxins Cry11Aa and Cry4Ba (RR ≤ 4.1). Early signs of altered susceptibility to Cry11Aa were detected in the last evaluations, suggesting that this toxin was a marker of the selection pressure imposed. RecBti larvae were also susceptible (RR ≤ 1.6) to the other control agents, temephos and diflubenzuron. The activity of the detoxifying enzymes α- and β-esterases, glutathione-S-transferases and mixed-function oxidases was classified as unaltered in larvae from two generations (F19 and F25), except for a β-esterases increase in F25.

**Conclusions:** Prolonged exposure of *A. aegypti* larvae to *Bti* did not evolve into resistance to the crystal, and no cross-resistance with temephos and diflubenzuron were recorded, which supports their sustainable use with *Bti* for integrated control practices. The unaltered activity of most detoxifying enzymes suggests that they might not play a major role in the metabolism of *Bti* toxins, therefore resistance by this mechanism is unlikely to occur. This study also highlights the need to establish suitable criteria to classify the status of larval susceptibility/resistance.

IIIM 6.3 Toxic or pathogenic effects on the crop or host which is to be protected

IIIM 6.4 Compatibility with products in authorized tank mixes and with other products that are applied under expected conditions of use

IIIM 6.4.1 Physical compatibility

No changes since last evaluation.

IIIM 6.4.2 Chemical compatibility

No changes since last evaluation.

IIIM 6.4.3 Biological compatibility

No changes since last evaluation.

**IIIM 6.4.4 Recommended interval between application of MPCP and chemical pesticide, to avoid loss of efficacy**

No changes since last evaluation.

IIIM 6.5 Contribution to risk reduction and integrated pest management strategies for the targeted crop or resource

No changes since last evaluation.

IIIM 6.6 Summary and assessment of data according to points 6.1 to 6.5

No changes since last evaluation.

IIIM 6.7 List of test facilities including the corresponding certificates

No changes since last evaluation.

Appendix 1: List of data submitted in support of the evaluation

| **Annex point** | **Author** | **Year** | **Title**  **Source (where different from company)**  **Company, Report No.**  **GLP or GEP status (where relevant)**  **Published or Unpublished** | **Data protection claimed Y/N** | **Owner** |
| --- | --- | --- | --- | --- | --- |
| IIIM 6.2.2/01 | Shelton, A.M., Sances, F.V., Hawley, J., Tang, J.D., Boune, M., Jungers, D., Collins, H.L., and Farias, J. | 2000 | Assessment of Insecticide Resistance After the Outbreak of Diamondback Moth (Lepidoptera: Plutellidae) in California in 1997  Journal of Economic Entomology Vol. 93, Issue 3, p. 931 - 936  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/02 | Tabashnik, B.E., Finson, N., Groeters, F.R., Moar, W.J., Johnson, M.W., Luo, K., and Adang M.J. | 1994 | Reversal of resistance to *Bacillus thuringiensis* in Plutella xylotella.  Proc. Natl. Acad. Sci. USA. Vol. 91, Issue 10, p. 4120 - 4124  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/03 | Huang H., Buschman, L.L., Higgins, R.A., and Mc Gaughey W.H. | 1999 | Inheritance of Resistance to *Bacillus thuringiensis* Toxin (Dipel ES) in the European Corn Borer  Science Vol. 284, Issue 5416, p. 965-967  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/04 | Gould, F., Anderson, A., Jones, A., Sumerford, J.D., Heckel, D.G., Lopez, J., Micinski,. S., Leonard, R., and Laster, M. | 1997 | Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*  Proc. Natl. Acad. Sci. USA. Vol. 94, Issue 8, p 3519-3523  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/05 | Liu, Y., B., Tabashnik, B.E., Dennehy, T.J., Patin, A.L., and Bartlett A.C. | 1999 | Development time and resistance to *Bt* crops  Nature, August 1999, Vol. 400, Issue 6744, p. 519  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/06 | Wirth, M.C., Georghiou, G.P., and Frederici, B.A. | 1997 | CytA enables CryIV endotoxins of *Bacillus thuringiensis* to overcome high levels of CryIV resistance in the mosquito, *Culex quinquefasciatus*  Proceedings of the National Academy of Sciences (PNAS), September 1997, Vol. 94, Issue 20, p. 10536-10540  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/07 | McGaughey, W.H. and Beeman, R.W. | 1988 | Resistance to *Bacillus thuringiensis* in Colonies of Indianmeal Moth and Almond Moth (Lepidoptera: Pyralidae)  Journal of Economic Entomology, February 1988, p 28-33, Vol. 81, Issue 1, p. 28-33  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/08 | Federici, B.A. and Bauer, L.S. | 1998 | Cyt1Aa Protein of *Bacillus thuringiensis* Is Toxic to the Cottonwood Leaf Beetle, *Chrysomela scripta*, and Suppresses High Levels of Resistance to Cry3Aa  Applied and Environmental Microbiology, November 1998, Vol. 64, Issue 11, p. 4368-4371  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/09 | Moar, W.J., Pustztai-Carey, M., Van Faassen, H., Bosch, D., Frutos, R., Rang, C., Luo, K., and Adang, M.J. | 1995 | Development of *Bacillus thuringiensis* CryIC Resistance by Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae)  Applied and Environmental Microbiology, June 1995, p 2086-2092, Vol. 61, Issue 6, p. 2086-2092  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/10 | Salama, H. and Matter, M | 1991 | Tolerance level to *Bacillus thuringiensis* Berliner in the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lep., Noctuidae)  Journal of Applied Entomology, December 1991, Vol 111, Issue 1-5, p. 225-230  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/11 | Janmaat, A.F. and Myers, J. | 2003 | Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*  Proceedings of the Royal Society B, November 2003, Vol. 270, Issue 1530, p. 2263-2270  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/12 | Rahardja, U. and Whalon, M.E. | 1995 | Inheritance of Resistance to *Bacillus thuringiensis* subsp. *tenebrionis* CryIIIA δ-Endotoxin in Colorado Potato Beetle (Coleoptera: Chrysomelidae)  Journal of Economic Entomology, February 1995, Vol. 88, Issue 1, p. 21-26  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/13 | Loke, S.R., Andy-Tan, W.A., Benjamin, S., Lee, H.L., and Sofian-Azirun, M. | 2010 | Susceptibility of Field-Collected Aedes aegypti (L.) (Diptera: Culicidae) to Bacillus thuringiensis israelensis and temephos  Tropical Biomedicine, July 2010, Vol. 27, Issue 3, p. 493-503  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/14 | Mascarenhas, R.N. and Boethel, D.J. | 1997 | Responses of field-collected strains of soybean looper (Lepidoptera: Noctuidae) to selected insecticides using an artificial diet overlay bioassay  Journal of economic entomology, October 1997, Vol. 90, Issue 5, p. 1117-1124  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/15 | Shelton, A.M., Sances, F.V., Hawley, J., Tang, J.D., Boune, M., Jungers, D., Collins, H.L., and Farias, J. | 2000 | Assessment of insecticide resistance after the outbreak of diamondback moth (Lepidoptera: Plutellidae) in California in 1997  Journal of Economic Entomology, Jun 2000, Vol. 93, Issue 3, p. 931-936  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/16 | Zhao, J.Z., Collins, H.L., Li, Y.X., Mau, R.F.L, Thompson, G.D., Hertlein, M., Andaloro, J.T., Boykin, R., and Shelton, A.M. | 2006 | Monitoring of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, indoxacarb, and emamectin benzoate  Journal of Economic Entomology, Feb 2006, Vol. 99, Issue 1, p. 176-181  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/17 | Zhao, J.Z., Li, Y.X., Collins, H.L., Gusukuma-Minuto, L., Mau, R.F.L., Thompson, G.D., and Shelton, A.M. | 2002 | Monitoring and characterization of diamondback moth (Lepidoptera: Plutellidae) resistance to Spinosad  Journal of Economic Entomology, Apr 2002, Vol. 95, Issue 2, p. 430-436  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/18 | van Frankenhuyzen, K., Nystrom, C.W., and Tabashnik, B.E. | 1995 | Variation in tolerance to Bacillus thuringiensis among and within populations of the spruce budworm (Lepidoptera: Tortricidae) in Ontario  Journal of Economic Entomology, Feb 1995, Vol. 88, Issue 1, p. 97-105  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/19 | Georghiou, G.P. and Wirth, M.C. | 1997 | Influence of exposure to single versus multiple toxins of Bacillus thuringiensis subsp. israelensis on development of resistance in the mosquito Culex quinquefasciatus (Diptera: Culicidae)  Applied and Environmental Microbiology, March 1997, Vol. 63, Issue 3, p. 1095-1101  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/20 | Fayad, N., Patiño-Navarrete, R., Kambris, Z., Antoun, M., Osta, M., Chopineau, J., Mahillon, J., El Chamy, L., Sanchis, V., and Awad, M.K. (2019) | 2019 | Characterization and Whole Genome Sequencing of AR23, a Highly Toxic *Bacillus thuringiensis* Strain Isolated from Lebanese Soil  Current Microbiology, September 2019, 76(12), p. 1503-1511  GLP: No  Published | N | Open literature |
| IIM 6.2.2/21 | Zhu, L., Peng, D., Wang, Y., Ye, W., Zheng, J., Zhao, C., Han, D., Geng, C., Ruan, L., He, J., Yu, Z., and Sun, M. | 2015 | Genomic and transcriptomic insights into the efficient entomopathogenicity of *Bacillus thuringiensis*  Scientific Reports, September 2015, 5(14129), p. 1-14  GLP: No  Published | N | Open literature |
| IIIM 6.2.2/22 | Da Silva Carvalho, K., Crespo, M.M., Araújo A.P., Santana da Silva, R., de Melo-Santos, V.M.A., Fontes de Oliveira, C.M, and Silva-Filha, M.H.N.L. | 2018 | Long-term exposure of Aedes aegypti to *Bacillus thuringiensis* svar. *israelensis* did not involve altered susceptibility to this microbial larvicide or to other control agents  Parasites & Vectors, December 2018, Vol. 11, Issue 1, p. 1-11  GLP: No  Published | N | Open literature |

Appendix 2: GAP table

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **1** | **2** | **3** | **4** | **5** | **6** | | **7** | **8** | **9** | **10** | **11** | | **12** | **13** | **14** |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | | **Application rate** | | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 1 | IT | Coniferous and deciduous forest and green areas (trees and shrubs in parks and gardens) | F | Lepidoptera caterpillars L1 to L4  *Choristoneura sp*. - CHONSP,  *Geometridae* - 1GEOMF,  *Hyphantria cunea* - HYPHCU  *Malacosoma neustria* - MALANE,  Stilpnotia salicis - LEUOSA,  *Euproctis chrysorrhoea* - EUPRCH,  *Lymantria dispar* - LYMADI,  *Lymantria monacha* - LYMAMO,  *Thaumetopoea pityocampa* - THAUPI,  *Thaumetopoea processionea* - THAUPR,  *Dendrolimus pini* - DENDPI,  *Dendrolimus superans* - DENDSU,  *Tortrix viridana* - TORTVI | Ground spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | | a) 1 - 4  b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | | a) 0.413 - 0.516 kg a.s/ha  b) 2.06 kg a.s./ha | 0 - 500 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha  Aerial application only by emergency permits (Avio). |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 2 | ES | Coniferous forest, Deciduous forest, Palm trees, shurbs and small ornamental trees | F | Lepidoptera caterpillars L1 to L4  Procesionaria, *Thaumetopoea processionea* - THAUPR  Procesionaria del pino, *Thaumetopoea pityocampa* - THAUPI  Lagarta, *Lymantria spp*. - LYMASP  Oruga del zurrón, *Euproctis chrysorrhoea* - EUPRCH  Tortrix, *Tortrix viridana* - TORTVI | Spray (ground and aerial application) | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | | a) 0.413 - 0.516 kg a.s/ha  b) 2.06 kg a.s./ha | Aerial application: no dillution  Ground application: 0 - 500 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 3 | ES | Coniferous forest, Deciduous forest, Palm trees, , shurbs and small ornamental trees | F | Lepidoptera caterpillars L1 to L4 | Spray (ground and aerial application) | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 1.5 - 2.5 L/ha  b) 10 L/ha | | a) 0.31 - 0.52 kg a.s/ha  b) 2.06 kg a.s./ha | Aerial application: no dillution  Ground application: 0 - 500 L/ha | - | Application rate in CFU:  a) 2.26 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 4 | LT | Forest | F | Lepidoptera caterpillars  *Lymantria monacha* - LYMAMO  *Dendrolimus pini* - DENDPI  *Tortrix viridana* - TORTVI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 2  b) 2 | 7 days | a) 2 - 2.5 L/ha  b) 5 L/ha | | a) 0.413 - 0.516 kg a.s/ha  b) 1.03 kg a.s./ha | - | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 5 | PL | Pine trees | F | *Lymantria monacha* - LYMAMO  *Dendrolimus pini* - DENDPI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 2.5 L/ha  b) 10 L/ha | a) 0.52 kg a.s/ha  b) 2.06 kg a.s./ha | - | - | Application rate in CFU:  a) 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 6 | PL | Deciduous forest | F | *Operophtera brumata* - CHEIBR  *Tortrix viridana* - TORTVI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 4  b) 4 | 5 days | a) 2.5 L/ha  b) 10 L/ha | a) 0.52 kg a.s/ha  b) 2.06 kg a.s./ha | UVL application: 0-10 L/ha,  application of high pressure (10 bar): 200 L/ha,  application of low pressure (2-3 bar): 600 L/ha. | - | Application rate in CFU:  a) 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 7 | PL | Deciduous forest | F | *Euproctis chrysorrhoea* - EUPRCH | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1 - 2  b) 2 | 14 days | a) 3 L/ha  b) 6 L/ha | a) 0.619 kg a.s/ha  b) 1.24 kg a.s./ha | UVL application: 0-10 L/ha,  application of high pressure (10 bar): 200 L/ha,  application of low pressure (2-3 bar): 600 L/ha. | - | Application rate in CFU:  a) 4.53 x 1013 CFU/ha  b) 9.06 x 1013 CFU/ha |

| **1** | **2** | | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 8 | | RO | Coniferous forest | F | Lepidoptera caterpillars L1 to L4  *Choristoneura spp.* - CHONSP  *Lymantria monacha* - LYMAMO  *Thaumetopoea pityocampa* - THAUPI  *Dendrolimus pini* - DENDPI | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | 0-10 L/ha (undiluted for ULV application) | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 9 | | RO | Deciduous forest | F | Lepidoptera caterpillars L1 to L4  *Hyphantria cunea* - HYPHCU  *Malacosoma neustria* - MALANE  *Stilpnotia salicis* - LEUOSA  *Euproctis chrysorrhoea* - EUPRCH  *Lymantria dispar* - LYMADI  *Thaumetopoea processionea* - THAUPR  *Tortrix viridana* - TORTVI  *Operophtera bru*mata - CHEIBR | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | 0-600 L/ha (undiluted for ULV application; high pressure application: 200L/ha and low pressure application 600 L/ha) | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 10 | HU | Deciduous forest species  (also on public areas) | F | Lepidopteran foliage pests  *Lymantria dispar* - LYMADI  *Hyphantria cunea* - HYPHCU  *Euproctis chrysorrhoea* - EUPRCH  *Aporia crataegi* - APORCR  *Thaumetopoea processionea* - THAUPR  *Tortrix viridana* - TORTVI  *Geometridae -* 1GEOMF  *Tortricidae* - 1TORTF  *Gracillariidae* - 1GRACF | Spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha  b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | Ground spray: 600 - 1500 L/ha  Aerial spray: 60 - 80 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 11 | HU | Pine species  (also on public areas) | F | Lepidopteran foliage pests  *Dendrolimus pini -* DENDPI *Rhyacionia buoliana* - EVETBU *Gracillariidae* - 1GRACF | Spray | When caterpillars are  visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | Ground spray: 600 - 1500 L/ha  Aerial spray: 60 - 80 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 12 | HU | Ornamental trees, bushes  (also on public areas) | F | Lepidopteran foliage pests  *Lymantria dispar* - LYMADI *Hyphantria cunea* - HYPHCU *Euproctis chrysorrhoea* - EUPRCH *Aporia crataegi* - APORCR *Thaumetopoea processionea* - THAUPR *Tortrix viridana* - TORTVI *Geometridae* - 1GEOMF *Tortricidae*  - 1TORTF *Gracillariidae -* 1GRACF *Dendrolimus pini* - DENDPI *Rhyacionia buoliana -* EVETBU | Spray | When caterpillars are  visible following egg hatch & foliage growth sufficient for deposition | a) 4 b) 4 | 5 days | a) 2 - 2.5 L/ha b) 10 L/ha | a) 0.413 - 0.516 kg a.s/ha b) 2.06 kg a.s./ha | Ground spray: 600 - 1200 L/ha  Aerial spray: 60 - 80 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 13 | DE | Coniferous forest, Deciduous forest | F | Lepidoptera caterpillars  L1 to L3 | Ground spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 2  b) 2 | 14 days | a) 2 - 2.5 L/ha  b) 5 L/ha | a) 0.413 - 0.516 kg a.s/ha  b) 1.03 kg a.s./ha | 600 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |
| 14 | DE | Coniferous forest, Deciduous forest | F | Lepidoptera caterpillars  L1 to L3 | Aerial spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 2  b) 2 | 30 days | a) 2 - 2.5 L/ha  b) 5 L/ha | a) 0.516 kg a.s/ha  b) 1.03 kg a.s./ha | 70 L/ha | - | Application rate in CFU:  a) 3.77 x 1013 CFU/ha  b) 1.51 x 1014 CFU/ha |

| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Use-No.** | **Member state(s)** | **Crop and/  or situation    (crop destination / purpose of crop)** | **F, Fn, Fpn  G, Gn, Gpn  or  I** | **Pests or Group of pests controlled    (additionally: developmental stages of the pest or pest group)** | **Application** | | | | **Application rate** | | | **PHI  (days)** | **Remarks:     e.g. g safener/synergist per ha** |
| Method / Kind | Timing / Growth stage of crop & season | Max. number  a) per use  b) per crop/ season | Min. interval between applications (days) | kg or L product / ha  a) min / max. rate per appl.  b) max. total rate per crop/season | g or kg as/ha   a) min / max. rate per appl.  b) max. total rate per crop/season | Water L/ha    min / max |
| 15 | DE | Ornamental trees | F | Lepidoptera caterpillars  L1 to L3 | Ground spray | When caterpillars are visible following egg hatch & foliage growth sufficient for deposition | a) 1  b) 1 | NA | a) 2 - 2.5 L/ha  b) 5 L/ha | a) 0.413 - 0.516 kg a.s/ha  b) 0.516 kg a.s/ha | 600 L/ha | - | Application rate in CFU:  a) 3.02 - 3.77 x 1013 CFU/ha  b) 3.77 x 1013 CFU/ha |

|  |  |  |  |
| --- | --- | --- | --- |
| **Remarks**  **columns:** | 1 Numeration necessary to allow references  2 Use official codes/nomenclatures of EU Member States  3 For crops, the EU and Codex classifications (both) should be used; when relevant, the use situation should be described (e.g. fumigation of a structure)  4 F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application  5 Scientific names and EPPO-Codes of target pests/diseases/ weeds or, when relevant, the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named.  6 Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench  Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated. |  | 7 Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 38263-3152-4), including where relevant, information on season at time of application  8 The maximum number of application possible under practical conditions of use must be provided.  9 Minimum interval (in days) between applications of the same product  10 For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products.  11 The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).  12 If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.  13 PHI - minimum pre-harvest interval  14 Remarks may include: Extent of use/economic importance/restrictions |

Appendix 3: Additional information provided by the applicant (e.g. detailed modelling data)

No changes since last evaluation.

Appendix 4: Summary of data on trials site and application details per use (EPPO code).

No changes since last evaluation.

Appendix 5: Summary of data on effectiveness trials per use (crop/harmful organism); (EPPO code)

No changes since last evaluation.

Appendix 6: Summary of detailed data on herbicide effectiveness trials.

No changes since last evaluation.

Appendix 7: Summary of phytotoxicity trials data in summary form

No changes since last evaluation.

Appendix 8 - Summary of available studies: Adverse effects on beneficial (arthropods other than bees) organisms.

No changes since last evaluation.

Appendix 9: Summary of data on succeeding crop

No changes since last evaluation.