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# Microbial Control of the Potato Tuber Moth (Lepidoptera: Gelechiidae)

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## ABSTRACT

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller), is considered the most damaging potato pest in the developing world. Larvae mine potato leaves and stems, but more importantly is the feeding damage in potato tubers, which also can cause rapid rotting in non-refrigerated storage. Insect-specific pathogens (biopesticides) offer control alternatives to chemical pesticides that provide a variety of benefits including safety for applicators, other natural enemies, the environment and food supply. The most researched and practically used for control of PTM are a granulovirus and the bacterium *Bacillus thuringiensis* Berliner. The PTM granulovirus (*Po*GV) is species specific and has the potential to play a key role in the management of PTM in stored tubers and in field crops. The virus kills infected larvae within 2-3 weeks. Application of *Po*GV for control of field populations of PTM has been relatively limited and the results have been variable. However, it provides very good protection of treated tubers, especially in non-refrigerated storage. *Bacillus thuringiensis* (*Bt*) is the only bacterium that has been evaluated for PTM control. *Bt* subsp. *kurstaki* (*Btk*) is the most commonly used against lepidopteran pests. *Btk* has been reported to be effective for control of PTM infestations under field conditions and in rustic stores. An integrated control approach comprising *Btk* applied at the beginning of the storage period in combination with early harvest has been effective and eliminated reliance on chemical pesticides. The implementation of biopesticides will ultimately depend on an increased awareness of their attributes by growers and the public.

**Keywords:** *Bacillus thuringiensis*, Baculovirus, biological control, Braconidae, entomopathogen, granulovirus, *Muscodor albus*, *Phthorimaea operculella*, *Po*GV, potato tuber moth, PTM, *Symmetrischema tangolias*, *Tecia solanivora*
**Abbreviations:** ***Bt***, *Bacillus thuringiensis*; ***Btk***, *Bacillus thuringiensis* subsp. *kurstaki*; **CIP**, Centro Internacional de la Papa; **IPM**, integrated pest management; **GPTM**, Guatemalan potato tuber moth; ***Po*GV**, PTM granulovirus; **PTM**, potato tuber moth

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## INTRODUCTION

In tropical and subtropical agroecosystems, the potato tuber moth (PTM) (*Phthorimaea operculella* (Zeller)) is considered the most damaging potato pest. Larvae mine both leaves and tubers, in the field and in storage making the pest a difficult target to control. PTM probably originated in the tropical mountainous regions of South America (Graf 1917) and has become a cosmopolitan pest of potato and other solanaceous crops like tomato (*Lycopersicon esculentum* (Miller)), tobacco (*Nicotiana tabacum* L.) and aubergine (eggplant) (*Solanum melogena* L.). In addition, wild species of the family Solanaceae, including important weeds in potato (e.g. black nightshade, *Solanum nigrum* L.) can also serve as host plants for PTM (Das and Raman 1994; Kroschel 1995). Today, its distribution is reported in more than 90 countries worldwide. The moth occurs in almost all tropical and subtropical potato production systems in Africa, Asia and Central and South America (Kroschel and Sporleder 2006). While it still can be of economic significance in more temperate environments, such as in Southern Europe and the Pacific Northwest of the United States, cold winters generally restrict its development and reduce its status as a pest (Sporleder *et al.* 2004, 2008a). However, global warming could change PTM population dynamics and geographical distribution in the future (Spor-

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leder *et al.* 2007a).

The traditional options for control of PTM prior to harvest comprise the use of several broad spectrum insecticides. This practice has caused a rapid build up in insecticide resistance in PTM populations (Richardson and Rose 1967; Cisneros 1984). The serious health threats of chemical pesticides to farmers, consumers and the environment has increased interest in the search for safer control alternatives through the development of Integrated Pest Management (IPM). This approach has been shown to successfully control this pest, thereby reducing or avoiding the use of insecticides (Kroschel 1995). Ecological approaches to IPM for PTM are based on an overall understanding of pest population dynamics supported by phenology modeling; yield loss assessments and the use of control thresholds for minimizing insecticide applications; habitat management and biological control; and special consideration for tuber storage management (Kroschel and Sporleder 2006).

Biological control of PTM or any other insect refers to the active use of natural enemies of the pest (parasitoids, predators, pathogens). The three biological approaches for pest insects are classical, augmentative and conservation biological control (DeBach 1964).

Classical biological control consists of the introduction of exotic antagonists for establishment and long-term control in those regions where an insect pest has been unintentionally introduced and not effectively controlled by native natural enemies. PTM has spread into many areas of Africa (Egypt, Kenya, Morocco, Tunisia), Asia (Bhutan, India, Indonesia, Nepal), Oceania (Australia, New Zealand) and North America (Mexico, United States) where classical biological could be implemented to support the sustainable management of this potato pest. The use of introduced parasitoids for PTM control has had mixed success. Among 18 different parasitoid species used for classical biological control of PTM, species in the families Braconidae (e.g., *Apanteles subandinus* Blanchard and *Orgilus lepidus* Muesebeck) and Encyrtidae (*Copidosoma koehleri* Blanchard) have been successfully established in several countries. In Zimbabwe, for example, releases have been so successful that PTM was eliminated as a significant potato pest (Mitchell 1978). On the other hand, although parasitism rates have been high in some locations, they have not been sufficient for adequate control of PTM. In some cases the introduction of highly specific parasitoids caused lower parasitism of PTM by native, less specific species.

Augmentative biological control includes inoculative releases of exotic natural enemies to support indigenous populations and inundative application of mass produced indigenous parasitoids and pathogens. Naturally occurring and inundatively applied insect pathogens (biopesticides) of PTM can significantly contribute to the control of this pest. Considerably more detail will be devoted to this subject in this review.

Conservation biological control is based on optimizing the controlling effects of natural enemies in the agroecosystem through cultural practices including habitat management, avoiding insecticide use or optimizing the timing of insecticide application. Horne (1990) reported that in areas of Australia where no chemical insecticides are used, naturalized parasitoids have become a very important factor limiting PTM. Their overall control potential became fully apparent after an IPM program was established that accounted for all potato pests (Horne and Page 2008). Successful control of insect pests through conservation of indigenous insect pathogens is broadly covered by Steinkraus (2007).

In this review we will present a comprehensive background on microbial control of PTM and its role in IPM. A diverse spectrum of microscopic and multi-cellular organisms (bacteria, fungi, viruses, protozoa, and nematodes) parasitize and kill insect pests of virtually every crop. Several of these agents have been developed as microbial pesticides (Burges 1981; Lacey *et al.* 2001; Kaya and Lacey 2007), some of which have been used to control certain insect pests of potato including PTM (von Arx *et al.* 1987; Hamilton and Macdonald 1990; Raman 1994; Cloutier *et al.* 1995; Kroschel *et al.* 1996b). Biopesticides have no preharvest interval and provide a variety of other benefits including safety for applicators, other natural enemies, the environment and human food supply (Laird *et al.* 1990; Hokkanen and Hajek 2003). Substantial effort has gone into the development of certain microbial agents for PTM control in several countries worldwide. The most researched and practically used are a granulovirus (Baculoviridae) and the bacterium *Bacillus thuringiensis* Berliner (*Bt*).

## GRANULOVIRUS AS A MICROBIAL CONTROL AGENT OF PTM

### Biological characterization and host range

A granulovirus (*Po*GV) that attacks PTM larvae has accompanied the moth from its South American center of origin to most countries where PTM has become established. Several surveys confirm the presence of *Po*GV in PTM populations in the Andean potato growing areas of South America (Alcázar *et al.* 1991, 1992a), Africa (Broodryk and Pretorius 1974; Laarif *et al.* 2003), the Middle East (Kroschel and Koch 1994; Kroschel 1995), Asia (Zeddam *et al.* 1999; Setiawati *et al.* 1999), Australia (Reed 1969; Briese 1981) and North America (Hunter *et al.* 1975). Several isolates and their origins are summarized by Sporleder (2003).

The name of the virus is derived from its granular appearance under high magnification. Each granule, also known as an occlusion body (OB) consists of a viral encoded protein (granulin) matrix in which a single rod-shaped, enveloped virion (nuleocapsid) is occluded (Tanada and Hess 1991; Federici 1997) (**Fig. 1**). The nucleocapsid consists of a protein coat containing the viral DNA genome. Following ingestion by PTM larvae, the proteinaceous coat or granulin is dissolved in the alkaline pH of the midgut liberating the nucleocapsids. The nucleocapsids pass through the peritrophic membrane and then fuse with the microvilli of the midgut epithelium. Infection of these cells is transient without the production of OBs (Federici 1997). Subsequently they invade a variety of host cells and produce hundreds of millions of OBs per larva. The larval fat cells are the predominant site of virus production. Ultimately, infected larvae die and become a source of inoculum for infection of other PTM larvae. Reed (1971) reported on the effect of virus concentration, temperature and larval age on the progression of disease in PTM. Most larvae die within 2-3 weeks of ingesting virus, but very high dosages of *Po*GV can cause death by toxicosis within 48 hours.

Like most granuloviruses (Tanada and Hess 1991; Federici 1997), *Po*GV has a fairly specific host range. Only PTM and certain other species in the family Gelechiidae are infected by the virus. The tomato moth *Tuta absoluta* (Meyrick) and the Guatemalan potato tuber moth (GPTM) *Tecia solanivora* (Povolny) are both susceptible to *Po*GV, but at lower levels than PTM (Zeddam *et al.* 2003a). Although *Po*GV has been isolated from the Andean potato tuber moth (APTM) *Symmetrischema tangolias* (Gyen) (Angeles and Alcázar 1995) it does not appear to affect this species for which no specific granulovirus could yet be identified (Zegarra *et al.* 2004). Additionally, Pokharkar and Kurhade (1999) reported no *Po*GV infectivity against 11 other lepidopteran species.

Sporleder (2003) assessed the activity of 14 geographical isolates of *Po*GV and found a wide range of activity covering several orders of magnitude. Using restriction endonuclease analysis (REN) of viral DNA, he found genomic polymorphisms in the "Kenya" and "Huaraz", Peru isolates using the restriction enzymes *Hind*III and *Eco*RI. Vickers *et al.* (1991) demonstrated minor differences among 8 geographically diverse *Po*GV isolates using REN. The profiles from the 8 isolates, including 5 from Peru and one each from Australia, India, and Tunisia revealed three distinct but closely related genotypes. One isolate from Peru

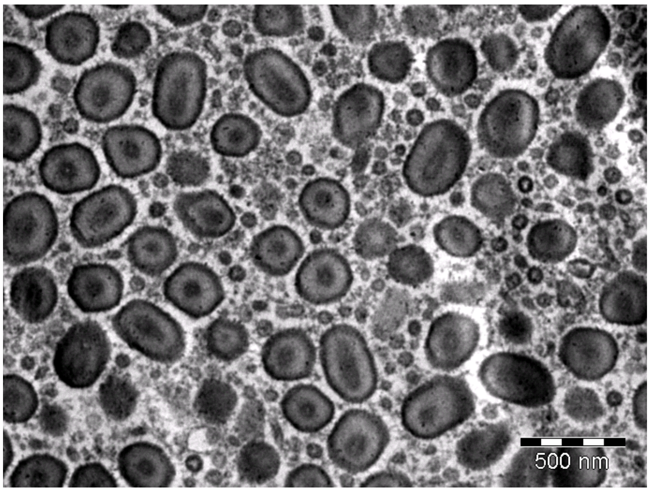


**Fig. 1 Cross and longitudinal sections of occlusion bodies of the potato tuber moth granulovirus.** Micrograph courtesy of Darlene Hoffmann, USDA-ARS, Parlier, CA.

was identical to one from India. Bioassays of three *Po*GV isolates from Indonesia revealed similar biological properties (Zeddam *et al.* 1999). The restriction pattern of the Indonesian Wonsosobo isolate varied only slightly from other *Po*GV isolates from different regions of the world (Zeddam *et al.* 1999). In contrast, Lery *et al.* (1998) demonstrated considerable genetic heterogeneity between a Tunisian isolate and isolates of *Po*GV from other regions. Kroschel *et al.* (1996a) reported similarity between an isolate from The Republic of Yemen and a Peruvian isolate from La Molina, Lima. Croizier *et al.* (2002) sequenced the genome of a Tunisian isolate which is 119,217 base pairs in length and selected 130 Open Reading Frames. Hence, in the future *Po*GV isolates might be more accurately compared by examining specific genes.

## Virus production

Methods for the *in vivo* production of *Po*GV are presented in Reed and Springett (1971), CIP (1992), Kroschel *et al.* (1996b), Sporleder *et al.* (2005) and others. Basically, the method employs the mass production of PTM followed by infection of neonate larvae by exposing them to tubers that have been treated by submersion in an aqueous suspension of triturated *Po*GV-infected larvae. Alternatively, PTM eggs can be dipped in *Po*GV suspensions, a methodology especially suitable to decrease variability and hence increase precision in bioassays for testing the biological activity of the virus (Sporleder *et al.* 2005). Larvae consume virus directly upon exiting the egg and are provided tubers in which to develop. Sporleder *et al.* (2005) recommend the use of purified virus for bioassays and virus production as a control for virus quality. For example, contamination with Microsporida could considerably reduce virus yields. Another production method proposed by Matthiessen *et al.* (1978) involved spraying virus suspensions onto infested potato plants in the field, collecting infested foliage after larvae become diseased, and separating them from foliage by exposure to heat. This method has not been used on a larger scale and does not appear to be competitive with those mentioned above. Lery *et al.* (1997) and Sudeep *et al.* (2005) reported on the establishment of PTM cell lines and demonstrated their utility for *in vitro* production of *Po*GV.

Sporleder (2003) and Sporleder *et al.* (2005, 2007b, 2008b) evaluated the effect of temperature, initial virus concentration, larval age and density per gram of potato on the yield of OBs. The number of virus infected larvae increased with increasing virus concentration with an optimal concentration of $10^9$ OBs/ml of suspension. Based on their studies, it was recommended that *Po*GV be propagated at temperatures of around 24°C. This temperature enables rapid larval development of PTM and minimizes mortality. Although larval weights are higher at cooler temperatures, this does not compensate for increased natural mortality and prolonged larval development. The optimal temperature and larval density for virus production was 24°C and 2 grams of potato/larva, respectively. Sporleder *et al.* (2007b) also found that the number of OBs produced per larva was highly correlated with larval age and weight. Pokharkar and Kurhade (1999) recorded 25°C as the optimal temperature for virus production.

For simple systems of virus multiplication, the effect of numbers of macerated *Po*GV infected PTM larvae on PTM development has been assessed. For high virus yield, the optimal virus concentration should not kill larvae before the third or fourth instar. Kroschel (1995) tested macerated infected fourth instars in a dilution series of one larva to 0.1, 1, 5 and 10 liters of water, respectively, which correspond to a titer of $10^8$ to $10^7$, $2 \times 10^6$, and $10^6$ OB/ml, respectively (assuming that one larva corresponds with $10^{10}$ OB). One larva per 5 l water ($2 \times 10^6$ OB/ml) produced the highest yields of virus-infected larvae.

Different researchers have reported a range of OBs produced per larval equivalent (LE) using different methods to estimate the number of OBs. Kroschel (1995) purified virus from 10,000 larvae, assessed the titer in the stock suspension by counts using a Neubaur counting chamber, and calculated $10^{10}$ OBs/LE. Arthurs *et al.* (2008a) counted 2.3 $\times 10^{10}$ OBs/LE by macerating 300 infected 4th instars in 50 ml of sterile water, followed by purification using a sucrose gradient dilution to 1 LE/ml and counting the OBs using dark field microscopy and a thin (10 µm) Petroff-Hausser counting chamber. Sporleder *et al.* (2008b) counted $5 \times 10^9$ OBs/LE on average but with high variability among fourth instar larvae. Based on data presented by Zeddam *et al.* (2003), each LE yielded $10^{10}$ OBs. Matthiessen *et al.* (1978) estimated 2-3 × $10^9$ OBs/LE.

## Biological activity

Knowledge of the relationship between pathogen concentration (or dose, e.g., OBs/ml of water or OBs/mg of larval body weight) and the host mortality response is essential for providing recommendations for field applications. The slope of the Probit-regression is especially important for economic determination and optimization of field dosages and the interpretation of field responses. The slope of the mortality response of PTM exposed to *Po*GV in field and laboratory experiments varies around 0.65 (Sporleder 2003; Kroschel and Sporleder 2006; Sporleder and Kroschel 2008) (**Fig. 2A**), in contrast to chemical insecticides, which have steeper slopes in dose-mortality relationships. The implications of a shallow slope are: proportionally lower increase in mortality rates for a given rise in dose; dosages to achieve high mortalities (>95%) might be difficult to achieve; but the advantage is that with significantly lower doses acceptable mortalities might be achievable. Sporleder *et al.* (2008b) conducted extensive bioassays over a 6-year period at temperatures ranging from 16 to 28°C. They reported that $LC_{50}$ values and slopes of probit-mortality curves were not significantly different between temperatures. **Fig. 2B** shows the mortality curve retransformed from the probit regression line (Kroschel and Sporleder 2006). In order to increase mortality responses from approximately 65% to approximately 85%, a 10-fold increase in *Po*GV concentration is necessary. For economic adjustments of optimal field rates, it may, therefore, be beneficial to apply *Po*GV at several intervals and at lower rates instead of targeting highest mortalities with one single application. Von Arx and Gebhardt (1990) studied the survival of PTM from egg to adult after exposure to 0.2, 0.02, and 0.002 *Po*GV-infected larvae or LE per kg of tubers. Survival was significantly affected after exposure to the two highest concentrations, but not at 0.002 LE. Generation time of survivors was not affected by the virus and fecundity was only reduced at the highest virus concentration. The intrinsic rate of increase of PTM

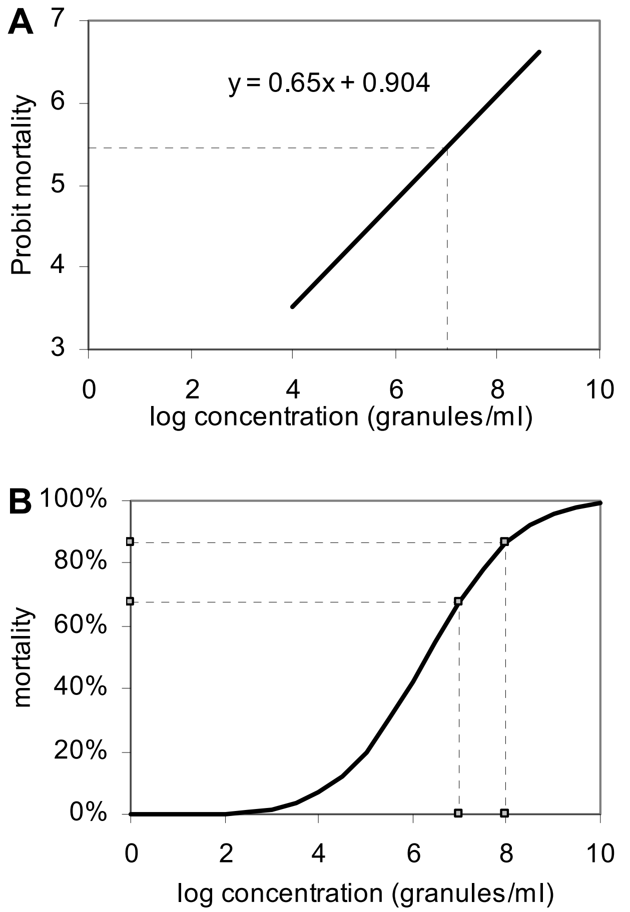


**Fig. 2 Concentration-mortality relationship between *Po*GV and its host *P. operculella* (neonate larvae).** (**A**) shows the Probit regression line with an average slope of 0.65 derived from several leaf-disc assays, and in (**B**) probit mortalities are retransformed into percentage mortalities. From: **Kroschel J, Sporleder M** (2006) Ecological approaches to integrated pest management of potato tuber moth *Phthorimaea operculella* Zeller (Lepidoptera, Gelechidae). *Proceedings of the 45th Annual Washington State Potato Conference*, Moses Lake, 7-9 February, 2006, pp 85-94, with kind permission from Washington State Potato Commission, Moses Lake, WA, USA, ©2006.

was only affected at the highest concentration. The activity of *Po*GV is highly variable in larvae due to larval age/weight (Sporleder *et al.* 2007b).

## Field application of *Po*GV

Application of *Po*GV for control of field populations of PTM has been relatively limited and the results have been variable. Reed (1971) and Reed and Springett (1971) conducted the first field trials with *Po*GV in Australia and found that an early application of virus (6275 LE/ha) could achieve effective control. They also observed that *Po*GV readily spread into untreated areas. Reed (1971) concluded that virus reached leaf mining larvae through the stomata and that wind and birds were responsible for spreading the virus. Field evaluations of *Po*GV in the Republic of Yemen were reported by Kroschel (1995) and Kroschel *et al.* (1996b) where two applications of *Po*GV at a dose of 5 × $10^{13}$ OBs/ha in 500 liters of water ($10^8$ OBs/ml) corresponded with approximately 10,000 virus-infected larvae/ha and resulted in 70% larval mortality. Typical symptoms (milky white coloration and reduced vitality) were observed in larvae 11 days after treatment and 70% mortality was noted 19 days after treatment. Ultimately, virus treatments resulted in up to 82.5% mortality of PTM. Zeddam *et al.* (2003b) proposed an application at a considerably lower rate (3 × $10^{12}$ OB/ha) in potato crops. Salah and Aalbu (1992) tested a *Po*GV suspension and powder preparation under field conditions in Tunisia. *Po*GV was applied to the surface of the soil in potato fields only incidentally reaching the plants. Field infestation of tubers by PTM was reduced by up to 73%. Kurhade and Pokharkar (1997) reported that *Po*GV applied at 5.5 × $10^{11}$ OBs/ha plus endosulfan (0.035%) provided effective control of PTM resulting in the lowest tuber infestation (6.9%) when compared to other insecticidal treatments. Salah *et al.* (1994) tested a combination of *Bt*, *Po*GV and extra irrigation for integrated control of PTM in Tunisian field trials. In some cases, the integration of microbial agents and cultural methods, such as extra irrigation proved to be more efficacious than conventional insecticides alone.

Applications of *Po*GV doses sufficient to cause over 95% mortality in the field are probably not economically feasible. For example, Arthurs *et al.* (2008a) reported good season long control of very high populations of PTM after 10 weekly applications of *Po*GV at $10^{13}$ OB/ha. PTM populations were reduced by 86-96% on pre-harvest foliage and 90-97% on tubers added to cages shortly before harvest. The results of Kroschel *et al.* (1996b) shown above (two applications of 5 × $10^{13}$ OBs/ha) resulted in slightly lower mortality than that reported by Arthurs *et al.* (2008b) where half the OBs/ha were applied throughout the growing season. Using Probit-regression curves derived from data reported by Kroschel *et al.* (1996b) and subsequent field experiments, 5 × $10^{13}$ OBs/ha is expected to result in approximately 85% mortality of neonate PTM larvae. In order to increase the efficacy of the application to provide 95% or even 99% mortality, a 10-fold and a 100-fold increase of the dosage would be necessary, respectively. On the other hand, 10 or 100-fold reduced rates would still result in 64% and 38% mortalities. This provides options for using *Po*GV as a relatively inexpensive partial suppression agent in potato fields through the use of low dosages per ha. In such an approach, the virus should be applied at short intervals, depending on the pest population growth potential in different agroecological zones. Specific treatment thresholds for such an approach will still need to be determined for each location (Kroschel and Sporleder 2006).

Ultraviolet (UV) radiation from sunlight can rapidly reduce the amount of *Po*GV available to larvae under field conditions (Kroschel *et al.* 1996a; Sporleder *et al.* 2001; Sporleder 2003; Arthurs *et al.* 2008a, Sporleder and Kroschel 2008). Different preparations of *Po*GV were investigated by Kroschel *et al.* (1996a) for their efficacy against PTM and their persistence on leaves and tubers in the field. They calculated a half-life of *Po*GV on tubers exposed to the sun to be 1.3 days. Mortalities of first instar larvae ranged from 43-49% when fed vegetation collected two days after treatment. Only 19.4-25.8% of larvae died when fed on foliage collected 8 days after virus application. Sporleder (2003) found that inactivation was initially very fast (first day with half-life times of 0.25-0.3 days) but slowed when about 95% of the virus was inactivated. Arthurs *et al.* (2008a) observed that early season applications of $10^{13}$ OBs/ha were highly effective for the first 24 hours (≥ 93% mortality), but there was a steady decline in activity over 10 days post-application due to UV inactivation of the virus.

A variety of adjuvants that have been used to protect other baculoviruses from UV inactivation were reviewed by Burges and Jones (1998). Sporleder (2003) Sporleder and Kroschel (2008) investigated the use of dyes, optical brighteners, antioxidants and insect host derived materials, for use in formulations for protecting *Po*GV against UV inactivation. He noted that the optical brightener 'Tinopal' and certain antioxidants (propyl gallate and phenylthiocarbamide, superoxide) protected the infectivity of irradiated virus. However, preparations of *Po*GV-infected larvae macerated in water were superior to other preparations in protecting the virus from UV irradiation (Kroschel and Koch 1996; Sporleder 2003; Kroschel and Sporleder 2006).

The possibility of the development of resistance to *Po*GV has been presented by Briese and Mende (1981, 1983) and Sporleder (2003). Briese and Mende (1981) noted differences in susceptibility to *Po*GV between field populations of PTM in Australia. Using a laboratory bioassay they compared the susceptibility of 16 field populations and observed a difference of 11.6 fold between the most and least susceptible populations. After serial exposure of susceptible PTM larvae to *Po*GV over 6 generations, Briese and Mende (1983) observed a 140 fold increase in the $LD_{50}$. Similar observations were made by Sporleder (2003). PTM larvae that survived exposure to virus concentrations corresponding to $LC_{50}$, $LC_{75}$, and $LC_{90}$ in the parent susceptible population were highly resistant to the virus after 12 generations. A single backcross with the susceptible population did not decrease the level of resistance. Based on the above reports, resistance management should be incorporated in control programs that regularly use *Po*GV.

### Use of *Po*GV to control PTM in potato stores

After harvest, tuber infestation by first instar larvae is hardly noticeable so that even with precautionary measures, infested tubers can be transferred to potato stores, where further propagation of the pest and infestation of the whole stock may take place. In the absence of refrigerated stores, complete damage to tubers can occur within a few months, if they are left untreated.

*Po*GV has been reported to provide very good protection of treated tubers, especially under non-refrigerated storage. A substantial amount of successful testing of *Po*GV has been conducted on stored tubers in the Andean countries (Peru, Ecuador, Bolivia, and Colombia) (Alcázar *et al.* 1992b; CIP 1992; Zeddam *et al.* 2003a, 2003b). *Po*GV has also been evaluated on stored tubers in several countries in the Middle East, Northern Africa, and Asia (Amonkar *et al.* 1979; Hamilton and Macdonald 1990; Islam *et al.* 1990; Ali 1991; Das *et al.* 1992; Setiawati *et al.* 1999; Kroschel *et al.* 1996a, 1996b). Because the virus is not exposed to UV degradation in storage, protection of tubers may last several months. A dust formulation, produced by selecting and grinding virus-infected larvae mixed with ordinary talcum, has been used at the rate of 5 kg/tonne of stored tubers (20 LE *Po*GV/kg talcum). Research showed that the granulovirus reduces damage in stores by 91 and 78%, 30 and 60 days after application (Raman and Alcázar 1990). Bioassays conducted at 25°C by Arthurs *et al.* (2008b) showed that *Po*GV suspended in water or mixed with carriers (talcum, sand, diatomaceous earth and kaolin clay), was highly effective in controlling neonate larvae in stored tubers. Aqueous suspensions of *Po*GV and talcum-formulated virus produced 100% larval mortality of neonate larvae on pre-infested tubers at concentrations as low as 0.00625 LE *Po*GV/kg tubers. Some carriers on their own (e.g. talc and diatomaceous earth) also produced significant mortality of control neonate larvae compared with dipping. Treatment of post-infested tubers with 0.4 LE *Po*GV/kg of tubers by dipping resulted in only 92% mortality.

For PTM control in potato storage, the virus is or has been commercially produced in Peru, Bolivia, Egypt, and Tunisia using low cost facilities for propagation. Further commercial development of *Po*GV is warranted based on the need for PTM management during the vegetative growth of potato, the potential for managing resistance to conventional insecticides, its safety, and potential for incorporation into IPM systems with minimal impact on beneficial non-target organisms.

## *BACILLUS THURINGIENSIS* AS A MICROBIAL CONTROL AGENT OF PTM

The only bacterium that has been evaluated for PTM control is *Bt*. It is a naturally-occurring bacterium that produces parasporal crystalline inclusions at the time of sporulation. These inclusions contain the proteinaceous toxins which cause disease in insects through the lysis of midgut epithelial cells (Beegle and Yamamoto 1992; Garczinski and Siegel 2007). Biopesticides based on *Bt* toxins are the most widely used of microbial pesticides and are commercially produced for use against a broad range of pests. These include coleopterans, dipterans and lepidopterans (Lacey *et al.* 2001), including PTM and other species that attack potato (Krieg *et al.* 1983; Hamilton and Macdonald 1990; Kroschel and Koch 1996; Lacey *et al.* 1999; Wraight and Ramos 2005; Wraight *et al.* 2007). Death can occur within a few hours to a few weeks of *Bt* application, depending on the insect species, age and the amount of *Bt* ingested. Although there are several different strains of *Bt*, each with specific toxicity to particular groups of insects, *Bt* subsp. *kurstaki* (*Btk*) is the most commonly used against lepidopterous insects. Several commercial formulations of *Bt* (Bio-T, Thuricide, Dipel, and others) have been developed for control of pest Lepidoptera since it was first commercialized (Beegle and Yamamoto 1992). Natural isolates of *Bt* were found within PTM's native range in Bolivia (Hernandez *et al.* 2005). Several strains were isolated from agricultural soils, warehouses, and tubers infested with PTM. Some of these isolates were shown to have equal or even greater toxicity when compared with a standard commercial strain of *Btk*, suggesting more effective indigenous strains of *Bt* could be developed for PTM control.

### Field application of *Bt*

*Bt* has been reported effective for control of PTM infestations under field conditions (Awate and Naik 1979; Broza and Sneh 1994; Kroschel 1995; Arthurs *et al.* 2008a). However, repeated applications have been required because *Bt* is degraded by UV light from the sun, and rain washes it into the soil (Salama *et al.* 1995b). Three consecutive applications of *Bt* ('Bio-T') at 8 day intervals were required to control PTM in an infested tomato crop in Israel (Broza and Sneh 1994). A high application volume (500 l/ha) was used to bring the active ingredient into the tunnels in the leaves where young larvae were mining. In field plot tests in India, foliar applications of *Bt* ('Thuricide' at 2 to 5 kg/ha) at 15-day intervals beginning 60 days after planting, were almost as effective at controlling PTM infestations as parathion and carbaryl (Awate and Naik 1979). In the Republic of Yemen, PTM infestations are very high. Kroschel (1995) tested *Bt* ('Dipel') over two seasons at two concentrations (0.2% and 0.3%) with three and four applications per potato season. In the control treatments PTM leaf infestation reached 26 and 35 mines per plant. Up until the plant yellowing stage, *Bt* application reduced PTM leaf infestation by 41% and 54% and final tuber infestation at harvest by 23% and 10%, respectively, compared to the control treatment. In comparison, the best results were achieved with the pyrethroid Fenvalerate (0.1%) which reduced leaf infestation by 100% and tuber infestation by 70%. Arthurs *et al.* (2008a) reported fairly good control of very high PTM populations with *Btk* but ten weekly applications of 1.12 kg/ha (Deliver®, Certis USA) were required throughout the growing season. A *Po*GV/*Btk* ($10^{13}$ OBs and 1.12 kg/ha) alternation was significantly more effective than *Btk* alone and as effective as *Po*GV at $10^{13}$ OBs/ha. In greenhouse and laboratory studies where *Bt* was applied to the soil to protect seedlings or tubers in pots, it retained its potency for up to 60 days (Amonkar *et al.* 1979).

### Use of *Bt* to control PTM in potato stores

*Bt* has also been widely tested to control PTM infestations under laboratory and storage conditions. Under laboratory conditions, PTM larvae are susceptible at differing degrees to various *Bt* subspecies including *kurstaki*, *thuringiensis*, *tolworthi*, *galleriae*, *kenyae* and *aizawai*, although the lethal concentration ($LC_{50}$) required increases with larval age (Salama *et al.* 1995a). For example, *Btk* ('Thuricide' HP) applied at 200 mg/kg potatoes reduced PTM survival from egg to adult emergence to 0.4%, compared with *Po*GV (0.8 to 34.7% depending on dosage) or controls (32.5%) (von Arx and Gebhardt 1990). In other laboratory studies, dust formulations of *Bt* (5000 IU/mg), along with permethrin (0.1%), prothiofos (1%) and rotenone (2.4%) provided good protection of potato tubers against PTM infestations and were more effective at controlling existing infestations compared with 1% chlorpyrifos (Hamilton and Macdonald 1990). In Egypt, another *Bt* preparation ('Dipel' 2X with 32000 IU/mg at 0.3% concentration) was also reported to be very effective in protecting tubers in stores, PTM infestation was eliminated compared with 100% infestation in untreated controls 60 days after treatment (Farrag 1998). In Tunisia, an integrated control approach comprising *Bt* applied at the beginning of the storage period in combination with cultural control (early harvest) eliminated the reliance on parathion sprays (von Arx *et al.* 1987). In cases when tubers had a high initial infestation (> 20%), *Bt* was replaced with a synthetic pyrethroid (permethrin). In tests in Indonesia, tubers treated with *Btk* ('Thuricide' at 2g/l) caused 79% larval mortality after 4 months of storage compared with 58% mortality of larvae on foliage in a screenhouse (Setiawati *et al.* 1999). In other studies, *Bt* subsp. *thuringiensis* (0.2% Bactospeine WP 16000 IU/mg) was reported ineffective at protecting tubers in storage, resulting in as much tuber damage as untreated controls (Das *et al.* 1992).

Formulation of *Bt* with various carriers has been reported by several researchers to improve *Bt* activity and/or to reduce product costs. *Btk* mixed with fine sand dust containing quartz provided effective control in tuber storage in the Republic of Yemen (Kroschel and Koch 1996). A very low proportion, 40 g *Btk* mixed with 960 g sand, applied to one tonne of stored potatoes proved to be efficacious. This treatment also controlled 96% of larvae that were already inside tubers. In Peru, Raman *et al.* (1987) reported that *Btk* ('Dipel') was effective in reducing feeding damage in storage when applied as a dust formulation. Formulations of *Btk* with various diluents were effective against neonate larvae. Arthurs *et al.* (2008b) demonstrated that tubers treated with 37.5 mg *Btk* WP in talcum or diatomaceous earth/kg tuber before infestation, resulted in 99% PTM larval mortality.

*Bt* also proved to be very effective in controlling the other species of the potato tuber moth complex, namely APTM and GPTM. This is especially important where these species co-exist as is the case in the Andes. The APTM, for which *Po*GV is not effective, is often the most prevalent PTM species in potato stores. A rate of 15 g of the commercial product 'Dipel' 2X mixed with one kg of talcum is recommended to protect 200 kg of tubers (Kroschel *et al.* 2009).

Other researchers suggest that *Bt* formulations in storage could be improved by the addition of plant extracts containing insecticidal properties. For example, extracts of *Atropa belladonna* L. and *Hyoscyamus niger* L. and *S. nigrum* plants reportedly decreased the $LC_{50}$ of *Bt* against PTM from 82 µg/ml to 43, 31 and 40 µg/ml, respectively (Sabbour and Ismail 2002).

## ENTOMOPATHOGENIC NEMATODES AND FUNGI

Entomopathogenic nematodes (EPNs) are insect-specific parasites in the genera *Steinernema* (Steinernematidae) and *Heterorhabditis* (Heterorhabditidae). These nematodes are obligately associated with symbiotic bacteria (*Xenorhabdis* spp. and *Photorhabdis* spp., respectively) which are responsible for rapidly killing host insects (Kaya and Gaugler 1993, Koppenhöfer 2007). After entering a host insect, the infective juvenile (IJ) stage of EPNs releases its symbiotic bacteria. In addition to killing the host, the bacteria digest host tissues and produce antibiotics to protect the host cadaver from saprophytes and scavengers. After two to three reproductive cycles, when host nutrients are depleted, IJs are produced and begin leaving the host insect. This stage is capable of immediately infecting a new host or may persist for months in the absence of a host (Kaya and Gaugler 1993; Koppenhöfer 2007). Applied and basic research conducted on EPNs over the past five decades has demonstrated their potential as biological control agents of a wide variety of insect pests (Grewal *et al.* 2005; Georgis *et al.* 2006). They have been commercially developed for the control of several economically important insect species. However, their use for control of PTM has only recently been investigated. Results of laboratory and field research conducted on EPNs and PTM reveal good potential for control of stages of the moth that enter or emerge from the soil (Lacey, unpublished data). At CIP, it was shown that L4 larvae of all species of the PTM complex are highly susceptible to *Heterorhabditis* sp. isolates from the high Andes of Ecuador and Peru (J. Alcázar, unpublished data).

Numerous species of entomopathogenic fungi are effective biopesticides of several insect pests (Goettel *et al.* 2005; Ekesi and Maniania 2007), including some key pests of potato (Lacey *et al.* 1999; Wraight and Ramos 2005). However, there is limited research on the feasibility of using fungi for PTM control. Laboratory studies on two common Hypocreales, *Metarhizium anisopliae* (Metschnikoff) Sorok., and *Beauveria bassiana* (Balsamo) Vuillmen indicate they have potential for control of PTM larvae, particularly younger larvae (Hafez *et al.* 1997; Sewify *et al.* 2000). Hafez *et al.* (1997) also demonstrated activity of *B. bassiana* against prepupae, pupae and adult PTM. Sewify *et al.* (2000) reported that the combination of *M. anisopliae* and *Po*GV resulted in synergistic larval control when a high concentration of the fungus was used with a low concentration of the virus.

The endophytic fungus, *Muscodor albus*, produces several volatile compounds (alcohols, esters, ketones, acids and lipids) that are biocidal for a range of organisms including plant pathogenic bacteria and fungi, nematodes and insects (Strobel *et al.* 2001; Worapong *et al.* 2001; Lacey *et al.* 2008; Riga *et al.* 2008). Adulticidal and larvicidal activity of *M. albus* was reported against PTM by Lacey and Neven (2006) and Lacey *et al.* (2008). PTM adults and neonate larvae were exposed to *M. albus* volatiles for 72 hours in hermetically sealed chambers. Mean percent mortalities of adult PTM in chambers with 15 and 30 g of formulated mycelia were 84.6% and 90.6%, respectively. Development to the pupal stage of PTM that were exposed as neonate larvae on tubers to 15 or 30 g *M. albus* formulation was reduced by 61.8% and 72.8%. Lacey *et al.* (2008) observed that the length of exposure to *M. albus* significantly affected mortality of larvae within infested tubers and their development to the adult stage. Exposure durations of 3, 7, or 14 days at 24°C followed by incubation at 27°C until emergence resulted in mortalities of 84.2%, 95.5% and 99.6%, respectively. Mortality of larvae was significantly reduced at 10 and 15°C. Most refrigerated storage temperatures for tubers depend on the market for the tubers (seed, fresh market, processing) and range from 3 to 10°C. Regardless of the final storage temperature, tubers are initially held at 10 to 16°C for 3 to 5 weeks for suberization (wound healing) at the beginning of storage, and the temperature is then lowered slowly to the long term holding levels (Knowles and Plissey 2008). Fumigation with *M. albus* during the initial holding period may be adequate to control neonates and young larvae (Lacey *et al.* 2008).

## USE OF BOTANICALS, SEX PHEROMONES AND PHYSICAL MEASURES FOR PTM CONTROL IN STORES

Other natural insecticides prepared by water extracts of *Azadirachta indica* A. Juss. seed provided relatively high levels of tuber protection from PTM (Kroschel and Koch 1996; Salama and Salem 2000). The foliage of some plants, such as *Eucalyptus* sp., *Lantana camara* L., and the native species *Schinus molle* L. and *Minthostachys* sp. in the Andean region, have some repellent effects and can be recommended as additional, complementary control with biopesticide treatments (Raman *et al.* 1987; Iannacone and Lamas 2003). Use of commercial sex pheromones to disrupt mating of PTM appears to be economic in potato stores and helps to monitor the pest during storage. Recently, attract-and-kill, which is a co-formulation of sex pheromones and a contact insecticide, developed for PTM and the APTM also showed high efficacy for PTM management (Kroschel and Zegarra 2007, 2008).

It is important to mention that for effective management of PTM during the full storage period, storage hygiene is of utmost importance to guarantee successful control with *Bt* and *Po*GV. In particular, physical control is needed to hinder new moths entering storage facilities and infesting young unprotected potato sprouts (Kroschel and Sporleder 2006). If this is not done, managing the moth in potato stores with alternative control measures (instead of systemic insecticides) will have significantly reduced effects.

## CONCLUSIONS

Natural enemies including parasites, predators and pathogens can exert substantial control of PTM populations, especially when little or no insecticide is used (Matthiessen and Springett 1973; Briese 1981; Kroschel and Koch 1994; Coll *et al.* 2000; Horne and Page 2008). It is likely that no single natural enemy species will provide stand alone control, but together they can be incorporated into an IPM program to regulate PTM in a complementary manner throughout the growing season, in the various stages of the life cycle and at various population densities of the moth. The fact that PTM has been distributed, together with potato, out of its range of origin, offers the opportunity to apply classical and augmentative biological control in several potato production agroecologies where it has been unintentionally introduced. For this purpose CIP maintains and studies parasitoids of PTM, all of neotropical origin (Kroschel *et al*. 2008). Complementary pathogen-parasitoid interaction warrants further attention with PTM, its parasitoids and *Po*GV in potato agroecosystems. For example, Kroschel *et al.* (1996b) surmised that parasitoids were slightly inhibited by application of $5 \times 10^{13}$ *Po*GV OBs/ha, but not by application of one tenth that amount of the virus. Ostensibly parasitoid larvae continued development in still living virus-infected PTM larvae. Parasitoids are better suited for exploiting uninfected hosts because of their abilities of search, whereas most pathogens, such as *Po*GV, require chance encounters. According to Begon *et al.* (1999), one of the most important aspects to consider in the integration of pathogens and parasitoids is the developmental stage of the host that is attacked. The fact that *Po*GV normally infects neonate larvae, while many parasitoids of PTM attack eggs and older larvae could enhance combined control.

*Po*GV has been successfully developed as a biopesticide product and is produced by national programs and used by farmers in some parts of the Andes to control PTM in potato stores. On the other hand, the market potential of *Btk* for PTM control in field crops and storage is not being fully exploited. Further research and development of *Btk* for PTM control is warranted especially considering its ease of production, existing commercial status for pest management, and its wider host range of all species in the PTM complex as well as other lepidopterans. Kroschel *et al*. (2009) suggested the production and commercialization of a *Btk*-talcum formulation for protecting potatoes during storage that is an effective re-formulation of commercial *Btk* products. This would make *Btk* cost effective for small scale farmers in developing countries.

An integration of biopesticides for PTM field control will depend on whether PTM is considered the only herbivore that has reached pest status in a potato agroecosystem. How an IPM program will consider all other pests and their control, including the judicious use of pesticides as well as the effect of environmental conditions on biopesticide infectivity and persistence will also require consideration. The successful management of microbial control agents will require: the selection of effective pathogen strains; development of formulations to improve field persistence; careful timing of application; and a better understanding of how they will fit into potato production systems. The implementation of biopesticides will ultimately depend on an increased awareness of their attributes by growers and the public (Lacey *et al.* 2001), who will be the main drivers for their use and commercialization.

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