Fruit, Vegetable and Cereal Science and Biotechnology ©2009 Global Science Books

Microbial Control of the Andean Potato Weevil Complex

Harry K. Kaya[1*] • Jesús Alcázar[2] • Soroush Parsa[3] • Jürgen Kroschel[2]

[1] Department of Nematology, University of California, Davis, CA 95616 USA
[2] Centro Internacional de la Papa, Apartado 1558, Lima 12, Peru
[3] Ecology Graduate Group, University of California, Davis, CA 95616 USA

Corresponding author: * **hkkaya@ucdavis.edu**

ABSTRACT

The Andean potato weevil consists of at least 14 species with 12 in the genus *Premnotrypes* and two in two other genera. The most important species attacking potato are *Premnotrypes suturicallus* Kuschel, *P. vorax* Hustache, and *P. latithorax* (Pierce). The weevil larva feeds and develops within the potato tuber resulting in yield loss. Although the weevil is native to the Andes, no parasitoids have been identified but predators like carabids affect the weevil population. In addition, entomopathogenic fungal and nematode species have been isolated in nature from these weevils. The fungus, *Beauveria bassiana* (Balsamo) Vuillmen, has been evaluated against the larvae and adults in the laboratory and field. Although effective under laboratory conditions, *B. bassiana* was not effective against weevil adults in the field because of the cold temperatures in the high Andes where potatoes are grown. It did show potential to control the weevil adults in potato storage areas, but its application did not provide sufficient benefits to farmers to adopt this biological control agent. On the other hand, the entomopathogenic nematode, *Heterorhabditis* species Alcázar-1, appears to be a potential candidate for biological control of the weevil. Its natural association with the Andean potato weevil complex, adaptation to cold, high virulence, superb host-finding abilities, high recycling potential and ease of mass rearing makes it a promising resource for Andean potato farmers. To be a practical control agent for large farms, investigations into large-scale production technologies for nematodes are required, whereas farmers with small farms could profit from the application of insect cadavers containing the nematode.

Keywords: *Beauveria bassiana*, entomopathogenic fungus, entomopathogenic nematodes, *Heterorhabditis*
Abbreviations: APW, Andean potato weevil; **asl**, above sea level; **CIP**, Centro Internacional de la Papa

CONTENTS

INTRODUCTION

The Andean potato weevil complex (APWs) (Coleoptera: Curculionidae), native to the Andes, consists of at least 14 monophagous species, 12 of which belong to the genus *Premnotrypes* and two genera, *Rhigopsidius* and *Phyrdenus*. The most important species are *Premnotrypes suturicallus* Kuschel in central Peru, *P. vorax* Hustache in northern Peru, Ecuador, Colombia and Venezuela, and *P. latithorax* (Pierce) in Bolivia and southern Peru. These three seemingly allopatric species have similar phenologies and behaviors in their respective areas of distribution (Alcázar and Cisneros 1997, 1999).

APWs occur between 2,800 and 4,700 m above sea level (asl), have one generation per year in Peru and Bolivia, and their development is synchronized with potato phenology (Alcázar and Cisneros 1999). With the onset of spring rainfall in October and November, the flightless adults that overwinter in the soil within pupal cells, emerge and walk from the previous season potato fields into newly planted potato fields. The females lay their eggs, and upon hatching, neonate larvae enter the soil and burrow into the developing potato tubers. Larval feeding and tunneling of the tubers result in significant tuber weight loss (Ortiz *et al*. 1996) and serve as portals of entry for plant-pathogenic fungi and bacteria that adversely affect the quality of the tubers. Moreover, moderate tuber damage reduces the commercial value of the crop by 50% (Ortiz *et al*. 1996).

Received: 24 June, 2008. Accepted: 30 September, 2008.

Invited Mini-Review

APW larvae pass through 4 or 5 larval instars depending on species. For example, *P. vorax* has 5 instars, whereas *P. suturicallus* has 4 instars (Alcázar and Cisneros 1997). The larvae remain in the tubers for most of the larval stage, but in early fall (April), the last instar larvae begin to leave the tubers to pupate in the soil. As many larvae remain in the tubers after harvest, some of them emerge to pupate in soil beneath potato piles before the potatoes are moved from the field site to covered storage areas (sheds or cellars) with soil floors. Consequently, larvae also pupate in the soil of storage units and adults emerge and potentially can disperse in the spring to potato fields (Alcázar and Cisneros 1999). Another important source of weevil infestation is from volunteer potato plants (Alcázar and Cisneros 1997).

Historically, control of the APWs by indigenous farmers has been by the sectoral fallow system that involved extended crop rotations and spatial separations between fields (Orlove and Godoy 1986; Ewell *et al*. 1990; Alcázar and Cisneros 1997). Current recommendations for APW management include early harvest, handpicking adult weevils from the potato plant, destroying volunteer plants, creating barriers with other crops [e.g., tarwi (*Lupinus mutabilis* Sweet.) and oca (*Oxalis tuberosa* Molina) and deterrent plants (e.g., mashua (*Tropaeolum tuberosum* Ruíz & Pavón)] to prevent weevil immigration into fields, and exposure of overwintering populations to the sun and chickens through plowing (Ortiz *et al*. 1996; Alcázar and Cisneros 1997). New and effective technologies tested in participatory research with farmers are the use of simple plastic barriers installed around potato fields before weevil emergence and migration (Kroschel *et al*. 2009). However, agricultural intensification and increasing quality demands from markets have motivated commercial growers to use chemical control (Peralta and Javier 1980), typically relying on systemic and highly toxic insecticides applied at planting (Ewell *et al*. 1990; Arica *et al*. 2006; Kühne 2007).

Despite APWs being endemic to the Andes, no weevil parasitoids have been found, but generalist insect predators commonly occur (Alcázar and Cisneros 1999; Loza del Carpio 1999; Alcázar 2004). The predators include one ant genus (*Eciton*) repor-ted to feed on larval weevils in storage, and three carabids (*Harpalus turmalinus* Ericchson, *Hylitus* sp., and *Notiobia* sp.) and one tenebrionid (*Metius* sp.) that prey on various life stages of weevils in the Andes. Recently, more detailed ecological studies have shown the importance of carabids – mainly of the genus *Blennidus* – as predators of APW. With regards to entomopathogens, fungi and nematodes have been identified as significant APW biocontrol agents. In this paper, our focus is on the potential use of entomopathogenic fungi and nematodes for the control of these potato pests. We also review a few other entomopathogens that have been isolated and/or evaluated against the APWs.

ENTOMOPATHOGENIC FUNGI

Species, taxonomy and natural weevil infection

A number of fungi including *Beauveria bassiana* (Balsamo) Vuillmen, *B. brongniartii* (Saccardo) Petch., *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Isaria* (*Paecilomyces*) *fumosorosea* (Wize), and *Fusarium* sp. have been isolated from various developmental stages of field-collected weevils (Rodríguez 1986; Alcázar 2004). Of all the fungi that have been isolated from APWs, *B. bassiana* has received the most attention as a potential biological control of this insect complex. Alcalá and Alcázar (1976) first reported on *B. bassiana* infecting larvae, pupae and adults of *P. suturicallus* in the Peruvian Andes (**Fig. 1**), but Torres *et al*. (1993) re-identified the entomopathogen as *B. brongniartii*. Later, Rehner (2005) indicated that *B. brongniartii* is of Eurasian origin. Subsequently, Kühne (2007) reported that the *B. brongniartii* isolates maintained at Centro Internacional de la Papa (CIP) in Lima, Peru under that species name has been re-classified to be *B. bassiana* by Dr. Teresa Ames.

Fig. 1 Adult of the Andean potato weevil, *Premnotrypes suturicallus*, killed by *Beauveria bassiana*. Black bar in the lower left corner = 1 cm.

Accordingly, in this paper, we use *B. bassiana* but place *B. brongniartii* in parenthesis if the paper referred to it as that species.

B. bassiana, like other Hypocreales that infect insects, invades its insect host through the integument. The conidia attach to the cuticle, germinate and penetrate into the host's hemocoel. The fungus proliferates in the hemocoel with host death resulting in 3 to 5 days at 25°C from a combination of actions including depletion of nutrients, invasion of organs and toxinosis. *B. bassiana* has a broad host range, is distributed worldwide, is easy to mass produce *in vitro*, is easy to apply the conidia using standard application equipment, has good efficacy against some soil insect pests, has the potential to recycle in the soil, and has many isolates which are amenable to genetic selection.

Cisneros and Vera (2001) surveyed the infection of APWs by *B. bassiana* (*B. brongniartii*) in fields in the region of Cusco and Huancayo, Peru, respectively, and found that 9 and 12.4% of the weevil population is naturally infected with the fungus. Under potato storage conditions, the level of natural infection was 11.4% in Cajamarca, 15.2% in Huancayo, and 27.1% in Cusco. The majority of the weevils were infected in the larval stage (71-92% depending on field or storage site), followed by pupae (7-23%) and lastly by adults (0-6%). These results indicate that this pathogen is probably very widely distributed and has importance in regulating the APW population under natural growing conditions of potato in the Andes.

Biological activity and characterization

In laboratory studies conducted in Colombia, *M. anisopliae* and *I. fumosorosea* killed 100% of *P. vorax* larvae in 8 and 10 days, respectively (Rodríguez 1986). In contrast, in the same country, separate treatments with *M. anisopliae* and *B. bassiana* caused only 53.3 and 43.4% mortality of *P. vorax* larvae at a concentration of 11×10^6 and 26×10^5 conidia/ml, but a combination of both fungi resulted in a mortality of 100% (Torres and Marina 1999).

In laboratory bioassays Vera *et al*. (1995b) studied the virulence of 5 Peruvian isolates of *B. bassiana* (*B. brongniartii*) against *P. latithorax* and showed that differences existed among the isolates. One isolate collected from the community of Racchi, Cusco, killed 100% of larvae, pupae and adults in 12, 15 and 30 days, respectively, at a concentration of 3.35×10^7 conidia/ml.

Cisneros and Vera (2001) assessed the biological activity of *B. bassiana* (*B. brongniartii*) at different temperature and moisture regimes. Larvae exposed to 2.5×10^8 conidia/ml and held for 8 days at a soil moisture of 10, 25, or 50% did not significantly affect virulence but temperature did. At 20°C, the fungus caused 100% larval mortality but at 4°C, it caused only 4% mortality. However, after three months of exposure to the fungus at 4°C, larval mortality reached 76-90% at all soil moisture regimes assessed. At 50% field soil

capacity, mycelial growth was more prevalent on the cuticle than conidial production, whereas at 25% field soil capacity, good conidial production occurred. The fungus survived in dried, fungal-killed insects when held at 12°C or lower, and mycelial growth and conidial production occurred in such insects when they were rehydrated more than a year later.

As noted by Kühne (2007), however, earlier studies conducted with *B. bassiana* had a number of problems including the use of field-collected weevils in which the homogeneity of their age is unknown or whether they were previously infected with fungi or other pathogens, bioassays were done with groups of insects rather than individually, and in field trials, the concentration of the applied fungus and their viability (i.e., quality) were unknown or not given. Moreover, it was not clear whether adequate control treatments were included in the study. Hence, Kühne (2007) conducted a series of standardized experiments by using laboratory-reared *P. suturicallus* under controlled conditions for the laboratory bioassays. Two isolates of *B. bassiana*, CIP40 and CIP56, were produced on a standard medium over a given period of time (14-21 days) at 20°C and the viability of the conidia was assessed. At 15°C, his study showed that the lethal concentration to kill 50% (LC_{50}) of the pre-pupae was 5.6×10^4 conidia/ml for CIP40 and 8.2×10^5 conidia/ml for CIP56 and the LC_{50} of the adults was 6.8×10^6 conidia/ml for CIP40 and 1.2×10^7 conidia/ml for CIP56. The lethal time to kill 50% (LT_{50}) of the pre-pupae or adult was assessed by using 1×10^6 conidia/ml. The LT_{50} varied from 12 to 23 days for pre-pupae and from 19 to 34 days for adults.

Kühne (2007) found that the optimal temperature for survival and oviposition for *P. suturicallus* was between 11 and 15°C, whereas the optimal temperature for *B. bassiana* growth and infectivity was closer to 20°C. For example, the LC_{50} of *B. bassiana* was 1600 times greater for weevil larvae at 13 than at 19°C.

Mass production

The good results achieved in controlling APWs with *B. bassiana* (*B. brongniartii*) stimulated researchers to develop effective methods for mass production of the fungus. Vera *et al*. (1995a) used the husks of Inca wheat (*Amaranthus caudatus* L.), quinoa (*Chenopodium quinoa* Willd.), wheat (*Triticum vulgare* L.), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) as substrate and achieved highest conidia yield with quinoa and barley with 2.25×10^7 and 1.75×10^7 conidia/g substrate. Studies by Cisneros and Vera (2001) later developed simple mass production schemes for *B. bassiana* that could be used at the small farm level. Uninfected larvae were collected from the field during the potato harvest period and used to infect larvae for mass production of the fungus. These larvae were (1) placed into soil naturally infested with conidia from the storage area, (2) placed into sterilized soil inoculated with *B. bassiana* conidia, or (3) immersed into a conidial suspension and then placed on wet paper. The results showed that 98% of the larvae exposed to a conidial suspension became infected, whereas 81 to 84% of the larvae in the infested soil became infected. Varying numbers of fungal-killed larvae (10, 20, or 30) were used to inoculate sterilized barley in plastic bags containing different volumes of water (i.e., 500, 600, 700, 800, 900, or 1000 ml) per kg of barley. The most efficient level of fungal production was at 700 ml of water per kg of barley, but there was no apparent difference in fungal production in relation to the number of fungal-killed larvae used for the inoculation.

Use of *B. bassiana* under potato storage and field conditions

Torres *et al.* (1993) were the first to report on the application of *B. bassiana* (*B. brongniartii*) at the rate of 2 kg/m^2 of the barley medium produced as described above on soils of potato storage areas in the Cusco region. That application achieved 48-99% mortality of *P. latithorax*. Other studies on potato storage were carried out by Cisneros and Vera (2001) using the same application rate at three different sites resulting in 50-96% mortality of *P. latithorax* in Cusco, 54-97% mortality of *P. suturicallus* in Huancayo, and 63-92% mortality of *P. vorax* in Cajamarca. These studies were very encouraging and showed the potential of using *B. bassiana* as a microbial control agent in potato storage against the three most important species in the APW complex.

First experiments to test the field efficacy of *B. bassiana* (*B. brongniartii*) (isolate Bbr-Per) to control *P. vorax* were conducted by Fernández and Colmenares (1997) in Venezuela at 2600 m asl. Potato plots (200 m^2) received one of the following treatments: overall plot application of 2×10^8 conidia/ml or 2×10^8 conidia/ml mixed with goat or chicken manure, and untreated control. The results showed significant differences in the numbers of damaged tubers between the treatments and control: 16.3, 22.4, 16.7 and 47.7%, respectively.

Kühne (2007) conducted cage and open field experiments at 3670 m asl to test the efficacy of *B. bassiana* under natural conditions. The cage experiment comprised the following treatments: two *B. bassiana* isolates, CIP40 and CIP56, a positive chemical control (carbofuran at the recommended rate), an untreated control and a control where no adult weevils were added. The fungus was grown on autoclaved rice in plastic bags for 2 to 3 weeks at 20°C, harvested, and applied at the rate of 1.3×10^{14} conidia/ha for the isolate CIP40 and 6.4×10^{14} conidia/ha for CIP56. In one fungal treatment, the conidia were sprayed onto the plant before the adults (4 males and 4 females) were released into the cage, and in another treatment, the adults were dipped into the same conidial suspension and then released into the cage. When the plants were mature, the tubers were assessed for damage. The results showed that tubers in the carbofuran treatment had no damage and tubers from the cage in which no adult weevils were added also were free of damage, whereas the untreated control and all *B. bassiana* treatments (sprayed plants and dipped adults) had tuber damage greater than 35%. In the open field experiment, Kühne (2007) applied *B. bassiana* at the rate of 9.7×10^{13} conidia/ha in an area infested with natural populations of *P. suturicallus*. The treatments were one application of *B. bassiana* at emergence of the potato plant, one application of *B. bassiana* at plant emergence and a second application 4 weeks later, one application of carbofuran at plant emergence and a second application 4 weeks later, and an untreated control. Tuber damage of the *B. bassiana* plots was greater than 80% and not different from the untreated control, whereas tuber damage of the carbofuran plots was less than 10%.

Potential of *B. bassiana* for Andean potato weevil control

The detailed studies by Kühne (2007) demonstrated that *B. bassiana* kills *P. suturicallus* under laboratory conditions but is not effective when applied in the field. One of the main reasons for the lack of good efficacy in the field may be the low temperatures at the high elevations. In contrast, Torres *et al*. (1993) and Cisneros and Vera (2001) obtained a high degree of infectivity of *B. bassiana* applied against weevil larvae under potato tuber storage situations. However, the use of *B. bassiana* in the storage area will remove only a limited percentage of weevil adults, but the surviving adults are not very likely to disperse into newly planted potato fields in the spring. An economic evaluation by Winters and Fano (1997), therefore, concluded that the benefits of using a biocontrol product based on *B. bassiana* for APW storage control are low and farmers are not expected to purchase the product in sufficient quantities to allow a market to develop.

ENTOMOPATHOGENIC NEMATODES

Taxonomy and species isolated in the Andes

Nematodes in the families, Mermithidae (Kaiser 1991), Steinernematidae and Heterorhabditidae (Kaya *et al*. 2006), commonly infect insects throughout the world. For the mermithids, very limited information is available against APW. Velasquez and Espinoza (2001) isolated an undescribed mermithid species from third- and fourth-instar *P. suturicallus* larvae from the Mantaro Valley (3,650 m asl), Peru. Although 12.5% of the weevil larvae were infected by the mermithid, the biological control potential of mermithid nematodes, in general, is limited because of their long life cycle, the long period of time to kill their hosts, their obligate parasitism requiring the need to rear their insect hosts, high cost of production, poor storage and poor natural dispersal, and an overall lack of knowledge of their biology, especially with those that are new to science.

In contrast to mermithids, the steinernematid and heterorhabditid nematodes show excellent biological control potential against APW. As background, these nematodes are called entomopathogenic because they are associated with mutualistic bacteria that can kill their insect hosts within 48 hours (Kaya and Gaugler 1993). The nematode/bacterium complex works together to kill their hosts. The genus *Steinernema* is associated with bacteria in the genus *Xenorhabdus*, whereas the genus *Heterorhabditis* is associated with bacteria in the genus *Photorhabdus*. In addition to the rapid mortality of the insect host, other positive attributes of these entomopathogenic nematode/bacterium complexes include a broad host range, safety to vertebrates and plants, worldwide distribution, ease to mass produce *in vivo* and *in vitro*, ease to apply the infective juvenile stage using standard application equipment, capability to search for their hosts in soil or in cryptic habitats, good efficacy against some soil insect pests, potential to recycle in the soil, compatible with many pesticides, and amenable to genetic selection.

Surveys to identify entomopathogenic nematodes in the Andes and evaluation of their potential in pest management started in the 1990s. Garzón *et al*. (1996) identified an undescribed native steinernematid in Colombia infecting *P. vorax*. Subsequently, in Peru, Alcázar and Kaya (2003) isolated an undescribed entomopathogenic nematode in the genus *Heterorhabditis*, designated as strain Alcázar-1, from last instar *P. suturicallus* larvae (**Fig. 2**) in soil from a commercial potato storage shed in Huasahuasi (2,750 m asl). With this discovery, a series of investigations were initiated to evaluate the potential of this isolate for the biological control of the APW. Further surveys continued in Peru and were initiated in Bolivia and Ecuador to search for new isolates in different agroecological zones. Today, collection of nematodes of the genera *Heterorhabditis* and *Steinernema* made from soils in high Andes and the Peruvian coast is maintained in CIP's collection. The existence of the two genera also has been confirmed for Ecuador and Bolivia (Hernandez 2006; J. Franco, pers. comm.).

Fig. 2 Dissection of a dead last instar larva of the Andean potato weevil, *Premnotrypes suturicallus*, showing the developmental stages of the entomopathogenic nematode, *Heterorhabditis* sp. (Alcázar-1). The arrow points to a second generation female nematode. Black bar in lower left corner = 1 cm.

Biological activity

One of the first studies to use nematodes against the APW complex was carried out by Garzón *et al*. (1996) against *P. vorax* in Colombia. They demonstrated that the LC_{50} for the last instar larvae was 26 infective juveniles for *Steinernema carpocapsae* (Weiser) strain 25, whereas it was 526 infective juveniles for an undescribed native steinernematid isolated in Colombia.

More detailed work was conducted by Parsa *et al*. (2006) with the Peruvian isolate, *Heterorhabditis* sp. strain Alcázar-1. The studies included the ability of Alcázar-1 to (1) infect and develop at the cold Andean temperatures, (2) infect different stages of *P. suturicallus*, (3) produce progeny within its host and hence to recycle, (4) protect potato tubers from infestation by neonate larvae, (5) search for hosts within a cryptic habitat, *i.e.*, within a tuber, and (6) determine its host range with other Andean weevils. These results are briefly summarized in the following section.

Temperature studies. Due to the unavailability of APW host, the surrogate insect host, the wax moth *Galleria mellonella* (L.), was used to determine the thermal range of infectivity for *Heterorhabditis* sp. strain Alcázar-1. The thermal range of infectivity was conducted at 10, 15, 20, 25, or 30°C and under a thermally fluctuating regime that mimicked soil temperatures in the high Andes (Junín, Peru). During the month of February, daily temperatures at the field site averaged 11.1°C with a maximum averaging 14.0°C and a minimum averaging 8.6°C. Therefore, this fluctuating temperature that averaged 11°C was programmed into a growth chamber and used to conduct the experiments. *G. mellonella* larvae were placed individually into a 24-well tissue culture plate and exposed to 50 Alcázar-1 infective juveniles. The results showed that at constant temperatures, Alcázar-1 killed *G. mellonella* larvae at all temperatures except 30°C. Median lethal times (LT_{50}) were 1.7 days at 25°C, 2.2 days at 20°C, 4.1 days at 15°C, and 11.8 days at 10°C. The LT_{50} was 7.2 days at the thermal fluctuation treatment averaging 11°C.

Infectivity against last APW instar larvae, pupae and teneral adults. The infectivity of Alcázar-1 to last instar *P. suturicallus*, obtained from a laboratory colony, was conducted at 15 and 20°C using individual larvae in 1.5 ml microcentrifuge tubes filled with 1 g of autoclaved sand and 50 µl distilled water. Six nematode concentrations ranging from 0 to 24 infective juveniles/larva were used. LC_{50} values at 20°C were 8.8 and 5.9 infective juveniles/larva at exposure times of 5 and 7 days, respectively. In comparison, LC_{50} values at 15°C were 31.7 and 9.2 infective juveniles/larva at the same exposure times. To verify the susceptibility of pupae and adults of *P. suturicallus* relative to the last larval instar, all these stages were exposed to 0 or 6 infective juveniles at 20°C. The results showed that all these stages were equally susceptible to Alcázar-1. Mortality of the last larval instar was 66.1%, of pupae was 64.7% and of the teneral adults was 52.1%.

Progeny production and recycling potential. To study Alcázar-1 reproductive potential in *P. suturicallus*, each last instar larva was weighed, exposed to 20 infective juveniles, and incubated at 20°C. Two days later, nematode-killed lar-

vae were transferred individually into White traps and progeny infective juveniles were counted. The number of infective juveniles emerging per gram of host was also estimated. The average production per cadaver was 97,817 ± 947 infective juveniles. The average larval weight was 102 ± 3 mg which gave an infective juvenile production per milligram of insect host of 947 ± 48.

Tuber protection assay from neonate larvae. A potato tuber weighing 14 ± 2 g was placed at the bottom of a 40 ml plastic cup which was then filled to the top with autoclaved, moist sand (-70 kPa) and either 0 (control), 25 infective juveniles/cm^2 or 50 infective juveniles/cm^2 were added to the soil surface in 200 µl distilled water. Ten recently hatched *P. suturicallus* larvae were then immediately added to the arena. After 7 days, the potatoes were removed from the cups, cleaned, and incubated for an additional three weeks, allowing growth of any established weevils in the tubers which facilitated visual evaluation of the damage. Nematode application resulted in a significant reduction in the number of tubers infested with weevils. At 25 and 50 infective juveniles/cm^2, 25% and 10%, respectively, of the tubers were infested, whereas in the control 85% of the tubers were infested.

Searching for larvae in the tuber. Ten neonate weevils were added to plastic cup arenas containing potato tubers and soil as described above and incubated for 5 days. After, tubers were extracted, placed at the bottom of a new cup to which moist sand, and either 0, 25 or 50 infective juveniles/cm^2 was added to the soil surface and the cups were incubated for 7 days. Following the treatment, potatoes were removed, cleaned and incubated for three weeks. Nematode application resulted in no infested tubers for either nematode treatment, whereas 70% of the control tubers were infested.

Host range studies with other Andean weevils. The APWs, *P. suturicallus*, *P. vorax* and *P. latithorax*, were individually exposed to 6 infective juveniles/last instar larva, whereas for the last instar of the oca weevil, *Adioristidius tuberculatus* Voss, a dose response assay was done. *P. suturicallus*, *P. vorax* and *P. latithorax* had a mortality of 59.7, 7.7 and 61.9%, respectively, at 7 days post-treatment. Dose response assays against *A. tuberculatus* resulted in a LC_{50} of 1.5 infective juveniles/larva 7 days post-treatment.

Field efficacy of *Heterorhabditis* sp.

Alcázar *et al*. (2007) evaluated the efficacy of *Heterorhabditis* sp. Alcázar-1 in controlling the APW, *P. suturicallus*, under semi-field and field conditions at 3,300 m asl in Junín, Peru. The semi-field experiments were conducted in field plots each covered with a 1 m^2 cage. In each plot four seed tubers were planted and after emergence, the cage was infested with 12 pairs (12 males and 12 females) of recently emerged *P. suturicallus*. The field experiment was conducted on a farm naturally infested by APW in 5 × 3 m (15 m^2) plots. The treatments consisted of (1) nematodes applied in aqueous suspension at a rate of 50 infective juveniles/cm^2, (2) nematodes applied within two nematode-killed *G. mellonella* larvae/plant (the last instar larvae were applied one week after infection at a rate of 8 cadavers/1 m^2 and 100 cadavers/5 m^2, respectively), (3) insecticide application with carbofuran at a rate of 2.5 ml/liter, and (4) untreated control with an application of water. In both experiments, the native nematode, *Heterorhabditis* sp., applied in suspension or as cadavers of *G. mellonella* larvae significantly reduced tuber damage and plant infestation by APW larvae compared to the control; in both experiments tuber damage was reduced by 65 and 41.4% and the larval infestation by 76.9 and 53.2%, respectively. The proportion of nematode-infected larvae reached 57.4% in the semi-field experiment and 34.9% in the field experiment, respectively.

Potential of *Heterorhabditis* sp. in APW control

The isolate Alcázar-1 has a higher reproductive capacity at lower temperatures (it does not reproduce above 25°C) reflecting an adaptation to the cold Andean temperatures. The ability of Alcázar-1 to kill *G. mellonella* larvae at a constant temperature of 10°C and under a fluctuating temperature averaging 11°C suggests that its infectivity satisfies thermal requirements for weevil control in the Andes. Although low soil temperatures can inhibit entomopathogenic nematode activity, effective suppression at temperatures as low as 9°C has been reported with cold-adapted heterorhabditid isolates tested against the black vine weevil, *Otiorhynchus sulcatus* (Fabricius) (Westerman and Zeeland 1989). In addition, low temperatures can induce infection latency after nematode penetration, allowing hosts to remain alive until better developmental conditions for the nematode and/or bacterial symbiont are reached (Brown *et al.* 2002). Our studies in potato fields in the Andes above 3,400 m asl showed that temperatures during the growing period of potato are sufficient to facilitate the infection of weevil larvae even though the field efficacy was not more than 41% after two applications of 50 infective juveniles/cm^2. Early weevil instars are both encountered and killed by the nematodes when they are searching for potato tubers in soil or they become infected after they are established inside the tubers. The ability of nematodes to kill a target host within-roots is not new as Jansson *et al*. (1990) and Mannion and Jansson (1993) demonstrated this previously with the sweetpotato weevil (*Cylas formicarius* Fabricius). The nematodes probably access the larvae inside the tubers through small entrance sites made by first instar weevils. However, under field conditions, these entrance sites may be sealed due to tuber growth, making established weevils impervious to future nematode infections. Accordingly, the window of opportunity for APW suppression might be shorter than suggested by results from the artificially infested tubers as demonstrated by Parsa *et al*. (2006).

Alcázar-1 is highly lethal to *P. suturicallus* overwintering stages, making it a candidate for applications in storage soils prior to storing potatoes. Median lethal concentrations for last instar larvae can vary significantly as a function of temperature and exposure time. In experiments with last larval instar of *P. suturicallus*, increasing assay temperature from 15 to 20°C resulted in a 5.4-fold increase in lethality. For the sweetpotato weevil, which is a highly susceptible host, Mannion and Jansson (1992) obtained LC_{50} values of 1.9 to 29.7 infective juveniles/larva 48 hours post-treatment at room temperature for 10 entomopathogenic nematode isolates. By contrast, even the most virulent of 13 entomopathogenic nematode isolates tested against larvae of the pecan weevil, *Curculio caryae* (Horn), caused less than 50% mortality at a concentration of 1,500 infective juveniles 13 days post-treatment at 25°C (Shapiro-Ilan 2001). Although differences in experimental parameters limit strict comparisons among these studies, they provide support for the high susceptibility of Andean weevils to Alcázar-1. However, the experiences with the use of the entomopathogenic fungi *B. bassiana* – described previously – have shown that farmers are very unlikely to adopt any technology for weevil control in storage facilities since stored tubers are already infested by APW at harvest time. The fact that storage facilities might be also a source of weevils migrating to potato fields may not warrant any treatments since previous potato fields are major weevil sources and potato storage sites are generally located quite a distance from the planting field. Further, in our recent studies we observed that rustic potato storage rooms at 3,800 m asl are too cold during the storage period in winter thus inhibiting the activity of nematodes to infect weevils.

Alcázar *et al*. (2007) suggested two approaches to use entomopathogenic nematodes for APW control: (1) reducing the APW population and tuber damage by the application of aqueous nematode suspensions during the potato-growing season, and (2) infecting fourth-instar larvae after harvest

especially in soils of former potato piles which are important overwintering and infestation sources of APW. To apply these approaches, a major problem for the Andean farmer is to have access to large quantities of entomopathogenic nematodes. The high reproductive potential of Alcázar-1 raises the possibility of producing nematodes at the community level in the Andes by providing the farmers with nematode inocula. Larvae collected from field after harvest or from potato storage areas could be infected and stored until the next season, assuming there is adequate longevity of the nematodes. The nematode-killed larvae may be in sufficient numbers to treat soils of the former potato piles and outer rows of potato fields, in which major weevil infestations and tuber damage occur. Applying entomopathogenic nematodes while in their host cadavers has several advantages such as the emergence of nematodes with superior dispersal (Shapiro and Glazer 1996), infectivity (Shapiro and Lewis 1999), and survival (Perez *et al*. 2003) compared with those applied through aqueous suspension. However, this application requires high amounts of nematode-killed larvae and our field results demonstrated that even the use of *G. mellonella* cadavers, which produce twice as much nematodes compared to APW larvae, were less effective compared to overall plot application using aqueous nematode suspensions (Alcázar *et al*. 2007). For large-field applications, investigations into large-scale production technologies for nematodes would be required. For small potato production systems, farmers of the high Andes could profit from a wider use of nematode-based bioinsecticides in high value crops, as suggested by Alcázar *et al*. (2007).

OTHER ENTOMOPATHOGENS

Besides the entomopathogenic fungi and nematodes, very little work has been done with other pathogens. There is a report that a cypovirus was isolated from *P. vorax* (Rodríguez 1986) but this finding has not confirmed. With regards to bacterial pathogens, Hernández *et al*. (2005) reported isolating *Bacillus thuringiensis* Berliner in Bolivia from potatoes infested with the APW, *Rhigopsidius tucumanus* Heller and from the larvae, pupae and adults of *R. tucumanus* and adults of *P. latithorax*. Nineteen of 27 (70.3%) infested potatoes were positive for *B. thuringiensis* and 23 of 46 (50%) of the APW (larvae, pupae and adults) had the bacterium. At least 11 different subspecies of *B. thuringiensis* associated with the APWs were isolated. On feeding the *B. thuringiensis* isolates to larvae and adults of *R. tucumanus* and *P. latithorax*, the isolates did not affect these stages, but many of them were lethal to larvae of the beet armyworm *Spodoptera exigua* (Hübner) and potato tuber moth *Phthorimaea operculella* Zeller. On the other hand, Gomez *et al*. (2000) cloned and expressed a Cry 3Aa protein from *B. thuringiensis* subsp. *tenebrionis* (= *sandiego*) in *Escherichia coli* (Migula) and found that the recombinant as well as the native protein were toxic to the APW, *P. vorax*.

CONCLUSIONS

Although the entomopathogenic fungus *B. bassiana* has shown potential to control APW in potato storage areas, its application has not proven sufficiently beneficial to warrant adoption by farmers. The thermal requirements of the fungus (see above) are probably the major factor limiting field application. On the other hand, *B. thuringiensis*, isolated from *R. tucumanus* were not lethal to APW larvae and adults. Instead, the entomopathogenic nematode isolate Alcázar-1 appears to be a potential candidate for APW biological control. Its natural association with the APW complex and other Andean weevil species, adaptation to cold, high virulence, superb host-finding abilities, high recycling potential and ease of mass rearing, make it a promising resource for Andean potato farmers.

ACKNOWLEDGEMENTS

We thank the McKnight Foundation, the Regional Fund for Agricultural Technologies (FONTAGRO) and the Government of Luxembourg for their research support against potato pests in the Peruvian Andes.

REFERENCES

Alcalá CP, Alcázar J (1976) Biología y comportamiento de *Premnotrypes suturicallus* Kuschel (Col.: Curculionidae). *Revista Peruana de Entomología* **19**, 49-52

Alcázar J (2004) Investigaciones en el control biológico del gorgojo de los Andes. *Memorias I Seminario Internacional y II Nacional de Control Biológico de Plagas y enfermedades de los cultivos.* Escuela Politécnica del Ejercito (ESPE), Facultad de Ciencias Agropecuarias, April, 2004, Quito, Ecuador, pp 64-67

Alcázar J, Cisneros F (1997) Integrated management for Andean potato weevils in pilot units. In: *Program Report 1995-96*, International Potato Center (CIP), Lima, Peru, pp 169-176

Alcázar J, Cisneros F (1999) Taxonomy and bionomics of the Andean potato weevil complex: *Premnotrypes* spp. and related genera. In: Arthur C, Ferguson P, Smith B (Eds) *Impact on a Changing World. Program Report 1997-98*, International Potato Center (CIP), Lima, Peru, pp 141-151

Alcázar J, Kaya HK (2003) Hallazgo de un nematodo nativo del genero *Heterorhabditis*, parasito del gorgojo de los Andes *Premnotrypes suturicallus*. *Abstracts XLV Convención Nacional de Entomología*, Huasahuasi, Junín, 1-5 December, Ayacucho, Peru, p 158

Alcázar J, Kroschel J, Kaya H (2007) Evaluation of the efficacy of an indigenous Peruvian entomopathogenic nematode *Heterorhabditis* sp. in controlling the Andean potato weevil *Premnotrypes suturicallus* Kuschel under field conditions. *XVI International Plant Protection Congress*, October 15-18, Glasgow, UK, pp 544-545

Arica D, Kroschel J, Forbes G, Saint Pere K (2006) Persistent organic pollutants (POPs) and hazardous pesticides in Andean farming communities in Peru. Final Report to the Canada POPs Fund, World Bank, The International Potato Center, Lima, Peru, 73 pp

Brown IM, Lovett BJ, Grewal PS, Gaugler R (2002) Latent infection: a low temperature survival strategy in steinernematid nematodes. *Journal of Thermal Biology* **27**, 531-539

Cisneros F, Vera A (2001) Mass-producing *Beauveria brongniartii* inoculum, an economical, farm-level method. In: *Scientist and Farmers: Partners in Research for the 21st Century. Program Report, 1999-2000*, International Potato Center (CIP), Lima, Peru, pp 155-166

Ewell PT, Fano H, Raman KV, Alcázar J, Palacios M, Carhuamaca J (1990) Andean weevil: an endemic pest in the highlands. In: *Farmer Management of Potato Insect Pests in Peru*, International Potato Center (CIP), Lima, Peru, pp 17-28

Fernández S, Colmenares X (1997) Evaluación de *Beauveria* spp. para el control de *Premnotrypes vorax* Hustache (Coleóptera: Curculionidae) en el cultivo de papa. *Agronomía Tropical* **47**, 249-257

Garzón M, Aza B, Jiménez J, Luque J (1996) Potencial del nematodo *Steinernema* sp. para el control biológico del gusano blanco de la papa. *Revista Colombiana de Entomologia* **22**, 25-30

Gomez S, Mateus AC, Hernandez J, Zimmermann BH (2000) Recombinant Cry3Aa has insecticidal activity against the Andean potato weevil, *Premnotrypes vorax*. *Biochemical and Biophysical Research Communications* **279**, 653-656

Hernández CS, Andrew R, Bel Y, Ferré J (2005) Isolation and toxicity of *Bacillus thuringiensis* from potato-growing areas in Bolivia. *Journal of Invertebrate Pathology* **88**, 8-16

Hernandez P (2006) Colección, patogenicidad y caracterización ecológica de nematodos parasitos de insectos en gusanos blanco (*Premnotrypes vorax* Hustache) en Ecuador. Masters thesis, Escuela Politecnica del Ejercito, Facultad de Ciencias Agropecuarias, Quito, Ecuador, 112 pp

Jansson RK, Lecrone SH, Gaugler RR, Smart GC Jr. (1990) Potential of entomopathogenic nematodes as biological control agents of sweet potato weevil (Coleoptera: Curculionidae). *Journal of Economic Entomology* **83**, 1818-1826

Kaiser H (1991) Terrestrial and semiterrestrial Mermithidae. In: Nickle WR (Ed) *Manual of Agricultural Nematology*, Marcel-Dekker, New York, pp 899-965

Kaya HK, Aguillera MM, Alumai A, Choo HY, de la Torre M, Fodor A, Ganguly S, Hazır S, Lakatos T, Pye A, Wilson M, Yamanaka S, Yang H, Ehlers R-U (2006) Status of entomopathogenic nematodes and their symbiotic bacteria from selected countries or regions of the world. *Biological Control* **38**, 134-155

Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. *Annual Review of Entomology* **38**, 181-206

Kroschel J, Alcazar J, Pomar P (2009) Potential of plastic barriers to control Andean potato weevil *Premnotrypes suturicallus* Kuschel. *Crop Protection* **28**, 466-476

Kühne M (2007) The Andean potato weevil *Premnotrypes suturicallus* ecology and interactions with the entomopathogenic fungus *Beauveria bassiana*. PhD thesis, Georg-August-University, Göttingen, Germany, 178 pp

Loza del Carpio AL (1999) Poblaciones y Ecología Alimentaria de Carabidos (Coleoptera) en Agroecosistemas del Altiplano. Thesis Magister Scientiae, Universidad Nacional del Altiplano-Puno

Mannion CM, Jansson RK (1992) Comparison of ten entomopathogenic nematodes for control of sweet potato weevil (Coleoptera, Apionidae). *Journal of Economic Entomology* **85**, 1642-1650

Mannion CM, Jansson RK (1993) Within-root mortality of *Cylas formicarius* (Coleoptera, Apionidae) by entomopathogenic nematodes. *Journal of Economic Entomology* **86**, 722-729

Orlove BS, Godoy R (1986) Sectoral fallowing systems in the Central Andes. *Journal of Ethnobiology* **6**, 169-204

Ortiz O, Alcázar J, Catalan W, Villano W, Cerna V, Fano H, Walker TS (1996) Economic impact of IPM practices on the Andean potato weevil in Peru. In: Walker TS, Crissman CC (Eds) *Case Studies of the Economic Impact of CIP Related Technologies*, International Potato Center (CIP). Lima, Peru, pp 15-31

Parsa S, Alcázar J, Salazar J, Kaya HK (2006) An indigenous Peruvian entomopathogenic nematode for suppression of the Andean potato weevil. *Biological Control* **39**, 171-178

Peralta T, Javier G (1980) Control integrado de las plagas de la papa en el Valle del Mantaro. *Revista Peruana de Entomologia* **23**, 133-137

Perez EE, Lewis EE, Shapiro-Ilan D (2003) Impact of the host cadaver on survival and infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) under desiccating conditions. *Journal of Invertebrate Pathology* **82**, 111-118

Rodríguez A (1986) Entompatogenos registrados en gusano blanco y pruebas de patogenicidad. In: Valencia L (Ed), *En memorias Curso Control Integrado de Plagas*, Bogata, Colombia, pp 9-17

Rehner SA (2005) Phyogenetics of the insect pathogenic genus *Beauveria*. In: Vega FE, Blackwell M (Eds) *Insect-Fungal Associations: Ecology and Evolution*, Oxford University Press, Oxford, UK, pp 3-27

Shapiro D, Glazer I (1996) Comparison of entomopathogenic nematode dispersal from infected hosts versus aqueous suspension. *Environmental Entomology* **25**, 1455-1461

Shapiro D, Lewis E (1999) A comparison of entomopathogenic nematode infectivity from infected hosts versus aqueous suspension. *Environmental Entomology* **28**, 907-911

Shapiro-Ilan D (2001) Virulence of entomopathogenic nematodes to pecan weevil larvae *Curculio caryae* (Coleoptera: Curculionidae) in the laboratory. *Journal of Economic Entomology* **94**, 7-13

Torres H, Ortega A, Alcázar J, Ames T, Palomino L (1993) Control biologico del gorgojo de los Andes (*Premnotrypes* spp.) con *Beauveria brongniartii*. *Guía de Investigación, CIP 8*, Centro Internacional de la Papa, Lima, Peru, 43 pp

Torres L, Marina A (1999) Biocontrol activity of entomopathogenic fungi against *Premnotrypes vorax* (Coleoptera: Curculionidae) through their individual or combined utilization. *Revista Colombiana de Entomología* **25**, 121-129

Velasquez OR, Espinoza SJC (2001) Un mermithidae (nematode), enemigo natural de *Premnotrypes suturicalllus* (Coleoptera: Curculionidae) en el cultivo de papa. *Proceedings of the XLIII Convencion nacional de entomologia*, 11/04-11/08/2001, Huancayo (Peru), Sociedad Entomologica del Peru, p 67

Vera A, Alcázar J, Arestegui A (1995a) Restos vegetales como substrato del hongo *Beauveria brongniartii* patógeno del Gorgojo delos Andes. *Revista Peruana de Entomologia* **38**, 91-94

Vera A, Alcázar J, Arestegui A (1995b) Patogenicidad del hongo *Beauveria brongniartii*, sobre el gorgojo de los Andes *Premnotrypes latithorax*. *Revista Peruana de Entomologia* **38**, 95-98

Westerman PR, Zeeland MG (1989) Comparison of *Heterorhabditis* isolates for control of *Otiorhynchus sulcatus* at low temperatures. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **54**, 1115-1123

Winters P, Fano H (1997) The economics of biological control in Peruvian potato production. Social Science Department, Working paper No. 1997-7, International Potato Center (CIP), Lima, Peru, 33 pp