Microbial Control of Potato Tuberworm in Potato Plants and Tuber Storage with Emphasis on Research Conducted in the Pacific Northwest of the United States

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Introduction

Potato tuberworm (PTW), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is widely recognized as a potato pest of global importance (Radcliffe 1982, Trivedi and Rajagopal 1992, Kroschel and Lacey 2008). Larvae mine in potato leaves and stems, but more important is the feeding damage in potato tubers, which also can cause rapid rotting in non-refrigerated storage. In the tropics and subtropics it is primarily a serious pest of stored tubers (Raman et al. 1987). If left untreated, PTW infestation of tubers stored under rustic conditions can be extremely high. When populations of PTW are very high, they can also be debilitating to potato plants. Although it is less commonly a potato pest in temperate regions, it can have periodic outbreaks causing significant damage to tubers (Sporleder et al. 2004, 2008a, De Bano et al. 2010). Because there is zero tolerance for insect parts in processed tubers, infestations, even relatively small ones, can result in substantial economic losses. For example, damage caused by PTW in the Pacific Northwest was especially high in 2003-2005 (Jensen et al. 2005, Rondon et al. 2006, 2008, De Bano et al. 2010), often requiring frequent application of broad spectrum insecticides (Schreiber and Jensen 2005).

Several natural enemies are reported from PTW including insects (parasitoids and predators) (Briese 1981, Horne 1990, Kroschel and Koch 1994, Kroschel 1995, Herman 2008a, Horne and Page 2008) and pathogens (Kroschel and Lacey, 2008, Lacey and Kroschel 2009). Naturally occurring and inundatively applied entomopathogens (biopesticides) can significantly contribute to control of PTW within an integrated control management (IPM) strategy. The advantages and disadvantages of using insect-specific pathogens as microbial control agents (MCAs) are presented by Kaya and Lacey (2007). In addition to efficacy, two important advantages of MCAs are their selectivity for targeted insects and safety for natural enemies and vertebrates (Laird et al. 1990, Hokkanen and Hajek, 2003). In this paper we review the most researched and practically used pathogens for control of PTW i.e. a granulovirus and the bacterium *Bacillus thuringiensis* (Bt).

Potato tuberworm granulovirus

The most effective MCA for PTW is a granulovirus (PoGV) in a family of viruses, the Baculoviridae (granuloviruses and nucleopolyhedroviruses), that infect and kill insects. The granuloviruses exclusively infect and kill several species of Lepidoptera. The name granulovirus is derived from their granular appearance under high magnification. Each granule contains a

single viral rod. After the granules, also known as occlusion bodies (OBs), are ingested by lepidopteran 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 OBs per larva. Several strains of PoGV have been isolated from PTW worldwide (Reed 1969, Broodryk and Pretorius 1974. Hunter et al. 1975, Briese 1981, Alcázar et al. 1991, 1992a, Vickers et al. 1991, Setiawati et al. 1999, Zeddam et al. 1999, Laarif et al. 2003) including the United States (Hunter et al. 1975). The virus is very selective and infects only PTW and some closely related species, such as the Guatemalan tuber moth, *Tecia solanivora* (Povolny). PoGV is prepared as an MCA by infecting PTW larvae with virus-treated tubers, after death grinding them for use in a diluted aqueous suspension for spraying or drying (Reed and Sringett 1971, Matthiessen et al. 1978, CIP 1992, Kroschel et al. 1996, Sporleder 2003). For dry application, the virus suspension is mixed with talc or another carrier and dried for application onto stored tubers (CIP 1992). A substantial amount of successful testing of PoGV has been conducted on stored tubers in Peru (Alcázar 1992b), Ecuador (Zeddam et al. 2003), Bolivia (Kroschel, personal communication), in the Middle East (Salah et al. 1994, Moawad et al. 1997, Farrag 1998), Northern Africa (Lagnaoui et al. al. 1997), Asia (Amonkar et al. 1979, Ali 1991, Kurhade and Pokharkar 1997, Das et al. 1992, Setiawati et al. 1999, Islam et al. 1990, Chandel et al. 2008), Australia (Hamilton and Macdonald 1990), and more recently the Pacific Northwest of the United States (Arthurs et al 2008a, 2008b, Lacey et al. 2010). In developing countries, it has been used primarily for control of PTW in the tuber storage. Research on the virus has also been conducted in potato plantings (Reed and Springett 1971, Briese and Mende 1981, von Arx et al. 1987, Salah and Aalbu 1992, Salah et al. 1994, Kroschel 1995, Kroschel et al. 1996, Arthurs et al. 2008a). Salah et al. (1994) tested a combination of Bt, PoGV and extra irrigation for integrated control of PTW in Tunisian field trials. In some cases, the integrated controls proved to be more efficacious than conventional insecticides. Factors that determine the larvicidal activity of PoGV have been presented by Pokharkar and Kurhade (1999), Sporleder (2003), Sporleder and Kroschel (2008), Sporleder et al. (2007, 2008b). The principal limiting factor under field conditions is solar degradation of the virus (Sporleder 2003, Sporleder and Kroschel 2008). Consequently, in areas on the plant that are not protected from direct sunlight, such as the surface of leaves, the virus can be deactivated within a few days.

Bacillus thuringiensis

Bt is the only bacterium that has been evaluated for PTW control. The crystal toxins produced at the time of sporulation act as stomach poisons. After ingestion by targeted insects, the toxin is solubilized and causes the lysis of midgut epithelial cells and ultimately death of the insects. Although there are several different strains of Bt, each with specific toxicity to particular groups of insects, Bt subsp. *kurstaki* (Btk) is most commonly used against Lepidoptera (Beegle and Yamamoto 1992, Garczynski and Siegel 2007). Btk has been reported to be effective for control of PTW infestations in field tests (Broza and Sneh 1994, Salah et al. 1994). However, repeated applications have been required because the toxin responsible for activity is inactivated by UV light from the sun, and rain washes it onto the soil. Btk has also been widely tested to control PTW infestations tuber storage (Kroschel and Koch 1996, Salama et al. 1995a, 1995b, von Arx and Gebhardt 1990, Hernandez et al. 2005, Arthurs et al. 2008a). 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).

Potato tuberworm granulovirus and *Bacillus thuringiensis* research in Washington State. Field studies. There are few insecticidal options for PTW control late in the growing season. We evaluated PoGV and Btk for season-long control of PTW on potato foliage in 2006 and 2007 (Arthurs et al. 2008a). Compared to untreated controls, 10 weekly applications of 10^{13} PTW occlusion bodies (OBs) per hectare (4 x 10^{12} OBs/acre) reduced PTW populations in replicated 1-m^3 field cages by 86–96% on pre-harvest foliage and 90–97% on tubers added to cages shortly before harvest. Infection rates of 82–95% of fourth instar larvae by PoGV were noted within individual larval cohorts. Equivalently timed Btk treatments (1.12 kg product/ha; one lb/acre) were significantly less effective at population suppression, with a only 36–76% reduction in larvae recovered from tubers added to cages. A PoGV/Btk alternation was significantly more effective than Btk alone and as effective as PoGV in 2007, but not in 2006. There was some evidence that reduced rate PoGV treatments (10% rate or 50% application frequency) were less effective than the standard program. There were no treatment effects on percentage of tubers growing in the ground that were infested at harvest, which remained comparatively low at ≤8.1%.

Bioassays were conducted to evaluate the residual activities of foliar deposits. Early-season applications were highly effective for the first 24 h (≥93% mortality) with a steady decline in activity over 10 days. A second application, applied later in the season, showed similar patterns, although in this case Btk was less persistent than PoGV, whereas both agents provided significant larval mortality compared with controls over 14 days. Both PoGV and Btk provide alternatives to manage field infestations of PTW prior to harvest, thus reducing the risk of tuber infestations in storage.

In 2008 we conducted large plot trials of PoGV against natural populations of PTW in potato plantings in Eltopia, WA and continued with caged tests in Moxee, WA (Lacey 2009). Large plot field trials in Eltopia assessed the efficacy of PoGV and combination of half rate PTW virus (820 virus infected larvae per acre) with half rate imidacloprid, virus alone and imidacloprid alone. The rationale of combining PoGV and imidacloprid was to investigate the possibility of the synergistic effect of the nicotinal insecticide and entomopathogens reported by other authors (Quintela and McCoy 1997). Tests started in mid growing season consisted of 5 bi-weekly applications of the virus until 2 weeks before vine kill. Results showed an 86% reduction of mines in plants treated with PTW virus, but only a 46% reduction of mines in imidacloprid treated plots. Tuber infestation was reduced by 90% in plots treated with PTW virus. There was no synergistic or additive activity of PTW virus and imidacloprid. We also monitored non-target impacts of the treatments. Figures 1 and 2 show the average numbers of beneficial and pest insects, respectively throughout the sampling period (July 1-September 19, 2008). No apparent effect of most of the treatments on beneficial insects was observed. None of the predators (big eyed bugs, damsel bugs, spiders) or parasitoids (Braconidae) in virus treated plots was negatively affected by virus. However, plots that received imidacloprid showed some decline in big eyed bugs. The gradual decline of parasitoids in all plots after July 21 and predators after August 20 is typical of natural enemies whose hosts have been reduced. The average numbers of pest insects (aphids, thrips, Colorado potato beetle [CPB], leafhoppers and other Homoptera) on each

sampling date was somewhat reduced in the imidacloprid plots predominantly due to the suppressing effect of the pesticide on the large population of CPB.

Small plot field evaluation of reduced applications (three) of PTW virus (full rate, 1645 infected larvae per acre) and Bt at the USDA research farm in Moxee started at mid-season. The highest mortality of PTW larvae (81%) in tubers was observed in plots treated with virus. Mortality of larvae in Bt-treated plots was negligible. Despite the high level of larval mortality within infested tubers in virus-treated plots, the infestation rate was not significantly different from controls.

Figure 1. Average number of predators and parasitoids on each of six sampling dates in control plots and in plots treated with PoGV, imidacloprid or an imidacloprid-PoGV combination. Eltopia, WA 2008.

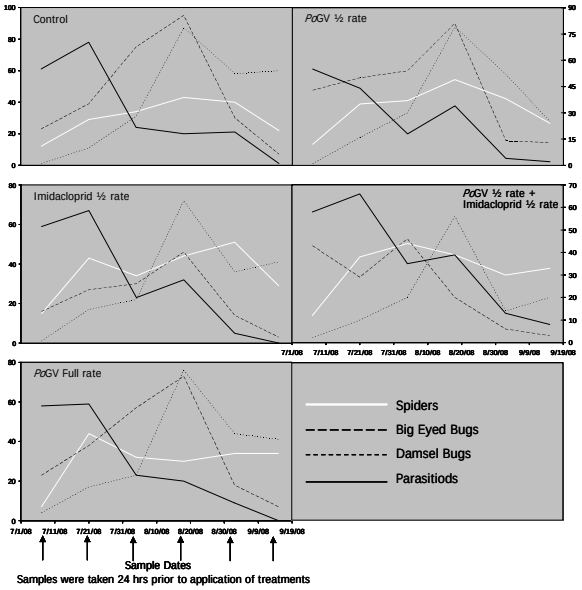
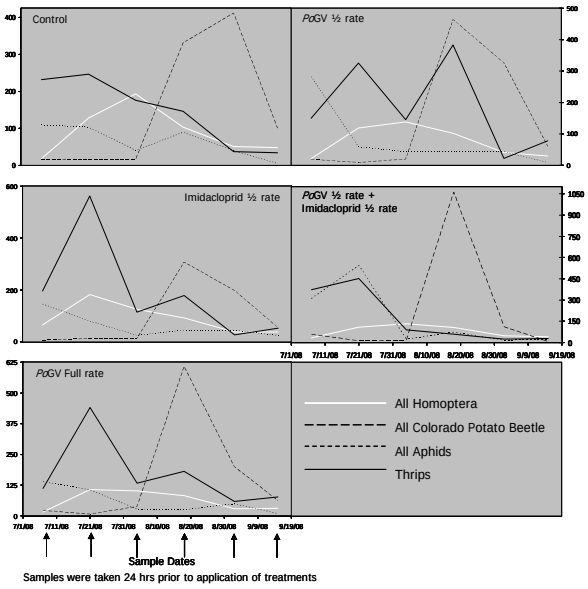


Figure 2. Average number of pest insects on each of six sampling dates in controls and in plots treated with PoGV, imidacloprid or an imidacloprid-PoGV combination. Eltopia, WA 2008.



PTW granulovirus and *Bacillus thuringiensis* research in Washington State. Storage studies. Liquid suspensions and dry formulations of PoGV derived from infected larvae and Btk were evaluated for control of the PTW in stored tubers (Arthurs et al. 2008b). Laboratory bioassays at 25°C demonstrated that both PoGV and a wettable powder (WP) formulation of Btk incorporated with carriers (water, talc, sand, diatomaceous earth, and kaolin clay), were effective against neonate larvae. Depending on the technique, 100% larval mortality was achieved at concentrations as low as 0.025 infected larval equivalents (LE) PoGV per kg tuber and 150 mg Btk WP per kg tuber. However, 100% mortality was never achieved with tests on pre-infested tubers, ostensibly due to the higher dosage required to kill older instars inside tubers. The most effective PoGV formulations were dipping (water) and talc, with dipping most effective for postinfestation treatments, causing up to 91.6% mortality at 0.4 LE per kg. There was no significant effect of formulation in the Btk treatments. The protective effects of residues were

also evaluated under longer-term storage conditions. Batches of tubers treated with PoGV or Btk via dipping (up to 0.1 LE and 150 mg WP per kg tuber) were stored in cages containing an initial potato PTW infestation (10% of tubers). Although potato PTW populations were reduced by up to 98.4% after 2 months at 25°C, no treatments prevented the development and reproduction of the F1 generation. The sprouting of stored tubers seemed to be a limiting factor for sustained control. No significant treatment effects were detected in similar cages held at 12°C for 4.5 months.

Treatment of tubers in rustic stores with the PoGV has been demonstrated to provide considerable protection of tubers. Research conducted by our group was the first to show the effects of PoGV for protection of tubers stored in refrigerated warehouse conditions (Lacey et al. 2010). Tubers were treated by dipping in aqueous suspensions of PoGV or water. An estimated 0.0819 LEs of virus or 1.88×10^9 OBs were deposited on each kg of tubers. They were held at 16°C for 11 days before lowering the temperature by 0.5°C per day until 10°C was reached. The tubers were stored at this temperature for 53 days. Mean numbers of infested tubers at the end of the assay was dependent upon both pre-infestation rate and virus treatment. Mean numbers of infested tubers in the control treatment was three tubers per chamber higher than in the virus treatment demonstrating that PoGV controlled larvae and minimized spread into un-infested tubers. Of the larvae that were retrieved in virus-treated infested tubers, the mean mortality was 87% compared to 37 % in controls.

Entomopathogenic fungi

Fungal MCAs and botanical insecticides have been investigated to a lesser extent than PoGV and Bt for control of PTW (Kroschel and Koch 1996, Hafez et al. 1997, Sewify et al. 2000, Sabbour and Ismail 2002, Chandel et al. 2008, Lacey and Arthurs 2008). An endophytic fungus, *Muscodor albus,* isolated from the bark of cinnamon trees was discovered to have biofumigant properties (Strobel et al. 2001).The fungus produces several volatile compounds (alcohols, esters, ketones, acids and lipids) that are biocidal for a wide range of pest organisms including plant pathogenic bacteria and fungi, plant parasitic nematodes, and insects (Strobel et al. 2001, Mercier and Jiménez 2004, Mercier and Manker 2005, Lacey et al. 2008, Riga et al. 2008). We studied the effects of these volatiles on 3 day old potato tuberworm larvae within infested tubers inside sealed chambers (Lacey et al. 2008). The length of exposure to *M. albus* significantly affected mortality of larvae, calculated as percentage of larvae failing to survive to the adult stage. For example, exposure durations of 3, 7, or 14 days at 24°C followed by incubation in fresh air at 27°C until emergence resulted in mortalities of 84.2, 95.5 and 99.6%, respectively. However, the longer exposures also increased levels of carbon dioxide (CO_2) that are unacceptable for tuber storage. Effects of *M. albus* on larval survival were also monitored at 10, 15 and 24°C, using an exposure duration of 7 days followed by incubation in clean air at 27°C to monitor moth emergence. Mortality of larvae was sharply reduced at the lower temperatures resulting in 50.8, 76.8, and 95.4% mortality, respectively. Tuber storage conditions, especially cooling rates, are discussed by Lacey et al. (2008) with respect to using *M. albus* as a fumigant without simultaneously producing unacceptable levels of CO_2 for tuber storage.

Conclusions

The biopesticides evaluated in our studies and in those of other researchers demonstrated effective control of PTW in storage and in the field under certain conditions. PoGV and Bt are safe to application personnel and the food supply and do not affect beneficial insects and other nontarget organisms. The further development and use of biopesticides will ultimately provide components for the integrated and sustainable control of PTW and other insect pests of potato (Cloutier et al. 1995, Lacey and Kroschel 2009, Lacey et al. 2009) and insect and mite pests in other agroecosystems (Lacey et al. 2001). In agroecosystems with a complex of insect and mite pests, highly specific biopesticides, such as PoGV will require combination with other methods of control, such as conservation and augmentation of natural enemies (Briese 1981, Horne 1990, Kroschel 1995, Herman 2008a), cultural control (Foot 1976, von Arx et al. 1987, Strand 2006, Chandel et al. 2008, Clough et al. 2008), use of soft pesticides (Nouri and Arfaoui 2008, Gentz et al. 2010), and judicious use of conventional chemical pesticides, especially the timing of applications (Strand 2006, Herman 2008b, Gentz et al. 2010). Although factors limiting the adoption and use of microbials include higher cost and requirement for repeated applications compared to most chemicals presently used, these costs could be offset by the premium price paid for food produced using a greener approach with concomitant environmental and food safety benefits. The implementation of biopesticides will ultimately depend on an increased awareness of their attributes by growers and the public, which will be the main drivers for their commercialization and use.

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