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

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The recent outbreak of the potato tuber moth (PTM), *Phthorimaea operculella*, in the Pacific Northwest has generated serious concerns regarding damage to tubers in the largest potato production area of North America (Jensen et al., 2005). The current options for control prior to harvest comprise the use of several broad spectrum insecticides (Schreiber and Jensen, 2005). However, the pre-harvest interval of most chemical insecticides does not permit trement of tubers just prior to harvest and consumption.

A diverse spectrum of microscopic and multicellular 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 (Kaya and Lacey, 2000; Lacey et al., 2001a), 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; Lacey et al., 2001b). Microbial control agents have no pre-harvest interval, are safe for application personnel, the food supply, and most non-target organisms including beneficial insects. Substantial effort has gone into the development of certain microbial agents for PTM control in several countries worldwide. In contrast, only limited attention has been paid to biopesticides for PTM control in the United States. In this overview we will present information regarding the development and potential of a virus, a bacterium, fungi, and nematodes for control of PTM.

Virus

A granulovirus (PoGV) that attacks PTM larvae has accompanied the moth from its South American center of origin to most countries where it has become established including the United States. Several surveys confirm the presence of the virus in PTM populations in the Andean potato growing areas of South America (Alcázar et al., 1991, 1992a; several isolates and their origins summarized by Sporleder, 2003), Africa (Broodryk and Pretorius, 1974; Laarif et al., 2003), the Middle East (Kroschel and Koch, 1994), Asia (Zeddam et al., 1999; Setiawati et al., 1999) Australia (Reed, 1969; Briese, 1981) and North America (Hunter et al., 1975). Other than the report by Hunter et al. (1975) of PoGV infected PTM larvae in California, no further research has been published on this virus in the United States.

The name of the virus is derived from its granular appearance under high magnification (Figure 1A). Each granule contains a single viral rod (Figure 1B). After the granules are ingested by PTM larvae, they dissolve in the alkaline midgut freeing the viral rods which attach to and pass through the membrane of the midgut epithelial cells. From there they invade a variety of host cells and produce hundreds of millions of rod-containing granules 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 larvae. Most larvae die within 2-3 weeks of ingesting virus, but very high dosages of PoGV can cause death by toxicosis within 48 hours.

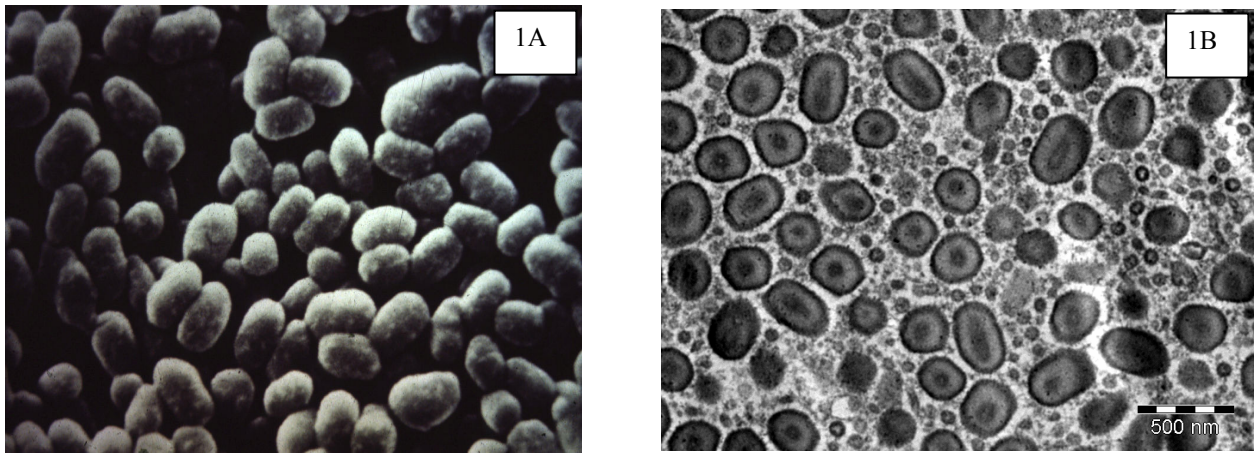

Figure 1. Granules of the potato tuber moth granulovirus (PoGV). 1A Intact PoGV granules. Scanning electron micrograph courtesy of the International Potato Center, Lima, Peru. 1B Cross and longitudinal sections of PoGV granules. Transmission electron micrograph courtesy of Darlene Hoffmann, USDA-ARS, Parlier, CA.

Sporleder (2003) assessed the activity of 14 geographical isolates of PoGV and found a wide range of activity covering several orders of magnitude. Vickers et al. (1991) demonstrated minor differences among 8 geographically diverse PoGV isolates using restriction endonuclease DNA analysis. Three distinct, but closely related genotypes of PoGV were revealed and the Peruvian isolate was readily distinguishable from granuloviruses of 5 other insect hosts (Vickers et al., 1991). Bioassays of 3 PoGV isolates from Indonesia revealed similar biological properties (Zeddam et al., 1999). The restriction pattern of the Indonesian Wonsosobo isolate varied only slightly from other PoGV isolates from different regions of the world (Zeddam et al., 1999). Kroschel et al. (1996a) reported similarity between an isolate from Yemen and a Peruvian isolate. In contrast, Lery et al. (1998) demonstrated considerable genetic heterogeneity between a Tunisian isolate and isolates of PoGV from other regions. Like most granuloviruses, PoGV has a fairly specific host range. Only PTM and certain other species in the same family (Gelechiidae) are infected by the virus. *Scrobipalpuloides absoluta* and *Tecia solanivora* are susceptible to PoGV, but at lower levels than PTM (Angeles and Alcázar, 1995, 1996; Zeddam et al., 2003). Although PoGV could be isolated from *Symmetrischema tangolias* it does not appear to affect this species (J. Kroschel, personal communication). Pokharkar and Kurhade (1999) reported no infectivity to 11 other lepidopteran species.

The natural incidence of PoGV in PTM populations has been documented in Australia and Yemen (Briese, 1981; Kroschel and Koch, 1994; Kroschel, 1995) where PTM larvae were infected at low frequencies. Kroschel and Koch (1994) observed that the majority of PTM mortality in Yemen was caused by braconid and ichneumonid parasitoids. Kroschel et al. (1996b) surmised that parasitoids were slightly inhibited by application of 5×10^{13} PoGV granules/hectare, but not by application of one tenth that amount of the virus. Parasitoid larvae ostensibly continued development in still living virus-infected PTM larvae. Complementary pathogen-parasitoid interaction with other insect pests was reviewed by Brooks (1993), Begon et al. (1999), Furlong and Pell (2005) and others and warrants further attention with PTM, its parasitoids and PoGV in potato agroecosystems in the Pacific Northwest of the United States. Parasitoids are better suited for exploiting uninfected hosts, particularly in cryptic habitats, because of their abilities of search, whereas most pathogens, such as PoGV, must wait for 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 stage of the host that is attacked. The fact that PoGV normally infects neonate larvae, while many parasitoids of PTM attack eggs and older larvae, could enhance combined control. Interaction of PoGV and natural enemies is not limited to parasitoids. Matthiessen and Springett (1973) made interesting observations on a bird, the silvereye (*Zosterops gouldi*), which is a predator of PTM and a potential vector of PoGV in Australia.

Under experimental conditions, von Arx and Gebhardt (1990) studied the survival of PTM from egg to adult after exposure to 0.2, 0.02, and 0.002 PoGV infected larvae or "larval equivalents" (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 was only affected at the highest concentration. It is evident from the above study and the natural incidence of PoGV in PTM populations, that in order to provide effective control of PTM populations (i.e. to reverse population increases and reduce damage caused by larvae in plants and tuber), inundative applications (e.g. through spraying) of the virus will be required.

Application of PoGV for control of field populations of PTM has been somewhat limited and the results have been variable. Reed (1971) and Reed and Springett (1971) conducted the first field trials of PoGV in Australia and found that an early application of virus (6275 LE/hectare) could achieve effective control. They also observed that PoGV 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. Salah and Aalbu (1992) tested a PoGV suspension and powder under field conditions in Tunisia. Virus 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 PoGV at 5.5×10^{11} granules/hectare plus endosulfan (0.035%) provided effective control of PTM resulting in the lowest tuber infestation (6.9%) compared to other insecticidal treatments. Salah et al. (1994) tested a combination of *Bacillus thuringiensis*, PoGV and extra irrigation for integrated control of PTM in Tunisian field trials. In some cases, the integrated controls proved to be more efficacious than conventional insecticides. Field evaluations of PoGV in Yemen were reported by Kroschel (1995) and Kroschel et al. (1996b) where applications of 5×10^{13} PoGV granules/hectare of potato plants resulted in significant control of PTM. 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.

In addition to effects of PoGV strain, methods of application, and concentration on efficacy, inactivation of the virus by the ultraviolet (UV) radiation in sunlight can rapidly reduce the amount of inoculum available to larvae under field conditions (Kroschel et al., 1996a; Sporleder et al., 2001; Sporleder, 2003). Different preparations of PoGV were investigated by Kroschel et al. (1996a) for their efficacy and persistence on leaves and tubers in the field. They calculated a half life of PoGV on tubers exposed to the sun to be 1.3 days. Mortalities of first instar larvae ranged from 43-49% when fed vegetation collected 2 days after treatment. Only 19.4-25.8% of larvae died when fed on foliage collected 8 days after virus application. A variety of adjuvants that have been used to protect other baculoviruses from UV inactivation were reviewed by Burges and Jones (1998).

Sporleder (2003) investigated the use of dyes, optical brighteners, antioxidants, insect host derived materials, and type of formulation for protection of PoGV from UV inactivation. He noted that the optical brightener Tinopal and certain antioxidants protected the infectivity of irradiated virus. However, PoGV-infected larvae macerated in water were superior to other preparations in protecting the virus from UV irradiation (Kroschel and Koch, 1996; Sporleder, 2003). PoGV has been reported by several researchers to provide very good protection of treated tubers, especially in non-refrigerated storage. A substantial amount of successful testing of PoGV has been conducted on stored tubers in the Andean countries (Peru, Ecuador, Bolivia, Colombia) where PTM and the potato are believed to have originated (Alcázar et al., 1992b; CIP, 1992; Zeddam et al., 2003). The virus 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). Because the virus is not exposed to UV degradation in storage, protection of tubers may last several months.

The potential for development of resistance to PoGV in PTM larvae has been presented by Briese and Mende (1981, 1983) and Sporleder (2003). Briese and Mende (1981) noted differences in susceptibility to PoGV 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 PoGV 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 that produced 50, 75, and 90% mortality 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 PoGV.

Methods for the *in vivo* production of PoGV are presented in Reed and Springett (1971), CIP (1992), Kroschel et al. (1996b), Sporleder (2003) 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 a aqueous suspension of triturated PoGV-infected larvae. Alternatively, PTM eggs can be dipped in PoGV suspensions (Sporleder et al., 2005). Larvae consume virus directly upon exiting the egg and are provided tubers in which to develop. Another production method involves spraying of virus suspensions onto infested potato plants in the field, collection of infested foliage after larvae become diseased, and separation of infected larvae from foliage using exposure to heat (Matthiessen et al., 1978). Sporleder (2003) presented information on the effect of temperature, initial virus concentration, larval age and density per gram of potato on the yield of granules. The number of virus infected larvae increased with increasing virus concentration with an optimal concentration of 10^9 granules/ml of suspension. The optimal temperature and larval density for virus production was 25°C (77°F) and 2 grams of potato/larva, respectively. Pokharkar and Kurhade (1999) also reported 25°C as optimal for virus production. 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 PoGV. The virus is or has been commercially produced in Peru, Bolivia, Egypt, and Tunisia. Although the PoGV is not commercially available in the United States, its development and registration is warranted based on the need for PTM control, the potential of PoGV as a method to slow development of resistance to conventional insecticides, its safety and potential for incorporation into IPM systems with minimal impact on beneficial non-target organisms.

Bacteria

The only bacterium that has been evaluated for PTM control is *Bacillus thuringiensis* (Bt). It is a naturally-occurring bacterium that produces crystal toxins (stomach poisons) which cause disease in insects through the lysis of midgut epithelial cells (Beegle and Yamamoto, 1992; Lacey et al., 2001a). Insecticides based on Bt toxins are the most widely used of microbial pesticides and are commercially produced for use against a broad range of pests including Coleoptera (beetles), Diptera (flies) and Lepidoptera (caterpillars), including species that attack potato (Krieg et al., 1983; Hamilton and Macdonald, 1990; Kroschel and Koch, 1996; Lacey et al. 1999, 2001b; Wraight and Ramos, 2005). Bt is considered ideal for pest management because of its specificity to pests and safety to humans and natural enemies of many crop pests. Typical agricultural formulations of Bt include wettable powders, liquid concentrates, dusts, baits, and others, and have been marketed under trade names such as Acrobe, Bactospeine, Certan, Dipel, Javelin, Leptox, Novabac, Thuricide and Victory. In order to be effective, Bt must be eaten by the feeding stage (larvae); it is ineffective against adult insects. 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 types of insects, Bt subsp. *kurstaki* is the most commonly used against lepidopterous insects. Natural incidences of Bt were noted within PTM's native range in Bolivia (Hernandez et al., 2005). Several strains were isolated from agricultural soils, warehouses, and tubers infested with PTM. Moreover, some of the isolates were shown to have equal or even greater toxicity compared with a standard commercial strain (HD-1), suggesting more effective indigenous strains of Bt could be developed for PTM control.

Bt has been widely tested to control PTM infestations under laboratory, field and storage conditions. At least 2 crystal proteins of Bt have activity against PTM, *e.g.* Cry1A(b) and Cry1B. 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, Bt subsp. *kurstaki* (Thuricide HP) applied at 200 mg/kg potatoes reduced PTM survival from egg to adult emergence to 0.4%, compared with PoGV (0.8-34.7% 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%) gave 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 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).

Bt has also been reported effective for control of PTM infestations under field conditions. However, repeated applications are required because Bt is degraded by UV light from the sun, and rain washes it onto 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 application of Bt (Thuricide at 2-5 kg/ha) at 15-day intervals beginning 60 days after planting, were almost as effective at controlling PTM infestations as parathion and carbaryl applied to the soil surface and resulted in average tuber yields of 9.3-10.7 tonnes/ha, compared with 6.7 tonnes without insecticides (Awate and Naik, 1979).

In many parts of the world, Bt and other non-chemical methods have been evaluated for post harvest control of PTM, which is a serious pest in traditional, non-refrigerated potato stores. In Yemen, Kroschel and Koch (1996) evaluated a range of low risk pesticides to protect tubers against PTM in storage. Bt mixed with fine sand and dusted on tubers was completely effective when applied before PTM eggs were deposited, but also controlled 96% of larvae that were already inside tubers. In Egypt, another Bt preparation (Dipel 2X at 0.3% concentration) was also reported to be very effective in storage, eliminating a PTM infestation 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 (over 20%), Bt was replaced with a synthetic pyrethroid (permethrin). In tests in Indonesia, tubers treated with Bt subsp. *kurstaki* (Thuricide at 2g/liter) 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 Peru, Raman et al. (1987) found Bt subsp. *kurstaki* (Dipel) was effective in reducing feeding damage in storage when applied as a dust formulation. Vegetable oil (1-2%) also reduced egg hatch but was phytotoxic, resulting in high levels of tuber rotting. However, 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). Other research suggests Bt may be improved through formulation with plant extracts containing insecticidal properties. For example, extracts of *Atropa belladonna* and *Hyoscyamus niger* and *Solanum nigrum* plants reportedly decreased the LC_{50} of Bt against PTM from 82 ug/ml to 43, 31 and 40 ug/ml, respectively (Sabbour and Ismail, 2002).

Emerging research suggests new ways in which Bt toxins might be used to control PTM. Studies have demonstrated the protective effects of Bt-modified potato lines on foliage and tubers from PTM damage in field and storage conditions (Davidson et al., 2004; Jansens et al., 1995; Westedt et al., 1998). Although planting transgenic potatoes may provide very effective PTM control options for growers, public acceptance of genetically modified food crops will be the ultimate deciding factor for widespread use of Bt potatoes.

Entomopathogenic Nematodes (EPNs)

EPNs are insect-specific parasites in the genera *Steinernema* (Steinernematidae) and *Heterorhabditis* (Heterorhabditidae) that are obligately associated with symbiotic bacteria (*Xenorhabdis* spp. and *Photorhabdis* spp., respectively) which are responsible for rapidly killing host insects. After entering a host insect, the infective juvenile stage of EPNs releases its symbiotic bacteria. In addition to killing the host, the bacteria digest host tissues, produce antibiotics to protect the host cadaver from saprophytes and scavengers. After two to three reproductive cycles, when host nutrients are depleted, infective juveniles 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.

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 control of several economically important insect species. However, their use for control of PTM has only recently been investigated. Results of research conducted on EPNs and PTM at the Yakima Agricultural Research Laboratory in Wapato, Washington reveal good potential for control of stages of the moth that enter or emerge from the soil.

Good potential of these nematodes is also envisioned for PTM control in potato cull piles. In depth research on the efficacy of *Steinernema* spp and *Heterorhabditis* spp. is planned for the 2006 season at field sites in Washington and Oregon.

Fungi

Numerous species of entomopathogenic fungi are effective microbial control agents of several insect pests (Goettel et al., 2005), 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 Hyphomycetes (Fungi Imperfecti), *Metarhizium anisopliae* and *Beauveria bassiana* indicate they have potential for control of 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 PoGV resulted in synergistic larval control when a high concentration of the fungus was used with a low concentration of the virus.

The fungus *Muscodor albus* produces a mixture of volatile organic chemicals that are toxic to a broad range of plant and human pathogenic fungi and bacteria. Several researchers have demonstrated the activity of *M. albus* for control of a variety of fungi and bacteria that cause plant disease and rot (Worapong et al., 2001, Stinsen et al., 2003, Mercier and Smilanick, 2005), but the insecticidal activity of this fungus has only recently been investigated. In studies conducted at YARL, PTM adults and neonate larvae were exposed to volatiles generated by *M. albus* mycelia on rye seeds plus water for 72 hours in hermetically sealed chambers (Lacey and Neven, 2006). Mean percent mortalities in adult moths exposed to 0, 15 and 30 g of fungal formulation were 0.9, 84.6, and 90.6%, respectively. Development to the pupal stage of PTM that were exposed as neonate larvae to 15 or 30 g of formulated *M. albus* mycelia was reduced by 61.8 and 72.8%, respectively relative to controls. Additional research on *M. albus* as a biofumigant for PTM control will continue in 2006.

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). It is likely that no single natural enemy species will provide stand alone control, but together they can 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. In addition to their utility for controlling PTM and other insect pests, insect-specific pathogens offer 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). The integration of insect-specific pathogens and nematodes into the potato agroecosystem will depend on their compatibility with other control agents, including pesticides and the effect of environmental conditions on their infectivity and persistence. Their successful utilization will require selection of effective pathogen strains and development of formulations to improve storage, application and persistence, careful timing of application, and a better understanding of how they will fit into potato production systems. Their implementation will ultimately depend on an increased awareness of their attributes by growers and the public (Lacey et al., 2001a).

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