The Andean potato weevil
Premnotrypes suturicallus

Ecology and interactions with the entomopathogenic fungus *Beauveria bassiana*

Dissertation
zur Erlangung des Doktorgrades
der Fakultät für Agrarwissenschaften
der Georg-August-Universität Göttingen

vorgelegt von
Magnus Kühne
geboren in Frankfurt am Main

Göttingen, Juli 2007

D 7

1. Referent:	Prof. Dr. S. Vidal
2. Korreferent:	Prof. Dr. H.-M. Poehling
Tag der mündlichen Prüfung:	19. Juli 2007

für Rocío

El Gorgojo de los Andes

Había una vez, en la provincia de Chimbivilcas, un agricultor muy poderoso, sus campos eran los mejores y los más grandes, sus cosechas de papa eran tan grandes que nadie podía igualarlo y esto se repetía todos los años.

Producía mucha papa, y toda esta papa la vendía o la cambiaba con otros productos, pero sus hijos nunca comieron esta papa; ellos solo veían cómo su poderoso padre producía más y más papa, pero ellos nunca pudieron comerla.

Y esto se repetía año tras año, y este poderoso agricultor cada vez tenía más papa, pero sus hijos nunca conocieron el sabor de esta papa, nunca disfrutaron del trabajo de su poderoso padre.

Hasta que una vez, sus hijos desaparecieron; y el padre los buscaba por todos sus terrenos y fuera de ellos, desesperadamente por que los quería mucho, los llamaba inútilmente, los hacía buscar con los demás agricultores, pero no los pudo encontrar.

Y el tiempo pasó, y llegó el momento de la siembra, y el agricultor preparó sus terrenos, llorando por la pérdida de sus hijos, pero tenía que producir más papa, por que esa era su vida, y preparó el estiércol, y dejó el campo listo para recibir la excelente semilla que él mismo producía.

Y abrió el almacén para sacar la semilla que tanto quería y encontró a sus hijos, y vio que sus hijos se habían comido la papa que él producía con desesperación, pero que jamás les hizo comer, y descubrió que sus hijos se habían convertido en ... el gorgojo de los Andes.

Oral tradition recorded by Luis Chacón in the community of Colquepata,
province De Santo Tomás (Chumbivilcas). Taken from Yábar Landa, 1994.

Translation:

The Andean Potato Weevil

Once upon the time, in the province of Chimbivilcas, there has been a powerful farmer, his fields have been the bests and biggest, the harvests of his potatoes have been so good, that no one could equal him and this happened every year.

He produced a lot of potatoes, and he sold all these potatoes or changed them for other products, but his sons never ate potato; they have only seen how their powerful father produced always more and more potato, but they never could eat them.

And this happened every year again, and this powerful farmer had each year more potatoes, but his sons never knew the taste of potato, never enjoyed the work of their powerful father.

Until once, when his sons disappeared; and the father searched for them all around his lands and further away, desperately because he liked them a lot, he called them in vain, he made the other farmers looking for them, but they could not find them.

And the time passed, and then came the time for planting, and the farmer prepared his fields, crying for the lost of his sons, but he also had to produce potatoes, for this was his life, and he prepared the manure, left the field ready to receive the excellent seed potatoes that he himself produced.

And he opened the store to take out the seeds that he liked so much and there he found his sons, and he saw that his sons had eaten the potatoes that he produced so desperately, but that he never had given them to eat, and y realized that his sons have been converted into ... the Andean potato weevil.

Table of Content

Summary

The Andean potato weevil (APW) is a complex of 14 species of the genera *Premnotrypes* and *Rhigopsidius*. It is one of the most important insect potato pests in the Andes from Venezuela to north-western Argentina above 2800 meter. The larvae feed inside the potato tuber causing severe damage. In a survey with over 600 farmers from all over Peru, average damages of 23% by APW are reported. Actually control is mainly based on the application of the insecticides Metamidophos and Carbofuran. The present studies have been carried out with *Premnotrypes suturicallus*, the dominant species in the central highlands of Peru.

Temperature dependent development and mortality rates of all life stages as well as adult longevity and fecundity were determined under laboratory conditions at constant temperatures. The non-linear thermodynamic model from Sharp and DeMichele was adapted to the data. Survival of all stages of APW and fecundity were maximal at temperatures between 11° C and 15° C. Especially oviposition was severely affected by temperature above 20° C. For soil stages, additionally the influence of soil humidity on development and mortality rates was determined. At approximately 8% soil water content, a threshold was observed below which development of pre-pupae and adult emergence was markedly retarded. A facultative dormancy could be suggested for pre-pupae to overcome the dry season. Under field conditions, the population development was studied along an altitude gradient from sea level to 4100 meter. Only few individuals survived at an altitude of 2400 meter (corresponding to 16.7° C) making it the lower distribution limit of *P. suturicallus*. These field studies confirmed the cold adaptation of the weevil as found under laboratory conditions.

Bioassays showing good pathogenicity of the entomopathogenic fungus *Beauveria bassiana* against pre-pupae and adult weevils in the laboratory could not be confirmed by cage and open field trials. Further experiments showed, that the median lethal concentration (LC_{50}) was 1600 times higher (respectively the pathogenicity lower) at 13° C than at 19° C. This means, that under the optimal environmental conditions for the weevil, the performance of the fungus could be substantially reduced. Additionally investigations aimed at studying the possibility to control neonate larvae in the soil with *B. bassiana*. LC_{50} values for larvae feeding in tubers were 10,000 times higher than for larvae kept in an empty container. By the determination of colony forming units on the larval cuticle, by scanning electron microscopy and by fluorescence microscopy, the loss of most (in many cases all) conidia from the cuticle of neonates moving through soil or feeding in potato tubers was proven. These results demonstrate, that environmental conditions and larval behaviour render it almost impossible to use *B. bassiana* successfully in the control of APW.

General Introduction

Potato is the main staple crop in the Andes on which especially subsistence farmers rely for their alimentation. Taking the harvested surface as a measure, it is the third most important crop in Peru (Instituto Nacional de Estadística e Informática 2007), while it is the most important crop in the highlands. Especially in Peru and Bolivia, its centre of domestication, a high diversity of native species and varieties are planted. A great part is produced for subsistence, but especially in areas with good market access, it is also an important source of income for the farmers. On the other side it is the crop with by far the highest costs and hence risks (apart of some vegetable crops of little importance) for the Andean farmers (Crissman et al. 1998).

The Andes present highly variable ecological zones with Tapia (1994) distinguishing 18 agroecological zones only for Peru, each with several production zones. In general, in their tropical part, the Andes are wetter towards the north (Ecuador, Columbia) and become dryer towards the south (Peru, Bolivia). From southern Ecuador to Bolivia, there is also a marked difference between the eastern and the western slopes. More rain is coming from the eastern Amazon rainforest while the western slopes towards the coastal desert present a dry climate.

Like the ecology, the agricultural systems too are quite divers. In more rural areas especially of southern Peru and Bolivia, a traditional community managed 5 to 10 years rotation system with wide spatial separations between potato plots within successive years, also called sectorial fallow system, is practiced (Orlove and Godoy 1986, Hervé 1994). It is reported, that under these conditions, even without further control measures, damage by the Andean potato weevil (APW) and other pests is much less than in individually managed systems (Ewell et al. 1994, Yábar Landa 1994). In most cases however, especially in areas with good market access, small or smallest plots are cultivated individually. In some more favourable areas, accessible to mechanized field tillage, larger production units can be found.

The Andean potato weevil is the most serious insect pest in the Andes causing on average up to 30% yield loss (Ewell et al. 1994, Raman 1994, Crissman et al. 1998). It is distributed above 2800 meter altitude from Venezuela, through Colombia, Ecuador, Peru and Bolivia to north-western Argentina. In fact, it is a complex of 14 weevil species of the genera *Premnotrypes* and *Rhigopsidius* (Coleoptera: Curculionidae) (Alcázar and

Cisneros 1999). Many authors include also the species *Phyrdenus muriceus* Germar (Coleoptera: Curculionidae) into the complex, even though it occurs at low altitudes all over south and central America (Wibmer and O'Brien 1986). Sometimes several other weevils that may be found in potato fields are included, although it has not been proven, that they cause any damage to the crop. Other important insect pests for the potato production in the Andes include the Guatemalan potato moth *Tecia solanivora* Povlony (Lepidoptera: Gelechiidae) which during the last decade became a very serious potato pest in the northern Andes, the potato tuber moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), the Andean potato moth *Symmestrischema tangolias* (Gyen) (Lepidoptera: Gelechiidae), cut worms of the family Noctuidae, flea beetles (*Epitrix* spp.) and blister beetles (*Epicauta* spp.) (Ewell et al. 1994, Sporleder 2003). Adult APW are flightless, migrating mostly from adjacent harvested fields into new potato fields. Their life cycle is closely synchronized with the phenology of the potato plant and well adapted to the cropping systems in the Andes. While night active adults feed on the potato leaves they do not cause significant damage. Severe damage is caused by the larvae developing inside the tubers. After maturation they leave the tubers and pupate in the soil (Alcázar and Cisneros 1999). Farmers deal with the damage giving them different usages from selling slightly damaged tubers on the market for half the price, preserving them as "freeze-dried" "chuños" or other traditional products, using them for their own consumption to feeding the most damaged tubers to the animals (Ewell et al. 1994).

Actually, chemical insecticide treatments are the most common way to control APW (Ewell et al. 1994, Crissman et al. 1998), but Cole et al. (1998) and Antle et al. (1998) evaluated in an interdisciplinary case study in Ecuador, how the indiscriminate use of pesticides impacts on the health and productivity of the farmers and their families. Therefore, attempts are made to reduce the quantity and toxicity of the insecticides used. One possibility is the use of biological control agents. The most extensively studied biological agent for controlling APW is the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. which has first been described parasitising APW (actually it was *P. suturicallus*) in 1976 (Alcalá C. and Alcázar S. 1976). Good results have been reported with applications of the fungus on the floors of potato stores to control mature larvae when they dig into the soil for pupation (Alcázar and Cisneros 1997), though limitations in the acceptance for the farmers of this form of application have been described (Winters and Fano 1997) and the efficacy to reduce yield losses is not clear. Therefore, other ways of application, especially in the field, need further attention.

Worldwide, *B. bassiana* is one of most frequently used entomopathogenic fungus for the biological control of insects. Several products have been developed to control major insects pests like the Colorado potato beetle *Leptinotarsa decemlineata*, the European corn borer *Ostrinia nubilalis*, or the coffee berry borer *Hypothenemus hampei*. Like most other entomopathogenic fungi, *B. bassiana* is used generally inundatively, with an augmentative approach (for fungi only few cases of classical biological control with the introduction of fungi from the order entomophtherales have been realized) (Hajek et al. 2001).

Beauveria bassiana belongs to the class of the Hyphomycetes, which belong to the Deuteromycota or fungi imperfecti. A characteristic feature of the genus *Beauveria* is the zig-zag rachis bearing the conidia. The main differentiation between the two most common *Beauveria* species *B. bassiana* and *B. brongniartii* is the shape of the conidia which is globose in the first case and more or less oval in the second case (see MacLeod (1954) and Domsch et al. (1980) for further details).

This entomopathogenic fungus is also able to survive saprophytically in the soil. Its host range is relatively wide with insects from several orders being parasitised. Conidia get passively attached to the insect cuticle. When the conidia germinate, they form germ tubes and appressoria which attach them firmly to the insect cuticle. The fungus then penetrates the different layers of the insect cuticle producing a series of extracellular enzymes, mainly proteases and peptidases. During the penetration process, the fungus has also to cope with a series of defence mechanisms of the insect. Inside the insect hemocoel, it grows vegetatively with yeast-like structures (blastospores) or hyphal bodies. The later lack complete cell walls, an adaptation, that seems to avoid non-self recognition by the host immune system. Host death is caused by invasion of its tissues after growth and replication in the hemocoel. *B. bassiana* produces several secondary metabolites of which the beauverolides and cyclosporins are the most important. No direct toxic effects of these compounds have been observed, but they are known to influence or suppress the host immune system. They may even be responsible for the specificity and virulence of the fungus (Vilcinskas and Götz 1999). After the host death, *B. bassiana* continues in its saprophytic phase to colonize completely the insect body, leaving it hard and mummified. It then grows out of the cadaver forming a white mycelium that will turn creamy in time and sporulates forming dry asexual spores or conidia (Hajek 1997). Conidia are passively dispersed by wind currents or direct contact of insects. They may survive for prolonged periods depending on environmental conditions. High temperatures and solar radiation are main abiotic factors affecting conidial survival while humidity influences principally the germination of conidia. Another source of mortality is germination without location of an adequate host (Goettel et al. 2000).

The research presented here was part of a project at the International Potato Center in Lima (Peru) financed by the government of the Grand-Duchy of Luxembourg. The general objective of the project was the development of a biological insecticide based on *B. bassiana*. Incomplete knowledge on factors influencing the life cycle of APW as well as inconsistencies in the pathogenicity of *B. bassiana* against the weevil led to the necessity for studies on the ecology of APW and on the interactions between the weevil and the fungus. All experiments were made with the species *P. suturicallus* Kuschel, the dominant species in the central highlands of Peru. In the first introductory part, a comprehensive review of all research activities concerning APW and a survey with over 600 farmers from all over Peru on the damage caused by APW and the measures taken to control it are presented. In the second part different aspect of the biology of APW are evaluated. The influence of temperature, humidity and altitude on survival, development time and fecundity are investigated. The third part deals with the pathogenicity of *B. bassiana* against different life stages of *P. suturicallus*, the influence of temperature on the pathogen and the interactions between the pathogen and neonate larvae.

References cited

Alcalá C., P. and J. Alcázar S. (**1976**). Biología y comportamiento de *Premnotrypes suturicallus* Kuschel (Col.: Curculionidae). Revista Peruana de Entomología **19**(1): 49-52.

Alcázar, J. and F. Cisneros (**1997**). Integrated Management for Andean potato weevil in Pilot Units. In: Program Report 1995-1996. B. Hardy and B. Smith (Eds). Lima, International Potato Center: pp. 169-176.

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

Antle, J. M., D. C. Cole and C. C. Crissman (**1998**). The role of pesticides in farm productivity and farmer health. In: Economic, environmental, and health tradeoffs in agriculture: Pesticides and the sustainability of Andean potato porduction. C. C. Crissman, J. M. Antle and S. M. Capalbo (Eds). Dordrecht (The Netherlands), Kluwer Academic Publishers: pp. 231-241.

Cole, D. C., F. Carpio, J. A. Julian and N. León (**1998**). Health impacts of pesticide use in Carchi farm populations. In: Economic, environmental, and health tradeoffs in agriculture: Pesticides and the sustainability of Andean potato porduction. C. C. Crissman, J. M. Antle and S. M. Capalbo (Eds). Dordrecht (The Netherlands), Kluwer Academic Publishers: pp. 209-230.

Crissman, C. C., P. Espinosa, C. E. H. Ducrot, D. C. Cole and F. Carpio (**1998**). The case study site: Physical, health, and potato farming systems in Carchi Province. In: Economic, environmental, and health tradeoffs in agriculture: Pesticides and the sustainability of Andean potato porduction. C. C. Crissman, J. M. Antle and S. M. Capalbo (Eds). Dordrecht (The Netherlands), Kluwer Academic Publishers: pp. 85-120.

Domsch, K. H., W. Gams and T.-H. Anderson (**1980**). Compendium of soil fungi. London (UK), Academic Press, Inc. 860pp.

Ewell, P. T., K. O. Fuglie and K. V. Raman (**1994**). Farmers' perspectives on potato pest management in developing countries: Interdisciplinary research at the International Potato Center (CIP). In: Advances in potato pest biology and management. G. W. Zehnder, M. L. Powelson, R. K. Jansson and K. V. Raman (Eds). St. Paul (MN, USA), APS PRESS: pp. 597-615.

Goettel, M. S., G. D. Inglis and S. P. Wraight (**2000**). Fungi. In: Field manual of techniques in invertebrate pathology. L. A. Lacey and H. K. Kaya (Eds). Dordrecht (The Netherlands), Kluwer Academic Publishers: pp. 255-282.

Hajek, A. E. (**1997**). Ecology of terrestrial fungal entomopathogens. In: Advances in microbial ecology. J. G. Jones (Ed.). New York, Plenum. Vol. 15: pp. 193-249.

Hajek, A. E., S. P. Wraight and J. D. Vandenberg (**2001**). Control of arthropods using pathogenic fungi. In: Bio-exploitation of filamentous fungi. S. B. Pointing and K. D. Hyde (Eds). Vol. 6: pp. 309-345.

Hervé, D. (**1994**). Desarollo sostenible en los Andes altos. Los sistemas de cultivo con descanso largo pastoreado. In: Dinamicas del descanso de la tierra en los Andes. G. Rivière (Ed.). La Paz (Bolivia), IBTA - ORSTOM: pp. 15-36.

Instituto Nacional de Estadística e Informática (**2007**). Perú en cifras. http://www.inei.gob.pe/. Accessed: 2007/05/05.

MacLeod, D. M. (**1954**). Investigations on the genera *Beauveria* Vuill. and *Tritirachium* Limber. Canadian Journal of Botany **32**(6): 818-890.

Orlove, B. S. and R. Godoy (**1986**). Sectoral fallowing systems in the central Andes. Journal of Ethnobiology **6**(1): 169-204.

Raman, K. V. (**1994**). Potato pest management in developing countries. In: Advances in potato pest biology and management. G. W. Zehnder, M. L. Powelson, R. K. Jansson and K. V. Raman (Eds). St. Paul (MN, USA), APS PRESS: pp. 583-596.

Sporleder, M. (**2003**). The granulovirus of the potato tuber moth *Phthorimaea Operculella* (Zeller). Characterisation and prospects for effective mass production and pest control. Weikersheim (Germany), Margraf Verlag. 196pp.

Tapia, M. (**1994**). Crop rotation and its management in the Peruvian Andes. In: Dinamicas del descanso de la tierra en los Andes. D. Hervé, D. Genin and G. Rivière (Eds). La Paz (Bolivia), IBTA - ORSTOM: pp. 37-53.

Vilcinskas, A. and P. Götz (**1999**). Parasitic fungi and their interaction with the insect immune system. Advances in Parasitology **43**: 268-313.

Wibmer, G. J. and C. W. O'Brien (**1986**). Annotated checklist of the weevils (Curculionidae *sensu lato*) of South America (Coleoptera: Curculionoidea). Gainesville (Florida), The American Entomological Institute. 563pp.

Winters, P. and H. Fano (**1997**). The economics of biological control in Peruvian potato production. Working Paper. Lima, International Potato Center**:** 33.

Yábar Landa, E. (**1994**). Manejo ecológico del gorgojo de los Andes. Lima, RAAA. 119pp.

Part one:

APW - A baseline

Biology and management of the Andean potato weevil - A review

Magnus Kühne, Jesús Alcázar, Stefan Vidal and Kerstin Jung

Abstract

The Andean potato weevil complex (APW), *Premnotrypes* spp. and *Rhigopsidius* spp. (Coleopteran: Curculionidae) is the main insect pest in potato in the Andes above 2800 meters. Farmers suffer regularly severe losses due to this pest. Even though attempts had been made to develop integrated pest managenemt strategies, control is almost exclusively relying on chemical insecticides. In this comprehensive review we are revising all available information on taxonomy and morphology, on its biology, ecology and behaviour, as well as on control and management strategies. Additionally some information on the development biology are brought together and reanalysed. Open questions are detected, that should be elucidated to improve the knowledge on this insect and to allow a successfull pest management.

Keywords: *Premnotrypes*, *Rhigopsidius*, *Phyrdenus*, taxonomy, morphology, distribution, control, behaviour, ecology, economic relevanz

Introduction

Although severe problems with regard to crop damage and losses of income for poor farmers caused by the Andean potato weevil prevail in the potato production in the Andean regions for many decades only few papers have been published on this pest species in international refereed journals. The Andean potato weevil (APW), distributed in the Andean highlands between Venezuela and northwestern Argentina, is in fact a complex of 15 species of the genera *Premnotrypes*, *Rhigopsidius* and *Phyrdenus*. Most studies dealing with APW are, with few exceptions, mainly focusing on the most important species *P. vorax*, *P. suturicallus*, *P. latithorax* and *R. piercei*.

Jansson (1992), in his review on the biological management of root and tuber damaging weevils stated that only limited information on APW biology and control has been available up to that date. Yábar Landa (1988, 1994) published overviews centered on *P. latithorax* and the Cusco area in southern Peru. Alcázar and Cisneros (1997, 1999) published two general overviews on IPM strategies and on the taxonomy and bionomics of APW focusing mainly on the studies run at or with support of the International Potato

Center (CIP) in Lima (Peru). Finally, Calderón et al. (2004) made a review on research done in Bolivia on *P. latithorax*, *R. piercei* and *Phyrdenus muriceus* which is to a great extent a summery of the annual reports of the activities of the Instituto Boliviano de Tecnología Agropecueria - Programa de Investigación de la Papa (IBTA-PROINPA).

Apart from a few articles in international referred journals, this review has been compiled using bachelor or master theses from various Latin American Universities and Institutes as well as information published in South American entomological journals. Additional data have been derived from bulletins published by national plant protection institutions and from project and program reports dealing with APW. Finally abstracts and papers from regional conferences in South American countries have been checked as well. All so far in Peru published data on APW should have been taken into account. A few references from Andean countries other than Peru could not be checked originally; however this information should be covered by other publications. Most of the references cited have been deposited at the International Potato Center (CIP) in Lima (Peru).

Table 1: Taxonomy of the Andean potato weevil complex

Order and family	Tribe and Subfamily	Genus and species	Author
Coleoptera: Curculionidae	Premnotrypini: Entiminae	*Premnotrypes solaniperda*	Kuschel (1956)
		P. latithorax	(Pierce) (1914)
		P. sanfordi	(Pierce) (1918)
		P. pusillus	Kuschel (1956)
		P. piercei	Alcalá (1979)
		P. suturicallus	Kuschel (1956)
		P. fractirostris	Marshall (1936)
		P. solani	Pierce (1914)
		P. vorax	(Hustache) (1933)
		P. solanivorax	(Heller) (1935)
		P. clivosus	Kuschel (1956)
		P. zischkai	Kuschel (1956)
	Rhytirrhinini: Rhytirrhininae	*Rhigopsidius tucumanus*	Heller (1906)
		R. piercei	Heller (1936)
	Cryptorrhynchini: Chryptorrhynchinae	*Phyrdenus muriceus*	(Germar) (1824)

Taxonomy

The Andean potato weevil is a complex of 12 species of the genus *Premnotrypes* (tribe Premnotrypini, subfamily Entiminae). However, other related species, like *Rhigopsidius piercei* and *R. tucumanus* (tribe Rhytirrhinini, subfamily Rhytirrhininae) and *Phyrdenus muriceus* (tribe Cryptorrhynchini, subfamily Cryptorhynchinae) (see Table 1) are generally included into the Andean potato weevil complex. In this review, *P. muriceus* will only be treated in passing, as it is doubtfull whether it should be included at all in the APW complex. As mentioned in the corresponding sections, it only occurs below 2000 meters altitude, is distributed all over tropical South and Central America and is in many area known as tomato pest (Fiedler 1941, Wibmer and O'Brien 1986, Morales Valles et al. 2003, Calderón et al. 2004). Some author included also species of the genera *Scotoeborus*, *Hyperodes*, *Adioristus*, *Lystroderes*, and *Naupactus* into the APW complex (Sánchez 1986, Alcázar 1995, Peña Villamil 2001); however, evidence is lacking that these species are able to complete their whole life cycle on potato (Alcázar and Cisneros 1999). Several other weevil species are frequently found in or around potato fields. *Amathynetoides nitidiventris* Hustache and *Adioristidius tuberculatus* Voss (new determination, formerly determined as *Microtrypes* sp.) attacking *Ullucus tuberosus* respectively *Oxalis tuberosa*, two Andean tuber crops, are often found in potato fields due to crop rotations and have been studied in detail (Aldana Yurivilca 2001, Aldana Yurivilca 2003). For *A. nitidiventris* and *A. tuberculatus* it has been established, that they do not cause damage to potato but are restricted to *U. tuberosus* respectively *O. tuberosa* (Alcázar et al. 1999).

Phyrdenus muriceus Germar and *Rhigopsidius tucumanus* Heller were described in 1824 (Alcázar and Cisneros 1999) respectively in 1906 (Morrone and Loiacono 1992). A revision of the genus *Phyrdenus* is presented by Fiedler (1941). Sasscer and Pierce (1913) described specimens as *R. tucumanus* which were later renamed by Heller (1936) as *R. piercei*. Van Emden (1952) considered both the same species, but this proposal was neither followed by Wibmer and O’Brien (1986) nor by Morrone and Loiacono (1992) who present a revision with key for the genus *Rhigopsidius*. As the latter publication was not known in Bolivia and Peru, all references from Bolivia and Peru refer to *R. tucumanus*. Though, following to Morrone and Loiacono (1992), the distribution of this species is restricted to Argentina. A redetermination of the specimens present in the collection of CIP in Lima and collected from different locations in southern Peru and

Bolivia revealed them as *R. piercei*. Therefore, for all references dealing with *Rhigopsidius* material from any other country than Argentina, we will in the following consider them as referring to *R. piercei*. Pierce (1914, 1918) describes the two genera *Premnotrypes* and *Trypopremnon* with the species *P. solani*, *T. latithorax* and *T. sanfordi* from specimens found in potato shipments from Peru arriving in the U.S. Kuschel (1956) made a revision of the tribe Premnotrypini, joining the former three species together with the meanwhile described *Solanophagus vorax* Hustache (1933), *Plastoleptops solanivorax* Heller (1935) and *Premnotrypes fractirostris* Marshall (1936) and includes five new species (*P. solaniperda*, *P. pusillus*, *P. suturicallus*, *P. clivosus* and *P. zischkai*) into the genus *Premnotrypes*. He describes in total 11 species and presents a key for their identification. Alcalá C. (1979) describes *P. piercei* completing 12 *Premnotrypes* species. More recently, Alcázar and Cisneros (1999) present a modified key to the species.

The genetic variation between 3 populations of *P. vorax* collected in Colombia has been investigated by González and Gómez C. (1989)without finding evidence for any genetic separation between these subpopulations.

Popular names used to identify the larvae and the adults of APW in the Andean countries are listed in Table 2. The Spanish designation "Gusano blanco" should not be translated with "white grub" as this generally refers to the larvae of Scarabaeidae, which in the Andean region are called "gusano arador".

Table 2: Local and popular names for the Andean potato weevil in South America. Most commonly used names are underlined (Agostini de Manero and Vilte 1982, Ewell et al. 1990, Yábar Landa 1994, Gallegos G. et al. 1997, Herrera 1997).

Country	Popular names for larvae	Popular names for adults
Colombia	Gusano blanco, Cusca	Cucarrón de la papa
Ecuador	Gusano blanco, Arrocino, mosco blanco, yurag kuro	
Peru	Papa Kuro, gusanera de la papa, kara casaca, Tuksa, Allpa Kuru, Ichu-Kuro, Shacra, Ita Kuru, Manko, Champa mayakuru, Gusano arroz, Kulo Kuru, Sajra kuro, Acsho Kuru, Barrenador	Gorgojo de los Andes, Picudo Andino de la papa, K'aracasaca, Papa Kuru mama, Uti-uti, Palla-palla Kuro, Cuhi-cuchi, Nabos Kuru, Jusipacha Kuru, Tanapusca, Ucha Kuru, Jachu-jachu, Laqha Kuru, Tankallo, Curco, Ticshincunca, Capacho Kuro, Capa Kuro, Ata Kuro, Zorro-zorro
Bolivia	Gusano blanco	Gorgojo de los Andes
Argentina	Chitupa	

Morphology

Morphological descriptions of all *Premnotrypes* species except of *P. piercei* are presented by Kuschel (1956). Other detailed studies of all life stages have been made by Muñoz Cáceres (1998) for *P. vorax*, Alcalá C. and Alcázar S. (1976) for *P. suturicallus*, Alcalá C. (1979) and Tovar Gamarra (1988) for *P. piercei*, Tisoc Dueñas (1989a) for *P. latithorax*, Gil Alvarez (1991) for *P. solaniperda*, Agostini de Manero and Vilte (1982) and Morrone and Loiacono (1992) for *R. tucumanus*, Morrone and Loiacono (1992) and Barea et al. (1996/1997) for *R. piecei* and Fiedler (1941) for *Phyrdenus muriceus*. Loiácono and Morrone (1991) describe the final larval stage of *R. piecei* and its difference to larvae of the genus *Premnotrypes*. The adult of all species are of brown, inconspicuous color making it vanish into the brown background of the soil (see Figure 1). The elytra are grown together making them flightless (except for *Phyrdenus muriceus*). They have more or less pronounced tubercles and scales on their elytra and measure roughly 5 to 9 mm long and 2.5 to 4.5 mm large. *P. pusillus* is the smallest species with 3.8 – 4.9 x 1.9 – 2.5 mm. The two species of *Rhigopsidius* are slightly bigger than the species of *Premnotrypes*. Small caduceus teeth that are lost after emergence, exist on the mandibles. The eggs are white to creamy in color and of cylindrical to oval shape with exception of the eggs of *R. piercei* with less elongated and more round eggs. Table 3 gives an overview of adult and egg sizes. Like typical Curculionidae larvae, APW larvae are legless and present setae over the whole body. Larvae and pupae are of a creamy white color. The pupae are free pupae forming a pupal chamber.

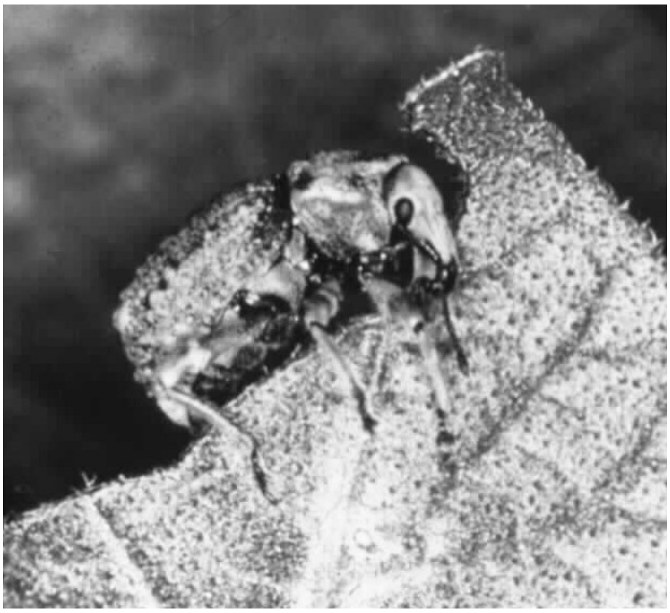

Figure 1: Adult of *Premnotrypes suturicallus* feeding on a potato leaf. ©International Potato Center

Table 3: Dimensions of adults and eggs of the species of the Andean potato weevil complex.

	adults long x large [mm]	eggs long x large [mm]	References
Premnotrypes vorax	5.7 - 8.5 x 2.2 - 3.9	1.1 - 1.45 x 0.3 - 0.5	(Kuschel 1956, Muñoz Cáceres 1998)
P. suturicallus	6 - 9.2 x 2.7 - 4.6	1 - 1.5 x 0.41 - 0.66	(Kuschel 1956, Alcalá C. and Alcázar S. 1976)
P. piercei	5.48 - 8.01 x 2.21 - 3.69	1.22 - 1.77 x 0.44 - 0.77	(Tovar Gamarra 1988)
P. latithorax	4.4 - 10.3 x 2 - 4.5	1.1 - 1.4 x 0.6 - 0.8	(Kuschel 1956, Tisoc Dueñas 1989a)
P. solaniperda	4.7 - 8.8 x 2.2 - 4.3	1 - 1.8 x 0.4 - 0.9	(Kuschel 1956, Gil Alvarez 1991)
P. solanivorax	6.3 x 3.5*	**	(Kuschel 1956)
P. fractirostris	5.5 - 6.3 x 2.9 - 3.4	**	(Kuschel 1956)
P. solani	6.2 - 7.6 x 3.2 - 4	**	(Kuschel 1956)
P. sanfordi	8 x 4.5*	**	(Kuschel 1956)
P. clivosus	5 x 2.1*	**	(Kuschel 1956)
P. zischkai	4.1 - 7 x 1.8 - 3.3	**	(Kuschel 1956)
P. pusillus	3.77 - 4.89 x 1.89 - 2.51	**	(Kuschel 1956)
Rhigopsidius tucumanus	5.77 - 11.2 x 3.01 - 5.08	0.8 - 1.2 x 0.7 – 1.0	(Agostini de Manero and Vilte 1982)
R. piercei	6.9 - 11.2 x 4.0 - 6.2	0.8 - 1.2 x 0.7 - 1.0	(Barea et al. 1996/1997)

*Only one specimen was measured.
**no data available.

All so far in detail studied species (see below) exhibit sexual dimorphisms. Sexes can generally be separated by using the following characteristics: females tend to be larger than males, ventrally the rear end - the apex of the fifth sternit - is pointed contrary to the blunt apex of the males and the declivity of the rear end of the elytra is more pronounced and set off to the dorsal part as compared to males (Alcázar and Cisneros 1999).

Geographical distribution

The species belonging to the APW complex occur in the Andes from Venezuela, Colombia, Ecuador, Peru, Bolivia and northern Argentina and Chile roughly between 2100 and 4500 meter altitude (Yábar Landa 1994, Alcázar and Cisneros 1999).

In Colombia the pest was first recorded in 1925 (Zenner and Posada O. 1968) while the first reference from Venezuela dates from 1964 for Táchira and 1971 for Mérida

(Angeles 1966, Angeles and Rodríguez D. 1971). It has been suggested that it may have been introduced through imported seed potatoes.

P. vorax occurs in Venezuela, Colombia and Ecuador where it is the only species as well as in northern Peru. In Peru 10 species of *Premnotrypes* are found with *P. vorax* being the dominant species in northern Peru, *P. suturicallus* in central Peru and *P. latithorax* in southern Peru. *P. piercei*, is of some importance in some areas of the central regions, and the same holds true for *P. solaniperda* in some areas in the south. Of minor importance are *P. sanfordi*, *P. pusillus*, *P. fractirostris*, *P. solani* and *P. solanivorax*. 4 species of *Premnotrypes* can be found in Bolivia with *P. latithorax* and *P. solaniperda* being the dominant species and *P. clivosus* and *P. zischkai* being of minor importance. *P. latithorax* occurs also in the Andes of northen Chile. The highest diversity of species is found in the central highlands of Peru. (Wibmer and O'Brien 1986, Alcázar and Cisneros 1999, Larraín S. 2003). *Rhigopsidius piercei* occurs in the arid high mountain zones of southernmost Peru, Bolivia and north-western Argentina and in a few places in northern Chile while *R. tucumanus* is restricted to Argentina were it has been recorded in the Andes between the Bolivian border and northern Patagonia (37° southern latitude) (Morrone and Loiacono 1992, Posadas 1998). *Phyrdenus muriceus* occurs below 2000 meters (Calderón et al. 2004) in most of tropical and sub-tropical South America east of the Andes, in Central America and in the southern part of North America as far north as Arizona (Fiedler 1941, Wibmer and O'Brien 1986) (Figure 2).

In Colombia *P. vorax* has been reported as low as 2100 meter and up to 3100 meter (López-Avila and Espitia-Malagón 2000) with reduced incidence below 2600 meter (Olivares Garces et al. 1973). In Venezuela it has been found between 2580 and 3600 meter (Niño et al. 2000). Reports for Peru (Carrasco Zamora 1961, Mellado 1961, Yábar Landa 1994) and Bolivia (Andrew et al. 1999b) for different *Premnotrypes* species vary from 2500 to 4700 meter Ewell et al. (1990) report for the central highlands of Peru an increase in damage by *P. suturicallus* between 3000 and 4000 meters and a sharp decline above 4000 meters. *R. piercei* is reported in Argentina between 2000 and 3900 meter with reduced incidence above 3000 meter where summer temperatures are below 14°C and winter temperatures below 4°C (Agostini de Manero 1993). The distribution of APW so far reported may be related to the presence of potato production, though its real distribution may be wider due to the presence of wild potato species.

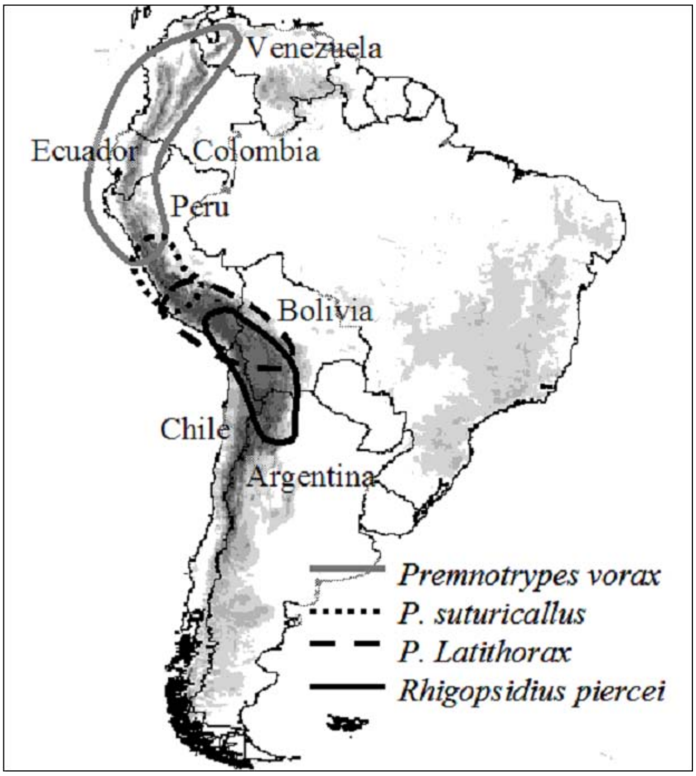

Figure 2: Distribution areas of the most frequently referred species of the Andean potato weevil complex. Additionally, *P. solanivorax* occurs in northern Peru, *P. fractirostris*, *P. piercei* and *P. solani* in central Peru, *P. pusillus* in central and southern Peru, *P. sanfordi* in southern Peru, *P. solaniperda* in southern Peru and northern Bolivia, *P. clivosus* and *P. zischkai* in Bolivia, *R. tucumanus* in northern Argentina and *Phyrdenus muriceus* in whole tropical South America east of the Andes as well as in Central America and the southern parts of North America (Wibmer and O'Brien 1986, Morrone and Loiacono 1992, Alcázar and Cisneros 1999).

Biology, behaviour and ecology

The species of the APW complex have most life history traits in common. Departures from these common traits will be discussed at the corresponding paragraphs. Species that have been studied are *P. suturicallus* (Alcalá C. and Alcázar S. 1976), *P. latithorax* (Tisoc Dueñas 1989a, Carvajal Pinto 1993), *P. vorax* (Muñoz Cáceres 1998), *P. piercei* (Tovar Gamarra 1988), *P. solaniperda* (Gil Alvarez 1991), *P. solanivorax* (Mellado 1961) and *Rhigopsidius piercei* (Agostini de Manero and Vilte 1982, Barea et al. 1996/1997). The biology and ethology of *Phyrdenus muriceus* was studied by Espul and Magistretti (1969). Some field observations have been made for *P. solani* (Wille T. 1952) and *P. pusillus* (Molleda M. 1965).

Adults

The elytra of adult APW are grown together making them flightless (except for *Phyrdenus muriceus*). Adults hide during the day at dark and humid sites below plant debris and soil clots mostly around the plant stem. The main activity period is between sunset and midnight when they are feeding on leaves, and copulation and oviposition takes place (Alcalá C. and Alcázar S. 1976, Gil Alvarez 1991, Muñoz Cáceres 1998). Adults leave half-moon shaped feeding traces on the leaf edge (except *Phyrdenus muriceus* who makes round holes). Some authors observed that adults of *P. vorax*, *P. suturicallus* and *P. piercei* are attracted by humidity or at least prefer humid sites (Alcázar Sedano 1976, Calvache Guerrero 1984, Tovar Gamarra 1988). The sex ratio in the species that have been studied varied between 0.71 and 1.72 males/females.

Oviposition

After emergence and copulation, there is a pre-oviposition period of around 10 days that may be extended to up to one month (see Table 4). Adults of *P. vorax*, *P. suturicallus* and *P. latithorax* lay eggs into dry straw or plant debris (Alcalá C. and Alcázar S. 1976, Tisoc Dueñas 1989b, Muñoz Cáceres 1998). Two studies on oviposition sites showed, that *P. vorax* has a clear preference for straw of *Stipa* sp. when compared to *Lolium perenne* L., *Triticum vulgare* VILL. and *Holcus lanatus* L. (Gallegos et al. 2004b) and for *Pennisetum clandestinum* Hochst. or fine potato stems when compared to wheat or barley straw and potato tubers or leaves (Navarro Mejía 1995). Carvajal Pinto (1993) for *P. latithorax* and Calvache Guerrero (1986a) for *P. vorax* observed that oviposition is reduced under dry air conditions respectively increases under wet conditions. Total number of eggs laid per female was recorded to be highly variable (Table 4) and the viability of the eggs varied between 82 and 99% (Tovar Gamarra 1988, Carvajal Pinto 1993, Salazar Benitez 1996, Muñoz Cáceres 1998). Oviposition was reduced to 0.1 to 0.2% if adults did not feed and it was still reduced to 11.8%, 1.4% and 2.8% when adults were feeding on *Brassica campestris*, *Bidens pilosa* and *Erodium* sp. respectively. However, no significant difference in viability was observed (Alcázar et al. 1995). Feeding adults with potato tubers significantly reduced oviposition as compared to adults fed with potato leaves (Valencia V. and Bohórquez 1994). For *P. vorax* Navarro Mejía (1995) determined, that one copulation led to lifelong oviposition without reduction of the total number of eggs, though in presence of more than 1 male oviposition was reduced. Several authors state that parthenogenesis occures in APW, with some data suggesting that virgin females dug out from pupating sites in the fiel are able to lay a reduced number of eggs (between very few to 36% of the number of eggs laid by non-virgin females) with a reduced viability (0 to 83% of the viability of eggs from non-virgin females) (Tovar Gamarra 1988, Gil Alvarez 1991, Alcázar et al. 1995, Muñoz Cáceres 1998). No studies exist with females reared individually in the laboratory.

First instar larvae

After hatching, first instar larvae dig into the soil to bore into the potato tubers. Navarro Mejía (1995) observed, that only after 12 h larvae begin to penetrate the tuber. In absence of tubers or if the population density is high, some larvae may occasionally feed on roots, stolons and even on the lower part of the stem (hypocotyl) (Calvache Guerrero (1986a) and Muñoz Cáceres (1998) for *P. vorax;* Villano Tárraga (1994) for *P. latithorax* and personal observations for *P. suturicallus*). However, in these cases a reduced fitness is reported (Catalán Bazán et al. 1993). Only *Phyrdenus muriceus* larvae feed regularly on roots instead of tubers (Alcázar and Cisneros 1999). Alcázar Sedano (1988) for *P. suturicallus*, Catalán Bazán et al. (1993) for *P. latithorax* and Navarro Mejía (1995) for *P. vorax* investigated the dispersal capacity of first instar larvae placing tubers at different depth and horizontal distances from the releasing point. When tubers were placed at a depth of 30 cm, 18 to 30 % of the larvae were able to reach the tubers in the soil; however only 1.5% (14% in the case of *P. vorax*) of the larvae were able to reach tubers at a depth of 40 cm. In horizontal direction, the percentage of larvae reaching the tubers decreased linearly from 62% at 5 cm to 5% at 30 cm. When larvae were placed 10 cm below the tubers in the soil no infestation occurred.

Larval stages and pupation

Larvae pass through 4 stages and complete their development within the tubers. *P vorax* is the only one that presents 5 larval stages (Torres Wills 1993, Muñoz Cáceres 1998, Peña Villamil 2001) although this could not be confirmed by Navarro Mejía (1995). Only the larvae of *P. pusillus*, the smallest weevil within the APW complex, feed superficially on the tubers without entering deeply into the tuber (Molleda M. 1965). Larvae leave the tubers only for pupation, which takes place in the soil. Up to 1% of larvae have been observed to pupate within the tuber. This may be a major cause of dissemination of weevils through seed tubers into previously weevil free areas (Wille T. 1952, Calvache Guerrero 1988a, Tisoc Dueñas 1989b, Gil Alvarez 1991) for *P. solani*, *P.*

vorax, *P. latithorax* and *P. solaniperda* respectively. Comparing storing conditions, Catalán Bazán et al. (1993) found that storing tubers on concrete floor led to 30% of larvae of *P. latithorx* pupating within the tuber while on earth soil only 3% pupated within the tuber. *R. piercei* and *R. tucumanus* are exceptional because the larvae always pupate within the tuber until emergence of the adults (Agostini de Manero and Vilte 1982, Andrew et al. 1999a). Third and forth instar larvae did not enter tubers offered to them and non-infested tubers did not become infested if stored together with infested ones (Catalán Bazán et al. 1993, Yábar Landa 1994).

In Peru intense population dynamics studies have been made for *P. vorax* and *P. latithorax*. Transition from pre-pupae over pupae to adult is described to be continuous over the dry season with pre-pupae present between February and September, pupae between April or June and September or October and adult remaining in pupal chamber from June or July to December. The population found in stores may be up to two months delayed in its development (Villano Tárraga 1994, Ravines Novoa 2002).

Studies with *P. vorax*, *P. suturicallus*, *P. piercei* and *P. latithorax* showed, that pupae are formed within a pupal chamber at depth between 10 and 30 cm or, if the soil is too much compacted as often encountered in stores, directly below the surface (Zenner and Posada O. 1968, Alcalá C. and Alcázar S. 1976, Tovar Gamarra 1988, Yábar Landa 1994, Vera Robles 2001, Niño et al. 2004). The depth of pupation may also be influenced by soil humidity (Yábar Landa et al. 2003).

Emergence of adults

The young adults remain in the pupal chamber for melanization. Most authors assume, that humidity or the onset of the raining period triggers the emergence of adults. This is suggested by rainfall data and populations dynamics of the weevil. Still, irrigation of pupation sites of *P. latithorax* resulted in only slight changes in the date of emergence by 1 to 5 days (Vallenas Bellota 1998). But in laboratory test, Valencia (1989) observed a strong response of adult *P. vorax* to irrigation, emerging few days after the initiation of irrigation. No measurements of initial and final soil humidity were made. As adults also emerge from stores where no rain enters, other stimulus must exist (Alcázar and Cisneros 1999).

In Colombia adult *P. vorax* emerge during the whole year with peaks in April-May and October-December while in Peru emergence take mainly place between October and

January (Villano Tárraga 1994, Yábar Landa 1994, Corporación Colombiana de Investigación Agropecuaria 2000, Ravines Novoa 2002). But Calderón and Herbas (1993 in: Calderón et al. 2004) found in Bolivia the onset of emergence of *P. latithorax* in stores beginning sporadically in September with a peak in October.

Migration of adults and attractants

Emerging adults remain in the field if potato plants are available or migrate to new potato fields (Alcázar and Cisneros 1999). Only *R. piercei* that pupates within the tubers is mainly brought into the field through the seed tubers. In migration performance experiments in Bolivia (*P. latithorax* and *P. solaniperda*) adults moved 1 m/h on short distances (up to 1 m) and 0.6 m/h on long distances (5 to 300 m) with retrieval of hardly 2.4 to 3.8% of the number of weevils released. At 500 meter distance no adults were recaptured (Chavez Ajata 1997). In absence of food, adult *P. vorax* may survive 1 to 4.5 months depending on local climatic conditions (Calvache Guerrero and Alvarado 1980, Gallegos G. et al. 1997) while *P. suturicallus* may survive for 80 days (Alcázar Sedano 1976).

Several authors mention the existence of pheromones either sexual or aggregative referring to observations of groups of adults clumped together. These aggregations normally occur at humid places. Recent laboratory studies with *P. suturicallus* revealed no significant preference of traps containing other weevils (coupled, virgin or feeding) as compared to control traps containing no adults. In field and laboratory experiments, using potato leaves as baits, adults of *P. vorax* and *P. suturicallus* were significantly attracted to these baits (Calvache 1985, Obando G. et al. 1988, Valencia 1989, Alcázar et al. 2004). However, it has been speculated that only foliage together with adults feeding on them may attract other adult weevils (Heath et al. 2001). New studies in Bolivia and Great Britain (in cooperation with CIP) with *P. latithorax* revealed two volatiles produced by potato leaves ((Z)-3-hexenol and (E)-2-hexenal also called leaf alcohol and leaf aldehyde) produced reaction in adult weevils and thus further studied for their potential as attractants will go on. Ríos et al. (2005) found that males of *P. suturicallus* are attracted by manure produced by females of the same species. On the other hand, a field study in Bolivia (though with incomplete design) revealed that only 5% of released adults orientated towards the next potato crop field (Esprella et al. 1996).

Adults tend to agglomerate in certain parts of the potato fields. In rotation fields where the flightless APW has to immigrate from outside the field, population density and subsequent damage is much higher at the borders and towards the infestation source while the population in the centre of the fields being as much as 20 to 30 time lower as on the border (Valencia 1988, Carvajal Pinto 1993, Catalán Bazán et al. 1993, Villano Tárraga 1994, Ravines Novoa 2002).

Field studies from Ecuador indicated that under conditions of permanent potato crops, adult population density varied little over the year; within one field adults were present from soil tilling to around 40 days after emergence of the crop and again one to three months after harvest (Gallegos 1989, Gallegos G. et al. 1997). Similarly, in Colombia the main population density was observed at the beginning of the cropping period between emergence and hilling (López-Avila and Espitia-Malagón 2000). However, the trapping results may have been influenced by the changes in the mircroecological contitions due to the developing crop which may have reduced the attractiveness of the traps. In Peru, with potato production mostly during at the rainy season from November/December to April, adults were observed in the potato fields between November (emergence of crop) and April (harvest) with a peak density in January (Villano Tárraga 1994, Sotelo Ravichagua 1996, Ravines Novoa 2002). In Bolivia, *P. latithorax* and *R. piercei* were both present from January to April (Calderón et al. 2004). In Venezuela too adults are mainly present after the onset of rain in March-April, though small numbers of adults were also captured during the dry season from November to February (Niño et al. 2004). Studies on population dynamics are presented for *P. vorax* in Venezuela (Niño et al. 2004), in Colombia (Galindo P. and Español Aragon 2004) and in northern Peru (Ravines Novoa 2002) and for *P. latithorax* in southern Peru (Villano Tárraga 1994) and in Bolivia (Carvajal Pinto 1993).

Development time

The life cycle varies considerable between the different potato growing regions in the Andes. Most species of the APW complex studied in Peru (including *P. vorax* in northern Peru) and Bolivia exhibit one generation per year. However, *P. vorax* in Colombia, Ecuador and Venezuela normally complete two to three generations per year. This pattern coincides with the general potato-growing pattern with one growing season in Peru and Bolivia (in absence of irrigation) and permanent potato growing in the wetter, northern part of the Andes. In some areas of Peru like the more humid, eastern slopes of the Andes where potatoes are grown the whole year round, simultaneous presence of all living stages of *P. suturicallus* within one plot (personal observations) suggests also more than one generation per year. *Phyrdenus muriceus* in the meso-thermal valleys of Bolivia and in Argentina has 2 generations per year and seems to be present throughout the whole year (Espul and Magistretti 1969, Andrew et al. 1999b, Novo et al. 2002, Calderón et al. 2004).

A summary of the life cycles studied with development times and mean temperatures and altitudes of studies sites are given in Table 4. Comparability of data is difficult as the exactitude of information given varies and most studies did not use controlled conditions. In some studies n was low. Nevertheless using the mean temperature or the altitude (given by the authors or completed with data from other databases) allows finding a satisfactory regression for *P. vorax*. Development rates from egg until the moulting to adult have been calculated from the data sets found in the literature (Figure 3). Data for *P. vorax* show a clear relation between development rate and temperature. The soil stages from pre-pupae to emerging adult may be considered together or apart by the different authors. Especially during this stages soil humidity may have a considerable influence on development. Even at low temperatures and on sites with only one generation per year observed in the field, total development time from egg to emerged adult varies between 185 and 300 days. This suggests, that under field conditions, other factors than temperature may influence the development.

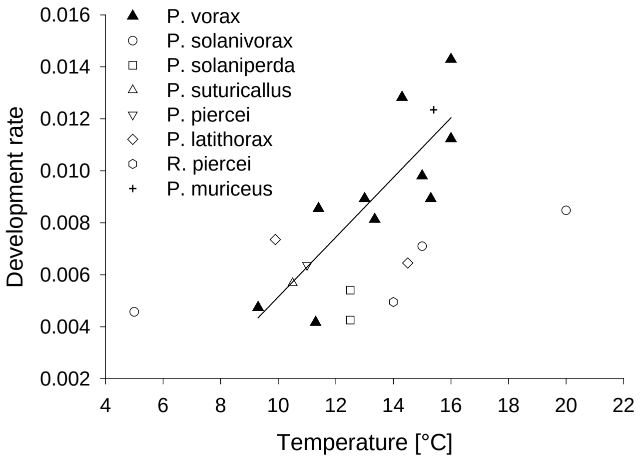

Figure 3: Development rates (1/development time in days) for the development from egg to the moulting to adult of different species of the Andean potato weevil complex at different temperatures. Linear regression is calculated for the data of *P. vorax* ($y = -0.0063 + 0.0011x$; $R^2 = 0.6680$)(for references see Table 4).

Table 4: Mean development time in days of live stages of different APW species under different conditions. [1](Zenner and Posada O. 1968, Zenner de Polanía 1986), [2](Calvache Guerrero and Alvarado 1980, Calvache Guerrero 1986a), [3](Calvache Guerrero 1988b), [4](Calvache Guerrero 1986a), [5](Garzón C. et al. 1996), [6](Villamizar Carvajal and Becerra Contreras 1983), [7](Salazar Benitez 1996), [8](Gallegos 1989), [9](Torres Wills 1993), [10](Niño et al. 2004) [11](Muñoz Cáceres 1998), [12](Mellado 1961), [13](Gil Alvarez 1991), [14](Alcalá C. and Alcázar S. 1976), [15](Tovar Gamarra 1988), [16](Tisoc Dueñas 1989b, 1989a), [17](Carvajal Pinto 1993), [18](Barea et al. 1996/1997), [19](Herbas et al. 1995 in: Calderón et al. 2004) *data taken from CIP databases, **data calculated by the authors.

species	*P. vorax*[1]	*P. vorax*[2]	*P. vorax*[3]	*P. vorax*[4]	*P. vorax*[5]	*P. vorax*[6]	*P. vorax*[7]	*P. vorax*[8]	*P. vorax*[9]	*P. vorax*[10]	*P. vorax*[11]
country	Colombia	Colombia	Colombia	Colombia	Colombia	Colombia	Colombia	Ecuador	Venezuela	Venezuela	Peru
site	Tibaitata (Cundinamarca)	Obonuco (Narino)	Manizales (Caldas)	Paramo de Letras (Caldas)	Bogota	Tunja (Boyaca)	Tunja (Boyaca)	Sta Catalina (Pichincha)	Plo Hondo (Tachira)	Mucuchíes (Mérida)	Chilimpampa (Cajamarca)
long (decimal)	-74.0333*	-77.3*	-75.5*	-75.3166*	-74.0833*	-73.3333*	-73.3333*	-78.55*	-71.918*	-70.9213*	-78.5011
lat (decimal)	4.7833*	1.2166*	5.0833*	5.0666*	4.6*	5.55*	5.55*	-0.3667*	8.2713*	8.7544*	-7.0514
altitude [m]	2560	2710	2100	3500	2530*	2690	2690	3058*	2134*	3100	3400
temp [°C]	14.3*	13	16	11.3*	16**	18.3	13.35*	11.4*	15	15.3	9.3
max temp [°C]	19.1*	21.3*	20.7*	15.9*	22		18.2*	13.5*		19	14.2
min temp [°C]	9.5*	10.9*	11.2*	6.7*	10		8.5*	9.2*		13.5	4.3
Egg	21	38	27	76	19		32	35	31	32	43
Larvae						**46**	**57**		**40**	**36**	**82**
Pre-pupae						5	15		14	19	36
Larvae (incl. pre-pupae)	**41**	**48**	**34**	**118**	**21**	**51****	**72****	**56**	**54****	**56**	**118****
Pupae	16	26	28	46	30	19	19	26	17	23	50
total until adult	**78**	**112**	**89**	**240**	**70**	**97****	**123**	**117**	**102**	**112**	**211**
Over wintering adult	18**	20	11	43		18	20	17	16	18	66
Total	**96****	**132****	**100**	**283****	**70**	**115****	**142**	**134****	**118****	**130****	**276**
Pre-oviposition	8**						29				10
Longevity	365**	365				> 120	154		350		180.5**
Oviposition period							88				119
Oviposition (eggs)	255						107				378

Species	*P. solanivorax*[12]					*P. solaniperda*[13]		*P. suturicallus*[14]	*P. piercei*[15]	*P. latithorax*[16]	*P. latithorax*[17]	*R. piercei*[18]	P. muriceus[19]
country	Peru					Peru		Peru	Peru	Peru	Bolivia	Bolivia	Bolivia
site	Laboratory					Illpa (Puno) (1st gen)	Illpa (Puno) (2nd gen)	Estac. Sta Ana (Huancayo)	Estac. Sta Ana (Huancayo)	Chinchero (Cusco)	Toralapa (Cochabamba)	Chinoli (Potosí)	Tarija
long (decimal)						-70.0666*		-75.233*	-75.233*	-72.0547*	-65.7166*	-65,3667	-64.75
lat (decimal)						-15.6666*		-12.029*	-12.029*	-13.3958*	-17.4333*	-19,6333	-21.5166
altitude [m]						3835		3280*	3280*	3760	3430	3450	2286*
temp [°C]	5	10	15	20	25	12.5		10.5	11	9.9	14.5	14	15.4*
max temp [°C]						13.4		16.2*	16.2*	16.4	27	20.6*	23.5*
min temp [°C]						11.2		1.2*	1.2*	3.2	6	4.7*	7.3*
Egg	84	60	27	22	0	43	47	33	27	48	65	38	12,5
Larvae						**47****	**44****	**46****	**36****	**34****	**31**	**65**	**37**
Pre-pupae						87	47	43	44	26	35	51	12,5
Larvae (incl. pre-pupae)	**93**	**96**	**80**	**70**	**0**	**134****	**91****	**89****	**78**	**60****	**66****	**116****	**49,5****
Pupae	42		34	26	0	58	47	54	52	28	24	48	19
total until adult	**219**		**141**	**118**	**0**	**235**	**185**	**176**	**157**	**136**	**155**	**202****	**81****
Over wintering adult								115	135	39	130		65
Total	**219**	**156**	**141**	**118**	**0**	**235****	**185****	**291****	**301**	**145**	**285****	**266**	**146**
Pre-oviposition								9	42	37		66	
Longevity	730	730	730	730	0	208.5**		142.5**	255	249	168**	136**	
Oviposition period								106	127	60**	156	46	
Oviposition (eggs)						129		631	521	161	565	39	

Table 5: Influences of alternative host plants on the life cycle of *P. latithorax*. *of reduced size. Data from Durán Auccatinco (2001a).

		adults feeding on leaves	eggs per female when feeding on plants	egg viability	recovering after infestation with 25 females and 25 males			recovering after infestation with 500 neonate larvae		
		[mm^2]	eggs	[%]	adults	pupae	larvae	adults	pupae	larvae
crops										
Potato	*Solanum tuberosum*	1064	227	73	23	181	28	319	3	0
Bean	*Vicia faba*	543	8	52	0	0	0	--	--	--
Oat	*Avena sativa*	54	0	--	0	0	0	--	--	--
Oca	*Oxalis tuberosa*	186	1	0	0	0	0	--	--	--
Ullucu	*Ullucus tuberosus*	32	0	0	0	0	0	--	--	--
Mashua	*Tropaeolum tuberosum*	631	1	0	2	0	3	--	--	--
Quinua	*Chenopodium quinoa*	121	1	0	0	0	0	--	--	--
weeds										
Turnip	*Brassica campestris*	420	21	66	2*	2*	0	10*	0	0
Dantlion	*Taraxacum officinalis*	781	3	41	0	0	5*	0	0	0
Kikuyu grass	*Pennisetum clandestinum*	44	0	0	0	0	0	--	--	--
Storksbill	*Erodium* sp.	69	1	21	2	0	0	--	--	--
Bur-Marigold	*Bidens pilosa*	24	1	0	0	0	0	--	--	--
Sorrel	*Rumex* sp.	0	1	83	0	0	0	--	--	--
??	nn	3	0	--	0	0	0	--	--	--

Rearing

Rearing attempts including the whole life cycle have so far resulted in low numbers of adults mostly due to a very high mortality during pre-pupal and pupal stages. Yábar Landa et al. (2005) found at 18 - 20°C mortalities of 61 to 86% for the different immature life stages of *P. latithorax*. The only successful rearing method with 58% survival from pre-pupae to adult is described by Garzón C. et al. (1996) at temperatures between 10 and 22°C and 60% relative humidity: Eggs within straw together with potato tubers were kept under humid conditions on sterilized soil and placed at dark as soon as larvae had hatched and entered the tubers. Mature larvae leaving the tubers were placed again on sterilized soil for pupating. Emerging adults were fed with tubers and potato leaves and dry straw was offered for oviposition. Rearing on artificial media have been studied but were not successful so far (Zenner de Polanía 1990). For bioassays with *Bacillus thuringiensis* toxins, Gomez et al. (2000) kept first instar larvae until the 5th day on a diet made of sterilized, smashed potato and agar containing methyl-paraben and ascorbic acid.

Host plants

Information on possible host plants of APW is given by a variety of authors, however, it seems that in many cases these records are based on random observations rather than detailed studies. Moreover, many authors refer to APW larvae to be observed on host plants other than potatoes even though it is difficult to distinguish APW larvae from those of several other weevil species present in the same ecosystems.

The most detailed study on host plants of *P. latithorax* is presented by Durán Auccatinco (2001a) including crop plants (*Solanum tuberosum* L., *Avena sativa* L., *Chenopodium quinoa* WILLD., *Vicia faba* L., *Oxalis tuberosa* MOLINA, *Ullucus tuberosus* LOZANO and *Tropaeolum tuberosum* RUIZ ET PAV.) and weeds (*Brassica campestris* L., *Taraxacum officinale* WIGGERS, *Pennisetum clandestinum* HOCHST., *Erodium* sp., *Bidens pilosa* L. and *Rumex* sp.). Feeding of adults on foliage was reduced for all plants to 0 to 73% of the feeding area on potato. Oviposition while feeding on the different plant species was in most cases reduced to less then 1% of the results for feeding on potato and in most cases no larvae hatched. After infestation of the plants with adult APW, only very few weevils completing their life cycle from oviposition to adults (of reduced size) could be recovered from *T. tuberosum*, *B. campestris* and *Erodium* sp. A summary of the results is given in Table 5. As these plants belong to taxonomically distant orders to *Solanum*, there may exist more suitable hosts for APW. Pilco Nina (1978) found *P. solaniperda* completing its life cycle on *Senecio* sp., *Stipa ichu* (RUIZ ET PAV.) and *Herodium cicutarum* (L'HÉR.), though the experimental conditions were not well described.

Plants cited as alternative feeding hosts for adults of *P. vorax* are: *Ullucus tuberosus* LOZANO, *Vicia faba* L., *Brassica campestris* L., *Trifolium repens* L., *Rumex crispus* L., *Rumex acetosella* L., *Pennisetum clandestinum* HOCHST., *Erodium cicutarium* L., *Galinsoga parviflora* CAV., *Drymaria* sp., *Siegesbeckia cordifolia* H.B.K., *Salvia palifolia* KUNTH, *Raphanus sativus* L., *Solanum nigrum* L., *Solanum caripense* HUMB. & BONPL. ex DUNAL and *Capsella bursa-pastoris* (L.) MED. (Calvache Guerrero and Alvarado 1980, Muñoz Cáceres 1998). For *P. suturicallus*, *Tagetes* sp., *Brassica campestris* L., *Vicia faba* L. and *Erodium cicutarum* L. are listed (Alcalá C. and Alcázar

S. 1976) whereas for *P. piercei* and *P. pusillus Oxalis tuberosa* MOLINA, *Ullucus tuberosus* LOZANO and *Tropaeolum tuberosum* RUIZ ET PAV., all of them being tuber crops of the Andes, are cited (Molleda M. 1965, Tovar Gamarra 1988).

Calvache Guerrero (1986a) found larvae of *P. vorax* feeding on *Drymaria* sp., *Galinsoga parviflora* CAV., *Brassica campestris* L., *Raphanus sativus* L., *Trifolium repens* L., *Solanum caripense* HUMB. & BONPL. ex DUNAL, *Siegesbeckia cordifolia* H.B.K. and *Veronica persica* POIRET; however, these larvae remain small and do not develop to adults. The same findings have been reported by Alcázar (1995) who found *P. suturicallus* larvae feeding on *Brassica campestris* L., *Erodium cicutarium* L., *Tagetes minuta* L., *Astragalus* sp., *Stipa ichu* (RUIZ ET PAV.) KUNTH, and *Senecio* sp. Together with other authors, the same author showed that under laboratory conditions and in fields highly infested by *P. latithorax* and *P. suturicallus* the other high Andean tuber crops *Oxalis tuberosa* MOLINA, *Ullucus tuberosus* LOZANO, *Tropaeolum tuberosum* RUIZ ET PAV. and *Lepidium meyenii* WALP. were not or only superficially damaged (Vera et al. 1994, Alcázar et al. 1999, Mayta et al. 2001). Alcázar and Cisneros (1999) state, that *Premnotrypes* spp. larvae can not survive on other plants than potato.

These findings give evidence that APW can only in exceptions and with reduced fitness complete its life cycle in absence of potato. The role of the different *Solanum* species is discussed below. It may be concluded, that APW is an oligophagous insect restricted mainly to tuber forming *Solanum* species. Populations of APW probably survive through their longevity and by feeding on volunteer and wild potato plants, which are always present in the Andean regions. This hypothesis may be corroborated by the fact that APW species maintain low, however still lasting populations even under potato rotation systems of 5 to 7 years that keep long distances between subsequence production sites. Even far away from any potato field, APW was found on wild *Solanum acaule* plants (personal observations by one of us). Though the possibility of few cases of larvae completing their life cycle on a few alternative host plants should be further considered. Especially the reproductive fitness of the resulting adults needs attention.

Physiology

Dávalos Vallejo (1997) studied the anatomy and physiology of the digestive gut of larvae of *P. latithorax* including the enzymatic activity and the protein content.

Table 6: Damage rates caused by the Andean potato weevil on potato tubers in the Andean region. *No indication whether insecticides were used.

Species and region	Mean damage [%]	Use of insecticide		References
		No	Yes	
Premnotrypes spp.				
Southern Peru	26	*		(Yábar Landa 1994)
	30		X	(Medina et al. 1999)
	31	X		(Alcázar et al. 1992)
	23		X	(Alcázar et al. 1992)
	30	*		(Delgado Mamani and Fernández Aquise 1993)
	8		X	(Vallenas Bellota 1998)
Central Peru	16	*		(Ewell et al. 1990)
	20	*		(Horton et al. 1980)
	38	*		(Carhuamaca Ticse and Ore Meza 1993)
Northern Peru	45 - 74	*		(Cerna 1993)
Colombia	10-25	*		(Alvarado et al. 1995)
Ecuador	41 - 45	*		(FORTIPAPA 1993)
Bolivia	30 - 47	*		(Chavez Ajata 1997)
	34	*		(Munro 1968)
	45	*		(Esprella et al. 1998)
	16-25	*		(Calderón 1993 in: Calderón et al. 2004)
	54-60	*		(Andrew and Herbas 1992 in: Calderón et al. 2004)
Rhigopsidius spp.				
Bolivia	4		X	(Herbas 1995)
	24	X		(Herbas 1995)
	28 - 85	X		(Barea et al. 1996/1997)
Argentina	24	*		(Agostini de Manero 1993)
Phyrdenus muriceus				
Bolivia	2-18	*		(Lino et al. 1995 in: Calderón et al. 2004)

Economic relevance

Raman et al. (1994) consider that, beside potato tuber moth, green peach aphid and leaf miner fly, APW is one of the mayor insect pests attacking potato in the developing world. In the Andes from Colombia to Argentina, it is in many cases considered the main insect pest in potato production above 3000 meter altitude (in Argentina above 2000 meter) (Ewell 1990, Calvache Guerrero 1991, Herbas Chavez 1992, Agostini de Manero 1993, Yábar Landa 1994, Ortiz et al. 1996). *Premnotrpes* spp is listed by the European and Mediterranean plant protection organization (EPPO) and by the Comité de sanidad vegetal del cono sur (COSAVE) as a quarantine pest with the potential to adapt to the respective climates, justifying post-entry quarantine procedures (COSAVE, OEPP/EPPO 1984). *Rhigopsidius* spp. though more easily brought in through tubers, is not listed.

Damages are principally caused by the larvae feeding on the tubers making them unmarketable and reducing yields by up to 50% and causing heavy commercial losses (Borda Velasco 1994, Ortiz et al. 1996). Adults cause only minor, characteristic half moon shaped damages to the border of the leaflets that does not affect crop development (Alcalá C. and Alcázar S. 1976, Barea et al. 1996/1997, Gallegos G. et al. 1997). But Zenner and Posada O. (1968) found that under laboratory conditions 5 adults may destroy completely a young potato plant within 3 days and Aréstegui P. (1976) affirms, that a high population in the young crop may cause complete defoliation. Durán Auccatinco (2001a) determined, that one adult may feed on 10 cm^2 during a life time of 5 months.

Mean damage rates vary between 16 and 45% (see Table 6) while in absence of the use of insecticides, up to 70 to 100% of the yield may be damaged leading farmers to abandon their fields (Ortiz et al. 1996). Damage may not be visible at harvest (especially at early harvest) and only become apparent after storage when larvae mature and leave the tubers (Yábar Landa et al. 1995). Ewell et al. (1990) indicate that in farmers' fields at harvest, 10% of 51 samples revealed over 50% damage. Above 4000 m damage rates by APW is significantly reduced (Tardieu et al. 1980, Ewell et al. 1990). In Bolivia, damage by APW was significantly lower during the July-December cropping season than during November-Mai season (Calderón, 1993 in: Calderón et al. 2004). Ortiz et al. (1993) calculated, that in southern Peru 31% of damaged tubers at harvest resulted in a lost of 276 US$/ha; a value quite similar to what is reported for Colombia (Torres Pérez and López-Avila 1997). On the other hand, damages produced by APW will reduce the commercial value of tubers by 22 to 50% (Gallegos G. et al. 1997). Price decrease for tubers with 25% of damage by APW was 35 to 48% in Ecuador (Waarts 2003). In Colombia farmers spend around 22 million US$ per year in insecticides to control APW, an amount which corresponds to 90% of the total insecticide costs in potato production in this country (Calvache Guerrero 1991, Trillos G. et al. 1993). In Ecuador and Colombia costs for insect pest control vary between 7.5 and 21.3% of total potato production costs (Gallegos G. and Avalos P. 1994/95, Corporación Colombiana de Investigación Agropecuaria 2000).

In Bolivia, below 2000 meter damage caused by Ph*yrdenus muriceus* varies between 2% in dry season crop production and 17 to 18% in rainy season crop production (Lino et al., 1995 in: Calderón et al. 2004). The adults attack the leaves and the stem of the potato plant and may cause its death. In the Córdoba region in Argentina and in Brazil it is also considered an important potato pest (Dos Santos et al. 1982, Novo et al. 2002), though in most part of tropical South America it is frequently referred to as tomato weevil attacking mainly tomato (Sibaja Chinchilla and Sanabria Ujueta 2002, Morales Valles et al. 2003) but damaging also other solanaceae like eggplant (*Solanum melongena* L.) and cocona (*Solanum sessiliflorum* DUNAL) (Ministerio de Agricultura del Perú, Novo et al. 2002).

Most of the time, damaged tubers, rejected by the market, are still used as seed potatoes, for direct consumption, for the production of chuños (a traditional freeze-dried like processing and conservation method) or for animal feeding, making the losses less pronounced for farmers producing for their own consumption than for market-orientated farmers. Subsistence farmer normally accept 20 to 30% damage as normal while for seed producers a damage rate of more than 10% is not acceptable (Ewell et al. 1990, Ortiz et al. 1996).

Some authors calculated damage thresholds; however, these calculations are mostly not based on experimental results. Recommendations vary between 2 and 5 adults per potato plant for *P. vorax* and *P. latithorax* (Ortiz Romero et al. 1996, Gallegos G. et al. 1997) while at other places no relation between captured adults and damage at harvest was observed (ICA 1986). For *Rhigopsidius piercei* a damage threshold of 2 larvae per 1 kg sample of seed tubers should not be exceeded (Vigiana and Serrano 2001).

Table 7: Control strategies against the Andean potato weevil and their efficacy. [1](FORTIPAPA 1993, Crespo V. et al. 1998, Gallegos G. et al. 2001, Catalán Bazán 2003, Calderón et al. 2004) ; [2](Torres et al. 1993, Zavala Aguirre 1995, Vera Robles 2001); [3](Vera Robles 1992, Peña Villamil et al. 1999b, Torres Torres and Marina Cotes 1999, International Potato Center, unpblished data); [4](Hernandez R. and Bohorquez B. 1992, Fernández and Colmenares 1997, Ortega 1998, Quispe Araujo 1999, Corporación Colombiana de Investigación Agropecuaria 2000, Peña Villamil et al. 2000); [5](Alcázar Sedano 1988); [6](Ñopo et al. 1998); [7](Chavez Ajata 1997, Medina et al. 1999); [8](Programa Andino Cooperativo de Investigación en Papa 1990); [9](Catalán Bazán et al. 1993, Alcázar et al. 1995, Cárdenas Flores 2000); [10](Gallegos G. and Avalos P. 1994/95, Suquillo et al. 1998, Niño et al. 2000, International Potato Center, unpubished data); [11](Gandarillas and Perpich 1992, Quispe Pérez 2001); [12](Catalán et al. 1993, Esprella et al. 1997 in: Calderón et al. 2004); [13](Carvajal Pinto 1993, Cerna 1993, Borda Velasco 1994, Barea et al. 1996/1997, Cárdenas Flores 2000).

Control measure	Way of application	Effective control	Comments	Ref.
Chemical insecticides		50-90%	Carbofuran, Triflumuron, Clopirifos, Fipronil, Difonate	1
Biological insecticides	Store	50-95%	*Beauveria* sp.; Control of next year generation	2
	Field	0-75%	Good results in laboratory, but until now, no consistent results in the field	3 (lab.) 4 (field)
Plant resistance	Conventional	Up to 90% in one clones of *S. andigena*	Not used in breeding programs	5
	Transgenic	100%	Not used in breeding	6
Crop rotation	Communal	80%		7
	Individual	67%		8
Early harvest		Up to 94% (12% per advanced week)	Established under southern Peruvian conditions	9
Shelter traps/bait crop		70 to 93%	Useful if mayor population of APW emerges before the crop	10
Border application of insecticides		69 to 94%	Minimum 3m border width, 50% of insecticides reduction	11
Ditches around field		70%	50% of insecticides reduction	12
Plant barriers		No effect	Plant species tested are *Oxalis tuberosa*, *Ullucus tuberosus*, *Tropaeolum tuberosum*, *Lupinus mutabilis* and *Alium* sp.	13

Control

Monitoring

So far no satisfactory monitoring method exists. Adults are inconspicuous and difficult to detect in the field and foliar damage is most of the time only seen if population density is already much too high. Still, nightly revision of plants or of the surrounding soil for adults may be the most usefull method. Due to the aggregated distribution of APW the field should be divided into 5 sectors for an adequate estimation of the APW population and for detecting the direction of immigration of the adults (Yábar Landa 1994). In areas with year around potato production like Ecuador or Colombia, the use of shelter traps, consisting of potato leaves with insecticide applications covered with a 50 x 50 cm piece of cardboard or jute, before emergence of the crop may help to assess APW population (Peña Villamil et al. 1999a). These shelter traps create humid and dark conditions favorable for sheltering of APW adults. They may also be used for monitoring when and from which side the weevils enter into the field. This may permit to reduce pesticide use by applying only to the indicated borders (Calderón et al. 2004). Pitfalls used by some authors gave unsatisfactory results after emergence of crop (Zenner de Polanía 1990).

A summary of the main control strategies and their efficacy to contol APW is shown in Table 7.

Chemical control

The most common control measure is the use of insecticides based on Metamidophos and Carbofuran (wettable powder or granulate) and to a lesser extent Cypermethrin and other pyrethroids as well as Aldicarp (personal data), though not always with satisfactory results (Ortiz et al. 1993, Calderón et al. 2004). Many papers refer to experiments using chemical insecticides with inconsistent data and in many times lacking an investigation strategy. In field experiments without or with a low number of repetitions, the unpredictable and heterogeneous distribution of APW makes the evaluation difficult. For application strategies one has to keep in mind the prolonged laps of time during which the pest migrates into the crop.

Carbofuran is the insecticide most frequently tested. It seems to give sufficient protection with over 50% damage reduction, even though there are still cases with up to 30% damage. Using Carbofuran as a standard, Triflumuron, Tefluthrin, Clorpirifos, Difonate, Lamda-Cyhalotrin and Fipronil were tested positively in the field (FORTIPAPA 1993, Crespo V. et al. 1998, Gallegos G. et al. 2001, Catalán Bazán 2003, Calderón et al. 2004). Tefluthrin (granulated), Diflubenzuron, Foxin, Diazinon, Izasophos and Dimilin did not result in sufficient APW control (Calvache Guerrero 1986b, Bejarano 1994, Gallegos G. and Castillo 1995). Laboratory trials with triflumuron and Diflubenturon resulted in 30 to 66% reduction of egg viability (Anonymous 1985, Carrasco Gamarra 1992, Vasquez Porras and Suarez Lopez 1997). Median lethal concentrations (LC_{50}) for Carbufuran, Permetrin, Metamidophos, Azinfos Metil and Azinfos Etil were 0.2, 0.5, 1.8, 6.3 and 10.6 mg/l respectively (Catalán Bazán 2003). Calderón et al. (2004) report the testing of many insecticides in Bolivia and recommend Fipronil and Lambda-Cyhalothrin for the control of *P. latithorax*, Lambda-Cyhalothrin, Furathiocarb, Stermin and Carbofuran for the control of *R. piercei* and Triflumuron and Lambda-Cyhalotrin for the control of *P. muriceus*. In all three cases applications should be made at crop emergence and at hilling. In Colombia an additional application at planting is recommended for late potato varieties (Gandarillas and Perpich 1992). A reduced application of Triflumuron only to the lower part of the potato plant and to every second row resulted in the same control as conventional applications (Gallegos et al. 2003a).

In a study on pest resistance against the insecticides Carbufuran, Permetrin, Metamidophos, Azinfos Metil and Azinfos Etil with field collected populations of *P. latithorax* and *P. suturicallus* from agricultural areas with high and with low insecticides input only slide and inconsistent differences were found (Catalán Bazán 2003).

Biological control

The main pathogen tested up to date is the fungus *Beauveria* sp. first reported in 1976 parasitising *P. suturicallus* (Alcalá C. and Alcázar S. 1976). In field collections, 1 to 12% of pupae were naturally infested with *Beauveria* sp., while in collections from the soil of potato stores 11 to 28% infestation was found. In general, prevalence is higher in stores than in fields (Villano Tárraga 1994, Cisneros and Vera 2001, Ravines Novoa 2002, Niño et al. 2004). In Colombia Torres Torres and Marina Cotes (1999) found 3% infection with *Beauveria brongniartii*, 4% with *B. bassiana* and 1% with *Metarhizium anisopliae*.

Bioassays concentrated mostly on mature forth instar larvae and on adults collected from the field. Depending on the isolate, LC_{50} for larvae varied between 3.2×10^3 and 7.8×10^7 conidia/ml with a strong increase of efficacy after 6 days and for adult between 5.5×10^6 (after 12 days) and 1.2×10^{11} conidia/ml (Peña Villamil et al. 1999b, Torres Torres and Marina Cotes 1999, Corporación Colombiana de Investigación Agropecuaria 2000, International Potato Center, unpubished data). Though Gallegos et al. (2003b) and Albarracin Triveño (1997) reached for some isolates 100% mortality after 10 respectively 15 days with around 1×10^7 conidia/ml. Eggs resulted being the least susceptible stage (Vera Robles 1992). Applying 1×10^7 conidia/ml, mortality of first instar larvae feeding on potato tubers and evaluated after one month was 12 to 51% (Castro Marín 1997). No activity of *Beauveria bassiana* on L1 larvae passing through inoculated soil was observed (FORTIPAPA 1993). Under laboratory conditions, *Metarhizium anisopliae* showed similar (Torres Pérez and López-Avila 1997, Torres Torres and Marina Cotes 1999, Corporación Colombiana de Investigación Agropecuaria 2000) or minor (Castro Marín 1997, Barriga et al. 2002, Gallegos et al. 2003b) activity than *B. bassiana* against APW adults and forth instar larvae. *Verticillium lecanii* too resulted less effective against forth instar larvae than *B. bassiana* (Ortega and Torres 1994). In greenhouse trials with soil applications of *B. brongniartii* in potted potato plants, adult mortality was up to 70%, though no data on tuber damage is given (Albarracin Triveño 1997).

Under storage conditions, satisfactory results have been found with 50 to 97% mortality (Torres et al. 1993, Zavala Aguirre 1995, Vera Robles 2001). In the stores, below the potato heap, conditions are much more favorable for the development of the fungus then under field conditions with low night temperatures and high day temperatures, low relative air humidity and strong sun radiations.

Due to poor experimental design, incomplete information about the doses, application methods and treatments like “farmer’s control” and/or incomplete analysis no consistent data on field trials with entomopathogenic fungus exist and comparing results is difficult. Even applying high doses, results vary between no effect (Hernandez R. and Bohorquez B. 1992, Quispe Araujo 1999, Pérez Rubiano 2002), little to medium effects (22-60% control) (Fernández and Colmenares 1997, Moreno Coronel 1998, Ortega 1998, Corporación Colombiana de Investigación Agropecuaria 2000) and satisfactory (75%) control (in this last case, control damage too was very low) (Peña Villamil et al. 2000).

Applying high doses under shelter traps (see above), led to 40 to 50% of adult mortality (Gallegos and Asaquibay 2004).

Gallegos G and Garcés (1999) tested some biological insecticides for their efficacy against adult *P. vorax* under laboratory conditions. Spinosad and Naturalis-L (with *Beauveria bassiana* as active ingredient) caused 95 and 80% mortality after 6 days while Raven (a.i.: *Bacillus thuringiensis kurstaki*) and Cryolite resulted in only 25% mortality.

The entomopathogenic nematode *Steinernema carpocapsae* was tested with an LC_{50} of 114 nematode/ml after 4 days for last instar larvae (Garzón C. et al. 1996). High concentrations of the nematode *Neoplectana carpocapsae* resulted in over 95% mortality at *P. vorax* (Amaya and Bustamante 1975). Alcázar and Kaya (2003) report another nematode (*Heterorhabditis* sp.) from larvae collected in potato stores. Under laboratory conditions, this nematode showed promising results, controlling APW larvae before significant damage incurred to the tubers. LC_{50} for pre-pupae was found to be below 6 infective juvenils, but also adults were found to be susceptible. Additionally, the cold tolerance of this nematodes was demonstrated in bioassays with *Galleria mellonella* (Parsa et al. 2006). Several isolates and pure Cry toxins of *B. thuringiensis* collected from different kinds of habitats had no or very low effect on adults and neonates of *P. vorax*, *P. latithorax* and *R. piercei*. Only the pure toxin Cry3A resulted in high mortality with *R. piercei* (Ferrandis et al. 1999, Uribe et al. 2003, Martínez O. and Cerón S. 2004, Hernández et al. 2005). Though, using the same toxin, Gomez et al. (2000) observed after 5 days a mortality of 43 to 49% on neonate larvae of *P. vorax* when 70µg/ml of the purified toxin was applied on the surface of an artificial medium. With the same method, Mateus Virviescas (1998) found a mortality of neonate larvae of 87% with a concentration of 1000µg/ml of the strain *B. thuringiensis var. tenebrionis* and of 48 to 61% with native isolates. Other entomopathogens tested on *P. vorax* are *Bacillus popilliae*, *B. lentimorbus*, *Geotrichum* sp. and *Spicaria* sp with no and uncertain effect (Amaya and Bustamante 1975).

Plant resistance

In most field experiments no consistent data were obtained with regard to the resistance of potato germplasms against APW. This is confirmed by observations from farmer fields where no differences in damage levels were found between any native or improved variety (Ewell et al. 1990). Out of four hybrid groups (397 clones in total) of

Solanum poliadenium, *S. berthaultil*, *S. chacoense* and *S. phureja*, Trillos G. et al. (1993) found 6 hybrid clones of *Solanum polyadenium* x *S. chacoens* and *S. polyadenium* x *S. phureja* with moderate resistance to *P. vorax;* all of them also contained high glycoalcaloide concentrations. In trials with *S. juzepczukii*, *S. stenotomum*, *S. ajanhuiri*, *S. andigena* (cultivated native potato species) and *S. acaule* (a wild potato specie) Alcázar Sedano (1988) found resistance in *Solanum acaula* to *P. suturicallus* with reduced adult feeding on leaves and a reduced oviposition rate and one clone of *S. andigena* exhibited a significantly higher mortality in APW larvae. Besides this, a relation between depth of tuberisation and damage caused by APW was established. Working with *P. latithorax*, out of 100 clones (58 clones of *S. andigena*, 8 of *S. stenotonum*, 3 of *S. curtilobum*, 1 of *S. Juzepczukii* and 30 not determined clones), Ramirez Ticona (1971) found 2 clones of *S. andigena* completely resistant and 7 more of the same species highly resistant (No replication was made of this trial). In all three cases resistance was not consistent over the species as other clones of *S. andigena* were highly susceptible, but was clone specific. Out of 575 accessions of the genbank of the International Potato Center evaluated by 2003 for APW resistance, most of them *Solanum andigena*, 21 are registered as resistant and 38 as moderately resistant. No correlation towards other evaluation data was found. Other studies evaluating commercial potato varieties in the field (Olivares Garces et al. 1973, Meza Rojas 1978, Romero Chávez 1983) and in the laboratory (Calderón et al., 1996 and Crespo et al., 1997 in: Calderón et al. 2004), found no effect of the variety on APW damage.

Ñopo et al. (1998) studied transgenic potatoes with different insecticidal genes of plant origin. One potato line with wheat alpha-amylase inhibitor and soybean trypsin inhibitor genes was resistant to attacks of *P. suturicallus* larvae, while 7 other line with other double gene constructs were moderately resistant.

Natural enemies

Little is known of species preying on APW. Yábar Landa (2001) studied four carabids collected in potato fields and found a high egg predations rate for *Bembidion* sp., a medium rate for *Metius* sp. and *Notiobia* sp. and no egg predation for *Mimodromius* sp. Other references mention various carabids (*Harpalus turmalinus*, *Metius* sp., *Stenolophus* sp. and *Pterostichus* sp.) and a darkling beetle (*Hylitus* sp.) as egg predators are and also some larvae predators (*Notiobia* sp. and *Meotachys* sp.) (Alcalá C. and Alcázar S. 1976, Delgado Mamani 1992, Yábar Landa 1994, Loza del Carpio 1999). Toads too have been regarded as important predators (Alcázar and Cisneros 1999), even though they have nowadays almost disappeared from potato fields, probably due to the use of chemical fertilizer and pesticides (Alcázar, personal communication). No parasitoids attacking APW are known (Alcázar and Cisneros 1999).

Agricultural control strategies

In traditional communitarian rotation systems, damage is normally below the economic threshold level. In this complex, so called sectorial fallowing systems, which are still in use in a significant part of remote southern Peru and in Bolivia, a large part of land of a community is divided into a few sectors with up to several hundreds of ha each. All these sectors pass through the same sequence of uses of generally 5 to 7 years and in any given year only one sector will have each cropping use (Orlove and Godoy 1986). This leads to considerable distances between the potato cropping sectors of consecutive years, a gap difficult to overcome by the flightless APW adults. For example, damage in such systems in southern Peru was below 10% even without any application of insecticides (Medina et al. 1999, Durán Auccatinco 2001b). In Bolivia, in one community with small plots (normally under 1 ha each) and individual rotation management 47% damaged tubers were found while in the adjacent community with communitarian rotation system only 8 to 12% damage was observed (Chavez Ajata 1997). Individual rotation systems suffer always of high pressure of immigrating pests, especially if plots are small (below 0.5 ha) as frequently encountered in the Andes. Nevertheless, in fields with rotation, mean damages of 22% have been observed as compared to 68% in fields without rotation (Programa Andino Cooperativo de Investigación en Papa 1990).

Under the conditions of one cropping period per year Catalán Bazán et al. (1993) and Cárdenas Flores (2000), found in the Cusco area and in southern Peru, a highly significant relation between harvesting date and tuber damage. In the first case, percentage of tubers damaged by APW increased from 1.2% to 10.8% and to 19.6% if harvested on March 25, on April 25 and May 15 respectively. In the second case, damage increased from 9.2% over 49.5% to 68,31% if harvested on April 10, on May 10 or May 30. When standardizing the data by putting the maximum damage for each study to 100%, both data sets produced parallel linear regressions with damage rates rising by 1.8% per day between end of March and end of May (Figure 4). Evaluating the harvest from 82 farmer fields Alcázar et al. (1995) found similar tendencies.

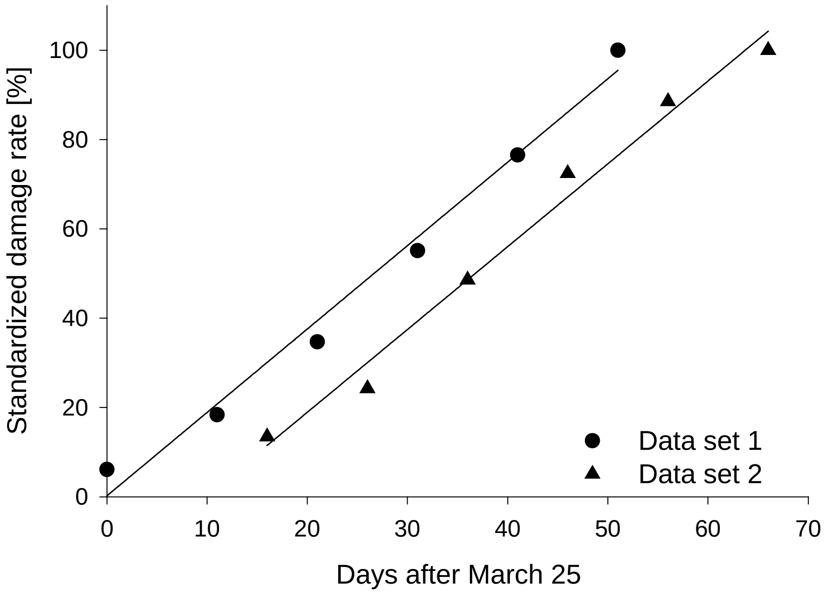

Figure 4: Relation between harvest date and tuber damage caused by the Andean potato weevil. Tuber damage has been standardized by setting the maximum damage of each data series to 100%. Linear regressions are $y = 0.2153 + 1.8679x$ ($R^2 = 0.9854$) for data set 1 (Catalán Bazán et al. 1993) and $y = -18.1743 + 1.8548x$ ($R^2 = 0.9851$) for data set 2 (Cárdenas Flores 2000).

Especially in Ecuador, Colombia and Venezuela the use of shelter or bait traps, as described above, distributed before planting and until emergence of the crop over the field in 10 to 12 m distance and bait crop (potatoes planted 3 to 4 weeks before the main crop with applications of insecticides) has given good results (Gallegos G. and Avalos P. 1994/95, Suquillo et al. 1998, Niño et al. 2000, Gallegos et al. 2004a). Under circumstances, where the mayor part of the adults emerges before the emergence of the crop, as it is the case in the northern part of the Andes, shelter traps may be a viable option. Under central Peruvian conditions, use of shelter traps during 8 to 9 weeks before emergence of the potato crop reduced damage rate by 70 to 75% (International Potato Center, unpubished data) but this requires late planting of the crop. Here, the recommendation to plant only when weevil number captured in shelter traps decreases may pose conflicts with the recommendation of early planting and harvest to escape late infestation and frost damage. Distributing straw of *Stipa ichu* as oviposition medium within the potato field and recollecting it after a maximum of 25 days, Gallegos, Asaquibay and Williams (2004b) reached a damage reduction of 30%.

Table 8: Comparison of the importance of infestation sources for populations of the Andean potato weevil. The number of pre-pupae, pupae and adult weevils found in the soil is quoted [1](Catalán Bazán et al. 1993), [2](Alcázar et al. 1992), [3](Salazar Benitez 1996), [4](Villano Tárraga 1994), [5](Cerna 1993), [6](Vera Robles 2001), [7](Alvarado et al. 1995), [8](Sotelo Ravichagua 1996), [9](Chavez Ajata 1997), [10](Crespo et al. 1995 in: Calderón et al. 2004), [11](Ortega 1998).

	Harvested field **[weevils/m^2]**	Tuber heaping site at harvest **[weevils/m^2]**	Pre-storage and storage area **[weevils/m^2]**	Abandoned field **[weevils/m^2]**	Volunteer plants **[plants/ha]**	Volunteer plants **[weevils/plant]**	Ref.
	2.5	706	1700	300	8500	9	1
	12-40		3000	300		9-15	2
	2.4-7.7		84.5				3
	62-318		56-397				4
	7	615	865-2000			12	5
	475	2681					6
	7.6-30						7
					6000		8
						27	9
		8540-10860	120				10
						9	11
means	88	3428	1225	300	7250	13.8	

For controlling the migration of weevils into the field, directed applications of insecticides only on border rows of the crop are recommended. This is applicable under any conditions as long as rotated fields are concerned. Applying insecticides 2 or 3 times (at emergence, hilling and 30 days later) on a border of 3 to 5 m around the crop, reduced damage by 69 to 94% and costs were reduced by 50% (Gandarillas and Perpich 1992, Catalán Bazán et al. 1993, Quispe Pérez 2001). Applying twice on only 1 m around the crop, Borda Velasco (1994) found no control effect. Digging ditches around the field before the emergence of the crop and applying insecticides into these ditches, gave comparable results (Catalán et al. 1993, Esprella et al., 1997 in: Calderón et al. 2004).

Several authors also mention the use other crops planted as barriers around the potato crop. Crops, which have been used as barriers are *Oxalis tuberosa*, *Ullucus tuberosus*, *Tropaeolum tuberosum*, *Lupinus mutabilis*, *Alium* sp. and graminae; however, non of these had a significant effect on the damage caused by APW (Carvajal Pinto 1993, Cerna 1993, Borda Velasco 1994, Vargas Oña 1994, Alcázar et al. 1995, Barea et al. 1996/1997, Gallegos et al. 2004a).

To prevent the establishment of too high pupal populations simple methods like heaping the harvested tuber up on plastic sheets are commonly recommended by the extension services. This prevents larvae abandoning the tubers to enter the soil for pupating in soil. Last instar larvae tend to leave tubers exposed to sun light within a few hours (Carrasco Zamora 1991).

Management of infestation sources is another important option to reduce APW population densities, though it usually requires the action of the whole farmer community to have an effect on the damage rate of APW. The harvested field, the sites for heaping up tubers at harvest, selecting or pre-storage sites, storage areas, abandoned fields (mostly abandoned for high weevil damage incidence) and volunteer plants have been identified as infestation sources (Table 8). Storage areas and heaping sites concentrate huge numbers of pupating/over-wintering weevils on small areas. These are easy to control by simple soil tillage. On the other hand, the number of pupae found in harvested fields, is probably higher, even though the concentration per square meter is lower. This and the fact that the fields are in general closer to the potato fields of the next cropping season then the storage area makes them probably the more important infestation source. To control these populations, soil tillage to exposing larvae/pupae and elimination of volunteer plants are general recommendations. In storage areas, the application of the entomopathogenic fungus *Beauveria* sp. is recommended (see above). Volunteer plants should be eliminated at the time of tuber formation, after oviposition to destroy eggs and larvae stages. Early elimination would cause adults to migrate towards other fields for oviposition (Catalán Bazán et al. 1993).

Besides the methods discussed above, some more measures are mentioned: Use of seed free of APW damage as this may reduce emergence and yield by 20 to 30% (Salazar Lermo et al. 1997, Frankhauser 1999). Last instar larvae tend to leave tubers when these are exposed to light. After ten days of storage under diffuse light conditions, the tubers were free of larvae. This assures, that seed tubers will be free of APW and will reduce the risk of a further dissemination of APW to formerly APW free sites (Yábar Landa 1986). Infested seed tubers are an important of dissemination of APW in Colombia (Corporación Colombiana de Investigación Agropecuaria 2000). In the case of *Rigopsidius* spp., which pupates within the tubers and seed tubers are the main infestation source, the use of clean seed is essential. Submerging seed tubers into insecticides reduced the incidence of *R. piercei* by 78% (Vigiana and Serrano 2001, Herbas, 1996 in: Calderón et al. 2004). Treatment of seed tubers with high temperatures or placing seed tubers into plastic backs made larvae leave the tubers and had similar or better effects than insecticides. Greening of tubers and treatment with chili had no effect (Bejarano et al., 1995, 1996 and Crespo et al., 1997 in: Calderón et al. 2004). Nightly handpicking of adult weevils and the use of chicken feeding on exposed larvae at harvest are other commonly recommended strategies. Some authors mention control effects of field applications of repellent plants like *Minthostachys* sp. (traditionally used by farmers in stores) (Ortega 1998), though mostly results are negative (Ortiz Romero 1997). Under laboratory conditions, 4-metoxibencilisotiocianate, a glucosinolate extracted from *Tropaeolum tuberosum* showed slight repellent effects on adults of *P. suturicallus* (Cañedo et al. 2005). Some farmers place pieces of meat into potato stores attracting ants of the genera *Eciton* and *Iridomirez* that feed on mature weevil larvae (Garmendia Lorena 1961, Delgado P. 1974; also personal data).

Implementation of different sets of IPM strategies in pilot units led to reductions of 45 to 80% of damage by APW (Esprella et al. 1998, Alcázar et al. 2001, Calderón et al. 2004) and a cost reduction of 124 to 287 US$/ha depending on how strongly the farmers were orientated towards the markets (Ortiz et al. 1996, Unda et al. 1999). Almost all of the formerly mentioned strategies are recommended in varying combinations depending on the country and the agro-ecological conditions.

Conclusions

This compilation gives an overview on the actual knowledge on APW from its whole distribution area. Many findings from references formerly only locally accessible are brought together and made accessible to a broader public.

The following points may need further attention: 1. More knowledge on the ecology of APW is needed for a better adaptation of control strategies. Existing studies were run under uncontrolled conditions leading to data difficult to interpret due to varying variables. Biotic and abiotic factors influencing the life history should be studies more in depth. Especially the influence of climatic conditions should be investigated through life cycle studies under controlled temperature and humidity conditions. 2. For a strategic planning of an integrated pest management a better understanding of the population dynamics is needed. Therefore, the migratory behaviour and capacity has to be studied on farmer community level. Its flightlessness should keep the range of actions of the insect limited. Additionally the effects of the cropping system and the still unclear role of possible alternative host plants on the population dynamics needs more research. 3. For the opportune application of control measures, a user-friendly monitoring system (may be based on the already used shelter traps) has to be developed. 4. Contradictory data is presented on the efficacy of the control with *Beauveria* spp. Especially field trials suffer from incomplete designe. Research should elucidate the interaction between both organisms to get tools to decide whether to continue or abandon investments in this biological control agent. 5. Research is needed on other biological control agents. Entomopathogenic nematodes need more attention. Parasitoids, especially egg-parasitoids, have never been looked for. A limiting factor for any biological agent may be the climatic conditions above 3000 meter and up to over 4000 meter altitude.

Acknowledgments

The authors are grateful to the staff of CIP library for their help in getting all the references and to the numerous other people who send us articles or theses from Peru, Venezuela, Colombia, Ecuador, Bolivia and Argentina.

References cited

Anonymous (**1985**). Control del gorgojo de los Andes, *Premnotrypes vorax*. Proceedings of the Programa Andino Cooperativo de Investigación en Papa, Reunion anual de evaluación, Informes tecnicos, 02/11-14/1985, Mérida (Venezuela), Programa Andino Cooperativo de Investigación en Papa.

Agostini de Manero, E. (**1993**). Distribution and degree of infestation of *Rhigopsidius piercei* Heller (Coleoptera: Curculionidae) in Jujuy province, Argentina. Revista de la Sociedad Entomológica Argentina **52**(1-4): 29-35.

Agostini de Manero, E. and H. Vilte (**1982**). Morphological and biological study of *Rhigopsidius tucumanus* Heller (Coleoptera: Curculionidae), a potato pest in the Quebrada de Humahuaca and the Puna of the province of Jujuy, Argentina. Revista Agronomica del Noroeste Argentino **19**(1-4): 5-42.

Albarracin Triveño, M. L. (**1997**). Pruebas preliminares del uso del hongo *Beauveria brongniartii* para el control de los gorgojos de los Andes *Premnotrypes latithorax* y *Rhigopsidius tucumanus*. Cochabamba (Bolivia), Thesis of the Universidad Mayor de San Simon**:** 95pp.

Alcalá C., P. (**1979**). Nueva especie del genero *Premnotrypes* Pierce, 1914 (Coleoptera: Curculionidae). Revista Peruana de Entomología **22**(1): 63-64.

Alcalá C., P. and J. Alcázar S. (**1976**). Biología y comportamiento de *Premnotrypes suturicallus* Kuschel (Col.: Curculionidae). Revista Peruana de Entomología **19**(1): 49-52.

Alcázar, J. (**1995**). Biología y comportamiento del gorgojo de los Andes. In: Memorias del II curso internacional de manejo integrado de plagas de papa. Curso de capacitación de distancia. E. N. Fernandez-Northcote (Ed.). Cochabamba, Instituto Boliviano de Tecnología Agropecueria - Programa de Investigación de la Papa: pp. 76-83.

Alcázar, J., R. Aldana and F. Cisneros (**1999**). Identidad de los gorgojos que atacan papa, oca, olluco y mashua. Proceedings of the XLI Convención nacional de entomología, programa y resumenes, 10/24-28/1999, Tumbes (Peru), Sociedad Entomologica del Perú.

Alcázar, J., W. Catalán Bazán, F. Zevallos G. and F. Inquiltupa A. (**1995**). Avances del proyecto manejo integrado del gorgojo de los Andes y polilla de la papa - Cusco: Informe anual. Cusco (Peru), International Potato Center**:** 64.

Alcázar, J. and F. Cisneros (**1997**). Integrated Management for Andean potato weevil in Pilot Units. In: Program Report 1995-1996. B. Hardy and B. Smith (Eds). Lima, International Potato Center: pp. 169-176.

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

Alcázar, J., F. Cisneros and A. Lagnaoui (**2001**). El Gorgojo de los Andes, *Premnotrypes* spp. en la region andina. Proceedings of the XLIII Convención nacional de entomología, 11/4-8/2001, Huancayo, Sociedad Entomologica del Perú. p. 169.

Alcázar, J., D. Hall, L. Crespo and C. Mendoza (**2004**). Capacidad de atracción de adultos dem gorgojo de los Andes *Premnotrypes suturicallus* Kuschel entre individuos de la misma especie y por hojas de papa. Proceedings of the XLVI Convención nacional de entomología., 11/07-11/2004, Arequipa (Peru), Sociedad Entomologica del Perú.

Alcázar, J. and H. Kaya (**2003**). Hallazgo de un nematodo nativo del genero *Heterorhabditis*, parásito del gorgojo de los Andes *Premnotrypes suturicallus*, en Huasahuasi, Junín. Proceedings of the XLV Convención nacional de entomología, 12/01-05/2003, Ayacucho, Sociedad Entomologica del Perú. p. 158.

Alcázar, J., K. V. Raman, W. Catalán and F. Cisneros (**1992**). Diseño y ejecución de un programa de control integrado del gorgojo de los Andes en el cultivo de papa en Huatata, Chinchero, Cusco. Unpublished Manuscript, INIAA - Cusco, Cusco (Peru).

Alcázar Sedano, J. G. (**1976**). Biología y comportamiento del "gorgojo de los Andes" *Premnotrypes suturicallus* Kuschel (Coleoptera: Curculionidae). Huancayo, Thesis of the Universidad Nacional del Centro del Perú**:** 108pp.

Alcázar Sedano, J. G. (**1988**). Evaluación de la Susceptibilidad de seis Especies Tuberiferas de *Solanum* al "Gorgojo de los Andes" *Premnotrypes suturicallus* KUSCHEL (Coleoptera: Curculionidae). Lima, Thesis of the Universidad Nacional Agraria La Molina**:** 199pp.

Aldana Yurivilca, G. S. (**2003**). Biología y medidas de control del gorgojo del ulluco *Amathynetoides nitidiventris* Hustache (Coleoptera: Curculionidae) en el departamento de Junín. Huancayo (Peru), Thesis of the Universidad Nacional del Centro del Perú**:** 132pp.

Aldana Yurivilca, W. R. (**2001**). Biología del gorgojo de la oca *Microtrypes* sp. (Coleoptera: Curculionidae) en el valle del Mantaro. Huancayo (Peru), Thesis of the Universidad Nacional del Centro del Perú**:** 130pp.

Alvarado, A., M. Palacios, O. Ortiz, J. Peñaloza and P. Corzo (**1995**). Validacion de un programa MIP para control del gusano blanco en Tunja, Boyacá, Colombia. Proceedings of the XVII Reunión de la

Asociación Latinoamericana de la Papa (ALAP), 07/9-15/1995, Mérida (Venezuela), Fondo Nacional de Investigación Agropecuarias. pp. 22-23.

Amaya, L. M. and E. Bustamante (**1975**). Control microbiológico de tres especies de coleopteros plagas del suelo en Colombia. Revista ICA **10**(3): 269-281.

Andrew, R., O. Barea, C. Bejarano and J. Vargas (**1999a**). Biología y Comportamiento del Gorgojo de los Andes *Rhigopsidius tucumanus*. Ficha Técnica. Cochabamba, Fundación PROINPA**:** 4.

Andrew, R., R. Calderón, L. Crespo and J. Herbas (**1999b**). Biología y Comportamento del Gorgojo de los Andes Premnotrypes spp. Ficha Técnica. Cochabamba, Fundación PROINPA**:** 4.

Angeles, N. d. J. (**1966**). Presencia del "Gorgojo de la papa", *Premnotrypes vorax* (HUSTACHE (Curculionidae-Coleoptera)) en la región Andina de Venezuela. Agronomía Tropical **16**(4): 295-298.

Angeles, N. d. J. and R. Rodríguez D. (**1971**). Nueva area de distribución en la region andina de Venezuela del "gusano blanco de la papa" *Premnotrypes vorax* HUSTACHE. Agronomía Tropical **21**(4): 345-346.

Aréstegui P., A. (**1976**). Plagas de la papa en Andahuaylas-Apurimac. Revista Peruana de Entomología **19**(1): 97-98.

Barea, O., R. Andrew and J. Vargas (**1996/1997**). Biology, damage, and chemical control of the Andean potato weevil, *Rhigopsidius tucumanus* Heller. Revista Latinoamericana de la Papa **9/10**(1): 96-105.

Barriga, E., P. Landázuri, P. Gallegos and R. Williams (**2002**). Pathogenicity evaluation of native *Beauveria* sp. and *Metarhizium anisopliae* to control *Premnotrypes vorax*. Revista Latinoamericana de la Papa **13**(1): 104-111.

Bejarano, C. (**1994**). Control químico del gorgojo de los Andes *Rhigopsidius tucumanus*. Proceedings of the III reunión nacional de la papa, 09/21-23/1994, Cochabamba (Bolivia), Instituto Boliviano de Tecnología Agropecueria - Programa de Investigación de la Papa. pp. 89-90.

Borda Velasco, E. G. (**1994**). Control del gorgojo de los Andes *Premnotrypes latithorax* en la producción de tuberculo-semilla de papa. Cochabamba (Bolivia), Thesis of the Universidad Mayor de San Simon**:** 65pp.

Calderón, R., J. Franco, O. Barea, L. Crespo, R. Esprella, C. Bejarano, J. Ramos, G. Iporre and R. Casso (**2004**). Desarrollo de componentes del manejo integrado del gorgojo de los Andes en el cultivo de la papa en Bolivia. Cochabamba (Bolivia), Fundación PROINPA - Papa Andina**:** 144.

Calvache Guerrero, H. (**1984**). Evaluación preliminar del uso de trampas para captura de adultos de *Premnotrypes vorax* (Hustache). Resultados de investigación. Pasto, Instituto Colombiano Agropecuario (ICA) - Centro Regional de Investigaciones "Obonuco"**:** 83-85.

Calvache Guerrero, H. (**1986a**). Aspectos biológicos y ecológicos del gusano blanco de la papa, *Premnotrypes Vorax* (Hustache). In: Memorias del curso sobre control integrado de plagas de papa. L. Valencia (Ed.). Bogotá, International Potato Center: pp. 18-24.

Calvache Guerrero, H. (**1986b**). Informe de avances 1985: Control de gusano blanco. Proceedings of the Programa Andino Cooperativo de Investigación en Papa. Memorias de la V reunión anual, 03/17-21/1986, Cochabamba, Programa Andino Cooperativo de Investigación en Papa. pp. 46-70.

Calvache Guerrero, H. (**1988a**). Colombia: Manejo del gusano blanco (*P. vorax*). Permanencia de larvas de *P. vorax* en papa almacenada bajo condiciones de luz difusa. Notipracipa **7**: 4-5.

Calvache Guerrero, H. (**1988b**). Informe técnico: Control de gusano blanco *Premnotrypes vorax* (Hustache). Proceedings of the Programa Andino Cooperativo de Investigación en Papa. Memorias de la reunión anual 1988, 05/16-20/1988, Cusco, Programa Andino Cooperativo de Investigación en Papa. pp. 27-47.

Calvache Guerrero, H. (**1991**). Barriers for the control of the Andean potato weevil (*Premnotrypes vorax* (Hustache)). Revista Latinoamericana de la Papa **4**(1): 22-35.

Calvache Guerrero, H. and L. F. Alvarado (**1980**). El gusano blanco de la papa, *Premnotrypes vorax* (Hustache) y su control. Pasto, Instituto Colombiano Agropecuario (ICA) - Centro Regional de Investigaciones "Obonuco"**:** 34.

Calvache, H. (**1985**). Capture of adults of *Premnotrypes vorax* (Hustache) with adults of the same species as attractant. Revista Colombiana de Entomología **11**(2): 9-14.

Cañedo, V., J. Alcázar, M. L. Guevara and E. Cosío (**2005**). Efecto de los glucosinolatos en la repelencia y alimentación de *Phthorimaea operculella* (Zeller) y *Premnotrypes suturicallus* Kuschel plagas del cultivo de la papa. Proceedings of the XLVII Convención nacional de entomología., 10/23-25/2005, Ica (Peru), Sociedad Entomologica del Perú.

Cárdenas Flores, J. Z. (**2000**). Efecto de fechas de siembra y cosecha en la infestación del gorgojo de los Andes *Premnotrypes latithorax* Kuschel en papa (*Solanum tuberosum* L.). Puno, Thesis of the Universidad Nacional del Altiplano**:** 96pp.

Carhuamaca Ticse, J. and F. Ore Meza (**1993**). Manejo integrado del gorgojo de los Andes (MIGA) en Huancayo. Proceedings of the Primer encuentro nacional de manejo integrado del gorgojo de los Andes, 16-17 December 1993, Lima, International Potato Center. p. 11.

Carrasco Gamarra, D. (**1992**). Ensayos con un inhibidor de sintesis de quitina para el control del "gorgojo de los Andes" *Premnotrypes latithorax* (P.)K. Cusco, Thesis of the Universidad Nacional de San Antonio Abad del Cusco**:** 136pp.

Carrasco Zamora, F. (**1961**). Sistemática y Biología del Gorgojo de los Andes *Premnotrypes latithorax* Pierce 1914 (Coleop.: Curculionidae). Revista Peruana de Entomología Agrícola **4**(1): 30-42.

Carrasco Zamora, F. (**1991**). Temperature and humidity as factors to control the Andean potato weevil. Revista Peruana de Entomología **34**: 69-70.

Carvajal Pinto, C. (**1993**). Biología, distribución geográfica, fluctuación poblacional y control del Gorgojo de los Andes *Premnotrypes latithorax* en la localidad de Aguirre. Cochabamba, Thesis of the Universidad Mayor de San Simon**:** 123pp.

Castro Marín, Y. (**1997**). Evaluación de cepas de *Beauveria* sp. y *Metarhizium* sp. para el control del gusano blanco de la papa *Premnotrypes vorax* (Hustache). Santa Fé de Bogotá (Colombia), Thesis of the Universidad Nacional de Colombia**:** 63pp.

Catalán Bazán, W. (**2003**). Evaluación de la susceptibilidad de dos poblaciones de *Premnotrypes latithorax* y *P. suturicallus* a cinco insecticidas organosintéticos. Lima (Peru), Thesis of the Universidad Nacional Agraria La Molina**:** 126pp.

Catalán Bazán, W., W. Villano T. and F. Inquiltupa A. (**1993**). Reunión anual sobre manejo integrado del gorgojo de los Andes (MIGA), Cusco - Perú. Proceedings of the Primer encuentro nacional de manejo integrado del gorgojo de los Andes, 16-17 December 1993, Lima, International Potato Center. p. 60.

Catalán, W., J. Alcázar and W. Villano (**1993**). Control del gorgojo de los Andes en campo de cultivo de papa usando barrera quimica en Chincheros, Cusco. Proceedings of the XXXV convención nacional de entomología, 11/07-11/1993, Arequipa (Peru), Sociedad Entomologica del Perú. p. 39.

Cerna, V. (**1993**). Reunión anual sobre manejo integrado del gorgojo de los Andes (MIGA), Cajamarca - Perú. Proceedings of the Primer encuentro nacional de manejo integrado del gorgojo de los Andes, 16-17 December 1993, Lima, International Potato Center. p. 35.

Chavez Ajata, C. R. (**1997**). Actividad migratoria y daño del Gorgojo (*Premnotrypes* spp.) en dos comunidades altiplanicas del departamento de La Paz. Oruro (Bolivia), Thesis of the Universidad Tecnica de Oruro**:** 118pp.

Cisneros, F. and A. Vera (**2001**). Mass producing *Beauveria brongniartii* inoculum, an economical, farm-level method. In: Scientist and farmer: Partners in research for the 21st century. Program Report, 1999-2000. Lima (Peru), International Potato Center: pp. 155-160.

Corporación Colombiana de Investigación Agropecuaria (**2000**). Generación y transferencia de tecnologías para el control biológico del gusano blanco *Premnotrypes vorax* y la polilla guatemalteca *Tecia solanivora* plagas de importancia económica del cultivo de la papa en Colombia. Bogotá (Colombia), Corporación Colombiana de Investigación Agropecuaria - Programa Nacional de Transferencia de Tecnología**:** 153.

COSAVE. Hojas de datos sobre organismos cuarentenarios para los paises miembros del COSAVE. Ficha Cuarentenaria: *Premnotrypes* spp. (Coleoptera: Curculionidae). http://www30.brinkster.com/cosave/base_ing.htm. Accessed: 26/11/2003.

Crespo V., L., R. Calderón and R. Andrew (**1998**). Control quimico del gorgojo de los Andes *Premnotrypes* spp. Proceedings of the XVIII reunión de la Asociación latinoamericana de la papa, 02/09-13/1998, Cochabamba, PROSEMPA. pp. 112-113.

Dávalos Vallejo, A. (**1997**). Estudio anatomico y fisiologico de la digestion del "gusano blanco de la papa" *Premnotrypes vorax* (Hustache): Coleóptera: Curculionidae. Quito (Ecuador), Thesis of the Pontificia Universidad católica del Ecuador**:** 104pp.

Delgado Mamani, P. (**1992**). Capacidad de predacion, en laboratorio, de tres carabidos sobre *Premnotrypes solaniperda* Kuschel. Proceedings of the XXXIV convención nacional de entomología, 11/08-12/1995, Lima (Peru), Sociedad Entomologica del Perú. p. 26.

Delgado Mamani, P. and N. Fernández Aquise (**1993**). Control biologico del gorgojo de los Andes con el hongo *Beauveria* spp. Proceedings of the Primer encuentro nacional de manejo integrado del gorgojo de los Andes, 16-17 December 1993, Lima, International Potato Center. p. 7.

Delgado P., M. (**1974**). Control quimico de los insectos que atacan a la papa almacenada. Avances en Investigación **4-5**(2/3): 11-12.

Dos Santos, H. R., M. A. L. Da Rocha, J. I. Zanon, A. R. Perri and C. B. Da Silva (**1982**). Test on the control of soil pests of potato with carbofuran. Ecosistema (Brazil) **7**(1): 19-27.

Durán Auccatinco, J. (**2001a**). Determinación de plantas cultivadas y no cultivadas como hospedantes de *Premnotrypes latithorax* (Pierce). Cusco (Peru), Thesis of the Universidad Nacional de San Antonio Abad del Cusco**:** 86pp.

Durán Auccatinco, J. (**2001b**). Evaluación de tuberculos andinos como hospedantes de *Premnotrypes latithorax* (Pierce) en el distrito de Chinchero. Proceedings of the Manejo iontegrado de plagas de los principales cultivos andinos. Memoria del seminario taller nacional y regional. Valle Sagrado de los Incas, June, 20-22 and October 21-23 2001, Urubamba - Cusco (Peru), ARARIWA. pp. 29-31.

Esprella, R., R. Calderón, J. Alcázar, J. Blajos and G. Thiele (**1998**). Manejo integrado del gorgojo de los Andes (*Premnotrypes* spp.) : La experiencia de Kollana. Proceedings of the XVIII reunión de la Asociación latinoamericana de la papa, 02/09-13/1998, Cochabamba, PROSEMPA. pp. 260-261.

Esprella, R., C. Chavez, L. Crespo, R. Calderón and J. Alcázar (**1996**). Estudio etológico y migración del gorgojo de los Andes *Premnotrypes* spp. en Cochabamba y La Paz. In: Infrome anual IBTA-PROINPA 1995-96. Cochabamba (Bolivia), Instituto Boliviano de Tecnología Agropecueria - Programa de Investigación de la Papa: pp. III.E.27-29.

Espul, J. C. and G. Magistretti (**1969**). Bioecología del gorgojo del tomate *Phyrdenus muriceus* (Germ.) y su control en la provincia de Mendoza. Revista de investigación agropecuaria **6**(5): 95-117.

Ewell, P. T. (**1990**). Socio-economic aspects of farmer-oriented research for potato IPM. In: Integrated pest Management for Tropical Root and Tuber Crops. Proceedings of the workshop on the global status of and prospects for integrated pest management of root and tuber crops in the tropics. S. K. Hahn and F. E. Caveness (Eds). Ibadan (Nigeria), International Institute of Tropical Agriculture: pp. 108-124.

Ewell, P. T., H. Fano, K. V. Raman, J. Alcázar, M. Palacios and J. Carhuamaca (**1990**). Farmer management of potato insect pests in Peru. Lima, International Potato Center. 77pp.

Fernández, S. A. and X. Colmenares (**1997**). Evaluación de *Beauveria* spp para el control de *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae) en el cultivo de la papa. Agronomía Tropical **47**(3): 249-257.

Ferrandis, M. D., R. Andrew, M. Porcar, J. Iriarte, V. Cosmao-Dumanoir, M.-M. Lecadet, P. Caballero and J. Ferré (**1999**). Characterization of *Bacillus thuringiensis* serovar *bolivia* (serotype H63), a novel serovar isolate from the Bolivian high valleys. Letters in Applied Microbiology **28**: 440-444.

Fiedler, C. (**1941**). Die amerikanische Rüßlergattung *Phyrdenus* Lec. (Curc. Cryptorhynch.). Zoologischer Anzeiger **134**(5/6): 123-137.

FORTIPAPA (**1993**). Informe anual: Compendio 1992-1993. Quito (Ecuador), INIAP**:** 61.

Frankhauser, C. (**1999**). Main diseases affecting seed degeneration in Ecuador: New perspectives for seed production in the Andes. Proceedings of the 14th Triennial Conference of the European Association for Potato Research, Sorrento (Italy), Assessorato Agricoltura Region Campania. pp. 50-51.

Galindo P., J. R. and J. A. Español Aragon (**2004**). Capture dynamic of *Premnotrypes vorax* (Coleoptera: Curculionidae) and the Guatemalan potato moth *Tecia solanivora* (Lepidoptera: Gelechidae) in traps with different types of attractants in a potato crop (*Solanum phureja*). Revista Colombiana de Entomologia **30**(1): 57-64.

Gallegos G., P., C. Asaquibay, R. Williams, M. Prado and J. Suquillo (**2001**). Control of the Andean weevil *Premnotrypes vorax* (Hustache) in potato (*Solanum tuberosum* L.) using Triflumuron. IPM CRSP Integrated Pest Management Collaborative Research Support Program. Eighth Annual Report 2000-2001. Blacksburg (VA), Virginia Polytechnic Institute and State University**:** 365-366.

Gallegos G., P. and G. Avalos P. (**1994/95**). Integrated control of *Premnotrypes vorax* (Hustache) by managing adult population and chemical control in the potato crop. Revista Latinoamericana de la Papa **7/8**(1): 55-60.

Gallegos G., P., G. Avalos P. and C. Castillo C. (**1997**). El gusano blanco de la papa en Ecuador: Comportamiento y control. Quito (Ecuador), INIAP. 34pp.

Gallegos G., P. and C. Castillo (**1995**). Control químico de *Premnotrypes vorax* mediante aplicaciones foliares. Quito (Ecuador), FORTIPAPA**:** 5.

Gallegos G., P. and S. Garcés (**1999**). Laboratory trials to determine control of Andean potato weevil using biorational insecticides. Arthropod Management Test **24**: 406.

Gallegos, P. (**1989**). Insectos plagas en el cultivo de la papa en el Ecuador. Proceedings of the Seminario taller: Aspectos entomológicos en el cultivo de la papa, 07/10-13/1989, Bogotá (Colombia), Programa Andino Cooperativo de Investigación en Papa. pp. 31-33.

Gallegos, P. and C. Asaquibay (**2004**). Estudios para la aplicación de cepas de *Beauveria* sp. en cultivo comercial para el control de gusano blanco *Premnotrypes vorax* H. In: Memorias de actividades del area de entomología ciclo; 2002-2003. P. Gallegos G. and C. Asaquibay (Eds). Quito (Ecuador), INIAP: pp. 7-14.

Gallegos, P., C. Asaquibay, Y. Cartagena, M. Freire and N. Chulde (**2004a**). Alternativas de manejo de gusano blanco (*Premnotrypes vorax*) en el cultivo de papa (*Solanum tuberosum*) bajo el sistema de labranza huacho rozado. In: Memorias de actividades del area de entomología ciclo; 2002-2003. P. Gallegos G. and C. Asaquibay (Eds). Quito (Ecuador), INIAP: pp. 15-32.

Gallegos, P., C. Asaquibay and R. Williams (**2003a**). Efectividad del inhibidor de quitina Triflumuron para el control del gusano blanco *Premnotrypes vorax* H. en el cultivo de la papa *Solanum tuberosum*. In: Actividades realizados en el ciclo agricola 2001-2002. Quito (Ecuador), INIAP.

Gallegos, P., C. Asaquibay and R. Williams (**2004b**). Desarollo de métodos de biocontrol de *Premnotrypes vorax* y polilla centroamericana de la papa *Tecia solanivora*. In: Memorias de actividades del area de entomología ciclo; 2002-2003. P. Gallegos G. and C. Asaquibay (Eds). Quito (Ecuador), INIAP: pp. 1-6.

Gallegos, P., E. Barriga, P. Landázuri and R. Williams (**2003b**). Desarollo de métodos de biocontrol de *Premnotrypes vorax*; evaluación de la patogenicidad de aislamientos nativos de *Beauveria* sp. y *Metarhizium anisopliae* para el control de *Premnotrypes vorax* en el laboratorio. In: Actividades realizados en el ciclo agricola 2001-2002. Quito (Ecuador), INIAP.

Gandarillas, A. and C. Perpich, Eds. (**1992**). PRACIPA: Avances y logros. Segunda fase, resumen 1987-1992. Cochabamba, IBTA. 18pp.

Garmendia Lorena, A. (**1961**). Observaciones sobre un posible método de control biológico de la gusanera de la papa depositada en almacén. Revista Peruana de Entomología Agrícola **4**(1): 76-77.

Garzón C., M. Y., B. O. Aza T., J. Jiménez G. and J. E. Luque Z. (**1996**). Potential of the nematode S*teinernema* sp. for the biological control of the Andean potato weevil. Revista Colombiana de Entomología **22**(1): 25-30.

Gil Alvarez, P. (**1991**). Biología y comportamiento de *Premnotrypes solaniperda* Kuschel (Coleoptera - Cuculionidae). Puno, Thesis of the Universidad Nacional del Altiplano**:** 72pp.

Gomez, S., A. C. Mateus, J. Hernandez and B. H. Zimmermann (**2000**). Recombinant Cry3Aa has insecticidal activity against the Andean potato weevil, *Premnotrypes vorax*. Biochemical and Biophysical Research Communications **279**(2): 653-656.

González, A. S. and P. L. Gómez C. (**1989**). Determinación de la variabilidad genética del gusano blanco de la papa *Premnotrypes vorax* (Hustache) por medio de separación electroforética. Proceedings of the Seminario taller: Aspectos entomológicos en el cultivo de la papa, 07/10-13/1989, Bogotá (Colombia), Programa Andino Cooperativo de Investigación en Papa. pp. 18-30.

Heath, J. J., J. Alcázar and A. Lagnaoui (**2001**). Mechanism of attraction in *Premnotrypes suturicallus* (Curculionidae). Proceedings of the The ESA 2001 Annual Meeting - 2001: An Enomological Odyssey of ESA, 09/12/2003-12/12/2003, San Diego, Entomological Society of America.

Heller, K. M. (**1935**). Ein neuer Rüsselkäfer aus peruanischen Kartoffeln. Arbeiten über physiologische und angewandte Entomologie aus Berlin-Dahlem **2**(4): 276-278.

Heller, K. M. (**1936**). Ergänzende Bermerkung über kartoffelschädigende Rüsselkäfer (Coloptera: Curculionidae). Arbeiten über physiologische und angewandte Entomologie aus Berlin-Dahlem **3**(4): 284-285.

Herbas Chavez, J. A. (**1992**). Estudio de los insectos del suelo que causan mayor daño en papa. Cochabamba, Thesis of the Universidad Mayor de San Simon**:** 99pp.

Herbas, J. (**1995**). Avances del manejo integrado del gorgojo de los Andes (*Rhigopsidius tucumanus*). In: Memorias del II curso internacional de manejo integrado de plagas de papa. Curso de capacitación de distancia. E. N. Fernandez-Northcote (Ed.). Cochabamba, Instituto Boliviano de Tecnología Agropecueria - Programa de Investigación de la Papa: pp. 24-25.

Hernández, C. S., R. Andrew, Y. Bel and J. Ferré (**2005**). Isolation and toxicity of *Bacillus thuringiensis* from potato-growing areas in Bolivia. Journal of Invertebrate Pathology **88**(1): 8-16.

Hernandez R., J. H. and C. A. Bohorquez B. (**1992**). Control microbial del gusano blanco de la papa (*Premnotrypes vorax*, Hustache) con el hongo *Beauveria brongniartii* Sacc. en areas paperas de Boyacá. Santa Fé de Bogotá (Colombia), Thesis of the Universidad Nacional de Colombia**:** 63pp.

Herrera, F. (**1997**). El Gusano Blanco de la Papa: Biología, Comportamiento y Prácticas de Manejo Integrado. Bogotá, Corporación Colombiana de Investigación Agropecuaria. 14pp.

Horton, D., F. Tardieu and M. Benavides (**1980**). Tecnología de la producción de papa en el valle del Mantaro, Perú: Resultados de una encuesta agro-económica de visita múltiple. Documento de trabajo. Lima (Peru), International Potato Center**:** 68.

ICA (**1986**). Informe de gerencia 1985. Bogotá (Colombia), Instituto Colombiano Agropecuario**:** 197.

Jansson, R. K. (**1992**). Biological approaches for management of weevils of root and tuber crops: A review. Florida Entomologist **75**(4): 568-584.

Kuschel, G. (**1956**). Revisión de los Premnotrypini y adiciones a los Bagoini. Boletin Museo Nacional de Historia Natural, Chile **26**: 187-235.

Larraín S., P. (**2003**). Plagas de la papa y su manejo. La Serena (Chile), Instituto de Investigaciones Agropecuarias. 110pp.

Loiácono, M. S. and J. J. Morrone (**1991**). Immature stages of *Rhigopsidius piercei* Heller (Coleoptera: Curculionidae). Elytron **5**: 23-29.

López-Avila, A. and E. Espitia-Malagón (**2000**). Plagas y benéficos en el cultivo de la papa en Colombia. Boletín técnico divulgativo. Bogotá, Corporación Colombiana de Investigación Agropecuaria**:** 35.

Loza del Carpio, A. L. (**1999**). Poblaciones y ecología alimentaria de carabidos (Coleoptera) en adroecosistemas del altiplano. Puno, Thesis of the Universidad Nacional del Altiplano**:** 129pp.

Marshall, G. (**1936**). New injurious Curculionidae (Col.) from South America. Bull. Entomol. Research **27**(3): 497-501.

Martínez O., W. and J. Cerón S. (**2004**). Binding of *Bacillus thuringiensis* proteins with brush border membrane vesicles of the digestive tract of the Andean potato weevil *Premnotrypes vorax* (Coleoptera: Curculionidae). Revista Colombiana de Entomología **30**(1): 51-56.

Mateus Virviescas, A. C. (**1998**). Caracterización de aislamientos nativos de *Bacillus thuringiensis* y estandarización de un método para la evaluación de toxicidad sobre el insecto plaga *Premnotrypes vorax* - Hustache. Santa Fé de Bogotá (Colombia), Thesis of the Pontificia Universidad Javeriana**:** 192pp.

Mayta, S., J. Alcázar, M. Holle and A. Lagnaoui (**2001**). Evaluacion de la susceptibilidad de hipocotilos de maca *Lepidium meyenii* (wild) al gorgojo de los Andes, *Premnotrypes* ssp. en condiciones de laboratorio. Proceedings of the XLIII Convención nacional de entomología, 11/4-8/2001, Huancayo, Sociedad Entomologica del Perú. p. 35.

Medina, C., W. Catalán, J. L. Cruz and H. Pumacahua (**1999**). El manejo integrado de plagas en el cultivo de la papa: Avances y dificultades. Proceedings of the Seminario Taller Internacional: Manejo integrado de plagas de los principales cultivos andinos, 03/23-26/1999, Urubamba (Peru), ARARIWA. pp. 55-60.

Mellado, G. (**1961**). Biología del gorgojo de los Andes y su control. Proceedings of the VII Convención Agronómica Regional de la Sierra Sur, 05/31-06/04/1961, Cusco, Asociación Peruana de Ingenieros Agrónomos. pp. 101-103.

Meza Rojas, D. A. (**1978**). Evaluación de plagas insectiles en variedades comerciales de papa. Huancayo (Peru), Thesis of the Universidad Nacional del Centro del Perú**:** 52pp.

Ministerio de Agricultura del Perú. Descripcíon de los productos forestales no maderables: Cocona. http://www.portalagrario.gob.pe/rrnn/rrnn_cocona.shtml. Accessed: 21.03.2006.

Molleda M., G. (**1965**). Contribución al estudio del *Premnotrypes pusillus* Kuschel (Col.: Curculionidae), en las zonas paperas de la parte alta de Cusco, Paucartambo, Chincheros, Huaypo, Pancarhauylla, Moray y Maras. Revista Peruana de Entomología **8**(1): 66-68.

Morales Valles, P., M. Cermeli, F. Godoy and B. Salas (**2003**). List of insect with relations to solanaceous plants found in the museum of insects of agricultural interest at CENIAP - INIA. Entomotropica **18**(3): 193-209.

Moreno Coronel, J. F. (**1998**). Eficiencia del hongo *Beauveria brongniartii* en el control del gusano blanco *Premnotrypes vorax* (Hustache) en el cultivo de papa en el municipio de Motavita-Boyacá. Tunja (Colombia), Thesis of the Universidad Pedagogica y Tecnologica de Colombia**:** 81pp.

Morrone, J. J. and M. S. Loiacono (**1992**). Revision of the genus *Rhigopsidius* HELLER (Insecta, Coleoptera, Curculionidae: Rhytirrhininae). Entomologische Abhandlungen Stattliches Museum für Tierkunde Dresden **54**(7): 129-139.

Muñoz Cáceres, M. (**1998**). Biología del "Gorgojo de los Andes" *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae). Cajamarca, Thesis of the Universidad Nacional de Cajamarca**:** 104pp.

Munro, J. A. (**1968**). Insects affecting potatoes in Bolivia. American Potato Journal **45**: 356-357.

Navarro Mejía, O. R. (**1995**). Estudio de algunos aspectos biológicos y de comportamiento del gusano blanco de la papa (*Premnotrypes vorax* Hustache). Santa Fé de Bogotá (Colombia), Thesis of the Universidad Nacional de Colombia**:** 117pp.

Niño, L., E. Acevedo and F. Becerra (**2000**). Control de adultos del gusano blanco de la papa con trampas de follaje. Fonaiap Divulga **67**: 41-43.

Niño, L., E. Acevedo, F. Becerra and M. Guerrero (**2004**). Some biological aspects and population fluctuation of the white tuberworm *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae) in Mucuchíes, Mérida state, Venezuela. Entomotropica **19**(1): 15-19.

Ñopo, L., J. Tenorio, A. Golmirzaie, V. Cañedo, A. Vera, C. Newell and W. Hamilton (**1998**). Resistance to Andean potato weevil in transgenic potato plants. Proceedings of the IX International congress on plant yissue and cell culture, 06/14-19/1998, Jerusalem, IAPTC. p. 165.

Novo, R. J., A. Viglianco and E. Vaudagna (**2002**). Effect of insecticides on the potato weevil *Phyrdenus muriceus* (Germ.) (Coleoptera: Curculionidae). Agriscientia **19**: 3-10.

Obando G., L., R. Velásquez C. and H. Calvache Guerrero (**1988**). Fluctuación de la población del gusano blanco de la papa (*Premnotrypes vorax* Hustache) durante el cultivo. Proceedings of the VI congresso internacional sobre cultivos Andinos, 05/30 - 06/02/1988, Quito, INIAP. pp. 501-508.

OEPP/EPPO (**1984**). EPPO data sheet on quaratine organisms No. 143. EPPO Bulletin **14**(1): 55-60.

Olivares Garces, N., P. Jimenez Espinosa, C. A. Cruz Gutierrez and P. M. Restrepo Osorio (**1973**). Estudio preliminar de reconocimiento y distribución geografica del nematodo dorado (*Heterodera rostochiensis* Wollenweber) y gusano blanco (*Premnotrypes vorax* Hustache) de la papa en Boyacá. Tunja (Colombia), Thesis of the Universidad Pedagogica y Tecnologica de Colombia**:** 62pp.

Orlove, B. S. and R. Godoy (**1986**). Sectoral fallowing systems in the central Andes. Journal of Ethnobiology **6**(1): 169-204.

Ortega, A. M. and H. Torres (**1994**). Eficiencia del *Verticillium lecanii* para controlar larvas del gorgojo de oca (GO) *Microtrypes* sp., en condiciones de laboratorio. Fitopatología **29**(1): 57.

Ortega, E. G. (**1998**). Alternativas de control químico, biológico y etológico del gorgojo de los Andes *Premnotrypes latithorax* (Coleoptera: Curculionidae) en el cultivo de papa *Solanum tuberosum*. Cochabamba (Bolivia), Thesis of the Universidad Mayor de San Simon**:** 82pp.

Ortiz, O., J. Alcázar, W. Catalán, W. Villano, V. Cerna, H. Fano and T. S. Walker (**1996**). Economic impact of IPM practices on the Andean potato weevil in Peru. In: Case studies of the economic impact of CIP - related technology. T. S. Walker and C. C. Crissman (Eds). Lima, International Potato Center: pp. 95-110.

Ortiz, O., J. Alcázar, W. Catalán and T. S. Walker (**1993**). Evaluación socioeconómica del primer año de aplicación del control integrado del gorgojo de los Andes (*Premnotrypes latithorax*) en Huatata, Cusco, Perú. Proceedings of the XVI Reunion de la ALAP, 03/22-26/1993, Santo Domingo (Dominican Republic), ALAP. pp. 13-14.

Ortiz Romero, R. (**1997**). Plantas con propiedades biocidas sobre el gorgojo de los Andes *Premnotrypes* sp. (Coleoptera: Curculionidae) en papa. Proceedings of the IX congreso internacional de cultivos andinos, 04/22-25/1997, Cusco, Universidad Nacional de San Antonio Abad del Cusco. p. 71.

Ortiz Romero, R., R. Bravo P., C. Ramirez C. and J. Quispe (**1996**). Umbral y nivel de dano economico del "Gorgojo de los Andes" en Puno. Proceedings of the XXXVIII convención nacional de entomología, 11/17-21/1996, Chincha (Peru), Sociedad Entomologica del Perú. p. 7.

Parsa, S., J. Alcázar, J. Salazar and H. K. Kaya (**2006**). An indigenous Peruvian entomopathogenic nematode for suppression of the Andean potato weevil. Biological Control **39**(2): 171-178.

Peña Villamil, L. A. (**2001**). Gusanos blancos de la papa: Biología y Manejo. Innovación & Cambio Tecnológico **2**(1): 29-33.

Peña Villamil, L. A., M. Aurelio Bolaños, B. Yépes Chamorro, J. Mena Huertas and J. Enríquez Insuasty (**2000**). Hongos entomopatógenos para el manejo del gusano blanco (*Premnotrypes vorax*) de la papa. http://www.redepapa.org/practicasculturalesred.html. Accessed: 26/11/2003.

Peña Villamil, L. A., B. Yepes Chamorro and M. A. Bolaños (**1999a**). Los Cultivos trampa: Una alternativa sostenible para el control del adulto de gusano blanco de la papa *Premnotrypes vorax*. Boletín de divulgación. Obonuco, Corporación Colombiana de Investigación Agropecuaria**:** 12.

Peña Villamil, L. A., B. Yepes Chamorro, M. A. Bolaños and J. Mena Huertas (**1999b**). Evaluación del control del gusano blanco de la papa mediante la utilización del hongo entomopatógeno (*Beauveria bassiana*). San Juan de Pasto (Colombia), Corporación Colombiana de Investigación Agropecuaria**:** 37.

Pérez Rubiano, C. C. (**2002**). Efecto de *Beauveria brongniartii* (Sacc) Petch sobre *Premnotrypes vorax* (Hustache) y otros insectos asociados al cultivo de papa en Ventaquemada, Boyacá. Tunja (Colombia), Thesis of the Universidad Pedagogica y Tecnologica de Colombia**:** 98pp.

Pierce, W. D. (**1914**). New potato weevils from Andean South America. Journal of Agricultural Research **1**(4): 347-352.

Pierce, W. D. (**1918**). Weevils which affect Irish potato, sweet potato, and yam. Journal of Agricultural Research **12**(9): 601-612.

Pilco Nina, J. (**1978**). Determinación de plantas hospederas al gorgojo de los Andes (*Premnotrypes solaniperda* Kuschel). Puno (Peru), Thesis of the Universidad Nacional Tecnica del Altiplano.

Posadas, P. (**1998**). Occurrence of the weevil genus *Rhigopsidius* (Coleoptera: Curculionidae) in Patagonia. Revista de la Sociedad Entomológica Argentina **57**(1-4): 90.

Programa Andino Cooperativo de Investigación en Papa (**1990**). Control de gusano blanco de la papa, (*Premnotrypes vorax*) (Hustache). In: II fase producción, Informe anual. Bogotá, Programa Andino Cooperativo de Investigación en Papa: pp. 21-38.

Quispe Araujo, U. (**1999**). Comparativo de formulaciones del hongo blanco (*Beauveria brongniartii* Alexopoulus) e insecticidas en el control del gorgojo de los Andes (*Premnotrypes* spp.) en papa. Puno (Peru), Thesis of the Universidad Nacional del Altiplano**:** 49pp.

Quispe Pérez, V. (**2001**). Efecto de barreras vegetales y químicas en el control del "gorgojo de los Andes" *Premnotrypes latithorax* (Pierce) Kuschel. Proceedings of the Manejo iontegrado de plagas de los principales cultivos andinos. Memoria del seminario taller nacional y regional. Valle Sagrado de los Incas, June, 20-22 and October 21-23 2001, Urubamba - Cusco (Peru), ARARIWA. pp. 9-11.

Raman, K. V., A. M. Golmirzaie, M. Palacios and J. Tenorio (**1994**). Inheritance of resistance to insects and mites. In: Potato genetics. J. E. Bradshaw and G. R. Mackay (Eds). Wallingford, CAB International: pp. 447-463.

Ramirez Ticona, J. E. (**1971**). Evaluación de la susceptibilidad al Gorgojo de los Andes: *Premnotrypes latithorax* (Pierce) Kuschel, en cien cultivares de papas nativas. Cusco, Thesis of the Universidad Nacional de San Antonio Abad del Cusco**:** 59pp.

Ravines Novoa, L. A. (**2002**). Dinámica poblacional del gorgojo de los Andes *Premnotrypes vorax*. Cajamarca (Peru), Thesis of the Universidad Nacional de Cajamarca**:** 56pp.

Ríos, A., D. Hall, J. Alcázar and L. Crespo (**2005**). Presencia de químicos atrayentes en el estiércol del gorgojo de los Andes *Premnotrypes suturicallus*. Proceedings of the XLVII Convención nacional de entomologia., 10/23-25/2005, Ica (Peru), Sociedad Entomologica del Perú.

Romero Chávez, J. (**1983**). Evaluación poblacional de plagas insectilces en diez variedades comerciales de papa. Huanuco (Peru), Thesis of the Universidad Nacional de Huanuco Mermilio Valdizán**:** 81pp.

Salazar Benitez, E. M. (**1996**). Ciclo biológico y dinámica poblacional del gusano blanco de la papa (*Premnotrypes vorax* Hustache) en el municipio de Motavita (Boyaca). Tunja, Thesis of the Universidad Pedagógica y Tecnológica de Colombia.

Salazar Lermo, G., J. Alcázar Sedano and J. Carhuamaca Ticse (**1997**). Influence of seed damaged by the Andean potato weevil on the yield in the Mantaro Valley, Peru. Revista Peruana de Entomología **40**: 87-88.

Sánchez, G. A. (**1986**). Plagas de la papa en el Perú. In: Principales plagas y enfermedades de la papa en el Perú. D. Untiveros O. (Ed.). Lima (Peru), INIPA: pp. 15-96.

Sasscer, E. E. and W. D. Pierce (**1913**). Preliminary report of the finding of a new weevil enemy of the potato tuber. Proceedings of the Entomological Society of Washington **15**(3): 143-144.

Sibaja Chinchilla, G. and C. Sanabria Ujueta (**2002**). Prinicpales plagas de insectos, ácaros y moluscos de cultivos agrícolas y forestales de Costa Rica. http://www.protecnet.go.cr/plagas/LISTADOentomolog%EDa.htm. Accessed: 21.03.2006.

Sotelo Ravichagua, J. M. (**1996**). Rol de las plantas voluntarias de papa como fuente de infestación del Gorgojo de los Andes. Huancayo, Thesis of the Universidad Nacional del Centro del Perú**:** 87pp.

Suquillo, J., P. Gallegos and F. Chamorro (**1998**). Validacion del sistema de control integrado del gusano blanco de la papa, *Premnotrypes vorax*, en el cultivo de la papa en la provincia del Carchi, Ecuador. Proceedings of the XVIII Reunión de la Asociación Latinoamericana de la papa, 02/9-13/1998, Cochabamba (Bolivia), PROSEMPA. pp. 262-263.

Tardieu, F., P. Alcalá C. and L. Tomassini V. (**1980**). Control químico del "Gorgojo de los Andes" (*Premnotrypes suturicallus* Kuschel) en el valle Mantero, Perú. Revista Peruana de Entomología **23**(1): 145-147.

Tisoc Dueñas, I. O. (**1989a**). Biological cycle of *Premnotrypes latithorax*, in Cusco (Peru), under laboratory conditions. Revista Peruana de Entomología **32**: 89-92.

Tisoc Dueñas, I. O. (**1989b**). Ciclo biologico de "Gorgojo de los Andes" *Premnotrypes latithorax* (Pierce 1914) (Coleoptera Curculionidae) en condiciones de Laboratorio. Cusco, Thesis of the Universidad Nacional de San Antonio Abad del Cusco**:** 73pp.

Torres, H., A. M. Ortega, J. Alcázar, T. Ames and L. Palomino (**1993**). Control Biológico del Gorgojo de los Andes (*Premnotrypes* spp.) con *Beauveria brongniartii*. Research guide. Lima, International Potato Center**:** 43.

Torres Pérez, R. and A. López-Avila (**1997**). Basic studies on the microbiological control of the Andean potato weevil (*Premnotrypes vorax*) with *Beauveria* spp. and *Metarhizium* sp. Revista Colombiana de Entomología **23**(1-2): 83-88.

Torres Torres, L. and A. Marina Cotes (**1999**). Biocontrol activity of entomopathogenic fungi against *Premnotrypes vorax* (Coleoptera: Curculionidae) through their individual or combined utulization. Revista Colombiana de Entomología **25**(3-4): 121-129.

Torres Wills, F. (**1993**). Ciclo biológico del gusano blanco de la papa *Premnotrypes vorax* en Pueblo Hondo - Estado Tachira. Proceedings of the V congreso latinoamericano y XIII venezolano de entomología, 07/04-08/1993, Nueva Esparta (Venezuela), Universidad Central de Venezuela. pp. 8-9.

Tovar Gamarra, A. T. (**1988**). Ciclo biológico y distribución geográfica en el valle del Mantaro del *Premnotrypes piercei* Alcala. Huancayo, Thesis of the Universidad Nacional del Centro del Perú**:** 116pp.

Trillos G., O., L. Valencia, R. Pineda C. and R. Martínez B. (**1993**). Evaluation of hybrids of *Solanum polyadenium* Grenm. for white worm resistance of potatoes (*Premnotrypes vorax* Hustache). Agronomia Colombiana **10**(2): 128-131.

Unda, J., V. Barrera and P. Gallegos (**1999**). Estudio de adopción e impacto económico del manejo integrado del gusano blanco (*Premnotrypes vorax*) en comunidades campesinas de la provincia de Chimborazo. In: Estudios de caso del impacto económico de la tecnología generada por el INIAP en el rubro papa. V. Barrera and C. C. Crissman (Eds). Quito (Ecuador), International Potato Center: pp. 33-72.

Uribe, D., W. Martínez and J. Cerón (**2003**). Distribution and diversity of *cry* genes in native strains of *Bacillus thuringiensis* obtained from different ecosystems from Colombia. Journal of Invertebrate Pathology **82**(2): 119-127.

Valencia, L. (**1988**). Field behavior of the adult Andean weevil (*Premnotrypes vorax* (Hustache)) in Colombia. American Potato Journal **65**(8): 503.

Valencia, L. (**1989**). The Andean potato weevil, *Premnotrypes vorax* (Hustache) in Colombia: I.- Behavior of adults in the field. Revista Latinoamericana de la Papa **2**(1): 57-70.

Valencia V., L. and C. Bohórquez (**1994**). Oviposition of the Andean potato weevil, *Premnotrypes vorax* (Hustache)(Coleoptera Curculionidae). Revista Colombiana de Entomología **20**(3): 165-167.

Vallenas Bellota, J. (**1998**). Informe sobre el manejo integrado del gorgojo de los Andes y polilla de la papa - Cusco - Peru. Chinchero, Agreement International Potato Center - Asociación ARARIWA**:** 38.

van Emden, F. (**1952**). On the taxonomy of Rhynchophora larvae: Adelognatha and Alophinae (Insecta: Coleoptera). Proceedings of the Zoological Society of London **122**(3): 651-795.

Vargas Oña, J. J. (**1994**). Biología, control químico y etológico del gorgojo de los Andes (*Rhigopsidius tucumanus*) bajo la influencia de Lequezana. Potosí (Bolivia), Thesis of the Universidad Autónoma Tomas Frías**:** 156pp.

Vasquez Porras, C. Y. and M. F. Suarez Lopez (**1997**). Evaluación del efecto del diflubenzuron sobre la oogenesis de las hembras y el desarrollo de las progenies del gusano blanco de la papa *Premnotrypes vorax* (Hustache). Tunja (Colombia), Thesis of the Universidad Pedagogica y Tecnologica de Colombia**:** 53pp.

Vera, A., J. Alcázar, F. Cisneros and W. Catalán (**1994**). Evaluacion de la susceptibilidad de los principales tuberculos andinos al gorgojo de los Andes. Proceedings of the XXXVI convención nacional de entomología, 11/06-10/1994, Iquitos (Peru), Sociedad Entomologica del Perú. pp. 7-8.

Vera Robles, A. (**1992**). Patogenicidad de cinco aislamientos del hongo *Beauveria brongniartii* (Sacc) Petch en el gorgojo de los Andes *Premnotrypes latithorax* Pierce (Coleoptera: Curculionidae). Cusco, Thesis of the Universidad Nacional de San Antonio Abad del Cusco**:** 99pp.

Vera Robles, A. (**2001**). El Hongo *Beauveria brongniartii* (Saccardo) Petch, su multiplicación y aplicación para el control del Gorgojo de los Andes *Premnotrypes spp.* (Coleoptera: Curculionidae). Lima, Thesis of the Universidad Nacional Agraria La Molina**:** 171pp.

Vigiana, A. and M. Serrano (**2001**). Manejo integrado de plagas en papa. Unpublished Manuscript, Universidad Nacional de Jujuy, Jujuy (Argentina): 24pp.

Villamizar Carvajal, T. and E. H. Becerra Contreras (**1983**). Biología, ciclo de vida y control químico del gusano blanco de la papa *Premnotrypes vorax* Hustache con distintas formulaciones del Lindano. Tunja (Colombia), Thesis of the Universidad Pedagógica y Tecnológica de Colombia**:** 115pp.

Villano Tárraga, W. V. (**1994**). Dinámica poblacional del "gorgojo de los Andes" *Premnotrypes latithorax* (Pierce) KUSCHEL en campo y almacén. Cusco, Thesis of the Universidad Nacional de San Antonio Abad del Cusco**:** 100pp.

Waarts, Y. (**2003**). The impact of the Andean weevil (*Premnotrypes vorax*) on potato-production in Ecuador. Wageningen, Thesis of the Wageningen University**:** 76pp.

Wibmer, G. J. and C. W. O'Brien (**1986**). Annotated checklist of the weevils (Curculionidae *sensu lato*) of South America (Coleoptera: Curculionoidea). Gainesville (Florida), The American Entomological Institute. 563pp.

Wille T., J. E. (**1952**). Entomología agricola del Perú. Lima, Ministerio de Agricultura del Perú. 544pp.

Yábar Landa, E. (**1986**). Control of Andean potato weevil storaging potatoes in diffuse light. Revista Peruana de Entomología **29**: 95-97.

Yábar Landa, E. (**1988**). Cultural practices integration for the control of the Andean potato weevil (*Premnotrypes* spp.). Revista Latinoamericana de la Papa **1**(1): 120-131.

Yábar Landa, E. (**1994**). Manejo ecológico del gorgojo de los Andes. Lima, RAAA. 119pp.

Yábar Landa, E. (**2001**). Respuesta funcional de algunos carabidae (col. adephaga) depredadores de huevos de gorgojo de los Andes: *Premnotrypes latithorax* (col. Curculionidae). Proceedings of the Manejo iontegrado de plagas de los principales cultivos andinos. Memoria del seminario taller nacional y regional. Valle Sagrado de los Incas, June, 20-22 and October 21-23 2001, Urubamba - Cusco (Peru), ARARIWA. pp. 21-23.

Yábar Landa, E., J. Alcázar Sedano, W. Cossio L., D. Farfán F. and S. Zúñiga F. (**2005**). Variabilidad genética en gorgojo de los Andes: *Premnotrypes* spp. (Col. Curculionidae). Cusco (Peru), Universidad Nacional de San Antonio Abad del Cusco - International Potato Center**:** 20.

Yábar Landa, E., A. Alfaro T., Y. Valdez and W. Cossio (**2003**). Population density and pupating depth of the Andean potato weevil: *Premnotrypes latithorax* (Col. Curculionidae) in fields and stores. Cantua **12**: 74-77.

Yábar Landa, E., A. Loayza, E. Guzmán, P. G. Valero, F. Flores L., M. A. Tapia and E. López (**1995**). Sistemas de almacenamiento en papa. In: Primer seminario-taller: Agroecologia en comunidades altoandinas: Problematica y posibilidades. E. Yábar Landa, E. Velarde Duran and O. Ochoa Mendieta (Eds). Sicuani (Peru), RAAA: pp. 40-51.

Zavala Aguirre, J. A. (**1995**). Biocontrol del gorgojo de los Andes *Premnotrypes suturicallus* Kuschel mediante el hongo entomopatogeno *Beauveria brongniartii* en condiciones de campo y almacenes de papas. Huancayo, Thesis of the Universidad Nacional del Centro del Perú**:** 94pp.

Zenner de Polanía, I. (**1986**). Guía general de manejo de plagas en el cultivo de la papa. Bogotá, Programa Andino Cooperativo de Investigación en Papa. 36pp.

Zenner de Polanía, I. (**1990**). Research and management strategies for potato insect pests in Colombia. In: Integrated pest Management for Tropical Root and Tuber Crops. Proceedings of the workshop on the global status of and prospects for integrated pest management of root and tuber crops in the tropics. S. K. Hahn and F. E. Caveness (Eds). Ibadan (Nigeria), International Institute of Tropical Agriculture: pp. 139-148.

Zenner, I. and L. Posada O. (**1968**). Generalidades sobre el gusano blanco de la papa *Premnotrypes vorax* (Hustache). Agricultura Tropical **24**(1): 33-40.

A survey on the incidence of the Andean potato weevil and its control in the Peruvian Andes

Magnus Kühne, Stefan Vidal and Oscar Ortiz

Abstract

Interviews with over 600 farmers from all over the Peruvian Andes covering 7 regions were analysed for this survey. Interviewed farmers were small holders planting in average 1/4 ha of potato. The Andean potato weevil (APW) is seen by most farmers as the most important problem in potato production apart from the plant disease late blight. Main harvest month was May though harvests occurred year around. In average, farmers reported damages of 23% caused by APW. Main damage incidences were found for the harvests during April to July, but some damages were reported for each month of the year. Significantly lower damage rates were reported for locations below 3000 meters altitude above sea level and above 3750 meters. Most frequently applied insecticides have been Metamidophos and Carbofuran, while 27% of the farmers did not use any insecticide.

Keywords: *Premnotrypes* spp., potato pest, damage rate, insecticides, altitude, Peru, late blight, *Phytophthora infestans*.

Introduction

Potato is one of the most important stable crops in the Andes. In 2002, in Peru on 270,815 ha 3,296,221 t of potato were harvested resulting in a yield of 12.2 t/ha at a price of 0.04 US$/kg making it the third crop after rice and corn in planted area. On the other side, price per kg in the field was one of the lowest for all agricultural products except for alfalfa and some tropical fruits (Instituto Nacional de Estadística e Informática 2003). At altitudes above 3500 meters above sea level, especially in remote areas, people rely to a great extent on potato for their alimentation. But potato is also the crop with the highest production costs. High seed potato prices, fertilizer requirements and labour costs result in 3 to 4 times higher costs per ha than other stable crops like wheat, barley or corn. Only some minor tuber crops require a similar investment (Sanchez Enriquez 1993; Bernet, personal communication). Crop losses translate into high economic losses and some time the loss of the complete yield. Most farmers in the Peruvian highlands are small holders producing mainly for their own consumption and sell the surplus to trade it for other basic food or school materials. Though it is an import source of income for those with better market accessibility.

The Andean potato weevil (APW) is distributed in the Andes from Venezuela to northwestern Argentina at altitudes above 2800 meters (Alcázar and Cisneros 1999). It encompasses a complex of 12 species of the genus *Premnotrypes* of which 10 occur in Peru as well as two species of *Rhigopsidius*. Adults are flightless and need potato to complete their life cycle. Infestation by APW occurs in the field with larvae feeding in the tubers making them inadequate for commercialisation. Farmers may use slightly damaged tubers for their own consumption, while heavily damaged tubers are fed to the animals (Winters and Fano 1997).

APW is one of the most important potato pests in the Andes (Ewell et al. 1994, Raman et al. 1994, Ortiz et al. 1996). Only late blight rivals APW in inflicting economic damage on potatoes production in this region (Ortiz et al. 1996). Interviewing 180 farmers in 1978 in the Cusco area in southern Peru, Franco et al. (1983) found, that at altitudes above 3000 meters framers perceived APW as the third most important insect problem after stem borer and *Epicauta* sp. In interviews with 270 farmers in the Rio Mantaro valley in central Peru, APW was by far the most frequently mentioned pest in potato (89% in mean against 44% for stem borers) (Franco and Horton 1979). In 1985 in a survey with 85 farmers covering both regions, farmers ranked APW as the main problem in potato (average rank 2.4 on a scale of 0-3) together with cut worms (Noctuidae) and followed by fungal diseases (2.3), potato tuber moth (PTM) (2.0), flea beetle (*Epitrix* sp.)(1.9), stem borer (1.9) and blister beetle (*Epicauta* sp.)(1.7) (Ewell et al. 1994).

Surveys on APW incidence and its control have mainly focused on only few villages or one area or date back more than 20 years. This survey attempts to actualise the information and cover a broader range of areas all over the Peruvian highlands. The present study focuses on the incidence of Andean potato weevil and its control. It includes information on late blight, which is considered to be the most important biotic problem in potato production in the Andes beside APW.

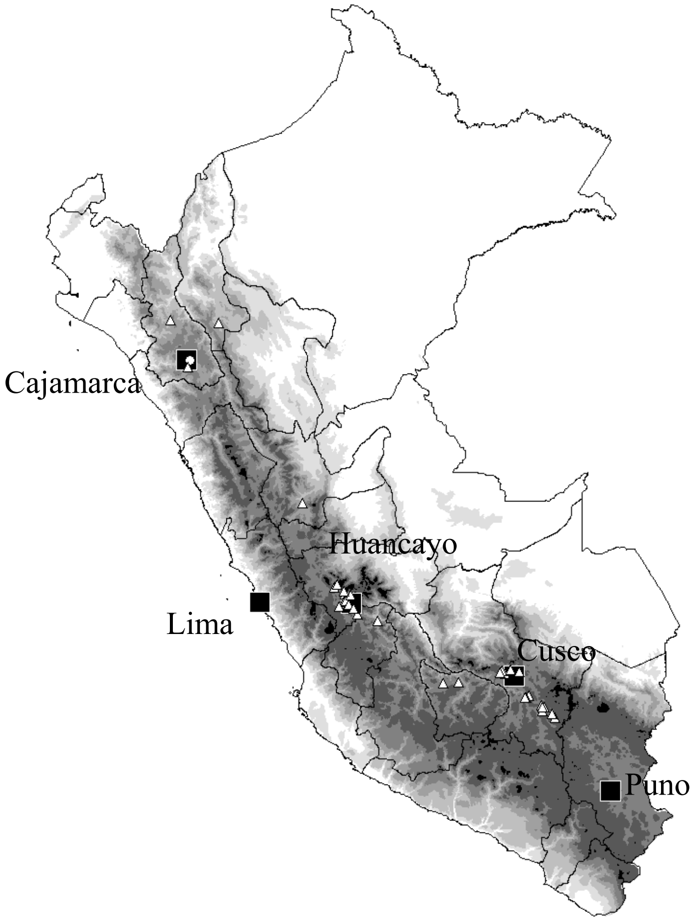

Figure 1: Interview locations in Peru. ○: Survey 1 location (near Cajamarca); △: survey 2 locations; ■: Main cities; symbols may overlap.

Material and method

Survey 1

In November 1999, a broad survey on potato production was run in Baños del Inca (7°10’S, 78°28’W) close to the departmental capital of Cajamarca in northern Peru (see Figure 1), situated between 2670 and 3900 meters above sea level. Part of the generated data is presented in this paper.

Eight farmer communities of the potato growing areas were visited and 92 farmers were interviewed (29 farmers having participated between 1992 and 1996 in an integrated pest management project which included training in the biology of the pests) during one hour with a questionnaire including pre-codified and open questions. Interviews were held in Spanish. Farmers for interviews were selected from a list of farmers of each community provided by community leaders in a previously organised meeting and introduction was through workers of the municipality.

Themes covered general characteristics of the farm and some socio-economic data, potato varieties and seed potato quality used, agronomic practices, use of inputs like fertilizer, insecticides and fungicides, storage practices and problems in potato production. More detailed questions were asked on knowledge on the biology and control measures for the Andean potato weevil, potato tuber moth and late blight and on the use of pesticides. The interviews focused on the previous cropping season (1998/99). This allowed getting specific information for each farmer rather than getting general responses, which would tend to make responses of the farmers similar to each other.

Survey 2

A one-page questionnaire in Spanish was elaborated with closed, pre-codified questions. To receive precise responses questions focused on one field and all labour and the harvest related to this field. Questions covered the following topics: location (5 entries), month of harvest and yield (5 entries), damage and control of the Andean potato weevil (6 entries) and of late blight (*Phytophthora infestans*) (5 entries) and 5 more entries on other activities in the field. Damages by APW were assessed using a scale with 11 steps from 0 to 100% and for late blight a scale with 6 steps from 0 to 100% was used.

In December 2001, questionnaires were distributed through a FAO - Farmer Field School project to the facilitators of 112 Farmer Field Schools (FFS) in the Peruvian highlands. Interviewers were not specially trained but received a one-page guide with instructions on how to fill in the questionnaire. Interviews were run between December 20th 2001 and May 24th 2002. Answers refer to the harvest period that preceded the interview.

628 filled in questionnaires were return from 76 highland communities covering 7 Peruvian regions. In 150 cases farmers responded twice, referring in the second interview to a second field and its management. In total, information on the APW incidence and management of 778 potato fields was analysed. Most interviews were held in the regions Cusco (218 interviews) and Junín (379). Other regions covered were Apurimac (34 interviews), Huancavelica (83), Huanuco (15), Cajamarca (37) and Amazonas (12) (Figure 1). For statistic analyses the statistical packages of SPSS version 10 and SYSTAT version 10 were used.

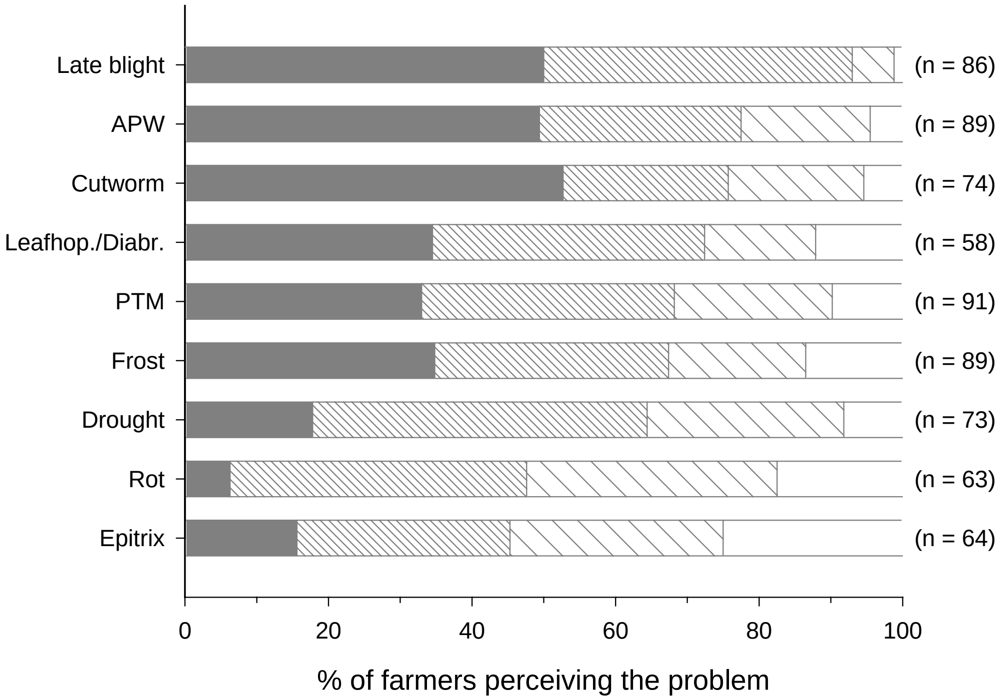

Figure 2: Problems in potato production as perceived by the farmers of Baños del Inca. ■ heavy problem; ▨ moderate problem; ▧ slight problem; □ no problem.

Results

Survey 1

The study showed that 39% of the farmers grow potato for own consumption. Only 12% sold more than 10 "arrobas" (one arroba corresponds to approximately 11.5 kg). Most people sell potato only to cover basic necessities. The most frequently mentioned problems in potato production in Baños del Inca are late blight followed by APW and cutworm (a noctuidae larvae) (Figure 2). The data may be slightly distorted because the farmers do not always identify correctly the pests. Late blight for example might be any burning of the foliage, of biotic or abiotic nature. *Diabotrica* sp. too could not be clearly differentiated in the interviews from other insect pests. The knowledge about the pests is quite variable. Of those farmers who had received IPM training, almost 100% new the life stages of APW and the relation between the larva and the adult. Of those without IPM training, this knowledge varied with 35% not knowing the adult weevil and 68% not knowing that larva and adult are the same pest. Damage by APW as reported by the farmers averaged 17.3% ranging from 0 to 100%. 51 out of 92 (55%) applied insecticides, of which 63% applied Metamidophos and 22% Carbofuran.

Survey 2

Locations of the interview sites were in median 3400 meters above sea level with 78.8% between 3250 and 3750 meters, 10% below 3000 m and 5% above 3750 meters above sea level. Indications of altitude refer to the altitude of the FFS or the village, which implies that real altitude of fields may differ considerable. The study area extended between 6°22' and 14°21.46' southern latitude in Peru (Figure 1).

Potato production:

Interviewed farmers planted in average 300 kg of potato, which corresponds roughly to an area of 0.25 ha (calculations are based on the assumption, that 1200 kg seed potatoes are used for planting 1 ha with approximately 90 cm between rows and 30 cm between each plant (Alcázar, personal communication). 10% of the farmers planted more than 1200 kg (1 ha) while 5% planted less than 59 kg (490 m^2)(n=675). Farmers harvested in mean 8.55 times the tuber quantity they planted which corresponded to a yield of 10.26 t/ha (5% trimmed mean was 7.92 t/ha). 5% of the farmers harvested over 23 t/ha and 5% harvested below 2.3 t/ha. No correlation was found between the amount of potato quantity planted and yield (Spearman correlation factor, $p < 0.05$), discarding a bias due to the size. May is the month when most farmer harvested (37.3%). Only very few farmers harvested during the second half of the year (generally the rainy season in most parts of Peru is from end of November until April) (Table **1**).

Table 1: Number of harvesting farmers for each month.

Month	n	%	Month	n	%	Month	n	%
Jan	46	5.9%	May	290	37.3%	Sep	23	3.0%
Feb	72	9.3%	Jun	88	11.3%	Oct	23	3.0%
Mar	44	5.7%	Jul	16	2.1%	Nov	14	1.8%
Apr	118	15.2%	Aug	13	1.7%	Dec	31	4.0%
						Total	778	100.0%

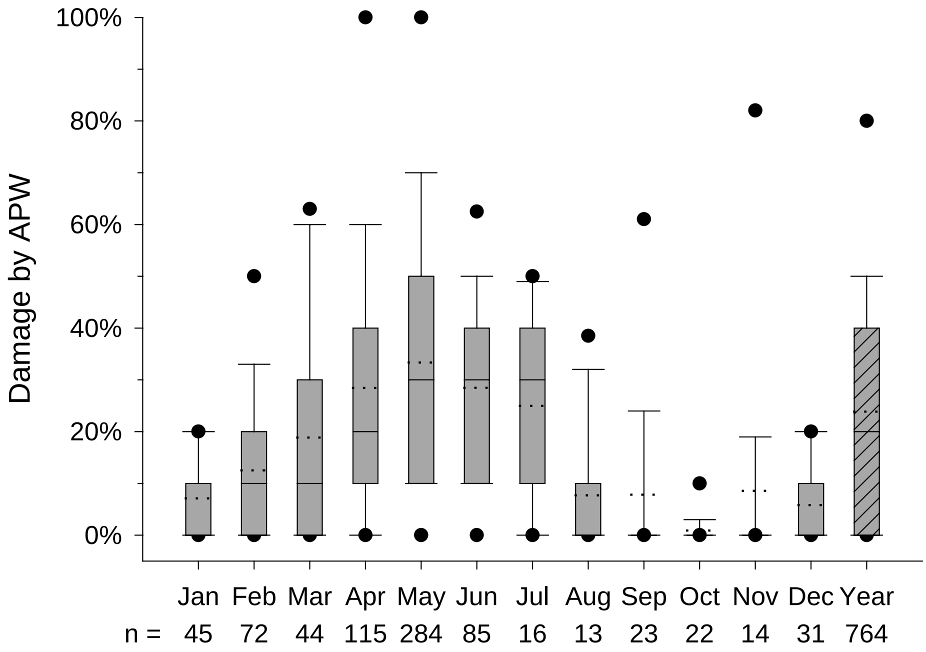

Figure 3: Damage caused by APW depending on month of harvest. The last box shows the results for the whole year. Boxes indicate interquartil range, whiskers indicate 10 and 90% percentiles, dots indicate 5 and 95% percentiles, solid lines within the boxes indicate the medians, dotted lines indicate the means. Numbers of cases for each month (n) are indicated on the x axes.

APW damage:

74% of the farmers mentioned occurrence of damage caused by APW. In mean, damage by APW reported by farmers was 23.8% ranging from 0% to 100% with a median of 20% and with 25% mentioning over 40% of damage (Figure 3). The month of harvest influenced significantly APW incidence. Farmers harvesting between April and July mentioned the highest damage rate while those harvesting during September to November mentioned almost no APW incidence. Nevertheless, APW damage occurred year around. APW damage was significantly lower at altitudes below 3000 meters and above 3750 meters above sea level (Figure 4). Differences between categories are significant (Kruskal-Wallis, $p < 0.05$). No correlation between latitude and APW damage was found (Spearman, $p < 0.05$). Equally no correlation between planting quantity and APW damage (Spearman, $p < 0.05$) was found, discarding a bias due to farm size.

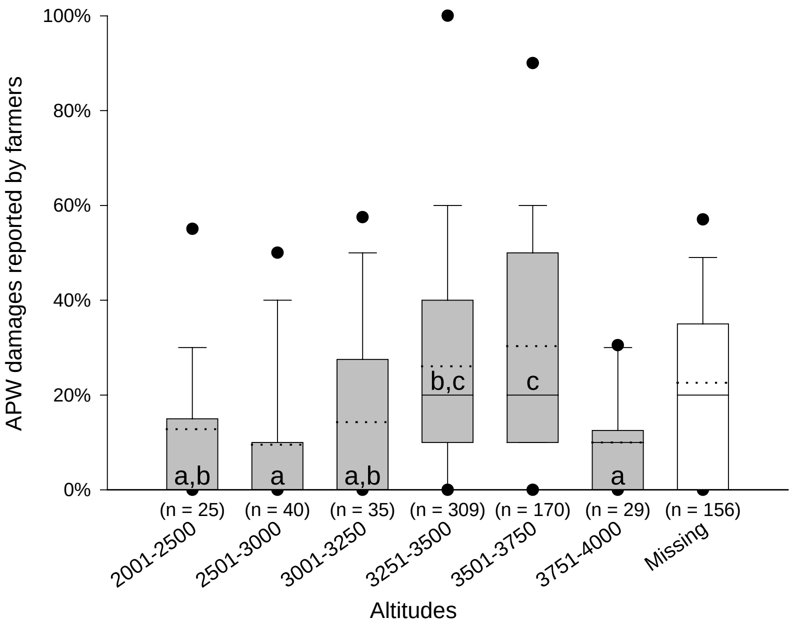

Figure 4: Damage caused by APW by altitude categories. The last category represents surveys without altitude indications. Boxes indicate interquartil range, whiskers indicate 10 and 90 percentiles, dots indicate 5 and 95% percentiles, solid lines within the boxes indicate the medians, dotted lines indicate the means. Same letters indicate no difference (Tukey, $p < 0.05$).

Insecticide applications:

73.3 % of the farmers applied some insecticide. Most farmers apply two or three times per season although some apply up to 8 times (see n in Figure 6). Most commonly used insecticides are Metamidophos (organophosphate) and Carbofuran (carbamate) followed by Cypermethrin (pyrethroid) (Figure 5). 41% of the farmers apply highly toxic products 30% extremely toxic products and 5% reported the use of banned products (following the classification of the Peruvian National Agricultural Health Service - SENASA). Comparing the number of insecticide applications with the reported damage caused by APW, a positive correlation can be observed with less damage mentioned by farmers not applying at all and higher damage rates for farmers applying more than 3 times (Figure 6). However, there is also a highly significant correlation between the amount of potatoes planted and the number of insecticide applications (Spearman, $p < 0.01$). This shows, that farmers with a large potato production apply more frequently. Grouping the insecticides by chemical class revealed, that farmers applying insecticides of the classes organochlorines and organophospahtes reported the highest damage rates (Figure 7).

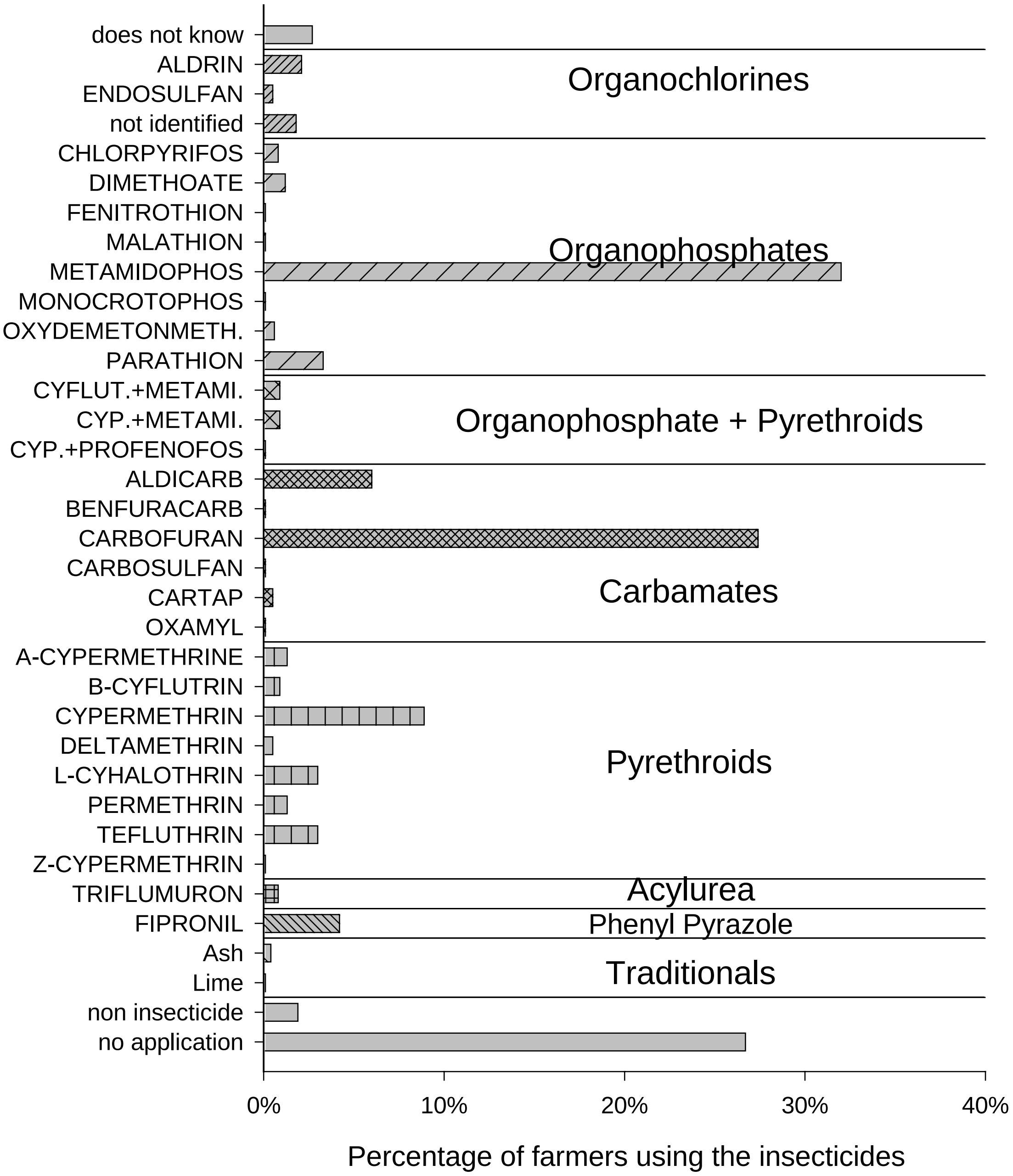

Figure 5: Insecticides and the chemical class they belong to used by the farmers. Percentages sum more than 100 because some farmer used more than one insecticide.

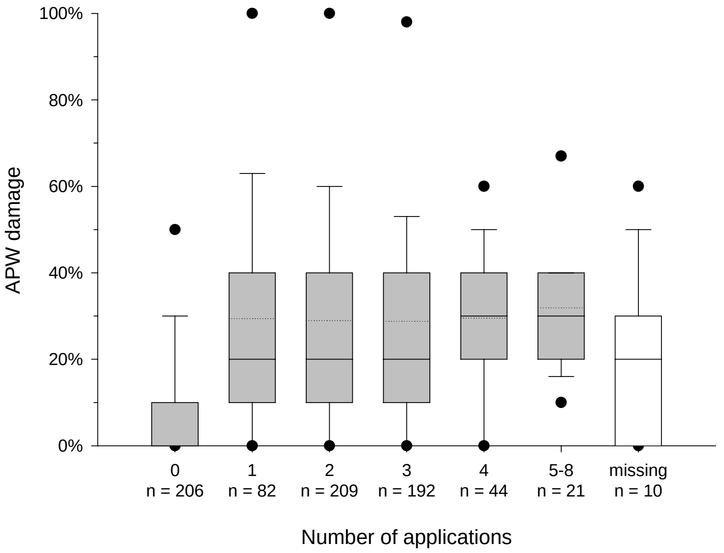

Figure 6: Damage caused by APW separated for the number of applications. The last box represents the surveys without any indication on the number of applications. Boxes indicate interquartil range, whiskers indicate 10 and 90 percentiles, dots indicate 5 and 95% percentiles, solid lines within the boxes indicate the medians, dotted lines indicate the means.

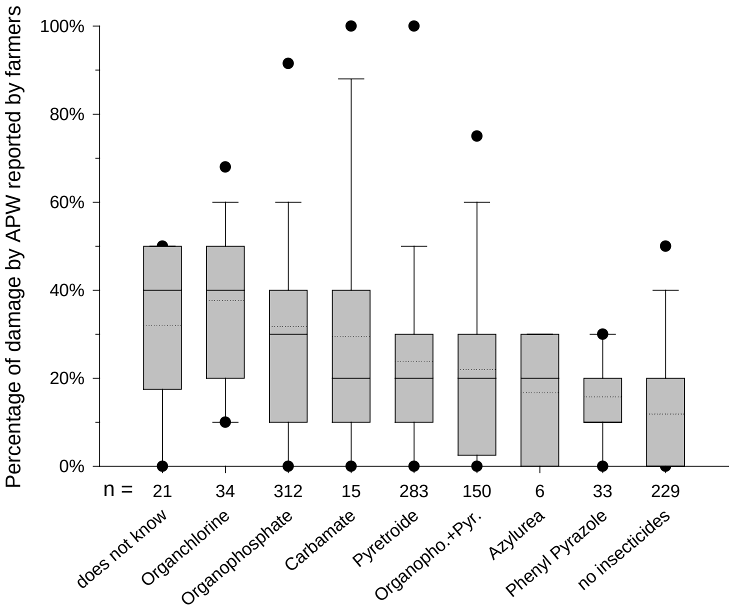

Figure 7: Relation between the chemical class of insecticides and the damage caused by APW. Boxes indicate interquartil range, whiskers indicate 10 and 90 percentiles, dots indicate 5 and 95% percentiles, solid lines within the boxes indicate the medians, dotted lines indicate the means.

Late blight damage and fungicide use:

Only 7.8 % of the farmers reported no damage by late blight. In a scale of 1 to 4 with 1 indicating low damage and 4 indicating high damage incidence, 39.8 % indicated 1, 16.2 % 2 and only 5.4 % and 1.6 % indicated 3 respectively 4. Most frequently used fungicides are Mancozeb, Propineb, Cymoxanil and Metalaxil, while 45.9% of farmers were not using any fungicide (Figure 8). Most farmers applied fungicides 2 (24% of the farmers) or 3 (14%) times per cropping season, 7% applied only once, 4% applied 4 times and 6% of the farmers applied 5 times or more up to a maximum of 15 applications. No difference in APW damage was observed between farmers using and those not using fungicides.

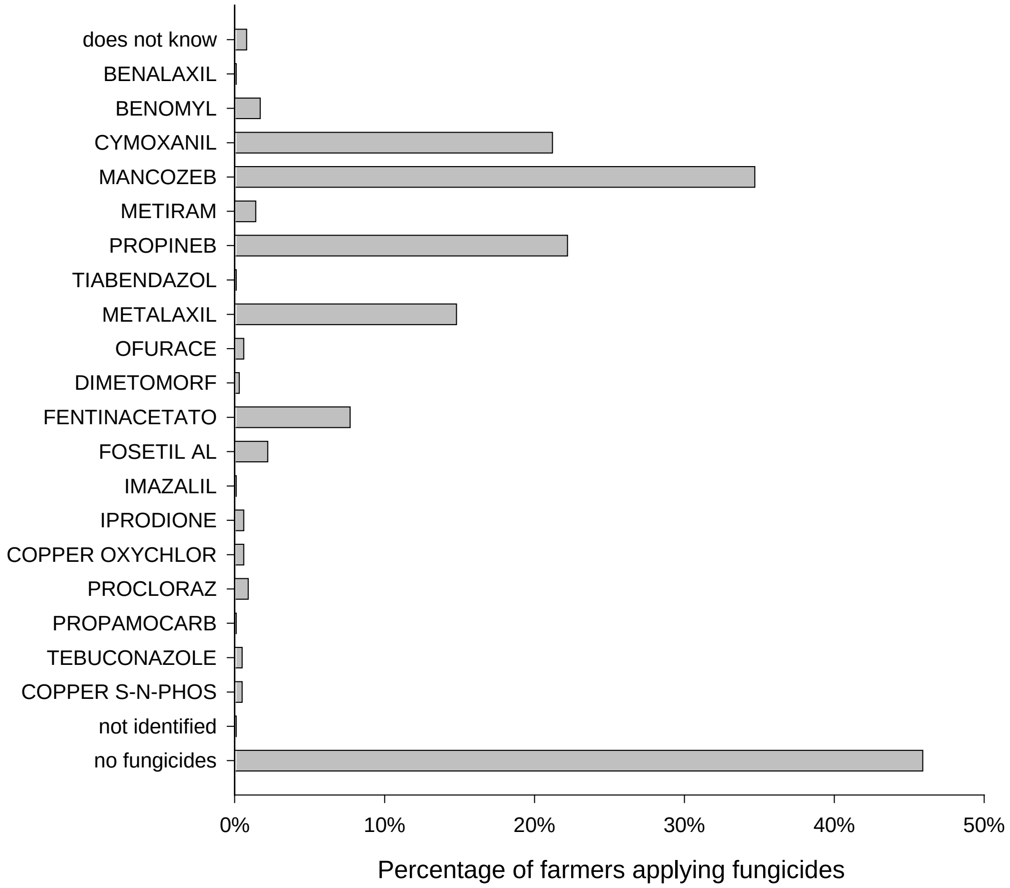

Figure 8: Fungicides used by the farmers. Percentages sum more than 100 because some farmer used more than one insecticide.

Discussion

APW damage:

The survey confirmed the importance of APW as a potato pest all over Peru. Average damage rate reported by the farmers was 23%. In other, regionally focused surveys the incidence of damage caused by APW varied greatly (Table 2, see also chap. 1). Analysing the data for a relation of APW damage incidence and altitude, we found a reduction of APW incidence below 3000 meters and above 3750 meters altitude while Ewell et al. (1990) found a sharp dropping of APW incidence above 4000 meters (only 4 surveys had been located above 4000 m). Up to 4000 meters they had found a slight increase of damage incidence with increasing altitude. Yábar Landa (1994) did not find any relation between altitudes and damage for altitudes between 3000 and 4000 meters. April to June were the months when most farmers harvested. The same was found by Franco and Horton (1979).

People have several ways to scope with the damage inflicted by APW: Tubers with APW damage are usually rejected from the market but are still used for direct consumption or for the elaboration of traditional “chuños” (a sort of freeze-dried potatoes). Traditionally, the largest tubers are selected for sale on the market, the smaller tubers are used for seed (with some tolerance to insect damage), damaged tubers are used for home consumption ("weevil larvae make potatoes sweeter"), seriously damaged tubers are fed to livestock and normally only a small part of the harvest is discarded (Ewell 1990). Value loss due to APW damage varied between 17 and 67% in a study made in the Cusco area in southern Peru (Ortiz et al. 1996).

Table 2: Summary of damage levels caused by APW as found in the literature.

region	APW specie	average damage	year of survey	reference
Ecuador	*P. vorax*	30%	1999	(Frankhauser 1999)
Cajamarca area	*P. vorax*	50%	1991	(Ortiz et al. 1996)
Huancayo area	*P. suturicallus*	16.4%	1986	(Ewell 1990)
Huancayo area	*P. suturicallus*	20%	1980	(Horton et al. 1980)
Cusco area	*P. latithorax*	26-28%	1994	(Yábar Landa 1994)

Insecticide use:

In the present survey Metamidophos and Carbofuran were by far the most common insecticides. Compared with earlier surveys a change in insecticide use has taken place. In 1978 in the Cusco area as well as in the Mantaro valley, Aldrin was the most frequently used insecticide followed by Parathion and Oxidemeton (the first two being banned now) (Horton et al. 1980, Franco et al. 1983). In 1986, most farmers in Mantaro valley spend between 40 and 170 US$ in insecticides (ranging from 0 to 400 US$). Of 176 farmers interviewed, 46% had used Carbofuran, 34% Aldrin, 33% parathion, 22% Metamidophos, 18% a mix of Aldrin with Parathion and 14% had used Dimethoate. In total 41 insecticides were mentioned (Ewell 1990). While in the present study 27% of farmers did not use any insecticide, Ewell (1990) found in 1985 only 4% not applying anything.

The great number of farmers still using highly and extremely toxic insecticides is especially concerning taking into account the finding of an interdisciplinary study run in the potato production area of Carchi in Ecuador (Crissman et al. 1998). The main finding of this study was, that the farmers and their households were highly contaminated with insecticides after applications. This affected the farmers' and their families' health. The effect on the economy and performance as well as on the intellectual capacity of the farmers and their equally exposed children was shown. The lack of awareness (typically statements are: "I feel a little bit ill after an application but that passed soon" or: "I am resistant to insecticides.") lets the farmer neglect basic security measures against intoxications.

The finding of higher damage reported by those farmers that apply more frequently insecticides might be explained by a build-up of resistance. But it might also be, that these farmers are more market oriented and less tolerant to damage by APW classifying more potatoes as damaged (especially as farmers with larger production applied more frequently). The high damage rate reported by farmers using organochlorines may be due to the fact, that most of these insecticides are banned and so farmers might be using adulterated products. The fact, that damage incidences by APW are independent of applications of fungicides let us rejects doubts that these applications may interfere with the activity of naturally in the soil occurring and APW infecting entomopathogenic fungi.

The data gathered in these surveys allowed getting an appraisal of the APW infestation over a wide range of locations in Peru and on the actual situation in the control measures used by the farmers. The analysis of the great number of interviews and locations represented was possible by reducing the amount of data collected in each interview. Questions arising from this survey should be clarified in more detailed interviews with farmers. These data represent also an update on national level in Peru on the importance and control of the Andean potato weevil, that will guide further intervention strategies.

Acknowledgments

We thank José Tenorio and all facilitators from the FAO-Farmer Field Schools project for the distributing, filling in and sending back of questionnaires and Marcelo Trebejo from the International Potato Center (CIP) in Lima (Peru) for digitalizing the data. These investigations were made at CIP and were possible due to the financial support by the Government of the Grand-Duchy of Luxembourg for the second survey and BeAF/GTZ for the first survey.

References cited

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

Crissman, C. C., J. M. Antle and S. M. Capalbo, Eds. (**1998**). Economic, environmental, and health tradeoffs in agriculture: Pesticides and the sustainability of Andean potato porduction. Natural resource management and policy. Dordrecht (The Netherlands), Kluwer Academic Publishers. 281pp.

Ewell, P. T. (**1990**). Socio-economic aspects of farmer-oriented research for potato IPM. In: Integrated pest Management for Tropical Root and Tuber Crops. Proceedings of the workshop on the global status of and prospects for integrated pest management of root and tuber crops in the tropics. S. K. Hahn and F. E. Caveness (Eds). Ibadan (Nigeria), International Institute of Tropical Agriculture: pp. 108-124.

Ewell, P. T., H. Fano, K. V. Raman, J. Alcázar, M. Palacios and J. Carhuamaca (**1990**). Farmer management of potato insect pests in Peru. Lima, International Potato Center. 77pp.

Ewell, P. T., K. O. Fuglie and K. V. Raman (**1994**). Farmers' perspectives on potato pest management in developing countries: Interdisciplinary research at the International Potato Center (CIP). In: Advances in potato pest biology and management. G. W. Zehnder, M. L. Powelson, R. K. Jansson and K. V. Raman (Eds). St. Paul (MN, USA), APS PRESS: pp. 597-615.

Franco, E. and D. Horton (**1979**). Produccion y utilizacion de la papa en el Valle del Mantaro, Peru: Resultados de la encuesta agro-economica de visita unica. Working Paper. Lima (Peru), International Potato Center**:** 118.

Franco, E., C. Moreno and A. Jorge (**1983**). Produccion y utilizacion de la papa en la region del Cuzco: Resultados de una encuesta de visita unica. Working Paper. Lima, International Potato Center**:** 103.

Frankhauser, C. (**1999**). Main diseases affecting seed degeneration in Ecuador: New perspectives for seed production in the Andes. Proceedings of the 14th Triennial Conference of the European Association for Potato Research, Sorrento (Italy), Assessorato Agricoltura Region Campania. pp. 50-51.

Horton, D., F. Tardieu and M. Benavides (**1980**). Tecnología de la producción de papa en el valle del Mantaro, Perú: Resultados de una encuesta agro-económica de visita múltiple. Documento de trabajo. Lima (Peru), International Potato Center**:** 68.

Instituto Nacional de Estadística e Informática (**2003**). Perú, compendio estadístico 2003. Lima (Peru), INEI. 924pp.

Ortiz, O., J. Alcázar, W. Catalán, W. Villano, V. Cerna, H. Fano and T. S. Walker (**1996**). Economic impact of IPM practices on the Andean potato weevil in Peru. In: Case studies of the economic impact of CIP - related technology. T. S. Walker and C. C. Crissman (Eds). Lima, International Potato Center: pp. 95-110.

Raman, K. V., A. M. Golmirzaie, M. Palacios and J. Tenorio (**1994**). Inheritance of resistance to insects and mites. In: Potato genetics. J. E. Bradshaw and G. R. Mackay (Eds). Wallingford, CAB International: pp. 447-463.

Sanchez Enriquez, R. (**1993**). Ecologia, produccion y desarrollo campesino. Lima (Peru), Convenio TINTA (CUSO-CEDEPAS-EDAC). 248pp.

Winters, P. and H. Fano (**1997**). The economics of biological control in Peruvian potato production. Working Paper. Lima, International Potato Center**:** 33.

Yábar Landa, E. (**1994**). Manejo ecológico del gorgojo de los Andes. Lima, RAAA. 119pp.

Part two:

APW - Ecology

Temperature depending development of the cold adapted Andean potato weevil *Premnotrypes suturicallus*

Magnus Kühne, Marc Sporleder and Stefan Vidal

Abstract

Temperature depending development and mortality rates for all life stages of the Andean potato weevil *Premnotrypes suturicallus* and adult longevity and fecundity were determined under laboratory conditions at constant temperatures. Data were adapted to the linear day-degree model as well as to the non-linear thermodynamic model developed by Sharpe and DeMichele (1977) and modified by Schoolfield et al. (1981) and Ikemoto (2005). The model has been validated under fluctuating temperatures in the laboratory and under field conditions. Larval development was well represented by the model while pre-pupae development showed high deviations, probably due to soil humidity conditions. Highest survival rates were found to be for all life stages between 11° C and 15° C. Larvae were the most susceptible stages to high temperatures with no survival at 25° C. The total development time from egg to adult varied from 70 days at 23° C to 500 days at 5° C. Including the maturation time of adults in the soil, the preovipostion period and the female age at 50% egg production, the medium generation times varied from 219 days at 15° C to 641 days at 5° C. Fecundity was highest at 13° C with over 250 eggs per female and lowest at 20° C. The results show, that *P. suturicallus* is a potato pest adapted to cold climate conditions.

Keywords: Development rate, thermodynamic model, Sharpe-DeMichele model, cold adaptation, potato pest, oviposition, longevity, life parameter.

Introduction

The Andean potato weevil (APW) is a complex of 14 species of the genera *Premnotrypes* and *Rhigopsidius* (Coleoptera: Curculionidae). It is distributed in the Andes above 2800 meter altitude between Venezuela and north-western Argentina where it is the main potato pest in these altitudes causing regularly 20 to 25% damage. Its altitudinal distribution extents up to 4500 meter, corresponding probably to the distribution limit of potato. Adults are night active and oviposit close to the plant stem into dry plant debris. Neonate larvae bore into the tubers where they remain until their development is accomplished passing through 4 larval stages. Mature larvae leave the tuber for pupation in the soil. Before the actual pupation a resting stage exists commonly referred to as pre-pupa. The freshly moulted adults remain in the soil for maturation.

After emergence, the flightless insects migrate to new potato fields (Alcalá C. and Alcázar S. 1976, Alcázar and Cisneros 1999).

Thorough understanding of the life cycle of a pest and the biotic and abiotic factors influencing this life cycle are crucial to develop population dynamic models. These again are important tools for decision taking in integrated pest management strategies by allowing predictions on population developments under varying environmental conditions. Life stage development as a consequence of climatic conditions influences for example the optimal timing of control measures (Sporleder et al. 2004). Especially in a heterogeneous ecosystem like the Andes with huge temperature and humidity variation due to altitudinal differences within small distances an understanding of the climatic factors influencing the development of a pest may give important clues for its control. To describe the relation between development rate (1/development time) and temperature, divers functions have been used. Normally models using development rate against temperature are preferred because they allow accumulating mean daily (or hourly) rates under fluctuating temperature conditions (Wagner et al. 1984). Models may focus on simplicity with few variables for easy application or more complex functions that describe reality more detailed and encompass a higher diversity of environmental conditions.

The life cycles and development times of several species of the APW complex have been studied under ambient conditions without temperature control. *P. suturicallus* has been studied by Alcalá C. and Alcázar S. (1976) . Under fluctuating temperatures with a mean of 10.5° C they found a mean development time from oviposition to adult of 176 days. This included 33 days for egg development, 46 for larvae, 43 for pre-pupae and 54 for pupae. Additionally, adult maturation until its emergence from soil took 115 days and pre-oviposition period was 9 days. Mean duration of the oviposition period was 106 days with in mean 631 eggs.

The present study proposes to determine the effect of temperature on the development rate and the mortality rates of the different life stages and the reproduction rate of *P. suturicallus* under controlled conditions.

Methods

Origin and rearing of the insect material

Weevils of all life stages were taken from a rearing colony at the International Potato Center (CIP) in Lima (Peru). Adult weevils had been collected from fields on the eastern slopes of the Rio Mantaro valley (province Concepcíon) in the central highlands of Peru with yearly new introductions into the rearing colony. Adults were kept in groups of 50 to 300 individuals in boxes with towel paper covering the bottom. For alimentation, halve potato tubers were added, generally of the variety Peruanita. For oviposition, 0.5 to 1 cm wide and 3 to 5 cm long stripes of several times tightly folded towel paper were added. Once per week egg clutches were collected and placed into petri dishes. Shortly before hatching, when head capsules became visible, petri dishes were sealed with parafilm to prevent escaping of the neonate larvae. The neonate larvae were placed within 48 h individually or in groups of maximum 3 into small containers (approximately 3 x 3 x 2.5 cm) containing a filter paper on the bottom and a small, halve potato tuber for alimentation. When the mature larvae abandoned the tuber, the tuber was removed and the larvae were either left for pupation in the same container or placed individually into microcentrifuge tubes containing sterilized soil with 10 to 15% water content for pupation. Rearing was kept in dark and at 18 to 20° C and produced approximately 3 generations per year.

Development times and mortalities

The effects of temperature and soil humidity on the life cycle of *P. suturicallus* were studied on separate cohorts for three life stages (eggs, larvae (stages within the potato tuber) and the stages staying in the soil (pre-pupae, pupae and maturing adult)). Temperature studies were run at fixed temperature between 5° C and 25° C. A sterilized and sifted loam soil was used for soil stage studies. The effective temperatures, as it was used in the analysis, were read from datalogger placed within the incubators during the experiments.

Eggs, oviposited maximum 24 h before the installation of the experiment, were placed in groups of 20 into petri dishes containing a filter paper and sealed with parafilm. Eggs were checked daily for hatched eggs. Neonate larvae of maximum 24 h age were placed in groups of 20 onto three potato tubers (variety Peruanita) within a container with a diameter of 12.5 cm and height of 12.5 cm. The containers were checked daily for mature larvae abandoning the tubers. Larvae were immediately weighted. Mature larvae that abandoned the tuber maximum 6 days earlier were placed into microcentrifuge tubes with sterilized and sifted soil with 8 to 12% water content. Evaluation took place three times a week, except for the 5° C treatment that was only evaluated once per week. Without opening the microcentrifuge tubes, moulting to pupa and to adult as well as adult melanization was checked. For the observation of cohorts throughout the whole cycle, eggs were placed individually in a small container onto potato tubers. Tubers were cropped at the bottom to assure easy access for the neonate larvae after hatching. Mature larvae leaving the tuber were placed with alcohol sterilized forceps into microcentrifuge caps as described above.

Development time was registered as the time until the next moult, except for larvae were the time until abandoning the tuber was registered. Survival of eggs and larvae was registered as the number of individuals passing on to the next stage while for pre-pupae and pupae mortality was registered directly.

Using the same methods, the established model was validated in a laboratory trial with fluctuating temperatures and in a field trial. In each case, development times for each life stage were observed for a cohort from egg to adult. Temperatures for the laboratory trial were programmed with 14 h at 21.8° C ± 1° C and 10 h at 4.6° C ± 0.5° C resulting in a mean temperature of 14.6 to 15.0° C. For the trial under field conditions, a box with the described containers was placed together with a datalogger at a shady place at the CIP field station in Huancayo (Peru) at 3311 meter altitude.

Oviposition and longevity

In different trials, couples of 1 male and 1 female or groups of 4 males and 4 females or 9 males and 7 females were placed together into containers with a diameter of approximately 12 cm. Potato tubers (variety Peruanita) and tightly folded strips of towel paper for ovipositions were added. Clutches of eggs were collected once a week and observed for viability. Trials were run at constant temperature between 4.9° C and 24.1° C. For the longevity, male and female survival time was registered at constant temperatures between 4.9 and 28° C. At –1.8° C, 35.3° C and 41.1° C evaluations were destructive, with separate groups of adults being observed for each evaluation date and means were calculated as median survival times by linear regression. The latest trial was also done with pre-pupae.

Model fitting

Development time was calculated as the median development time and analysed with two different models. For its simplicity, the linear day-degree model

$$\frac{1}{D} = -\frac{t}{k} + \frac{1}{k}T \qquad (1)$$

where D, T, t and k represent the duration of development (days), the temperature (° C), the estimated developmental zero (° C) and the effective cumulative temperature (degree-day, ° D), was adapted calculating the linear regression. From this, the theoretical lower development threshold (t) and the day-degrees necessary for the full development (k) of each stage was estimated (inverse of the slope of the regression). However, the linear model is only valid within a central temperature range. At higher and lower temperatures, development rate deviates from the linear regression. For this reason, a second model is applied, the non-linear thermodynamic model developed by Sharpe and DeMichele (1977) and modified by Schoolfield et al. (1981). This Model is based on the following assumptions: 1. The development rate is determined by a single hypothetical rate-controlling enzyme. 2. The development rate is proportional to the product of concentration of the active enzyme and its rate constant. 3. The rate controlling enzyme is reversibly inactivated at high and low temperatures, but a constant total concentration (active and inactive) is maintained independent of them. The supposed rate-controlling enzyme is regulated by the kinetics of a reaction following Eyring's absolute reaction rate theory. This model describes the non-linear response of development rates at high and low temperatures as well as the linear response at intermediate temperatures and is believed to be the most suitable model for the prediction of development times (Wagner et al. 1984). It was adapted by Ikemoto (2005) for the use with insects with low and high optimum development temperatures.

$$\mathrm{r} = \frac{\rho \frac{[T]}{[T_c]} \exp\left[\frac{\Delta H_A}{R}\left(\frac{1}{[T_c]} - \frac{1}{[T]}\right)\right]}{1 + \exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{[T_L]} - \frac{1}{[T]}\right)\right] + \exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{[T_H]} - \frac{1}{[T]}\right)\right]} \qquad (2)$$

r is the median development rate (1/D), T the absolute temperature (K), R the universal gas constant (1.987 cal/deg/mol), T_C the reference temperature assuming no enzyme deactivation (K), ρ the development rate at T_C (1/days), ΔH_A the enthalpy of activation of reaction (cal/mol), ΔH_L and ΔH_H the change in enthalpy associated with low respectively high temperature inactivation of enzymes (cal/mol) and T_L and T_H the temperature at which enzymes are half low respectively high temperature inactivated.

For solving non-linear regressions, it is important to choose adequate initial values for the parameters as these may have great influence on the results. We chose initial values of parameters using the method described by Ikemoto (2005). For T_C, values in the centre of the linear part between 12 and 15° C were chosen, for T_L, the value of t from the linear model was taken, ΔH_A was calculated estimating the slope on the Arrhenius plot (ln development rate versus inverse of temperature expressed in Kelvin) and ΔH_H, T_H and ΔH_L were found by graphical fitting. Using these initial values, the values for these six parameters were estimated using the solver in the Microsoft Excel tool menu minimizing the second-order Akaike's Information criteria for small samples (AICc) for the model (Suguira 1978, Burnham and Anderson 2002). ρ is estimated directly from T_C using the linear model. In the case that no data points exist for the lower or the upper end of the temperature range of development (non-linear regions), the second respectively the third term of the denominator of Formula 2 can be deleted. The number of parameters is reduced by 2 and the model will ignore the low respectively high temperature enzyme inactivation (Schoolfield et al. 1981). For the determination of the temperature range where the linear model is applicable, the model described by Ikemoto and Takai (2000) was used. Development time was multiplied by the corresponding temperature and plotted against the development time. Graphically, data points deviating from the linear tendency were excluded.

For validating the model, the development times observed under fluctuating temperatures were compared with the development rates calculated using the thermodynamic model with the parameters determined for each life stage. Based on hourly measured temperature data, development rates per day were estimated, divided by 24 and the results added up to the median time necessary until the transition to the next stage. This was done for each life stage with the corresponding temperatures and the results were backtransformed into development time.

Table 1: Estimated parameters of the linear and the thermodynamic models fitted to the median development rates of immature life stages of *Premnotrypes suturicallus*.

Model	**Parmeter**	**Egg**	**Larvae**	**Pre-pupae**	**Pupae**
linear	**intercept (-t/k)**	-0.02177	-0.01418	-0.03805	-0.01405
	slope (1/k)	0.0039	0.0027	0.0051	0.00357
	r^2	0.9191	0.9701	0.7322	0.9780
	t = T_{min} (° C)	5.55	5.26	7.40	3.94
thermo-dynamic	ρ	0.0324	0.0208	0.0277	0.0361
	T_C (K)	286.96	286.12	285.93	287.21
	[=° C]	[13.81]	[12.97]	[12.78]	[14.06]
	ΔH_A	19238.4	20524.9	21864.3*	13670.1
	T_L (K)	276.0	273.5	273.4	274.4
	[=° C]	[2.86]	[0.23]	[0.28]	[1.21]
	ΔH_L	-99973.55	-100000	-33700*	-42800*
	T_H (K)	299.2	296.3	298.4	299.8
	[=° C]	[26.02]	[23.13]	[25.22]	[26.61]
	ΔH_H	46743.078	50000.002	80000*	95600*
	r^2	0.9489	0.9684	0.8698	0.9954

*values fixed.

Results

Development under constant temperature conditions

Development rates of all immature life stages increased significantly with rising temperatures. Within the temperature range between approximately 10° C and 20° C, the development rate increases linearly with rising temperature. Above 20° C development rate was reduced, while below 10° C, development rate was increased as compared to the linear model. These deviations from the linear tendency could very well be represented with the thermodynamic model. In all cases, the thermodynamic model with 6 parameters could be fitted to the observed data (Figure 1). The estimated parameters for both models are shown in Table 1. Theoretical low temperature development thresholds calculated from the linear model were, 5.55° C, 5.26° C, 7.40° C and 3.94° C for eggs, larvae, pre-pupae and pupae respectively. The median degree-days (° D = inverse of the slope of the linear model) for the transition to the next stage within the temperature range of linear development were 256.4° D, 370.4° D, 196.1° D and 280.1° D for eggs, larvae, pre-pupae and pupae respectively, summing to 1103° D for the development from egg to adult. For

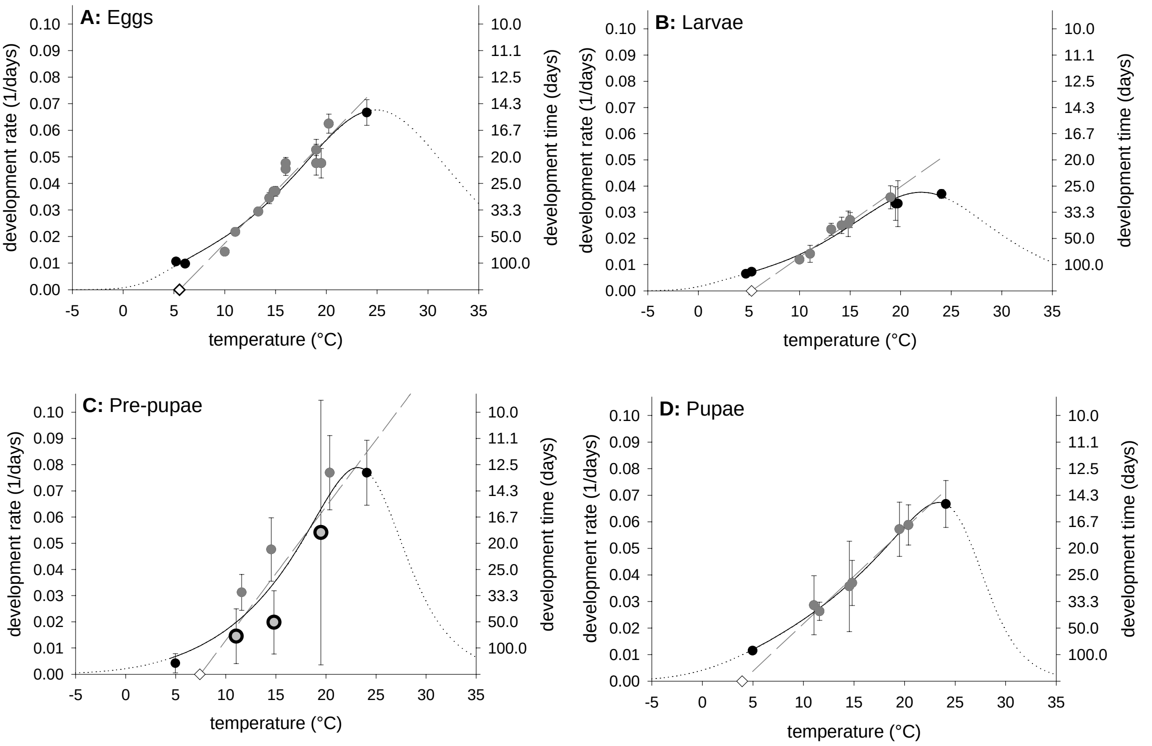

Figure 1: Temperature dependent median development rates of *P. suturicallus* eggs (A), larvae (B), pre-pupae (C) and pupae (D). Black solid lines represent development rates predicted by the thermodynamic model, dotted lines indicate extrapolations of the same model not supported by data, grey dashed lines represent the linear model, dots show observed data, grey dots show observed data used for linear model and white diamonds indicate the theoretical lower development threshold (for parameters see Table 1). Bars indicate standard deviations of medians. In graphic C, grey dots with black border are from one cohort (see text for further explanations).

one cohort, the data points of the development rate of pre-pupae are all below the regression line and show very high standard deviations (Figure 1C).

Backtransforming the development rates of the models for each life stage into development time (days), summing them up and retransforming the sum into development rates, results in the development rate model for one generation of *P. suturicallus* (Figure 2A). Developing times vary between almost 500 days at 5° C and a minimum of 70 days at 23° C. Observations of cohorts over the whole development cycle show similar tendencies, though resulted in most cases in lower development rate (longer development time). No regression model could be fitted to the maturation and melanization process of adults. This process was shortest at 14.5° C with 9 days, while it was prolonged at low temperatures with 11 days and 21 days at 11.6° C respectively 5° C and at higher temperatures with 16 and 18 days at 20.4° C respectively 24.1° C. Survival rate were above 90% except for 20.4° C with a survival rate of 84% and 24.1° C with a significantly reduced survival rate of 21%. The influence of temperature on the weight of mature larvae is shown in Figure 3. Maximum weight is reached when developing at a temperature of 13° C. For each temperature separately a lower larval weight was observed with longer development time.

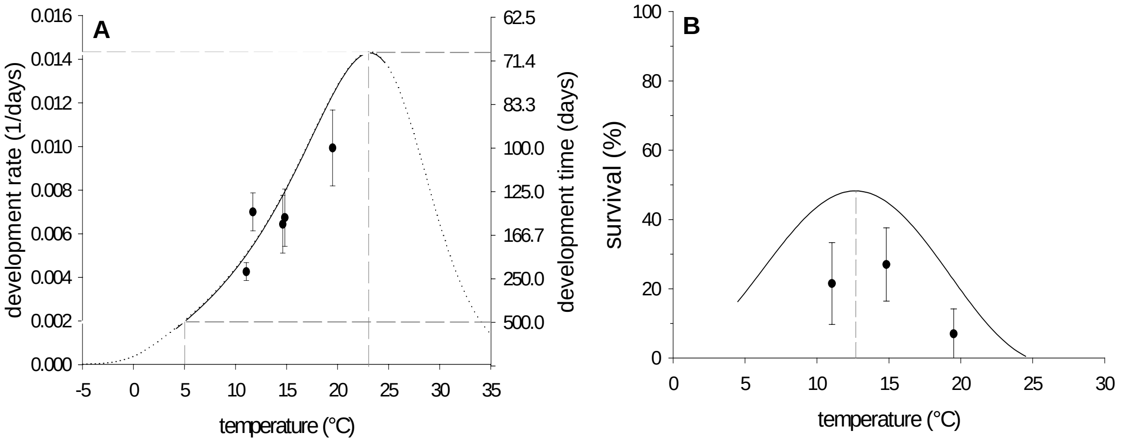

Figure 2: Temperature dependent development rate (A) and survival (B) for one generation of *P. suturicallus*. Functions represent models based on the summing of the separate models for each life stage. Dotted line indicates extrapolation of model not supported by data; drop lines show development time (days) and corresponding temperature at maximum development rate and at 5° C (A) respectively maximum survival temperature (B). Data points show values from cohorts observed from egg to adult.

Validation of model

The development times predicted by the thermodynamic model match in some cases quite well the observed development times while in other cases they do not match (Table 2). The most marked deviation is found for pre-pupae in the laboratory trial. But also for egg development time estimated is for both trials too long. The datalogger for recording the temperature conditions in the field failed for over a month at the beginning of the field trial in December. For this time laps, temperatures have been extrapolated using the first six days of recorded data. This may explain the deviation of observed egg development durations in the field trial from expected duration as temperature in December can be assumed to be lower than in January.

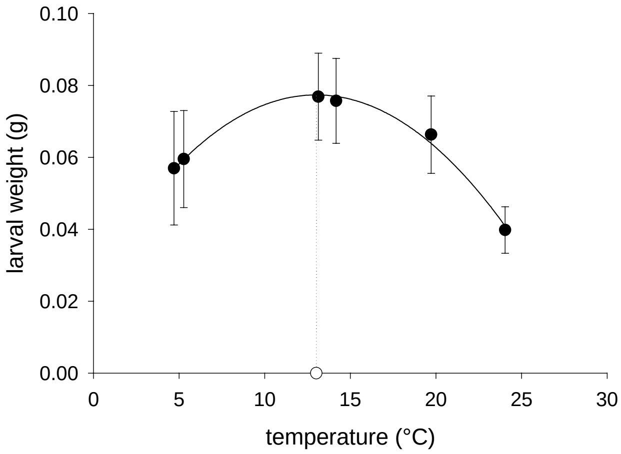

Figure 3: Temperature dependent weight of mature larvae of *P. suturicallus* after development at constant temperatures. Fitted curve is a second grade polynomial regression ($y = 0.0267 + 0.0078x + 0.0003x^2$; $r^2 = 0.9905$). Bars indicate standard deviations of medians. The white symbol indicates the temperature of maximum larval weight.

Table 2: Observed median development time of *P. suturicallus* eggs, larvae, pre-pupae and pupae at fluctuating temperatures in the laboratory (14 hours at 21.8° C and 10 hours at 4.6° C) and under field conditions, and the corresponding development times predicted by the thermodynamic model.

		egg	larvae	pre-pupae	pupae	cycle
Laboratory	*mean temperature (° C)*	*14.81*	*14.69*	*14.94*	*14.61*	
	(min – max temp. (° C))	*(3.74 - 25.17)*	*(4.15 - 22.48)*	*(4.15 - 22.48)*	*(3.31 - 22.48)*	
	observed dev. time (days)	30	35	59.5*	25	154
	predicted dev. time (days)	24.9	40.8	21.0	23.3	109.9**
Field	*mean temperature (° C)*	*13.01*	*13.16*	*12.81*	*12.40*	
	(min – max temp. (° C))	*(5.81 - 24.40)*	*(5.40 - 25.17)*	*(4.15 - 24.40)*	*(1.17 - 23.63)*	
	observed dev. time (days)	38*	47	30	28	141.5
	predicted dev. time (days)	32.8	48.2	33.8	31.1	146.0**

*see text for possible explications of deviations.

**sum of development times of life stages.

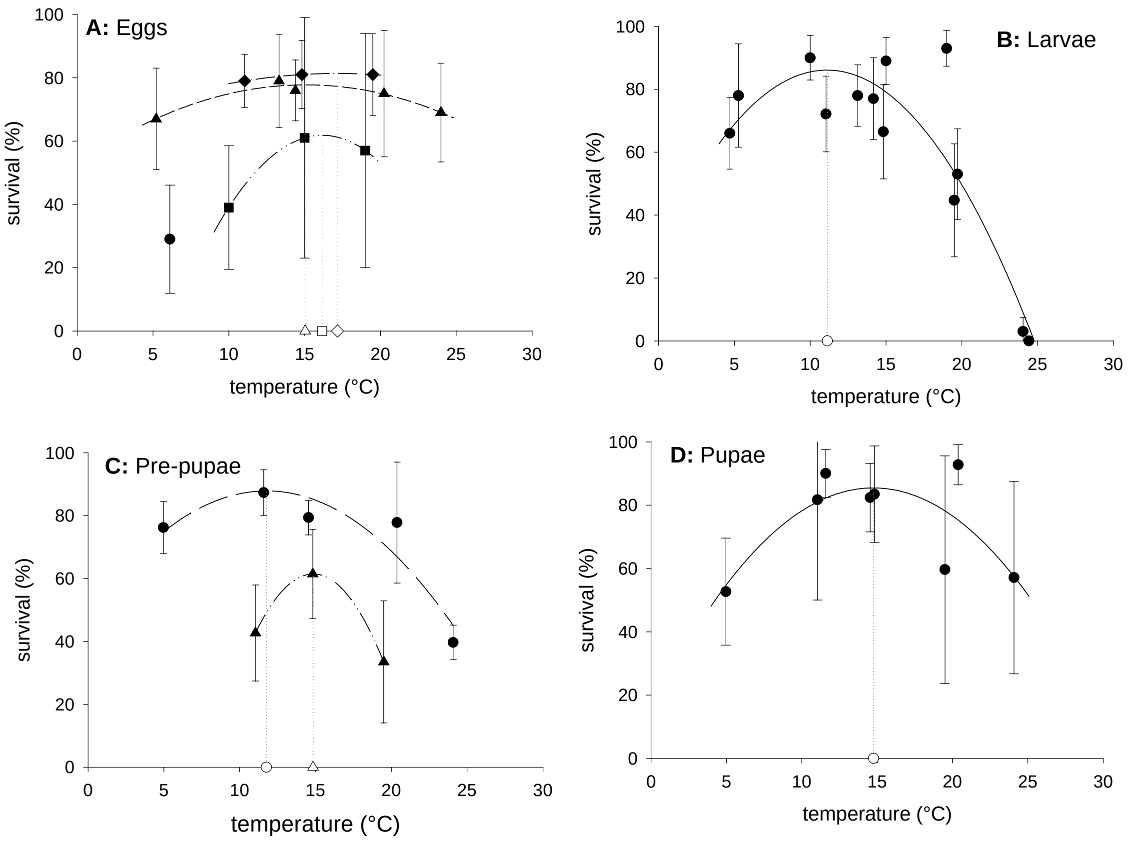

Figure 4: Temperature dependent survival rates of *P. suturicallus* eggs (A), larvae (B), pre-pupae (C) and pupae (D). Fitted models are second grade polynomial regressions (for parameters see Table 3). Bars indicate standard deviations of means. White symbols indicate temperature of maximum survival. In (A) and (C) same symbols represent data of cohorts taken from the same rearing batch and installed on the same date.

Immature mortality

The survival patterns of the different life stages at different constant temperatures are shown in Figure 4 (for parameters see Table 3). The results for eggs varied between the different insect batches and repetitions of the experiment but the tendencies were always the same. Temperatures of maximum survival varied between 15° C and 17° C, though within each experiment run, egg survival showed only little influence by temperature. Larvae survival was strongly influenced by temperature. Highest survival rates were at 11.1° C with only a slight reduction in survival at low temperatures but a strong reduction at high temperatures. No survival was observed at over 24.4° C. The data for pre-pupae too varied between the different insect batches and repetitions of experiment. The highest survival rates were found at 11.8° C respectively 14.8° C.

Influence of temperature on survival was less pronounced than for larvae. The same holds true for pupae survival rates.

Summing up the survival (%) of the models of each life stage, results in the survival model for one generation of *P. suturicallus* (Figure 2B). In the cases of eggs and pre-pupae with high variability in data, models used for the calculation of the generation model were those based on a maximum of data points (trial 2 for eggs and trial 1 for pre-pupae). Maximal survival under laboratory conditions was 48% at 12.7° C. Observations of cohorts over the whole development cycle show a similar tendency but lower survival rates.

Adult life span and fecundity

Between 4° C and 28° C female life span diminished linearly from 221 days to 12 days (Figure 5). Above 30° C survival was less than 1 day, while at –1.8° C females survived in mean for 20 days. The results for males were similar to those for females. Pre-pupae supported freezing at -1.8° C for over 40 days while high temperatures above 33° C were supported for less than a day.

Table 3: Estimated parameters for second grade polynomial regressions ($y = y_0 + m_1x + m_2x^2$) fitted to the survival rate of life stages of *P. suturicallus*. In the case of eggs and pre-pupae, the model was fitted to the temperature of each trial repetition separately.

Life stage	**Trial**	y_0	m_1	m_2	r^2
Egg	1	-95	19.4	-0.6	1
	2	52.84	3.30	-0.11	0.9459
	3	62.93	2.15	-0.06	1
Larvae		29.19	10.24	-0.46	0.8177
Pre-pupae	1	48.78	6.64	-0.28	0.8634
	2	-223.58	38.38	-1.29	1
Pupae		15.48	9.49	-0.32	0.5871

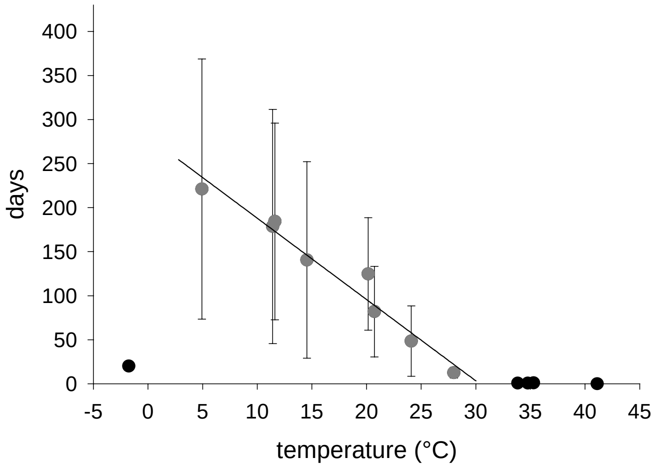

Figure 5: Temperature dependent longevity of *P. suturicallus* females. Dots show observed data, grey dots show observed data used for linear regression and bars indicate standard deviations of means. Fitted linear regression is $y = 280.35 - 9.23x$, $r^2 = 0.9583$.

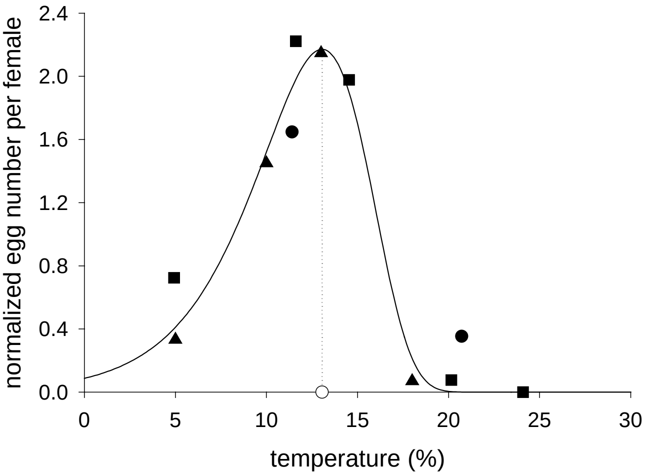

Figure 6: Temperature dependent fecundity of female *P. suturicallus*. Egg numbers per female are normalized for each trial separately (egg number/mean egg number). Same symbols represent data of one trial, i.e. of cohorts taken from the same rearing batch and installed on the same date. The white symbol indicates the temperature of maximum fecundity. A 4 parameters Weibull regression was fitted to the whole data set (Formula 3) with $a = 2.171$, $b = 18622.021$, $c = 5989.938$, $x_0 = 13.052$ and $r^2 = 0.9489$).

Female fecundity was approximated with a 4-parameter Weibull regression (Figure 6).

$$y = a\left(\frac{c-1}{c}\right)^{\frac{1-c}{c}}\left[\frac{x-x_0}{b}+\left(\frac{c-1}{c}\right)^{\frac{1}{c}}\right]^{c-1} e^{-\left[\frac{x-x_0}{b}+\left(\frac{c-1}{c}\right)^{\frac{1}{c}}\right]^{c}+\frac{c-1}{c}} \qquad (3)$$

The maximum fecundity was observed at 12.9° C. Due to high variations in oviposition between cohorts used at different dates, fecundity has been normalized by dividing the number of eggs per female of one temperature through the mean number of eggs per female of all temperature of the same cohort. At 5° C fecundity was still 15 - 33% of the maximum fecundity but increasing temperatures above 15° C had detrimental effects on fecundity. At 18° C and 20° C only a minimum oviposition could be observed ceasing completely at 24° C. Cumulative fecundity graphics are shown in Figure 7. Maximum egg number per female was 255 at 11.6° C, while minimum egg number was 6 at 20.2° C respectively 0 at 24.1° C. 50% egg production is in most cases reached at an age corresponding to 50 - 60% of the median survival time. Only at high temperatures this point is reached earlier. At 11 to 15° C, 90% egg production is only reached at an age of 145 to 170% of the median survival time indicating that many female die without reaching their maximum egg production. At lower and at higher temperatures the point of 90% egg production is reached earlier in female live. The pre-oviposition period extended from approximately 10 days at 14.5° C, over 30 days at 11.6° C to 45 days at 4.9° C. Adding to the immature development time a constant for the duration of melanization of adults in the soil (15 days) and the female age at 50% egg production (58% of female longevity including the pre-oviposition period), the medium generation duration changes from 641 days at 5° C to 347 days at 10° C and 219 days at 15° C.

Multiplying the generation survival rate for each temperature with the corresponding values of the Weibull regression for the female fecundity generated the net reproduction rate. The highest reproduction rate per generation was reached at 12.9° C with 42.8 female eggs (supposing a relation of 1:1 male to female) per individual of the original cohort. It fell below 1 at temperatures above 18.5° C (Figure 8).

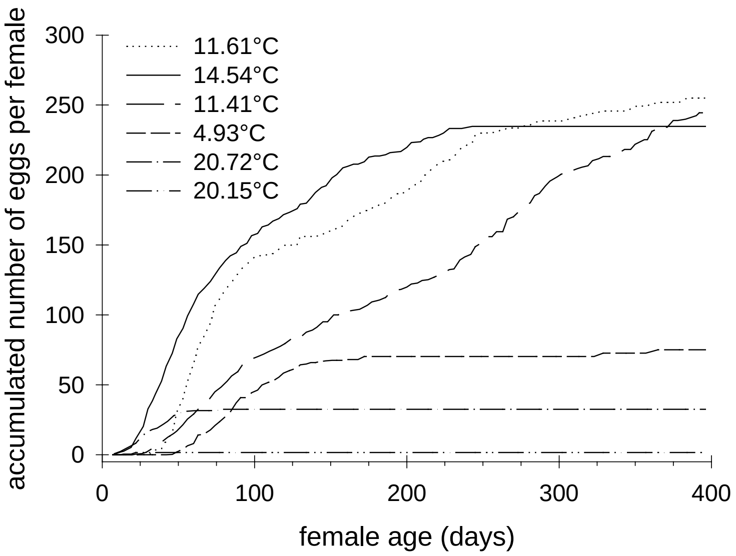

Figure 7: Cumulative fecundity for *P. suturicallus* at different incubation temperatures.

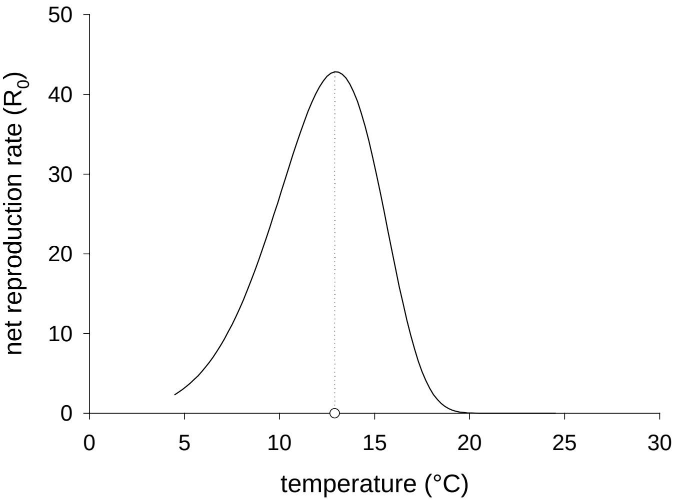

Figure 8: Temperature depending net reproduction rate of *P. suturicallus* with indication of the temperature of maximum reproduction.

Discussion

The presented data show that the temperature range for the development of *P. suturicallus* respectively the maximum survival and reproduction temperatures are low compared to most other insects. With a temperature optimum for survival and fecundity of 12 to 14° C and with a reproduction rate becoming zero at temperature even below 20° C this potato pests surly can be said to be cold adapted. Additionally, pre-pupae and adults support freezing temperatures for several days. Cold adaptation in insects of cold climates means, that their metabolic rate is higher at a given low temperature than in related forms from warmer regions (Downes 1965, Addo-Bediako et al. 2002). But many authors refer to cold adaptation as the capability to support freezing by freezing-tolerance or supercooling, a feature that should be called cold hardiness (Sømme 1989). This has to be separated from the capability to maintain metabolic functions at relatively low temperatures above 0° C. Gaston and Chown (1999) state, that most terrestrial arthropods have a critical maximum temperature between 35 and 50° C, clearly above the maximum survival temperature of *P. suturicallus* (Figure 5). Even arthropods from artic and sub-arctic areas seem to support higher temperatures (Hodkinson et al. 1996, Klok and Chown 1997, 1998, 2003), though the trial methods were different. It has also to be differentiated between the theoretical optimum temperature as found in the laboratory and the realized temperature range in the field (realized niche). Under field conditions, a species may be displaced from sites corresponding to its optimum temperatures by competitors, predators or pathogens. In this case, it will escape to suboptimal conditions if it counts with an appropriate tolerance (Begon et al. 1990). For *P. suturicallus*, the temperatures for optimal survival and reproduction match quite well the temperatures found in the field with an average temperature of 9.9° C and average day maximum and minimum temperatures of 19.1° C and 4.9° C (Figure 9).

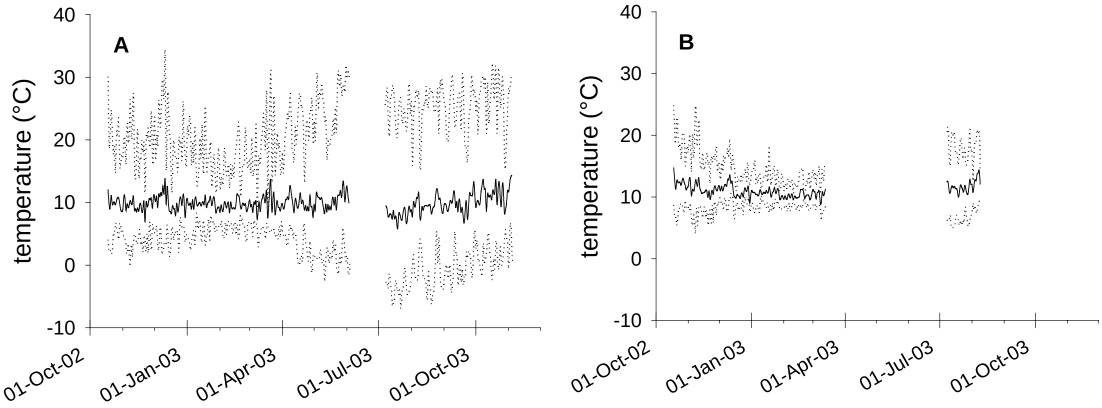

Figure 9: Climatic conditions in a potato field at 3730 meter above sea level measured 5 cm above ground (A) and 10 cm below ground (B). Solid lines indicate day means and dotted lines indicate day maximums and day minimums. The reduced amplitudes from mid of December to March correspond to the time when sensors where shaded by the potato plants.

No other comparable studies were found treating insects from cold climates of tropical highlands with diurnal rather than seasonal temperature cycles. Only cold-season insects from temperate climate zones that make diapauses during summer and develop, reproduce and are active during winter months show comparable temperature ranges for development. Topp and Kirsten (1991) and Topp (2003) describe the winter moth *Operophtera brumata* and some Cholevinae beetles with optimum temperature for reproduction of around 10° C and a significantly reduced reproduction at 15° C. Development rates for *O. brumata* show a similar temperature range as found for *P. suturicallus*.

Alcalá C. and Alcázar S. (1976) made a study on the development biology of *P. suturicallus* life stages with insect material collected in the same area as for the present study. His study was run under temperatures fluctuating between 1.2° C and 16.2° C with a mean temperature of 10.5° C. For most of the life stages he observed shorter development times than the model would predict for a constant temperature of 10.5° C (Table 4). This may be due to less exact temperature measurements, but also to the fluctuating temperature regime during the experiment while the results for the model are based upon a constant temperature of 10.5° C. With the minimum temperature below the temperature range of linear development rate, the development time will be reduced as compared to the linear regression (Wagner et al. 1984, Worner 1992, Bryant et al. 1999, Georges et al. 2005). Only for pupae Alcalá C. and Alcázar S. (1976) found longer developing times. The same authors found also a sex ratio of 1.2 male/female and a much higher fecundity with a mean of 631 eggs/female ranging from 101 to 934 eggs/female.

Table 4: Development times (days) of *P. suturicallus* life stages at fluctuating temperatures with an average of 10.5° C found by Alcalá C. and Alcázar S. (1976) and predicted by the model for a constant temperature of 10.5° C.

live stage	(Alcalá C. and Alcázar S. 1976)	model predictions		
		predictions	confidential limits	
eggs	33	47.2	31.2	96.8
larvae	46	67.9	47.2	121.6
pre-pupae	43	54.9	20.5	--
pupae	54	40.7	34.3	50.4
total cycle	176	210.6	133.1	--

Table 5: Parameters of the linear model calculated for *Premnotrypes vorax* based on the data taken from different studies from Colombia, Venezuela, Ecuador and northern Peru (Zenner and Posada O. 1968, Garzón C. et al. 1996, Niño et al. 2004). Several other studies have only be presented as thesis or on conferences (see chap. 1).

life stage	intercept	slope	r^2	min. dev. threshold (° C)
eggs	-0.016	0.0036	0.5021	4.5
larvae + pre-pupae	-0.014	0.0025	0.3404	5.5
pupae	-0.001	0.0031	0.3184	0.3
total cycle	-0.003	0.0009	0.5426	3.2

Most of the studies made on other species of APW were made on *P. vorax* in Venezuela, Colombia, Ecuador and northern Peru. The development times were mostly observed under ambient, fluctuating temperature conditions. Data of these studies have been analysed and compared with the data found in the present study (Table 1 and Table 5). All temperature conditions have been within the temperature range of linear development rate. For the development rate of eggs and of the complete cycle, the linear regressions of *P. vorax* are almost parallel to those of *P. suturicallus*. The slope of the joint development rate for larvae and pre-pupae is slightly steeper while for pupae it is slightly gentler for *P. vorax*. At all stages the regression line for the development rate of *P. vorax* was slightly shifted upwards, which corresponds to a shorter development times and a lower theoretical minimum development thresholds. Under uncontrolled conditions, several studies on other *Premnotrypes* species have been presented. Only few have been published (Tisoc Dueñas 1989, Barea et al. 1996/1997, Alcázar and Cisneros 1999), others have only be presented as thesis or on conferences (see chap. 1). All species show similar life cycles (except for *P. vorax* with 5 larval stages instead of 4)(see chap. 1). Studies on other *Premnotrypes* species should be run to understand better the actual and potential distributions of the APW complex and the species.

Comparing the thermodynamic model established for the development times of *P. suturicallus* with observed data under fluctuating temperature regimes, the predicted data fit in most cases quite well the observed data while in some cases the deviate. The marked deviations of pre-pupae development time in the laboratory trial may be due to inadequate soil humidity conditions. Under constant temperature conditions, pre-pupae development was found to be highly variable. It seems to be strongly influenced by other factors like soil humidity (see chap. 4). For larvae, whose development is not influenced by other climatic factors like humidity, the model gives a good approximation of the real situation in the field.

It is important to keep in mind, that for predicting the development period of the insect, the real conditions in the microhabitat of the different life stages should be taken into consideration. This especially, as insect behaviour at high altitudes tends to reduce exposure to extreme temperature (Dillon et al. 2006). The same author gives examples of insects experiencing considerable higher temperature for its development than the ambient temperature. In contrast APW seems to avoid high temperatures. Adult APW are feeding during the first half of the night on the leaves of the potato plant and hide during the day below soil clots preferring humid places (Alcázar and Cisneros 1999) avoiding the extreme temperatures that may reach from freezing temperatures up to 34° C (Figure 9). At humid places, cold due to evaporation will damp high day temperatures. Similar behaviours have been described for other high altitude insect species (many of them carabids beetles) and may reduce the risk of overheating and desiccation by the strong radiation and dry air during daytime and of freezing by too cold temperatures during the second half of the night (Sømme 1989, Sømme et al. 1996). Larvae develop within the tubers and pre-pupae and pupae stay in the soil, regularly at depths of 10 to 30 cm (Alcalá C. and Alcázar S. 1976) where they avoid both temperature extremes. This is due to the buffering effect of soil where the amplitude of temperature fluctuation decreases exponentially with depth, so that the amplitude at 30 cm depth, depending on soil density, water content, plant cover and other factors, may be reduced to only 5% of the soil surface amplitude (Marshall and Holmes 1988). This behaviour of the weevil allows him experiencing a narrower temperature range than climate data would suggest. Still, the temperature range for development of *P. suturicallus* fits well to the climatic data.

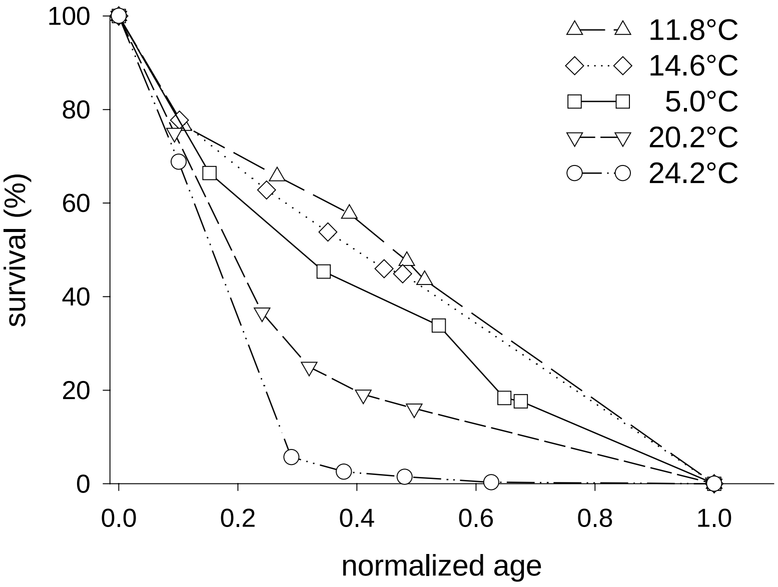

Figure 10: Survivorship curves of *P. suturicallus* at different temperatures. Time is expressed as normalized age (age at moulting devided by longevity) for each temperature. Data points correspond for each temperature to the following sequence of events: oviposition of egg, hatching of larva, abandoning of tubers (pre-pupa), moulting to pupa, moulting to adult, melanized adult, adult death.

Joining the survival rate found for each life stage, the survivorship curves as found under laboratory conditions are close to type II survivorship curves as described by Deevey (1947) (see also Begon et al. (1990)) with more or less constant mortality rates over the whole life time at low and medium temperatures (Figure 10). Only egg mortality is slightly higher. At high temperatures (above 20° C) the survivorship curve is of type III (concave) with larval survivorship most severely affected by high temperatures. The time until emergence during which adults may remain in the soil has been ignored for these calculations because it is mainly influenced by soil humidity (see chap. 4)

Another indicator for fitness may be body size (Stearns 2000). Here we took mature larval weight as a measure of body size. Results show a peak at similar temperatures than highest survival rate, suggesting a correlation between weight and fitness. With reduced weight at high as well as at low temperatures, this contradicts the idea of the temperature-size rule that states that organisms developing at lower temperature would grow larger then individuals of the same species grown at higher temperatures (Atkinson 1994, Angilletta et al. 2004). But (Dillon et al. 2006) found in a literature review, that this rule only applies to species distributed along latitudinal gradients, while along altitudinal gradients insects do not tend to have larger body size in higher and cooler environments or, as stated by Sømme (1989) even tend to smaller size. In our studies we also found, that larval weight diminished with longer development time similarly to the studies of Klingenberg and Spence (1997) with water insects. A negative correlation between developing time and body size rather than a trade-off between both variables was found. Even though we measured in the present case the fresh weight, this should not lead to a bias. Larvae developing inside the potato tuber are exposed to the same humidity condition at any temperature, though no dehydration effects should occur.

Acknowledgements

We owe special thanks to Marcelo Trebejo of the International Potato Center (CIP) in Lima (Peru) for maintaining the APW rearing and helping in the evaluations on the experiments. We thank also Marc Sporleder of CIP for his support in the calculation of the models. This research was done at CIP and was made possible by funding of the Government of the Grand-Duchy of Luxembourg.

References cited

Addo-Bediako, A., S. L. Chown and K. J. Gaston (**2002**). Metabolic cold adaptation in insects: a large-scale perspective. Functional Ecology **16**(3): 332-338.

Alcalá C., P. and J. Alcázar S. (**1976**). Biología y comportamiento de *Premnotrypes suturicallus* Kuschel (Col.: Curculionidae). Revista Peruana de Entomología **19**(1): 49-52.

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

Angilletta, M. J., T. D. Steury and M. W. Sears (**2004**). Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. Integrative and Comparative Biology **44**(6): 498-509.

Atkinson, D. (**1994**). Temperature and organism size - a biological law for ectotherms. In: Advances in Ecological Research. M. Begon and A. H. Fitter (Eds). London (UK), Academic Press, Inc. Vol. 25: pp. 1-58.

Barea, O., R. Andrew and J. Vargas (**1996/1997**). Biology, damage, and chemical control of the Andean potato weevil, *Rhigopsidius tucumanus* Heller. Revista Latinoamericana de la Papa **9/10**(1): 96-105.

Begon, M., J. L. Harper and C. R. Townsend (**1990**). Ecology - individuals, populations, communities. Oxford (GB), Blackwell Scientific Publications. 960pp.

Bryant, S. R., J. S. Bale and C. D. Thomas (**1999**). Comparison of development and growth of nettle-feeding larvae of Nymphalidae (Lepidoptera) under constant and alternating temperature regimes. European Journal of Entomology **96**(2): 143-148.

Burnham, K. P. and D. R. Anderson (**2002**). Model selection and multimodel interference. A practical information-theoretic approach. New York (USA), Springer. 488pp.

Deevey, E. S. (**1947**). Life tables for natural populations of animals. Quarterly Review of Biology **22**(4): 283-314.

Dillon, M. E., M. R. Frazier and R. Dudley (**2006**). Into thin air: Physiology and evolution of alpine insects. Integrative and Comparative Biology **46**(1): 49-61.

Downes, J. A. (**1965**). Adaptations of insects in the arctic. Annual Review of Entomology **10**: 257-274.

Garzón C., M. Y., B. O. Aza T., J. Jiménez G. and J. E. Luque Z. (**1996**). Potential of the nematode S*teinernema* sp. for the biological control of the Andean potato weevil. Revista Colombiana de Entomología **22**(1): 25-30.

Gaston, K. J. and S. L. Chown (**1999**). Elevation and climatic tolerance: a test using dung beetles. Oikos **86**(3): 584-590.

Georges, A., K. Beggs, J. E. Young and J. S. Doody (**2005**). Modelling development of reptile embryos under fluctuating temperatures regimes. Physiological and Biochemical Zoology **78**(1): 18-30.

Hodkinson, I. D., S. J. Coulson, N. R. Webb and W. Block (**1996**). Can high arctic soil microarthropods survive elevated summer temperatures? Functional Ecology **10**(3): 314-321.

Ikemoto, T. (**2005**). Intrinsic optimum temperature for development of insects and mites. Environmental Entomology **34**(6): 1377-1387.

Ikemoto, T. and K. Takai (**2000**). A new linearized formula for the law of total effective temperature and the evaluation of line-fitting methods with both variables subject to error. Environmental Entomology **29**(4): 671-682.

Klingenberg, C. P. and J. R. Spence (**1997**). On the role of body size for life-history evolution. Ecological Entomology **22**(1): 55-68.

Klok, C. J. and S. L. Chown (**1997**). Critical thermal limits, temperature tolerance and water balance of a sub-antarctic caterpillar, *Pringleophaga marioni* (Lepidoptera: Tineidae). Journal of Insect Physiology **43**(7): 685-694.

Klok, C. J. and S. L. Chown (**1998**). Interaction between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). Journal of Insect Physiology **44**(7-8): 615-628.

Klok, C. J. and S. L. Chown (**2003**). Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. Biological Journal of the Linnean Society **78**(3): 401-414.

Marshall, T. J. and J. W. Holmes (**1988**). Soil physics, Cambridge University Press. 374pp.

Niño, L., E. Acevedo, F. Becerra and M. Guerrero (**2004**). Some biological aspects and population fluctuation of the white tuberworm *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae) in Mucuchíes, Mérida state, Venezuela. Entomotropica **19**(1): 15-19.

Schoolfield, R. M., P. J. H. Sharpe and C. E. Magnuson (**1981**). Non-linear regression of biological temperature-dependent rate models based on absolut reaction-rate theory. Journal of Theoretical Biology **88**(4): 719-731.

Sharpe, P. J. H. and D. W. DeMichele (**1977**). Reaction kinetics of poikilotherm development. Journal of Theoretical Biology **64**(4): 649-670.

Sømme, L. (**1989**). Adaptations of terrestrial arthropods to the alpine environment. Biological Reviews of the Cambridge Philosophical Society **64**(4): 367-407.

Sømme, L., R. L. Davidson and G. Onore (**1996**). Adaptations of insects at high altitudes of Chimborazo, Ecuador. European Journal of Entomology **93**(3): 313-318.

Sporleder, M., J. Kroschel, M. R. Gutierrez Quispe and A. Lagnaoui (**2004**). A temperature-based simulation model for the potato tuberworm, *Phthorimaea operculella* Zeller (Lepidoptera; gelechiidae). Environmental Entomology **33**(3): 477-486.

Stearns, S. C. (**2000**). Life history evolution: successes, limitations, and prospects. Naturwissenschaften **87**(11): 476-486.

Suguira, N. (**1978**). Further analysis of the data by Akaike's information criterion and the finite corrections. Communications in Statistics, Theory and Methods **A7**(1): 13-26.

Tisoc Dueñas, I. O. (**1989**). Biological cycle of *Premnotrypes latithorax*, in Cusco (Peru), under laboratory conditions. Revista Peruana de Entomología **32**: 89-92.

Topp, W. (**2003**). Phenotypic plasticity and development of cold-season insects (Coleoptera: Leiodidae) and their response to climatic change. European Journal of Entomology **100**(2): 233-243.

Topp, W. and K. Kirsten (**1991**). Synchronization of pre-imaginal development and reproductive success in the winter moth, *Operophtera brumata* L. Journal of Applied Entomology **111**(2): 137-146.

Wagner, T. L., H.-I. Wu, P. J. H. Sharpe, R. M. Schoolfield and R. N. Coulson (**1984**). Modeling insect development rates: a literature review and application of a biophysical model. Annals of the Entomological Society of America **77**(2): 208-225.

Worner, S. P. (**1992**). Performance of phenological models under variable temperature regimes: Consequences of the Kaufmann or rate summation effect. Environmental Entomology **21**(4): 689-699.

Zenner, I. and L. Posada O. (**1968**). Generalidades sobre el gusano blanco de la papa *Premnotrypes vorax* (Hustache). Agricultura Tropical **24**(1): 33-40.

Humidity depending development and dormancy of the Andean potato weevil

Magnus Kühne, Stefan Vidal and Jürgen Kroschel

Abstract

The Andean potato weevil complex is a main insect pest in potato production in the Andes above 2800 meter altitude. Under controlled laboratory conditions, the influence of soil humidity on development and mortality of pre-pupae and pupae of *Premnotrypes suturicallus* as well as on emergence of adults and of relative air humidity on oviposition were studied. A threshold of approximately 8% soil water content (30% of the field capacity of the soil) was established for pre-pupae development and adult emergence. Higher humidity levels had no influence on both while lower humidity levels delayed them markedly. Pupae development was not influenced by soil humidity. A facultative dormancy is proposed for the pre-pupal stage and the so called ‘over wintering adult’ in the soil. This allows the weevil to adapt to the variable rain regimes found in the Andes and enables the weevil to survive a prolonged dry season. Oviposition was highest at moderate relative humidity levels while it was reduced below 50% and above 90% relative humidity.

Keywords: *Premnotrypes suturicallus*, potato pest, soil humidity, development rate, adult emergence, dry season, relative humidity, oviposition.

Introduction

The Andean potato weevil (APW) is a complex of 12 species of the genus *Premnotrypes* and two species of the genus *Rhigopsidius* (Coleoptera: Curculionidae). All species occur in the Andes above 2800 meter altitude between Venezuela and north-western Argentina. It is the main potato pest in the Andes causing regularly 20 to 25% damage (see chap. 2 and Alcázar and Cisneros (1999)). Adults are night active and oviposit close to the potato plant stem into dry plant debris. After hatching, neonate larvae bury into the soil and bore into the tubers. They feed in the tuber until their development is accomplished passing through 4 larval stages. At maturation, larvae leave the tuber for pupation in the soil. Before the pupation, last instar larvae remain in a rest stage commonly referred to as pre-pupae. After pupation, the freshly moulted adults melanize in its pupal chamber in the soil. At the end of this process adults may still remain for a more or less prolonged period in the pupal chamber, often referred to as ‘over wintering’ adult. The onset of rain after a dry season has been identified as a probable factor influencing emergence. After emergence, the flightless insect migrates into new potato fields (Alcalá C. and Alcázar S. 1976, Alcázar and Cisneros 1999).

Thorough knowledge of the life cycle of a pest and the biotic and abiotic factors influencing this life cycle are important tools for decision-making in integrated pest management strategies. Especially in a heterogeneous ecosystem like the Andes with huge temperature and humidity variation due to altitudinal differences within small distances an understanding of the factors influencing the development of a pest may give important clues for its control. Within the distribution area of the APW complex, pronounced differences in rain regimes and humidity are found. In general, the Andes are wetter towards the north (Ecuador and Colombia) and become dryer towards the south (Peru and Bolivia) with marked dry seasons. Additionally, in Peru and Bolivia a similar change can be observed from the wet eastern slopes of the Andes facing the Amazon basin to the dry western slopes facing the costal desert.

Tauber et al. (1998) stressed the importance of moisture in the determination of the seasonal ecology of insects. Though it is a widely neglected factor. This may be due to the fact that most phenological studies are made in temperate zones were temperature and photoperiod are prominent cues for seasonality. Another reason may be the difficulty in controlling soil humidity and changes in humidity within the soil or pupal chamber without disturbing the insect. Some studies looked at the humidity or water dependent egg and embryonic development (Wardhaugh 1980, Régnière et al. 1981, Tanaka 1987, Pires et al. 2000), but few looked at pupae (Tauber et al. 1994). Denlinger (1986) reviewed the presence of dormancy among tropical insects focusing on warm tropical climates and considered dry-season dormancy of mayor importance in the tropics.

A general study on the biology of *P. suturicallus* has been presented by Alcalá C. and Alcázar S. (1976). While the influence of temperature on the life cycle of APW has been studied in detail at another place (see chap. 3), the present study proposes to determine the effect of humidity on the development rate and the mortality rates of the different life stages as well as the reproduction rate of *P. suturicallus* under controlled conditions in the laboratory.

Methods

The origin and rearing of the insect material

All life stages used in the trials were taken from a rearing colony at the International Potato Center (CIP) in Lima (Peru). Adult weevils had been collected from fields on the eastern slopes of the Rio Mantaro valley (province Concepcíon, central Peru) with yearly new introductions into the rearing colony. The rearing was kept at 18 to 19° C in dark and produced approximately 3 generations per year. For the rearing methods, see chap. 3.

Development times and mortalities

The effects of soil humidity on the life cycle of *P. suturicallus* were studied on separate cohorts for the life stages staying and developing in the soil (pre-pupae, pupae and maturating adult). For these trials, we used a sterilized and sift loam soil collected from the same field as the original adults for the rearing colony. Temperature was kept constant at 15° C.

At a maximum of 6 days after abandoning the potato tuber, mature larvae were placed into microcentrifuge tubes with sterilized and sieved soil. Soil humidity was set to 5 humidity levels on a range of 0.5% to 24.9% soil water content. This corresponded to 1.4 to 91.4% of the soil field capacity which was reached at 25.1% water content. After addition of the necessary quantities of water, a sample of each treatment was weighted and dried at 80° C until weight stability for exact water content determination. Evaluation took place three times a week. Without opening the microcentrifuge tubes, moulting to pupae and to adult as well as adult melanization and mortalities were checked.

Adult emergence

Pupae were placed individually in sterilized and sieved loam soil of different levels of water contents. Water contents were set to 1.7, 6.3, 8.9, 15.1 and 18.6% of soil dry weight, which corresponded to 6.2, 23.3, 32.9, 55.7 and 68.7% of the field capacity of the soil with a water content of 27.1% at field capacity. Exact water contents were determined as described above. The soil was placed up to a height of approximately 2.5 cm into 16 ml glass vials, with pupae placed at 2 cm depth. The vials were incubated at 15° C. Evaluation consisted in weekly checking the vials for the appearance of the adults on the surface of the soil. After 15 weeks the trial was stopped. For each treatment 14 pupae were used.

Oviposition and longevity

In different trials, groups of 4 males and 4 females or 9 males and 7 females were placed together into containers with a diameter of approximately 12 cm. Potato tubers (variety Peruanita) and tightly folded strips of towel paper for ovipositions were added. Clutches of eggs were collected once a week, placed in petri dishes containing a filter paper and observed for viability. The air humidity was set to values from 44 to 95% relative humidity. To reach this humidity conditions, trial containers were placed into bigger container containing at the bottom dishes with saturated salt solutions. For the different humidity levels potassium hydroxide, calcium nitrate and potassium chloride solutions were used. The effective humidity was read from a datalogger placed into each container. The containers were incubated at 19° C.

Results

Responses in development time of soil stages on soil humidity were different depending on the stage. Pre-pupae development time was not influenced by soil humidity above 8.8 % soil humidity (32% of field capacity). But at lower humidity, development time was significantly prolonged by up to 6 times (Figure 1A). Development of pupae was not significantly influenced by soil humidity, though a slight tendency towards reduced development time at low humidity can be observed (Figure 1B).

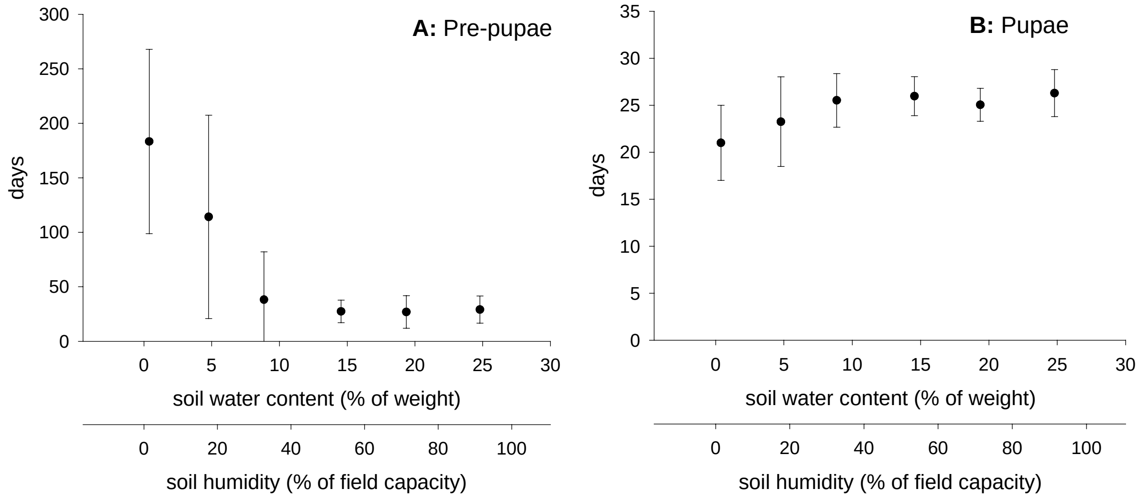

Figure 1: Soil humidity dependent development times (days) of soil stages of *P. suturicallus* pre-pupae (A) and pupae (B). Bars indicate standard deviations of medians.

Adult emergence was significantly influenced by soil humidity showing a marked threshold between 6.5 and 8.5% soil humidity (Figure 2). Below this threshold, no or very few adults emerged and for those emerging, time until emergence was prolonged by approximately 3.5 times. Above this threshold no delaying or acceleration of emergence was observed. The reduced number of emerged adults at high soil humidity was due to death by bacterial or fungal infections.

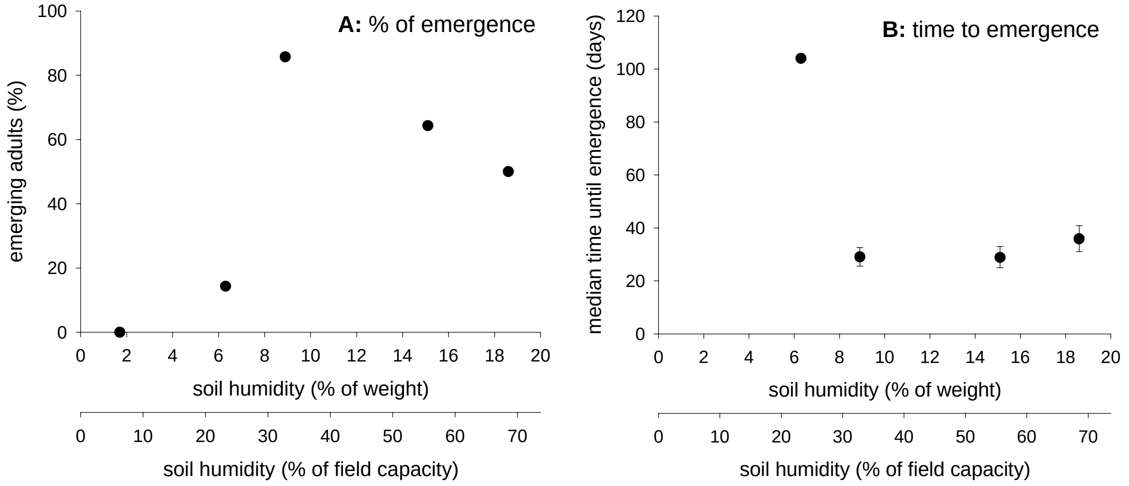

Figure 2: Soil humidity dependent adult emergence of *P. suturicallus*. Percentage emerging of total number installed (A) and median time until emergence (B). Bars indicate standard deviations of medians.

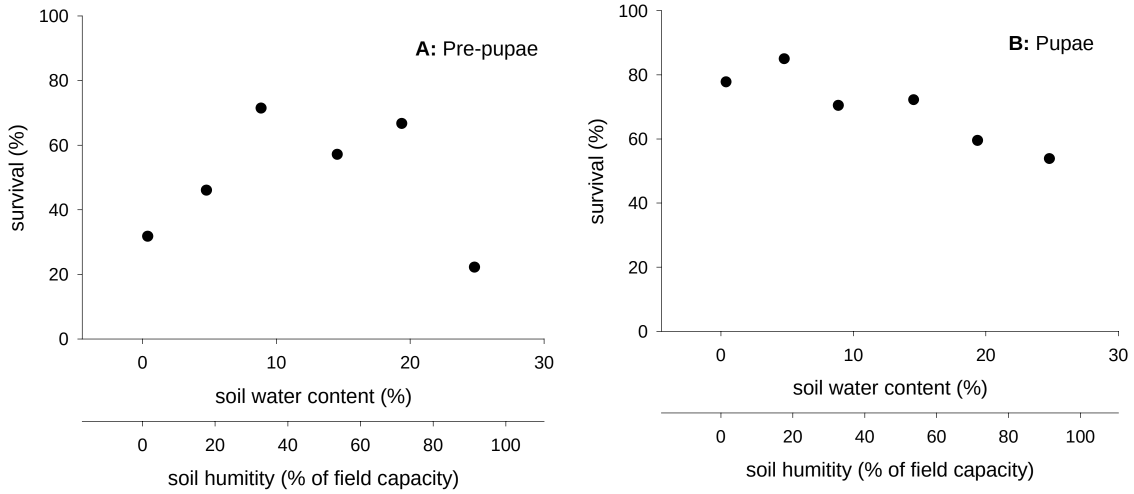

Figure 3: Soil humidity dependent survival rate of *P. suturicallus* pre-pupae (A) and pupae (B).

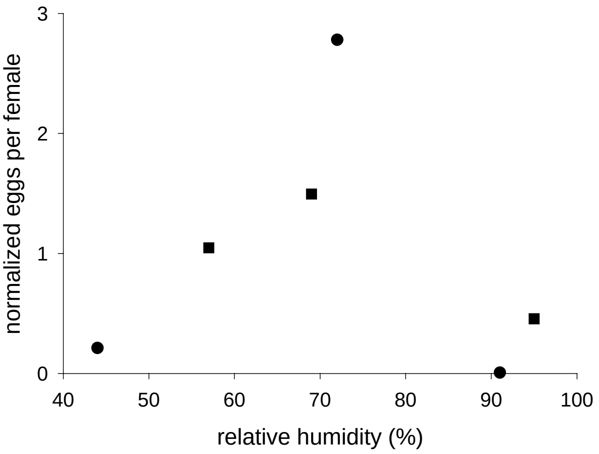

Figure 4: Humidity dependent fecundity of female *P. suturicallus*. Egg numbers per female are normalized for each trial repetition separately (egg number divided by mean egg number). Same symbols represent data of cohorts taken from the same rearing batch and installed on the same date.

Survival of pre-pupae was reduced at low and high soil humidity conditions, while pupae survival was only slightly reduced in humid soil (Figure 3). Mortality at high soil humidity was mostly due to bacterial or fungal infections while mortality at low humidity may be a result of prolonged duration and desiccation.

Oviposition was influenced by relative humidity of the air (Figure 4). Egg numbers per female was highest at moderate levels of around 70% relative humidity and was reduced at low and high relative humidity.

Discussion

Soil humidity respectively dryness has a great influence on the life cycle of *P. suturicallus*. A threshold of around 8% soil humidity was found for the development of the pre-pupal stage. Above this threshold, soil humidity did not affect development time while development was significantly delayed below. A prolonged pre-pupae stadium can also be observed in the field. Digging into the very dry soil below a potato heap in stores reveals commonly the presence of pre-pupae even 4 to 5 months after harvest. A similar threshold as for pre-pupae was found for the emergence of adults from soil. It has already been assumed, that the onset of rain after the dry season acts as a stimulus for the emergence of adult APW, though other factors are suspected as adults emerge also from the soil of roofed stores (Alcázar and Cisneros 1999). In fact no studies exist on the soil humidity neither in stores nor in fields and its variation throughout the year. In experiments without measurement of the effective soil humidity Valencia (1989) had shown a relation between the onset of rain and emergence.

The data presented in this study show, that dry soil conditions induce dormancy in pre-pupae of APW. In soil with humidity level above the threshold, pre-pupae stage is short. Emergence of adults on the other side is triggered by soil humidity. Though it is not yet clear how moulding to pupae and adult is affected by changes in moisture, dormancy is possible either as pre-pupae or as adult before emergence. This makes soil humidity an important factor for the determination of the length of the life cycle. This facultative dormancy allows *P. suturicallus* to adapt to climates of a wide range of humidity conditions, a faculty especially useful in the heterogeneous environment of the Andes.

Moisture as an important cue for development of dormancy has been emphasized especially for the tropics (Tauber et al. 1998). Though also for Colorado potato beetle, an insect distributed in temperate climates, the influence of soil moisture respectively rainfall on adult emergence has been shown (Tauber et al. 1994). Apart from adult emergence, most studies on the influence of soil moisture on life history traits of soil stages of insects look at the onset of rainfall stimulating pupation in diapausing larvae (Denlinger 1986). In fact in the case of APW, we cannot determine with certainty, how far an increase of soil humidity induces development to pupae or a decrease induces dormancy in pre-pupae or absolute humidity levels control the development. The difficulty consists in making controlled changes in soil humidity without disturbing the pre-pupae. Though, at the end of the present research project we observed in the permanent rearing, that APW also pupates without soil, placing the pre-pupae in small receptacles containing a peace of filter paper that absorbs the surplus of condensation water forming inside the receptacle. This could allow controlling and varying the air humidity and investigate more in detail the influence of moisture on soil stage development. The influence of temperature on the development of pre-pupae to pupae was shown to be continuously without any marked threshold (see chap. 3).

Observations in the field seem to confirm the adaptability of *P. suturicallus* to different climate conditions. In the central Andean valley of the Rio Mantaro, with a marked dry season *P. suturicallus* produces only one generation per year (Alcalá C. and Alcázar S. 1976). On the eastern slope of the Andes where soils retain moisture during the whole year, the presence of all life stages (eggs, young larvae, mature larvae, pre-pupae, pupae, melanizing adults and free adults) of the same species on the same plot and time suggests more generations per year (Kühne and Alcázar, personal data). A similar situation can be supposed for *P. vorax* with one generation per year observed in northern Peru, and 2 or 3 generations in Ecuador and Colombia with rain during most of the year (Alcázar and Cisneros 1999). Unfortunately no soil humidity measurements have been made in these cases.

The plasticity in resting time seems to be a good adaptation to the conditions under which its host plant grows. In the humid areas with more than one APW generation per year, potato is grown year around. Even under the dry conditions of central Peru a few potato sprouts may already emerge under favourable local conditions during the first part of the dry season in July or August. The water required for germination of the sprouts is covered by the water content of the potato tuber itself, though for root growth and emergence of the plant moisture is required (Beukema and van der Zaag 1990, Diepenbrock et al. 1999). In the same way, probably using some moisture left in the soil, a very small number of adult weevils have been captured in the field during the dry season (Kühne, personal data). The high variability in the development time of pre-pupae, especially under dry soil conditions (Figure 1A), may indicate a sort of cohort splitting for risk spreading (Begon et al. 1990).

Additionally it has been shown that relative humidity of the air affects the oviposition rate of females. Reduced oviposition under dry weather conditions has also been reported for *P. vorax* in non-published studies (see chap. 1). Such medium air humidity as found for the highest oviposition rate are generally found during the first half of the night, the time of highest activity of the adults (Alcalá C. and Alcázar S. 1976). Through its behaviour of feeding during the first half of the night on the plant and hiding during the day at humid places under soil clots adult weevils may reduce the risk of overheating and desiccation by the strong radiation and dry air during daytime and of freezing by too cold temperatures during the second half of the night (Sømme 1989, Sømme et al. 1996).

Acknowledgements

We owe special thanks to Marcelo Trebejo of the International Potato Center (CIP) in Lima (Peru) for maintaining the APW rearing and helping in the evaluations on the experiments. This research was realized at CIP and was made possible through funding by the Government of the Grand-Duchy of Luxembourg.

References cited

Alcalá C., P. and J. Alcázar S. (**1976**). Biología y comportamiento de *Premnotrypes suturicallus* Kuschel (Col.: Curculionidae). Revista Peruana de Entomología **19**(1): 49-52.

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

Begon, M., J. L. Harper and C. R. Townsend (**1990**). Ecology - individuals, populations, communities. Oxford (GB), Blackwell Scientific Publications. 960pp.

Beukema, H. P. and D. E. van der Zaag (**1990**). Introduction to potato production. Wageningen, Pudoc. 207pp.

Denlinger, D. L. (**1986**). Dormancy in tropical insects. Annual Review of Entomology **31**: 239-264.

Diepenbrock, W., G. Fischbeck, K.-U. Heyland and N. Knauer (**1999**). Spezieller Pflanzenbau. Stuttgart (Germany), Verlag Eugen Ulmer. 523pp.

Pires, C. S. S., E. R. Suji, E. M. G. Fontes, C. A. Tauber and M. J. Tauber (**2000**). Dry-season embryonic dormancy in *Deois flavopicta* (Homoptera: Cercopidae): roles of temperatures and moisture in nature. Environmental Entomology **29**(4): 714-720.

Régnière, J., R. L. Rabb and R. E. Stinner (**1981**). *Popillia japonica* (Coleoptera: Scarabaeidae): Effect of soil moisture and texture on survival and development of eggs and first instar grubs. Environmental Entomology **10**(5): 654-660.

Sømme, L. (**1989**). Adaptations of terrestrial arthropods to the alpine environment. Biological Reviews of the Cambridge Philosophical Society **64**(4): 367-407.

Sømme, L., R. L. Davidson and G. Onore (**1996**). Adaptations of insects at high altitudes of Chimborazo, Ecuador. European Journal of Entomology **93**(3): 313-318.

Tanaka, S. (**1987**). Changing responses to temperature and moisture of diapausing and developing eggs of *Allonemobius fasciatus* (Orthoptera: Gryllidae). Journal of Insect Physiology **33**(9): 635-641.

Tauber, M. J., C. A. Tauber and J. P. Nyrop (**1994**). Soil moisture and postdormancy emergence of Colorado potato beetles (Coleoptera: Chrysomelidae): descriptive model and field emergence patterns. Environmental Entomology **23**(6): 1485-1496.

Tauber, M. J., C. A. Tauber, J. P. Nyrop and M. G. Villani (**1998**). Moisture, a vital but neglected factor in the seasonal ecology: hypotheses and tests of mechanisms. Environmental Entomology **27**(3): 523-530.

Valencia, L. (**1989**). The Andean potato weevil, *Premnotrypes vorax* (Hustache) in Colombia: I.- Behavior of adults in the field. Revista Latinoamericana de la Papa **2**(1): 57-70.

Wardhaugh, K. G. (**1980**). The effects of temperature and moisture on the inception of diapause in eggs of the Australian plague locust, *Chortoicetes terminifera* Walker (Orthoptera: Acrididae). Australian Journal of Ecology **5**(2): 187-191.

Survival of the Andean potato weevil *Premnotrypes suturicallus* along an altitudinal gradient

Magnus Kühne, Stefan Vidal and Jürgen Kroschel

Abstract

The survival of different life stages of the Andean potato weevil *Premnotrypes suturicallus* Kuschel (Coleoptera: Curculionidae) has been studied in the field along an altitude gradient from sea level to 4100 meters in the Andes of central Peru (10° to 12° southern latitude). Controlled numbers of individuals of different life stages of the weevil were released into cages. Maximum survival for all stages was found to be between 3000 and 4000 meters above sea level. The lower limit for survival was at approximately 2400 meters, corresponding to an average temperature of 15.5° C to 16.7° C. At this altitude a small number of adults was able to develop. Only eggs and young larvae were able to survive at lower altitudes. At high altitudes no clear reduction of survival could be observed. The data confirm the results on temperature depending development of the same species under laboratory conditions (see chap. 3). *P. suturicallus* is adapted to the cold climates of the Andean highlands. A survival or dispersion to lower altitudes seems not possible.

Keywords: *Premnotrypes suturicallus*, potato pest, temperature, cold adaptation, altitude gradient.

Introduction

Premnotrypes suturicallus belongs to the Andean potato weevil (APW) complex, which includes 14 species of the genera *Premnotrypes* and *Rhigopsidius* (Coleoptera: Curculionidae). The 12 *Premnotrypes* species are distributed in the Andes from Venezuela to Bolivia above 2800 meters altitude, while the 2 *Rhigopsidius* species occur in north-western Argentina, Bolivia and the southern Altiplano of Peru. Their altitudinal distribution extends up to 4500 meters, corresponding probably to the distribution limit of potato. It is the main potato pest in these altitudes causing regularly 20 to 25% damage (Alcázar and Cisneros 1999). The species used in the present study, *P. suturicallus*, is the dominant species of the central highland of Peru. The adults are night active and oviposit close to the plant stem into dry plant debris. Neonate larvae dig into the soil and bore into the potato tubers where they feed until their development is accomplished passing through 4 larval stages. Mature larvae leave the tuber for pupation in the soil. After emergence, the flightless adults migrate into new potato fields (Alcalá C. and Alcázar S. 1976).

An important way of dispersion of the weevil is probably through harvested potato tubers. Some early maturing larvae leave the tubers already within the field, some will leave the tubers when they are exposed to sun during and shortly after harvest and some are brought together with the tubers into the stores or to the market (Alcázar and Cisneros 1999). Especially the last option may accelerate dispersion. Most larvae will pupate in soil (in the case of *Rhigopsidius* generally all larvae pupate within the tuber), however, it is reported, that small numbers of *Premnotrypes* weevils pupate within the tuber. In the case of *P. latithorax* this number has been increased up to 30% if potatoes were stored in stores with concrete floor (see chap. 1). Yábar Landa (1994) considers the transport of seed potatoes from one area to another as a main mean of dispersal of the weevil. Nevertheless, even though large quantities of potatoes are continuously brought to lower areas for consumption and as seed, and larvae may regularly be found in these tubers, APW has never spread to low altitudes. On the other side, potato production extends down to the costal areas of Peru. It is not clear whether this limited distribution is due to the physiology of the weevil or to competition, predation and/or parasitation.

The question arose, where the potential lower distribution limits for APW are. This question gets more importance, considering that APW is a quarantine pest not only for Europe but also for the southern South American countries like Chile and Argentina (COSAVE, OEPP/EPPO 1984). For export, it may be of importance to know, whether potatoes harvested on the Peruvian cost can be guaranteed to be free of APW.

In previous studies (Kühne and Vidal, in prep.) it has already been shown under laboratory conditions, that *P. suturicallus* is adapted to cold temperatures similar to those prevailing in the highlands of Peru. In the present research, the survival of different life stages has been studied in the field along an altitudinal gradient from sea level to 4100 meters altitude.

Table 1: Locations of the experiment for the establishment of an altitude gradient.

sites	Trial	altitude [m]	longitude (W)	latitude (S)	location in the Andes
La Molina (Lima)	2	251	76°57.181'	12°04.639'	costal desert (west)
San Ramon	1 + 2	849	75°21.408'	11°07.637'	rain forest (east)
Oxapampa-Cantarizu	2	1794	75°23.103'	10°38.972'	eastern slope
Mariscal Castillas	1 + 2	2375	75°03.585'	11°34.864'	eastern slope
Huasahuasi	1 + 2	2799	75°38.682'	11°16.049'	eastern slope
Carita	1 + 2	3225	75°41.199'	11°14.878'	eastern slope
Santa Ana	2	3311	75°13.425'	12°00.489'	central valley
La Libertad	2	3738	75°18.640'	11°50.512'	central valley
Ogobamba	1 + 2	3797	75°41.735'	11°15.510'	eastern slope
Chicche	2	4112	75°17.413'	11°49.004'	central valley

Methods

The origin of the insect material

All life stages of *P. suturicallus* for the experiments were taken from a rearing colony at the International Potato Center (CIP) in Lima (Peru). Adult weevils had been collected from fields on the eastern slopes of the Rio Mantaro valley (province Concepcíon, central Peru) with yearly new introductions into the rearing colony. The rearing was kept in dark and at 18 to 20° C producing approximately 3 generations per year. For details on the rearing method see chap. 3.

Altitude gradient

The effect of altitude on the population development was studied under semi-field conditions on locations distributed along an altitude gradient from 200 and 4100 meters above sea level (Table 1). At each site a datalogger was installed recording every hour the temperature. To avoid interferences and loss of plant material due to late blight infections, the late blight resistant potato clone CIP 387164.4 was used.

First trial: 2 or 3 potato plants were transplanted from the greenhouse to the trial site into a cage of approximately 1 x 1 x 1 meters made of wind cutting mesh with 36% shading effect. Meshes were relatively wide to allow for adequate air circulation within the cage and prevent the weevil from escaping. At each site 3 cages were installed and 7 females and 7 males from the laboratory colony were liberated into each cage. If necessary, weeds were eliminated. After maturation of the potato plants, the dry shots were eliminated and the tubers were evaluated for damage on a scale of 0 to 4 (0 = no damage; 1 = ¼ damaged, 2 = ½ damaged; 3 = ¾ damaged; 4 = completely damaged). The damage index was calculated using the Townsend and Heuberger (1943) formula

$$i.u. = \frac{\sum(nv)}{iN} \qquad (1)$$

with i.u. = damage index (%), n = number of tubers in each category, v = values of category, i = value of highest category and N = total number of tubers. After harvest traps were installed for capturing the emerging adults. Traps were a combination of pit falls and shelter traps consisting of a beaker buried up to the edge into the soil and containing a funnel fitting exactly over the opening of the beaker. A small wooden board was placed over the beaker resting on soil clots or small stones leaving a thin slit for access. The wooden board offered an attractive shelter for the adult weevils during the day while the funnel prevented adults fallen into the beaker from escaping. Traps were checked regularly.

Second trial: The life cycle was divided into three subtrials. For the first subtrial, 50 newly laid eggs from the laboratory colony deposited inside dry straw, were liberated on top of a pot dug up to the edge into the soil, filled up with soil and containing potato tubers at 20 cm depth. The straw was fixed to a small stick to avoid it being blown away by the wind. Evaluation took place after 4 to 6 weeks checking for larvae found within the tubers. The result gave the survival rate from eggs to larvae. For the second subtrial, potato tubers with 50 almost mature larvae from the laboratory colony were placed at a depth of approximately 20 cm into the soil. An emergence cage and traps were placed on the soil above. Traps were regularly evaluated for adults. The results gave the survival rate from mature larvae to emerging adult. For the third subtrial, 3 males and 3 females from the laboratory colony were liberated into similar cages as described for the first trial containing one potato plant. 3 to 4 weeks after maturing of the plant the number of larvae, pre-pupae and pupae within the tubers and in the soil was evaluated. The result gave the number of mature larvae produced per female. The tuber damage was evaluated as in the first trial. Combining the results of subtrial 1 with those of subtrial 3, the oviposition rate per female was calculated. For each subtrial, 4 cages have been installed at each trial site. Durations until evaluation were adapted to the local temperature conditions (Table 2).

Table 2: Installation and evaluation dates at the different experimental sites and mean temperatures (°C) during the trial.

	Trial I (complete cycle)			Trial II (divided cycle)				
	instal. date	eval. date	temp. (° C) *(range*)*	instal. date	eval. date			temp. (° C) *(range)*
					subtrial I (eggs liberated)	subtrial II (larvae liberated)	subtrial III (adults liberated)	
La Molina (Lima)				13/10	12/11	17/01**	17/01**	21.4 *(17.2 - 29.6)*
San Ramon	06/02	19/05	24.2 *(18.4 - 35.1)*	22/10	17/11	01/02**	01/02**	25.7 *(20.0 - 36.7)*
Oxapampa-Cantarizu				19/10	25/11	31/01**	31/01**	18.4 *(14.7 - 25.2)*
Mariscal Castillas	14/02	31/05	15.5 *(10.1 - 24.5)*	19/10	16/11	21/01**	21/01**	16.7 *(11.4 - 25.8)*
Huasahuasi	05/02	01/02**	14.0 *(8.4 - 24.7)*	20/10	13/12	01/02**	28/03**	15.2 *(9.7 - 24.5)*
Carita	05/02	01/02**	11.2 *(6.3 - 20.6)*	20/10	13/12	01/02**	28/03**	11.9 *(7.5 - 20.3)*
Ogobamba	05/02	28/03**	8.1 *(3.2 - 17.1)*	20/10	13/12	01/02**	28/03**	9.6 *(4.0 - 19.9)*
Santa Ana				18/10	10/12	02/02**	28/03**	13.5 *(6.5 - 24.4)*
La Libertad				18/10	06/01**	02/02**	28/03**	10.4 *(4.0 - 21.3)*
Chicche				18/10	06/01**	02/02**	28/03**	7.7 *(2.6 - 17.2)*

* mean daily minimum and mean daily maximum temperatures.

** + 1 year

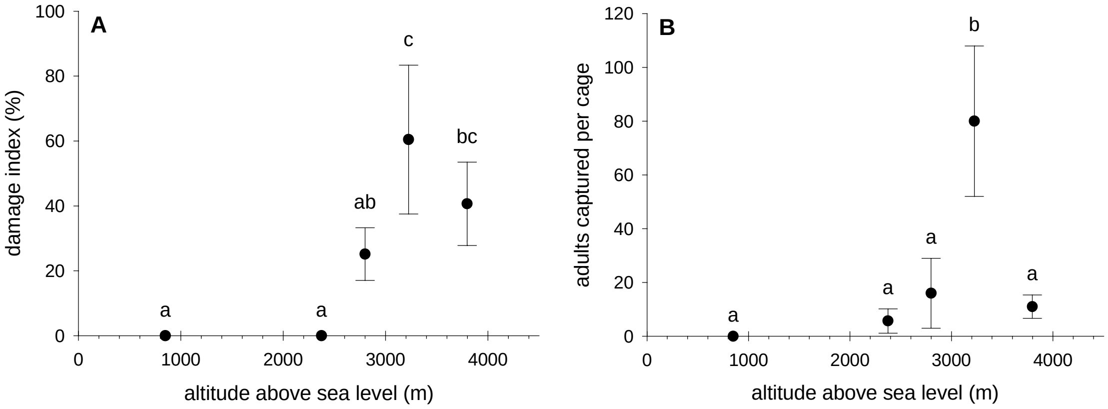

Figure 1: Influence of an altitudinal gradient on potato tuber damage caused by *P. suturicallus* (A) and on the population development of the same pest (B), measured as the number of adult weevils captured after the release of 7 couples of adult weevils (Trial I). Different letters indicate significance (Tukey, $p < 0.05$); bars indicate standard deviations.

Results

Table 2 shows the temperature conditions at the different experimental sites. In both trials the lower limit for the development of *P. suturicallus* was around 2400 meters altitude, (corresponding to 16.7° C) with no or very little survival or potato tuber damage (Figure 1A and Figure 2D). In the first trial some adults were able to develop at 2400 meters at average temperatures of 15.5° C while in the second trial, with 16.7° C in average no adults developed. Only for the egg to larvae stages a very small number survived even at 1800 meters (18.5° C)(Figure 2A). In all cases a peak in survival could be observed at around 3200 meters (ca. 12.0° C). No clear tendency towards a reduction of survival could be observed at high altitudes. Nevertheless, for the number of adults captured after the liberation of larvae (trial 2, Figure 2B) as well as after a whole cycle development (trial 1, Figure 1B) a slight reduction of survival could be observed above 3700 meters. The lack of survival at the site Santa Ana (3311m) in Figure 2A and C as well as the extremely high number of adults captured in Chicche (4112m) in Figure 2B may be artefacts. High variations between cages result in few significant differences between sites. Dividing the number of larvae recovered after liberation of adults (Trial 2, subtrial II) by the percentage of larvae recovered of liberated eggs (Trial 2, subtrial I), the oviposition rate could be estimated (Figure 3)(the sites La Molina, San Ramon and Santa

Ana are not represented as no larvae were recovered after liberation of eggs). The fitted 4-parameter Weibull regression

$$y = a\left(\frac{c-1}{c}\right)^{\frac{1-c}{c}}\left[\frac{x-x_0}{b}+\left(\frac{c-1}{c}\right)^{\frac{1}{c}}\right]^{c-1} e^{-\left[\frac{x-x_0}{b}+\left(\frac{c-1}{c}\right)^{\frac{1}{c}}\right]^{c}+\frac{c-1}{c}} \qquad (2)$$

shows a peak at around 3700 meters altitude corresponding to 10.5° C.

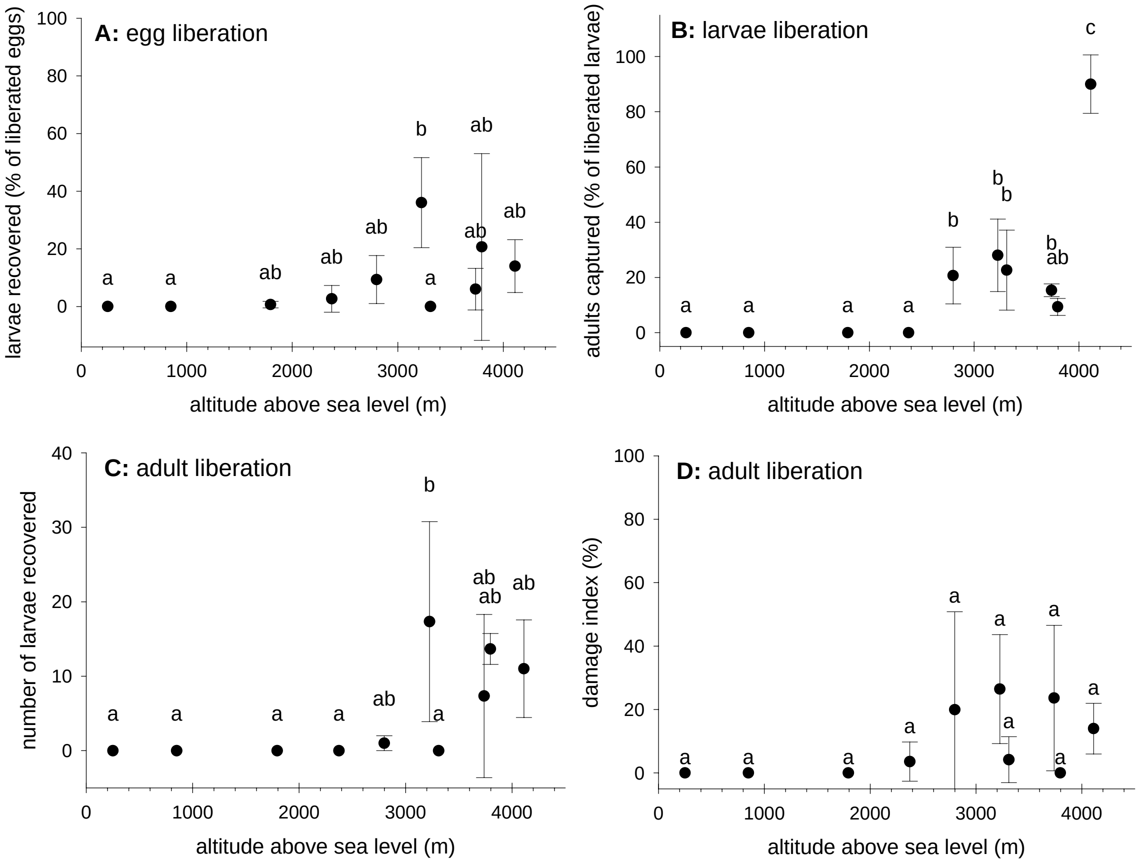

Figure 2: Influence of an altitudinal gradient on the survival and development of different phases of the life cycle of *P. suturicallus*: Eggs to larvae (A); pre-pupae to pupae and the emergence of adults (B); survival and fecundity of adults and survival of eggs to larvae (C), as well as on the damage caused by the pest (D)(Trial II). Different letters indicate significance (Tukey, $p < 0.05$); bars indicate standard deviations.

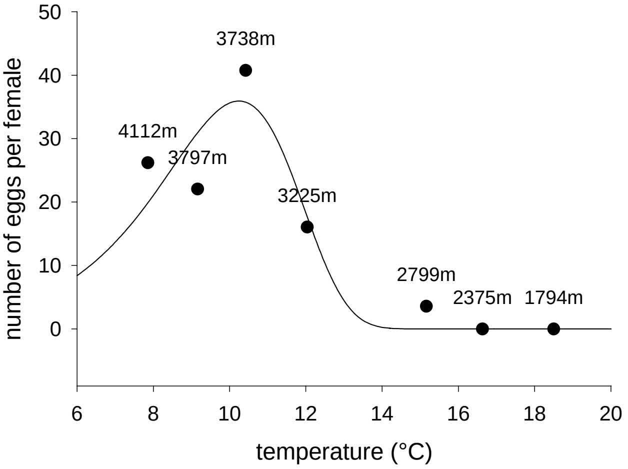

Figure 3: Temperature dependent egg production of *P. suturicallus* along an altitudinal gradient. Corresponding altitudes are indicated together with data points for egg number per female versus temperature. A 4 parameters Weibull regression was fitted to the egg number versus temperature data set (Formula 2) with a = 35.93, b = 17559.18, c = 9748.50, x_0 = 10.25 and r^2 = 0.8876).

Discussion

A lower limit for the survival of *P. suturicallus* along an altitude gradient could be fixed at around 2400 meters above sea level. Under the trial conditions, this corresponded to 16.7° C. At low altitudes, it cannot be completely ruled out, that some damages may have been caused by other insects. At altitudes above 3700 meters, a tendency to reduced survival rates could be observed. These data confirm the results found for the same species under laboratory conditions (see chap. 3). However, the maximum temperatures for survival of the different life stages of *P. suturicallus* seem to be shifted toward lower temperatures. In the field, survival was impossible at altitudes below 2400 meters or temperatures above 16.7° C while under laboratory conditions temperatures above 20° C were supported by a reduced number of weevils. The temperatures for the maximum survival rate were found to be the same as under laboratory conditions. Like in the laboratory trials, eggs were least susceptible to high temperatures. The lower maximal temperature limit found in the field data as compared to the laboratory data may be explained with other factor than temperature, e.g. the presence of pathogens. Especially entomopathogenic fungi like *Beauveria bassiana*, which are regularly found affecting pre-pupae of *P. suturicallus*, are more pathogenic under temperatures above 15° C (see chap. 7). As for the survival rates, the maximum reproduction rate is also slightly shifted towards lower temperatures and the maximum temperature for egg production is lower than in the laboratory. The data are also reflected by the results from a survey including over 600 farmers all over Peru (see chap. 2) where a significantly reduced damage incidence was reported for altitudes above 3750 meters and under 3000 meters. Ewell et al. (1990) found a reduced damage incidence only above 4000 meters.

The presented field data confirm the adaptation of this important potato pest to cold climates. The other way around, laboratory data found in chap. 3 show, that the survival distribution found in the present study is primary due to physiological adaptation and only to a lesser extent to competition, predation and/or parasitation. The realized niche of *P. suturicallus* is only little smaller than its potential niche. However, temperatures used for direct comparison are average temperatures. But it may be, that the maximum day temperatures act as limiting factor. Of cause, other abiotic factors than temperature may also influence survival. Dillon et al. (2006) discusses some factors linked to altitude that may influence directly insect development or indirectly by affecting its temperature budget (e.g. dry air, radiation, air density, convection and oxygen partial pressure).

It has been shown, that *P. suturicallus* cannot survive or reproduce in the field under temperature conditions above 16° C or at altitude below 2400 meters. Similar studies should be made with other species of the APW complex to make general statements on the distribution limits of APW.

Acknowledgements

We owe thanks to Marcelo Trebejo, Adan Vega and Carlos Torpoco of the International Potato Center (CIP) in Lima, Huancayo and San Ramon (Peru) for helping supervising and evaluating the field sites. This research was done at CIP and was made possible through project funding by the Government of the Grand-Duchy of Luxembourg.

References cited

Alcalá C., P. and J. Alcázar S. (**1976**). Biología y comportamiento de *Premnotrypes suturicallus* Kuschel (Col.: Curculionidae). Revista Peruana de Entomología **19**(1): 49-52.

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

COSAVE. Hojas de datos sobre organismos cuarentenarios para los paises miembros del COSAVE. Ficha Cuarentenaria: *Premnotrypes* spp. (Coleoptera: Curculionidae). http://www30.brinkster.com/cosave/base_ing.htm. Accessed: 26/11/2003.

Dillon, M. E., M. R. Frazier and R. Dudley (**2006**). Into thin air: Physiology and evolution of alpine insects. Integrative and Comparative Biology **46**(1): 49-61.

Ewell, P. T., H. Fano, K. V. Raman, J. Alcázar, M. Palacios and J. Carhuamaca (**1990**). Farmer management of potato insect pests in Peru. Lima, International Potato Center. 77pp.

OEPP/EPPO (**1984**). EPPO data sheet on quaratine organisms No. 143. EPPO Bulletin **14**(1): 55-60.

Townsend, G. R. and J. W. Heuberger (**1943**). Methods for estimating losses caused by diseases in fungicide experiments. Plant Disease Reporter **27**: 340-343.

Yábar Landa, E. (**1994**). Manejo ecológico del gorgojo de los Andes. Lima, RAAA. 119pp.

Part three:

Pest - pathogen Interactions

Susceptibility of the Andean potato weevil to the entomopathogenic fungus *Beauveria bassiana* in laboratory and field trials

Magnus Kühne, Jesús Alcázar, Kerstin Jung, Dietrich Stephan and Stefan Vidal

Abstract

The Andean potato weevil is one of the most important potato pests in the Andes above 2800 meter and its current control is essentially based upon chemical insecticides. We tested the possibilities of a control with the entomopathogenic fungus *Beauveria bassiana*. Medium lethal concentrations varied between 5.6 x 10^4 and 8.2 x 10^5 conidia/ml for pre-pupae depending on the isolate and 6.8 x 10^6 and 1.2 x 10^7 for adults at 15° C under laboratory condition. The medium lethal time with an inoculation of 1 x 10^6 conidia/ml varied between 12 and 23 days for pre-pupae and 19 and 34 days for adults. Application of *B. bassiana* in cages as well in open field with concentrations corresponding to approximately 1 x 10^{14} to 6 x 10^{14} conidia/ha had no effect on the damage incidence caused by *P. suturicallus*. Nor could we observe long-term effects on the weevil population like higher pathogen prevalence or pupal mortality.

Keywords: *Premnotrypes suturicallus*, potato pest, bioassays, LC_{50}, LT_{50}, field applications.

Introduction

The Andean potato weevil (APW) is the most damaging insect potato pest in the highlands of the Andes. In mean it causes damages of 20 to 25%, though complete yield lost may occur (Ortiz et al. 1996, Alcázar and Cisneros 1999). APW is a complex of 12 species of the genus *Premnotrypes* and 2 species of the genus *Rhigopsidius*. It is distributed above 2800 meter in the Andes from Venezuela to north-western Argentina. Below 3000 meter, in most cases, no significant damage is observed. The present research was conducted with the species *P. suturicallus*, the dominant species in the highlands of central Peru.

The damaging life stage is the larval stage feeding inside the potato tubers. Adults make only minor, characteristic half moon shaped damages to the potato leaves with generally no effects on the plant development (Alcalá C. and Alcázar S. 1976). The commercial value of potato tubers damaged by APW is reduced by 22% to 50% and 90%

of the insecticides applied in potato fields are directed against APW (Calvache Guerrero 1991). Applications of chemical insecticides in the field are the main, and in many cases only, control measure used by the farmers against APW. They are mainly directed against adult weevils. Insecticides most commonly used are Metamidophos and Carbofuran (wettable powder or granulate) and to a lesser extent Cypermethrin and Aldicarp (see chap 2). In an extensive interdisciplinary study with agronomists, social scientists and medical scientists, Crissman et al. (1998) established a strong relation between the use of these highly toxic pesticides and their detrimental effects on the health and intellectual capacities of the farmers and their families. These are principally due to a lack of appropriate training of the farmers. Therefore attempts are being made to reduce the quantity and toxicity of the agrochemicals used.

An important field of research has been the use of the entomopathogenic fungus *Beauveria* spp. It has first been reported parasitising APW in 1976 (Alcalá C. and Alcázar S. 1976). Infested cadavers of adults are regularly found in the field, though in storage areas the infestation rates seem to be higher. The most affected life stage are mature larvae or pre-pupae, followed by pupae and the less affected adults (Torres Torres and Marina Cotes 1999, Cisneros and Vera 2001, Niño et al. 2004). Many laboratory studies on the pathogenicity of the fungus against mature larvae and adults as well as field applications have been conducted with inconsistent results, though only few have been published (Vera Robles et al. 1995, Fernández and Colmenares 1997, Torres Torres and Marina Cotes 1999). Eggs have been found to be little affected by the fungus (Vera Robles et al. 1995). However several methodological errors have been committed. For pathogenicity bioassays in the laboratory, field collected larvae or adults have been used that may have been naturally infected with the fungus and no homogeneity of age is given. Additionally larvae or adults were not kept individually for the bioassays, but in most cases in groups of 10 or 20. For the field trials, normally no exact concentration of the applied fungus is indicated and its quality has not been controlled. Also, the heterogeneous distribution of the weevil in the field leads frequently to a higher variation within the treatments than between the treatments.

The standard method for the assessment of the pathogenicity of any product is the probit analysis (Finney 1971). The transformation to probit units changes the normal sigmoid mortality curve into a linear regression. To avoid a bias through the highly variable values at the extremes (low and high mortality), the central mortality range (around 50% mortality) receives a higher weight. Therefore the median lethal dose (LD_{50}) or median lethal concentration (LC_{50}) and the slope of the regression line are the biological most meaningful parameters. If the regression lines are parallel on a logarithmic dose (or concentration) scale, the relative potency of different substances can be estimated (in the case of non-parallelism, the comparison would only be valid for the LC_{50} but not for other values on the regression line like for example the LC_{90} or the LC_{99}, which are of special interest for applications).

In this study, thoroughly conducted bioassays with *B. bassiana* on mature larvae and on adults are presented. Additionally, greenhouse trial with potted plants and field trials with controlled weevil populations in cages as well as open field trial have been run.

Methods

The origins of insect and fungi material

The insect material for the laboratory and the greenhouse trials as well as for the caged trials in the field was taken from a laboratory colony kept at the International Potato Center (CIP) in Lima. Insects were reared at 18 to 20° C and dark conditions as described in chap. 3. Original weevils were collected from the eastern slopes of the Rio Mantaro valley in central Peru with yearly new introductions from the field.

The isolates of *Beauveria bassiana* used in the trials were taken from the CIP collection of entomopathogenic fungi isolated from field collected cadavers of adults and pre-pupae of APW (*P. suturicallus*, *P. vorax* and *P. latithorax*). Formerly registered as *B. brongniartii*, they have been re-determined as *B. bassiana* by Dr. Teresa Ames at CIP. They have been stored at 4 - 8° C on potato-dextrose-agar (PDA) and were regularly passed through APW pre-pupae for reactivation. Before using them in the assays, they were less then 5 times subcultured on artificial medium. Based on results of earlier run bioassays at the International Potato Center, 2 isolates, that had shown high pathogenicity against *P. suturicallus* and *P. latithorax* have been selected for the present investigation. CIP56, isolated in the year 1997 from a *P. vorax* pre-pupa, was selected for presenting the lowest LC_{50} values against pre-pupae of *P. suturicallus*. CIP40, isolated in the year 2000 from a *P. suturicallus* adult, was selected for being an isolate with high pathogenicity against APW pre-pupae (low LC_{50}) and showing the best growing performance at low temperature (see chap. 7). Additionally the *Metarhizium* sp. isolate W54 isolated in the year 2003 from a *P. suturicallus* adult was used for comparison in some bioassays.

Laboratory trials

The fungi were grown on phyto-yeast-medium (PYM)(2% glucose, 0.5% soy peptone, 0.5% yeast extract and 1.8% agar) at 20° C until full sporulation (14 - 21 days). Conidia were then scraped and washed from the surface of the medium with 0.1% Tween 80 (0.5% Tween 80 for *Metarhizium* spp.). The suspensions were placed for approximately 3 min. into an ultrasonic bath to disrupt spore clumps. Spore concentrations were estimated using an improved Neubauer haemocytometer and 5 or 6 concentrations of 1 x 10^3 or 1 x 10^4 to 1 x 10^7 or 1 x 10^8 conidia/ml were prepared adding 0.1% respectively 0.5% Tween 80. The concentration of 1 x 10^6 conidia was re-determined and used as the reference concentration for the statistical analysis. For the determination of the viability of the conidia used, the germination rate was assessed applying 100 µl of approximately 5 x 10^6 conidia/ml to PYM agar and incubating them for 24 h at 20° C. A few drops of lactophenol cotton blue were placed on each plate to stop growth after 24 h. In 5 sections of each plate, 100 conidia were counted and determined whether they germinated or not.

20 or 30 adults or pre-pupae of *P. suturicallus* were inoculated by submerging them individually for ca. 3 seconds into the conidia suspension. For control, adults or pre-pupae were treated with 0.1% (respectively 0.5%) Tween 80 only. Adults or pre-pupae were then placed individually into small containers (approximately 3 x 3 x 2.5 cm) containing a piece of filter paper at the bottom. The inoculated weevils were incubated at 15° C and darkness. Evaluations of mortality were made 1 or 3 times per week during 60 days. For the determination of the LC_{50} the evaluation data of the 20th postinoculation day (approximately) were used. Concentration-response relation was analysed with probit analysis (using the statistical package SPSS 10.0.7) estimating medium lethal concentrations (LC_{50}), slope of regression and fiducial limits. Normal probit analysis requires independence between the responses of the different concentration levels tested. In the case of multiple observations of the same group of insects after several time intervals, this is not given. An adapted analysis for serial time-mortality data is needed for the determination of the medium lethal time (LT_{50}) (Throne et al. 1995). The corresponding probit program developed by Throne (1996) for Mathematica software as well as the programs for comparing the slopes and estimating the relative potency were

downloaded from the website www.ars.usda.gov/pandp/people/people.htm?personid=5643.

Greenhouse trial

Different ways of spore uptake by the adult weevils were tested. Potatoes (variety Maria Reiche) were planted in pots in the greenhouse and covered with a cage to avoid escaping of the weevils. *B. bassiana* isolate CIP40 was grown on PYM agar for 20 days at 20° C and a suspension with 1 x 10^8 conidia/ml was prepared. The viability of the fungus was determined as described above.

4 treatments were installed: For treatment 1, 450 g of soil were inoculated with 20 ml of the *B. bassiana* suspension (resulting in 4.4 x 10^6 conidia/g of soil) and added 1 cm high on top of the existing soil in the plant pots. For treatment 2, 20 ml of the *B. bassiana* suspension were applied by spraying to each potato plant, while the soil was covered with a plastic sheet to avoid a contamination of the soil. After spraying, the plastic sheet was withdrawn. For treatment 3, adult weevils from the laboratory colony were inoculated 3 weeks earlier with *B. bassiana* isolate CIP40 and placed in a sealed petri dish for full sporulation of the fungus. 2 cadavers with sporulating *B. bassiana* were placed close to the plant stem. For treatment 4, adult weevils were directly inoculated with the *B. bassiana* suspension by submerging them for 3 sec in the conidia suspension before liberating them on the potato plant. In the control treatment, no application of *B. bassiana* was made. Each treatment was replicated 5 times.

On each plant, 10 adults were liberated. Plants were kept at natural light conditions with a mean temperature of 17.5° C and a mean relative humidity of 78.5%. After 5 days, adults were recollected from the pots and placed individually into small containers. Forceps used for collecting were sterilized after each individual collection. Containers with adult weevils were incubated at 20° C and evaluated once per week for mortality and fungus development.

For the determination of the effectively applied amount of fungus conidia, composed samples of 3 subsamples of soil for treatment 1 and of 3 leaflets for treatment 2 were taken and washed in 10 ml of 0.1% Tween 80. For treatment 3, after recollection of the weevils the sporulating adult cadavers were washed with 1 ml of 0.1% Tween 80. The suspensions were diluted and 100 µl were applied to selective medium (2% glucose, 0.5% soy peptone, 0.5% yeast extract, 1.8 % agar, 0.005% cycloheximide, 0.01% dodine, 0.01% streptomycin and 0.005% tetracyclin) (Beilharz and Parbery 1982, Liu et al. 1993). After incubation during 21 to 28 days at 20° C the colony forming units (CFU) were counted. To determine the number of CFU per leave area, the surface area of the leaflets was determined following the method described by O'Neal et al. (2002) using the computer program Scion Image v. alpha 4.0.3.2 downloaded from the web site www.scioncorp.com.

Field trials

To assess the natural infestation rate of adults, 100 living adults were collected from six different fields on the eastern slopes of the Rio Mantaro valley in central Peru at altitudes between 3800 and 3950 meters, an area with commonly high APW incidence. On the selected fields, potatoes have been grown the year before and spontaneous potato plants were present. The adults were placed individually in sealed petri dishes and incubated at 15° C in the laboratory. They were observed over 2 months for development of *Beauveria* sp.

The efficacy of *B. bassiana* isolates CIP40 and CIP56 was tested in a semi-field trial with the release of a defined number of adult weevils in cages. Approximately 1 x 1 x 1 m big cages with wind cutting mesh of 36% shading effect. allowing adequate air circulation, were installed in a field with loam soil at 3670 meter altitude in the same area as described above. In each cage one potato plant (variety Yungay) grown in the greenhouse was transplanted. 8 adult *P. suturicallus* weevils (4 males and 4 females) were released into each cage. In one cage a datalogger was installed recording the temperature every hour. For each of the two tested *B. bassiana* isolates, one treatment with fungus applied by spraying to the plant immediately before releasing the weevils and one treatment with adults submerged into the fungus suspension before their release were installed. The fungus was grown for two to three weeks on autoclaved rice in plastic backs at approximately 20° C after which the concentration of the conidia per gram of rice was determined. For the application in the cages, the rice were washed with 0.1% Tween 80 and water was added resulting in concentrations of 6.3×10^7 conidia/ml for CIP40 and of 3.2×10^8 conidia/ml for CIP56. 200 ml were applied by knapsack to each plant (cage) and the same suspension was used for submerging the adults. The concentrations corresponded to an application of 1.3×10^{14} conidia/ha for CIP40 and of 6.4×10^{14} conidia/ha for CIP56. As positive control, one treatment with Carbofuran application in the recommended concentration was installed. In the control treatment nothing was applied and in an additional negative control treatment no weevils were released to control for a possible natural infestation with APW. At maturation of the potato plants, tubers were harvested and evaluated for APW damage using the Townsend and Heuberger (1943) formula

$$i.u. = \frac{\sum(nv)}{iN} \qquad (1)$$

with i.u. = damage index (%), n = number of tubers in each category, v = values of category, i = value of highest category and N = total number of tubers.

The *B. bassiana* isolate CIP40 was also tested in a field trial without controlled APW population. In the same area as the above trial at 3830 meter altitude a field with loam soil was chosen which presented a high incidence of APW in the preceding year. This assured an infestation from within the field and by this, a potentially random distributed of the APW population. Depending on an immigrating APW population would lead to a high population at the borders of the field from where the migration occurs and a low population respectively absence of APW on the opposite border and centre of the field (Valencia 1988, Alcázar and Cisneros 1999). The field was divided into 16 plots of 58 m^2 each and 1 m wide spaces between each plot. The central 6.5 m^2 of each plot were used for evaluation. Potatoes (variety Peruanita) were planted 30 cm apart with 90 cm between the rows. In the centre of the field, a datalogger was installed recording hourly the soil temperature at surface and in 10 cm depth. Before the installation of the field trial in each plot, the number of adult APW was assessed on 5 squares of 1 m^2 and a randomly distributed APW population was confirmed. 4 treatments were installed randomly in the plots: Treatment 1 consisted in 1 application of *B. bassiana* at emergence of the potato plants, treatment 2 of 1 application at emergence and a second application 4 weeks later and in treatment 3, the insecticide Carbofuran was applied at emergence and 4 weeks later. In the control treatment nothing was applied. Applications of *B. bassiana* were done as described for the cage trial with concentrations corresponding to 9.7×10^{13} conidia/ha. At plant maturation, tubers were harvested and damage was determined using Formula 1.

To assess the effects of the applications on the APW population, the infestation rate of the APW population with *B. bassiana* was assessed, as described for the assessment of the natural infestation rate. 20 adult weevils from each plot were collected before the first application and 1 week after both applications. Additionally, from one month after the first application on during 12 months, the population of APW was monitored in each plot using a combination of shelter traps and pit falls. These consisted of a plastic beaker dug up to the top edge into the soil with a funnel on top to avoid escaping of the trapped insects. Above the beaker, a wooden board was placed, leaving a slit of maximum 0.5 cm for access, making it an attractive shelter place for the weevils. Approximately 4 months after harvest, during the second half of September and before the onset of the next rainy season, the APW population within the soil was assessed in each plot. Finally the concentration of *B. bassiana* in the soil was monitored before and after the applications of the fungus. Therefore, at several dates a composite sample of 5 subsamples was taken from each plot and washed with 0.1% Tween 80. After 2 min of sedimentation liquid samples were taken just above the sediment, diluted, applied to selective medium (see above for recipe), incubated for 21 to 28 days at 20° C and evaluated for the number of CFU.

A second field was planted but potatoes did not germinate due to lack of rain. Parallel field trials with the same design and 2 applications of *B. bassiana* isolate CIP40 or the insecticide Carbofuran were run at 4 sites distributed throughout Peru: Huancayo and Huasahuasi in central Peru with the presence of *P. suturicallus* as the dominant APW species and Cusco and Puno in southern Peru with *P. latithorax* and *P. solaniperda* as the dominant APW species.

Results

Laboratory trials

The viability of the conidia was in all trials and for all isolates between 89 and 99%. The LC_{50} of the tested isolates varied for adults between 6.8 x 10^6 and 2.4 x 10^8 conidia/ml after 18 days (Table 1) and for mature larvae between 5.6 x 10^4 and 2.4 x 10^6 after 19 days (Table 2). LC_{50} values decreased after additional days of incubation. In all cases, the *B. bassiana* isolate CIP40 showed the lowest LC_{50} respectively the highest pathogenicity and the *Metarhizium* sp. isolate W54 was the least pathogenic with a 33 to 50 time lower relative potency than isolate CIP40. Mature larvae were more susceptible than adults though this was less pronounced for the isolate CIP56. On the other side, the slopes of the regressions lines were steeper for adults, indicating, that an increase of the concentration has a stronger influence on adult mortality than on larvae mortality.

The results of the repeated observations of mortality in time are shown in Table 3 and Table 4. The medium lethal time (LT_{50}) was shortest for the isolate CIP40 and for mature larvae with almost 11.8 days, while it took 25 to 27 days for killing half of the adults at a concentration of 9.2 x 10^5 conidia/ml and 17 to 19 days at 9.2 x 10^6 conidia/ml. In this case, the slopes of the regression lines were steeper for the mature larvae.

Table 1: Concentration dependent response of *P. suturicallus* adults to *B. bassiana* with the parameters of the probit regression line, the median lethal concentration (LC_{50}) and the potency as compared to the most effective isolate of each trial.

trial	isol.	n	common slope (± SE)	LC_{50} (conidia/ml)	(95% fiducial limits)	χ^2	Paralel. test P	relative potency	(95% confid. limits of rel. potency)
I				at 18 days from inoculation					
	C40	20	1.25 (± 0.20)	6.8 x 10^6	(2.9 x 10^6 - 1.4 x 10^7)	5.26	0.138	1	
	C56	20		1.2 x 10^7	(5.4 x 10^6 - 2.6 x 10^7)			0.58	(0.18 – 1.64)
	W54	20		2.4 x 10^8	(7.5 x 10^7 - 9.3 x 10^8)			0.03	(0.00 – 0.18)
				at 24 days from inoculation					
	C40	20	1.36 (± 0.31)	2.0 x 10^6	(5.5 x 10^5 - 4.3 x 10^6)	5.98	0.364	1	
	C56	20		4.1 x 10^6	(1.4 x 10^6 - 8.8 x 10^6)			0.48	(0.10 – 1.37)
	W54	20		1.2 x 10^8	(4.3 x 10^7 - 4.2 x 10^8)			0.02	(0.00 – 0.17)
II				at 22 days from inoculation					
	C40	30	1.35 (± 0.22)	1.3 x 10^6	(7.1 x 10^5 - 2.3 x 10^6)	4.86	--	--	
	C56			contaminated					

Table 2: Concentration dependent response of mature larvae of *P. suturicallus* to *B. bassiana* with the parameters of the probit regression line, the median lethal concentration (LC_{50}) and the potency as compared to the most effective isolate of each trial.

trial	isol.	n	common slope (± SE)	LC_{50} (conidia/ml)	(95% fiducial limits)	χ^2	Paralel. test P	relative potency	(95% confid. limits of rel. potency)
I				at 18 days from inoculation					
	C40	20	0.75 (± 0.11)	5.6 x 10^4	(9.6 x 10^3 - 2.6 x 10^5)	19.36*	0.645	1	
	C56	20		8.2 x 10^5	(1.4 x 10^5 - 4.9 x 10^6)			0.07	(0.00 – 0-76)
	W54	20		2.4 x 10^6	(4.3 x 10^5 - 1.8 x 10^7)			0.02	(0.00 – 0.34)
				at 24 days from inoculation					
	C40	20	0.79 (± 0.12)	4.1 x 10^4	(1.2 x 10^4 - 1.1 x 10^5)	13.79	0.553	1	
	C56	20		1.1 x 10^6	(3.3 x 10^5 - 3.6 x 10^6)			0.04	(0.00 – 0.26)
	W54	20		1.4 x 10^6	(4.5 x 10^5 - 4.8 x 10^6)			0.03	(0.00 – 0.20)
II				at 22 days from inoculation					
	C40	30	0.67 (± 0.08)	3.3 x 10^4	(1.3 x 10^4 - 7.8 x 10^4)	10.69	1.000	1	
	C56	30		3.1 x 10^5	(1.3 x 10^5 - 7.5 x 10^5)			0.11	(0.02 – 0.41)

*A heterogeneity factor was applied in the calculation of the fiducial limits.

Table 3: Time dependent response of *P. suturicallus* adults to *B. bassiana* with the parameters of the probit regression line, the median lethal time (LT_{50}) and the potency as compared to the most effective isolate.

trial	isol.	n	slope (± SE)	LT_{50} (days)	(95% fiducial limits)	χ^2	Paralel. test P	relative potency	(95% confid. limits of rel. potency)
at concentrations of 9.2 x 10^{5} ± 2.00 x 10^{5} conidia/ml									
I	C40	20	0.056 (± 0.012^{a})	24.7	(15.5 – 33.9)	16.36	0.069	1	
	C56	20	0.030 (± 0.010^{ab})	34.3	--	47.15	< 0.001*	0.72	(0.30 – 1.14)
	W54	20	0.008 (± 0.005^{b})	101.0	--	80.88	< 0.001*	0.24	(0.01 – 0.48)
II	C40	30	0.044 (± 0.010^{a})	27.2	--	24.70	< 0.001*	0.91	(0.48 – 1.34)
at concentrations of 9.2 x 10^{6} ± 2.00 x 10^{6} conidia/ml									
I	C40	20	0.056 (± 0.016^{a})	18.6	--	70.86	< 0.001*	0.91	(0.36 – 1.45)
	C56	20	0.074 (± 0.021^{ab})	22.2	--	18.30	0.004*	0.76	(0.40 – 1.12)
	W54	20	0.047 (± 0.015^{b})	41.6	(27.5 – 54.7)	5.39	0.225	0.41	(0.22 – 0.59)
II	C40	30	0.066 (± 0.015^{a})	16.9	--	9.69	0.008*	1	

The slopes with different letters are different at a 0.05% level.

*A heterogeneity factor was applied in the calculation of the fiducial limits.

Table 4: Time dependent response of mature larvae of *P. suturicallus* to *B. bassiana* with the parameters of the probit regression line, the median lethal time (LT_{50}) and the potency as compared to the most effective isolate.

trial	isol.	n	slope (± SE)	LT_{50} (days)	(95% fiducial limits)	χ^2	Paralel. test P	relative potency	(95% confid. limits of rel. potency)
at concentrations of 1 x 10^{6} conidia/ml									
I	C40	20	0.082 (± 0.021^{b})	11.8	--	39.42	< 0.000*	1	
	C56	20	0.028 (± 0.007^{a})	31.6	--	30.58	< 0.000*	0.37	(0.09 – 0.61)
	W54	20	0.050 (± 0.010^{ab})	30.9	(25.0 – 37.2)	5.02	0.028*	0.38	(0.15 – 0.61)
II	C40	30	0.143 (± 0.022^{c})	17.8	(15.1 – 20.7)	2.99	0.224	0.66	(0.29 – 1.02)
	C56	30	0.034 (± 0.007^{a})	15.6	--	62.73	< 0.000*	0.75	(0.06 – 1.45)

The slopes with different letters are different at a 0.05% level.

*A heterogeneity factor was applied in the calculation of the fiducial limits.

Greenhouse trial

The viability of the applied fungus was over 99%. The effectively applied amount of conidia was 3.0 x 10^5 ± 6.0 x 10^4 conidia per gram of soil for treatment 1, 8.9 x 10^3 ± 4.3 x 10^3 conidia per cm^2 of leave surface for treatment 2 and 2.4 x 10^6 ± 8.8 x 10^5 conidia per cadaver for treatment 3. After 26 days of incubation, only the treatments with direct inoculation of the adults (treatment 4) and with placement of sporulating cadavers (treatment 3) showed a mortality of around 90% significantly different from the control (Figure 1). Treatment 2 with spray application to the leaves showed a higher but not significantly different mortality as the control. After 50 days of incubation, the mortality was in all treatments significantly higher than in the control and the mortality in treatment 2 showed similar values as treatment 3 and 4 (almost 100%). Mortality in treatment 1 (soil inoculation) was still significantly lower than in the other treatments.

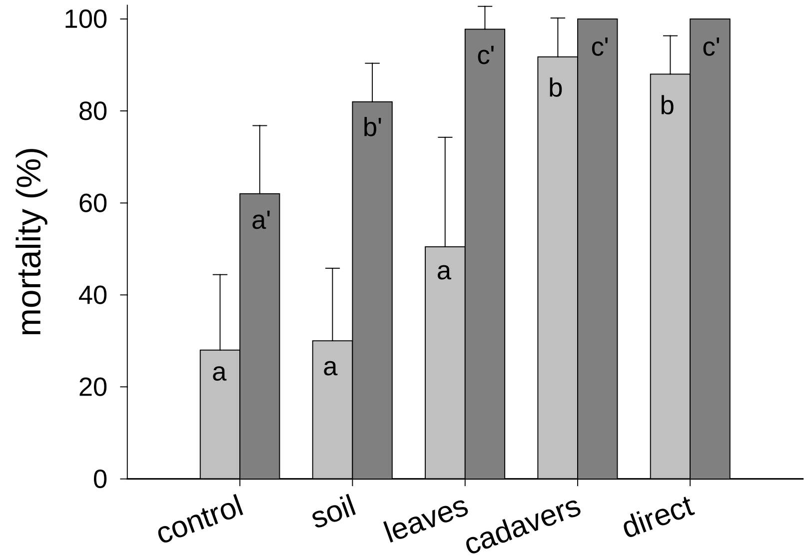

Figure 5: Mortality of *P. suturicallus* adults after 5 days of exposure to different ways of application of *B. bassiana* and after incubation at 20° C for 26 (□) or 50 days (■). For each incubation period separately, different letters indicate significant difference (Tukey, $p < 0.05$). Bars indicate standard deviations of means.

Field trials

Natural infestation rate of *P. suturicallus* adult field populations with *B. bassiana* was 12.3% (median) ranging from 6.0 to 57.8%. In half of the cases the infestation rate was between 6 and 8%. In few cases (1% (median) ranging from 0 to 2.9%) infestations with *Metarhizium* sp. were observed.

The viability of the *B. bassiana* conidia applied in the field trials was 73%. The mean temperature during the cage trial and the field trial was 9.8° C (max 24.4° C, min 1.2° C) respectively 8.7° C (max 19.8° C, min 1.6° C).

In the cage trial with defined weevil populations as well as in the open field trial, no effects of the *B. bassiana* applications on the damage rate by APW could be observed (Figure 2 and Figure 3). The damage rates in the cage trial were slightly lower for the treatments with applications of the fungus as compared to the control, but this difference was not significant. No difference was found between the two isolates applied. In the field trial, the damage rates for the *B. bassiana* applications were even higher then in the control. On the other hand, the treatments with applications of Carbofuran showed in both trials a very high efficacy. In the parallel run field trials on 4 other sites with the same application method for *B. bassiana* similar results were found.

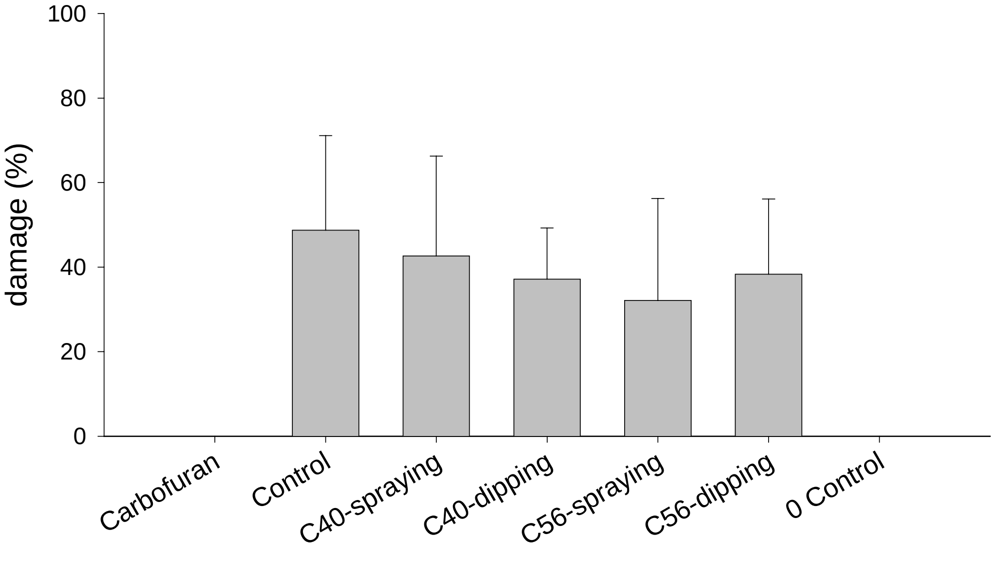

Figure 6: Tuber damage rates caused by *P. suturicallus* with application of *B. bassiana* in cages with defined weevil populations. 0 control: no weevils were liberated. Bars indicate standard deviations of means.

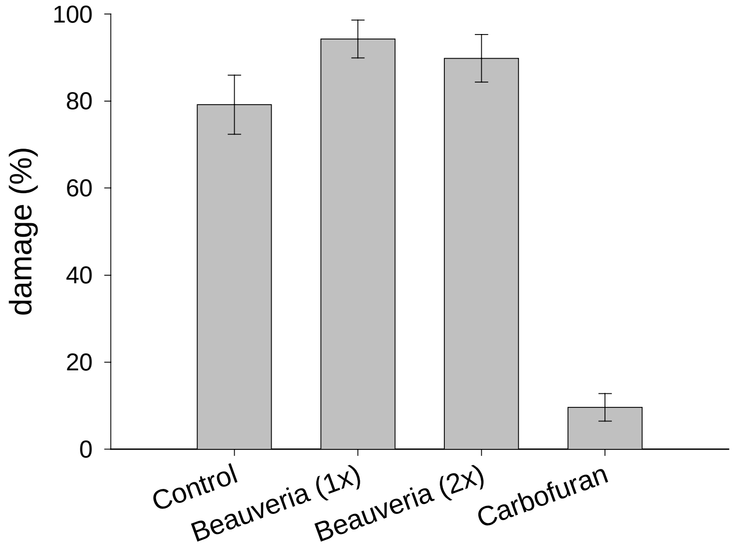

Figure 7: Tuber damage rates caused by *P. suturicallus* with application of *B. bassiana* in the field. Bars indicate standard deviations of means.

No significant changes could be observed for the infestation rates of the weevil populations with *B. bassiana* after each fungus application as compared to the situation before the trial. Monitoring the adult population until no more adults were captured (August 17th), no difference was observed in the number of adults captured in plots with *B. bassiana* applications and the control while in the treatment with insecticide applications, the number of adults was significantly reduced (Table 5). During the next vegetation period beginning with the first adults captured (September 15th) until the peak of APW population (December 23rd) no significant difference was observed between the treatments. The assessment of the soil population of APW at the end of the dry season too did not reveal any significant differences between the treatments neither for the number of living individuals nor for the number of individuals infested with *B. bassiana* (Table 5). This is mainly due to the high variability of the data. Nevertheless, a clear tendency with a much lower population in the treatment with insecticide applications and slightl lower populations in the plots with *B. bassiana* applications can be observed. Concerning the persistence of *B. bassiana* in the soil, the concentration of the fungus has dropped after 30 days to the levels found before the application, though these data too are highly variable (Table 6).

Table 5: Mean (± standard deviations) number of adult weevils captured per plot and collecting date after the applications until no more adult was captured (August 17th) and during the next vegetation period starting when the first adult was captured (September 15th) as well as mean number of soil population at the end of the dry season.

	Adult population[1]				Soil population[2]	
treatment	after applications		next season		end of dry season	
Cabofuran	1.6 ± 3.0	a	17.3 ± 31.0	a	2.8 ± 4.8	a
1 x *Beauveria*	12.1 ± 17.6	b	23.0 ± 34.8	a	9.2 ± 5.1	a
Control	14.9 ± 20.6	b	33.9 ± 60.3	a	14.2 ± 16.2	a
2 x *Beauveria*	15.3 ± 19.7	b	34.0 ± 55.4	a	12.5 ± 4.5	a

For each column, same letters indicate no significances ([1]Games-Howell, [2]Tukey $p < 0.05$).

Table 6: Colony forming units per gram of soil before and after the application of *B. bassiana*.

	day	n	CFU per g of soil*	
before application	0	12	2.1×10^3	a
after application	0	8	1.9×10^4	c
	8	8	8.7×10^3	abc
	14	8	1.3×10^4	bc
	30	8	3.3×10^3	ab
	36	4	2.5×10^3	a

*numbers are back transformed means of log of original data.

different letters indicate significance (Tukey, $p < 0.05$).

Discussion

Laboratory trials

The time to reach adequate mortalities is relatively long and LC_{50} values are relatively high for *P. suturicallus*. This is especially true for adults. Comparable data on the medium lethal dose (LC_{50}) and medium lethal time (LT_{50}) exist only for other species of the APW complex. These have been lower than the results found in the present research. In bioassays run earlier at CIP, LC_{50} values found for mature larvae of *P. latithorax* varied from 1.1×10^2 to 1.2×10^6 conidia/ml after 7 days and LT_{50} values varied between 5 and 7 days for 1×10^6 conidia/ml. Values for *P. suturicallus* are so low, that they can only be explained by inadequate trial conditions. For *P. vorax*, LC_{50} for adults were found to be 5.5 x 10^6 conidia/ml after 12 days (Torres Torres and Marina Cotes 1999). It should be kept in mind, that the present bioassays were run at 15° C, a relatively low temperature for the fungus, while the other authors do not indicate the incubation temperature.

In studies with different insects in most cases lower LC_{50} values were found (for example Leland et al. (2005), Neves and Hirose (2005) and Liu and Bauer (2006) with values ranging from 1x 10^3 to 6.3 x 10^7 conidia/ml after 5 to 7 days for bugs, the coffee berry borer and a beetle). However in studies with adults of other weevils species, values reported are similar or even higher (for example Chikwenhere and Vestergaard (2001) and France et al. (2002) with values of 8.2 x 10^5 conidia/ml after 18 days and 4.9 x 10^4 to 1.5 x 10^5 conidia/ml after 35 days). The same holds true for the LT_{50} values. Most of these studies have been run at temperatures of 24 to 28° C, only France et al. (2002) worked at 18° C. Under this perspective the values found in the present study are acceptable and justify ongoing experiences.

Greenhouse trial

Mortality of adults through secondary pickup was significantly lower than by direct inoculation of the adults except for the case of infestation through sporulating cadavers. This may be due to a higher virulence of the fungus grown on the host instead of artificial medium or to the strategic location in the cavity around the plant stem where adults like to hide during the day. Other authors too found lower mortalities for secondary pickup through foliage applications (Fernandez et al. (2001) with Colorado potato beetle). The conidia concentrations at the foliage applications were relatively high with 8900 conidi/m^2. Other authors found values between 18 and 4000 conidia/m^2 leading to 50% mortality after foliage application against different insects species (Wraight et al. (1998) for white fly, Ugine et al. (2005) for trips and Liu and Bauer (2006) for beetles).

Field trials

The fungus *B. bassiana* was found in each of the studied field populations. Observations in the field confirm, that in APW infested potato fields, adults with infections of *B. bassiana* can readily be found. The high value of prevalence of over 50% in one of the cases indicates the event of en epizooty. For other coleopterans similar natural infestation rates have been observed (for example the cockchafe *Melolontha*

melolontha with a general prevalence of the fungus *B. brongniartii* of 7.5 to 15% and epizooties with up to 60% infections (Zimmermann 1998) or the water hyacinth weevil with 5% and 38% infected adults (Chikwenhere and Vestergaard 2001)).

Considering the results from the laboratory and from the greenhouse, the absence of any effect on the APW population in field application may surprise. But other studies with field applications of *B. bassiana* against APW also found only little to no control effect (Fernández and Colmenares 1997, for other unpublished studies see Kühne et al., in perp.). The negative result of the present field trials is emphasized by the absence of any long-term effect on the weevil population.

A possible reason may be an inadequate application method or formulation of the fungus, though this would not explain the lack of effects found in the caged trial with direct inoculation of the adults through submergence. This method is comparable to the one used in the laboratory and greenhouse trials. Another possible reason could be the long duration until the infection leads to death. This may allow a high reproduction of the weevil before death (see chap 3 for fecundity curves of *P. suturicallus*). A third reason, and probably the most important, may be the low field temperature during the trials as compared to the conditions for the laboratory trials as well as the high solar radiation in the highlands of the Andes (Goettel et al. 2000). Generally, 23 to 25° C is considered the temperature for optimal growth of *B. bassiana*. At decreasing temperature, growth and virulence of the fungus are reduced. However, activity of *B. bassiana* isolates has been described for temperatures below 8° C (Fargues et al. 1997).

The fungus concentrations of 1×10^{14} to 6×10^{14} conidia/ha applied in the field trials are within the range used for other pest insects. Anderson et al. (1988) and Watt and LeBrun (1984) obtained significant reductions in Colorado potato beetle (*Leptinotarsa decemlineata*) populations with 5×10^{13} respectively 2.8×10^{14} conidia/ha while Hajek et al. (1987) observed only in one third of the trials significant control with 5×10^{13} conidia/ha and Cantwell et al. (1986) found significant control of the same pest only with 7.5×10^{15} conidia/ha but not with 7.5×10^{14}. Significant cockchafer (*Melolontha melolontha*) control was reached with 4×10^{13} to 3.7×10^{14} conidia/ha of *B. brongniartii* (Wille et al. 1962, Keller et al. 1997, see also Zimmermann 1998).

While results for the pathogenicity of *B. bassiana* against the Andean potato weevil *P. suturicallus* were satisfactory under laboratory and greenhouse conditions, no effect had been detected at field applications of the fungus. Similar situation have been observed for other pest-pathogen systems (Dorschner et al. 1991, Inglis et al. 1997). Inglis et al. (1997) showed, that environmental conditions and not pathogen virulence were responsible for these results. Climatic conditions in the highland of the Andes may be adverse for entomopathogenic funi. Further studies should clarify the effect of environmental factors, especially temperature on *B. bassiana* and on its interaction with *P. suturicallus*.

Acknowledgements

Especial thanks to Marcelo Trebejo from the International Potato Center (CIP) in Lima (Peru) for maintaining the APW rearing and to Adan Vega from CIP-Huancayo for his invaluable help in the field trials. We thank Kerstin Jung and Dietrich Stefan from the Federal Biological Research Centre for Agriculture and Forestry (BBA) in Darmstadt (Germany) for their advices on everything concerning entomopathogenic fungi. Thanks also to Denny Fliegner from the Max Plank Institute for Dynamics and Self-Organization in Göttingen for an introduction into the use of the Matematica software. The present studies were made at CIP and were possible due to the financial support by the government of Luxembourg.

References cited

Alcalá C., P. and J. Alcázar S. (**1976**). Biología y comportamiento de *Premnotrypes suturicallus* Kuschel (Col.: Curculionidae). Revista Peruana de Entomología **19**(1): 49-52.

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

Anderson, T. E., D. W. Roberts and R. S. Soper (**1988**). Use of *Beauveria bassiana* for suppression of Colorado potato beetle populations in New York state (Coleoptera: Chrysomelidae). Environmental Entomology **17**(1): 140-145.

Beilharz, V. C. and D. G. Parbery (**1982**). Dodine: A selective agent for certain soil fungi. Transactions of the British Mycological Society **79**(3): 507-511.

Calvache Guerrero, H. (**1991**). Barriers for the control of the Andean potato weevil (*Premnotrypes vorax* (Hustache)). Revista Latinoamericana de la Papa **4**(1): 22-35.

Cantwell, G. E., W. W. Cantelo and R. F. W. Schroder (**1986**). Effect of *Beauveria bassiana* on underground stages of the Colorado potao beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). The Great Lakes Entomologist **19**(2): 81-84.

Chikwenhere, G. P. and S. Vestergaard (**2001**). Potential effects of *Beauveria bassiana* (Balsmo) Vuillemin on *Neochetina bruchi* Hustache (Coleoptera : Curculionidae), a biological control agent of water hyacinth. Biological Control **21**(2): 105-110.

Cisneros, F. and A. Vera (**2001**). Mass producing *Beauveria brongniartii* inoculum, an economical, farm-level method. In: Scientist and farmer: Partners in research for the 21st century. Program Report, 1999-2000. Lima (Peru), International Potato Center: pp. 155-160.

Crissman, C. C., J. M. Antle and S. M. Capalbo, Eds. (**1998**). Economic, environmental, and health tradeoffs in agriculture: Pesticides and the sustainability of Andean potato porduction. Natural resource management and policy. Dordrecht (The Netherlands), Kluwer Academic Publishers. 281pp.

Dorschner, K. W., M.-G. G. Feng and C. R. Baird (**1991**). Virulence of an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) to the hop aphid, *Phorodon humuli* (Homoptera: Aphididae). Environmental Entomology **20**(2): 690-693.

Fargues, J., M. S. Goettel, N. Smits, A. Ouedraogo and M. Rougier (**1997**). Effect of temperature on vegetative growth of Beauveria bassiana isolates from different origins. Mycologia **89**(3): 383-392.

Fernandez, S., E. Groden, J. D. Vandenberg and M. J. Furlong (**2001**). The effect of mode of exposure to *Beauveria bassiana* on conidia acquisition and host mortality of Colorado potato beetle, *Leptinotarsa decemlineata*. Journal of Invertebrate Pathology **77**(3): 217-226.

Fernández, S. A. and X. Colmenares (**1997**). Evaluación de *Beauveria* spp para el control de *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae) en el cultivo de la papa. Agronomía Tropical **47**(3): 249-257.

Finney, D. J. (**1971**). Probit analysis. Cambridge (UK), Cambridge University Press. 333pp.

France, A., M. Gerding and A. Sandoval (**2002**). Pathogenicity of chilean isolates of *Beauveria bassiana* to adults of *Asynonychus cervinus* (Coleoptera: Curculionidae). Agricultura Técnica (Chile) **62**(4): 489-496.

Goettel, M. S., G. D. Inglis and S. P. Wraight (**2000**). Fungi. In: Field manual of techniques in invertebrate pathology. L. A. Lacey and H. K. Kaya (Eds). Dordrecht (The Netherlands), Kluwer Academic Publishers: pp. 255-282.

Hajek, A. E., R. S. Soper, D. W. Roberts, T. E. Anderson, K. D. Biever, D. N. Ferro, R. A. LeBrun and R. H. Storch (**1987**). Foliar applications of *Beauveria bassiana* (Balsamo) Vuillemin for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae): An overview of pilot test results from the northern United States. The Canadian Entomologist **119**(11): 959-974.

Inglis, G. D., D. L. Johnson and M. S. Goettel (**1997**). Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vuillemin against grasshoppers. The Canadian Entomologist **129**(1): 171-186.

Keller, S., C. Schweitzer, E. Keller and H. Brenner (**1997**). Control of white grubs (*Melolontha melolontha* L.) by treating adults with the fungus *Beauveria brongniartii*. Biocontrol Science and Technology **7**(1): 105-116.

Leland, J. E., M. R. McGuire, J. A. Grace, S. T. Jaronski, M. Ulloa, Y.-H. Park and R. D. Plattner (**2005**). Strain selection of a fungal entomopathogen, *Beauveria bassiana*, for control of plant bugs (*Lygus* spp.) (Heteroptera : Miridae). Biological Control **35**(2): 104-114.

Liu, H. and L. S. Bauer (**2006**). Susceptibility of *Agrilus planipennis* (Coleoptera : Buprestidae) to *Beauveria bassiana* and *Metarhizium anisopliae*. Journal of Economic Entomology **99**(4): 1096-1103.

Liu, Z. Y., R. J. Milner, C. F. McRae and G. G. Lutton (**1993**). The use of dodine in selective media for the isolation of *Metarhizium* spp. from soil. Journal of Invertebrate Pathology **62**: 248-251.

Neves, P. M. O. J. and E. Hirose (**2005**). *Beauveria bassiana* strains selection for biological control of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera : Scolytidae). Neotropical Entomology **34**(1): 77-82.

Niño, L., E. Acevedo, F. Becerra and M. Guerrero (**2004**). Some biological aspects and population fluctuation of the white tuberworm *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae) in Mucuchíes, Mérida state, Venezuela. Entomotropica **19**(1): 15-19.

O'Neal, M. E., D. A. Landis and R. Isaacs (**2002**). An inexpensive, accurate methode for measuring leaf area and defoliation through digital image analysis. Journal of Economic Entomology **95**(6): 1190-1194.

Ortiz, O., J. Alcázar, W. Catalán, W. Villano, V. Cerna, H. Fano and T. S. Walker (**1996**). Economic impact of IPM practices on the Andean potato weevil in Peru. In: Case studies of the economic impact of CIP - related technology. T. S. Walker and C. C. Crissman (Eds). Lima, International Potato Center: pp. 95-110.

Throne, J. E., D. K. Weaver, V. Chew and J. E. Baker (**1995**). Probit analysis of correlated data: Multiple observations over time at one concentration. Journal of Economic Entomology **88**(5): 1510-1512.

Torres Torres, L. and A. Marina Cotes (**1999**). Biocontrol activity of entomopathogenic fungi against *Premnotrypes vorax* (Coleoptera: Curculionidae) through their individual or combined utulization. Revista Colombiana de Entomología **25**(3-4): 121-129.

Townsend, G. R. and J. W. Heuberger (**1943**). Methods for estimating losses caused by diseases in fungicide experiments. Plant Disease Reporter **27**: 340-343.

Ugine, T. A., S. P. Wraight and J. P. Sanderson (**2005**). Acquisition of lethal doses of *Beauveria bassiana* conidia by western flower thrips, *Frankliniella occidentalis*, exposed to foliar spray residues of formulated and unformulated conidia. Journal of Invertebrate Pathology **90**(1): 10-23.

Valencia, L. (**1988**). Field behavior of the adult Andean weevil (*Premnotrypes vorax* (Hustache)) in Colombia. American Potato Journal **65**(8): 503.

Vera Robles, A., J. Alcázar Sedano and A. Aréstegui P. (**1995**). Pathogenicity of the fungus *Beauveria brongniartii* on the Andean potato weevil *Premnotrypes latithorax*. Revista Peruana de Entomología **38**: 95-98.

Watt, B. A. and R. A. LeBrun (**1984**). Soil effects of *Beauveria bassiana* on pupal populations of the Colorado potato beetle (Coleoptera: Chrysomelidae). Environmental Entomology **13**(1): 15-18.

Wille, H., T. Wikén and P. Bovey (**1962**). Ergebnisse der in der Schweiz in den Jahren 1954 und 1955 durchgeführten Freilandversuche zur mikrobiellen Bekämpfung des Engerlings von *Melolontha melolontha* L. Entomophaga **7**(2): 161-174.

Wraight, S. P., R. I. Carruthers, C. A. Bradley, S. T. Jaronski, L. A. Lacey, P. Wood and S. Galaini-Wraight (**1998**). Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii*. Journal of Invertebrate Pathology **71**(3): 217-226.

Zimmermann, G. (**1998**). The entomopathogenic fungus *Beauveria bassiana* (Sacc.) Petch and experiences in its use for biological control of the European field and forest cockchafer. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes **50**(10): 249-256.

Influence of temperature on the growth and pathogenicity of *Beauveria bassiana*, pathogen of the Andean potato weevil

Magnus Kühne, Jesús Alcázar, Kerstin Jung, Dietrich Stephan and Stefan Vidal

Abstract

The Andean potato weevil *Premnotrypes suturicallus* is a main insect pest of potato in the Andes under climatic conditions with high diurnal temperature variations. Adults and prepupae infected with the entomopathogenic fungus *Beauveria bassiana* are readily found in the field. Under laboratory conditions, several native isolates of the fungus were grown at temperatures from 4.2° C to 28° C. Only Isolate CIP40 germinated and showed growth below 5° C and performed also well at high temperatures. This isolate and CIP56, which had shown the lowest medium lethal concentration (LC_{50}) in previously run bioassays, were used for pathogenicity tests on mature larvae of *P. suturicallus* at different temperatures. LC_{50} values increased 1600 and 2000 times for both isolates with temperatures decreasing form 19° C to 13° C. Medium lethal time (LT_{50}) increased 2.5 and 3.9 times. Changes in growth rate with varying temperature were closely related to changes in LT_{50} making the growth rate a good indicator for the estimation of pathogenicity at different temperatures. The optimum temperatures for survival and fertility of *P. suturicallus* as shown by Kühne et al. (in prep.) match the temperatures found in the field and are much lower as the optimum temperature of the fungus. The tested isolates cannot be expected to perform satisfactorily under field conditions.

Keywords: *Premnotrypes suturicallus*, entomopathogenic fungi, biological control, potato pest, radial growth, bioassay, LC_{50}, LT_{50}.

Introduction

Potatoes (*Solanum* spp.) are a main staple crop in the Andes on which especially subsistence farmer rely for their alimentation. The Andean potato weevil (APW) is a complex of 12 species of the genus *Premnotrypes* and 2 species of the genus *Rhigopsidius* (Coleoptera: Curculionidae) distributed from Venezuela to northwestern Argentina above 2800 meter altitude. It is the most important insect pest of potatoes. causing regularly 20 to 25% damage, though complete yield lost may occur (Ortiz et al. 1996, Alcázar and Cisneros 1999). The present studies were conducted with *P. suturicallus* Kuschel the dominant species in the central highlands of Peru.

Control of APW is mainly restricted to the application of chemical insecticides and here especially to Carbamates, Metamidophos and to a lesser extent Cypermethrin and Aldicarp (Kühne et al., in prep.) which lead to important health problems among the farmers and their families (Cole et al. 1998).

The entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. was first reported parasitising APW in 1976 (Alcalá C. and Alcázar S. 1976). Since then several studies were made showing good pathogenicity of the fungus against larvae and adults under laboratory conditions. But in field applications little to no effect on the weevil population and on potato tuber damage was observed (Kühne et al, in prep.). Similar discrepancies between laboratory and field trials as well as between different field trials are reported from other *B. bassiana* - pest systems (Dorschner et al. 1991, Inglis et al. 1997, Martin et al. 2000). This may be due to two main reasons: Either the exposure methods (e.g. application method, formulation) are inadequate in the field trials and/or the difference in environmental factors between laboratory and field are responsible. James et al. (1998) showed that the environmental conditions have a large impact on fungus efficacy. Generally it has been recognized, that temperature, humidity and solar radiation are environmental constrains to the use of entomopathogenic fungi (Goettel et al. 2000).

As most other entomopathogenic fungi, *B. bassiana* has a wide range of temperature tolerance for survival. High temperatures above approximately 35° C may kill them, while it can be stored over long periods at sub-zero temperature. Though growth is in most cases only observed as low as 5 to 8° C and the optimal temperatures for growth and infection are generally between 20 and 25° C (Ferron 1967, Fargues et al. 1997, Yeo et al. 2003, Quesada-Moraga et al. 2006). Several studies focused on the limitations of fungus activity at low or high temperatures due to environmental conditions or due to insect behaviour like thermoregulation in the case of grasshoppers (Doberski 1981, Hywel-Jones and Gillespie 1990, Martin et al. 2000, Davidson et al. 2003, Yeo et al. 2003, Leland et al. 2005).

In the here presented study, the influence of temperature on the growth of pre-selected isolates of *B. bassiana* was assessed. Thereafter the pathogenicity of the best performing isolates was tested at various temperatures against mature larvae of *P. suturicallus*.

Methods

12 isolates of *B. bassiana* were taken from the collection of entomopathogenic fungi of the International Potato Center (CIP) in Lima (Peru). Most of them had been isolated from *P. suturicallus* specimens collected in the Mantaro valley in the central highlands of Peru, except for CIP47 and CIP53 that had been isolated from *P. latithorax* collected in the Cusco area in the southern highland of Peru and CIP56 and CIP66 isolated from *P. vorax* collected in the Cajamarca area in the northern highlands of Peru. CIP40, CIP53 and CIP66 had been isolated from adult weevils while all other were collected from mature larvae (pre-pupae). The isolates have been stored at 4 to 8° C on potato-dextrose-agar (PDA) and were regularly passed through APW pre-pupae for reactivation. Before using them in the assays, they were less then 5 times subcultured on artificial medium. The isolates have been pre-selected from the collection based on the results of previously run bioassays on the pathogenicity against pre-pupae of *P. suturicallus* and *P. latithorax*.

The *P. suturicallus* larvae for the bioassays were taken from a rearing colony maintained at CIP. Insects were reared as described in chap. 3 at approximately 18° C producing 3 generations per year. Weevils had been collected on the eastern slopes of the Rio Mantaro valley in central Peru with yearly new introductions from the field.

Fungus growth

Conidia suspensions with a concentration of 1×10^7 conidia/ml were prepared for each isolate. For each isolate, 2 agar petri dishes (phyto-yeast-medium (PYM): 2% glucose, 0.5% soy peptone, 0.5% yeast extract and 1.8% agar) were inoculated with 100µl of suspension and incubated during 5 days at 20° C until the surface was completely covered with mycelium. The colonies were in full growth phase however not sporulating. Agar plugs of 7 mm diameter were cut using a flame-sterilized cork borer. This plugs were placed with the mycelium downside in the centre of a new petri dish with PYM + 0.01% streptomycin medium (5 repetitions for each isolate), one plug per dish. The petri dishes were then incubated for 10 days at 5.6° C, 9.2° C, 14.3° C, 20.8° C or 28.1° C. Every second day, two perpendicular diameters of the growing colony were measured with a ruler. For the analysis of the growth, the surface of each colony was determined using its average diameter of which the surface of the original mycelium disc was subtracted.

In a second trial, a hole of 7 mm diameter was made into an agar dish with PYM + 0.1% streptomycin medium. A conidia suspension of 5 x 10^5 conidia/ml was prepared for those isolates that performed best at low temperatures in the first trial. 50 µl of this suspension were applied to the hole in the agar dish and incubated for 20 days at 4.2° C, 13.1° C, 14.1° C, 17.3° C and 19.7° C. In this case conidia had to germinate before growth could be measured. Evaluation and analysis was done similarly as described above. For the analysis, the statistical package SPSS 10.0.7 was used.

Pathogenicity

The isolates CIP40 and CIP56 were grown on PYM medium for 14 to 21 days at 20° C until full sporulation. Conidia were washed from the medium with 0.1% Tween 80 and the suspensions were placed for 3 min into an ultrasonic bath to disrupt spore clumps. The conidia concentrations were estimated using an improved Neubauer haemocytometer and 5 concentrations from 1 x 10^3 to 1 x 10^7 conidia/ml were prepared. The quality of the conidia suspension was assessed determining the germination rate of the conidia. Therefore, 100 µl of the suspension with approximately 1 x 10^6 conidia/ml were applied to PYM medium and incubated for 24 h at 20° C. After 24 h a few droplets of lactophenol cotton blue were placed on each plate to stop germination and 100 conidia were counted for germination and non-germination in each of 5 fields per plate.

For each concentration and temperature, 30 larvae were submerged for approximately 3 seconds into the conidia suspension, placed individually into small containers (approximately 3 x 3 x 2.5 cm) containing a piece of filter paper at the bottom and were incubated at 13° C, 15° C and 19° C. For each temperature the same number of larvae were treated with a 0.1 % Tween 80 solution as control. Evaluations took place approximately once per week.

The medium lethal concentration (LC_{50}), the regression slope and the potency for each temperature were determined applying probit analysis (Finney 1971) using the statistical package SPSS 10.0.7. For the evaluation the data of the 20th post-inoculation day were used. For the estimation of the medium lethal time (LT_{50}) an adapted analysis for serial time-mortality data with multiple observations of the same group of insects was used (Throne et al. 1995). The program files for use with Mathematica software was downloaded from the website www.ars.usda.gov/pandp/people/people.htm?personid=5643.

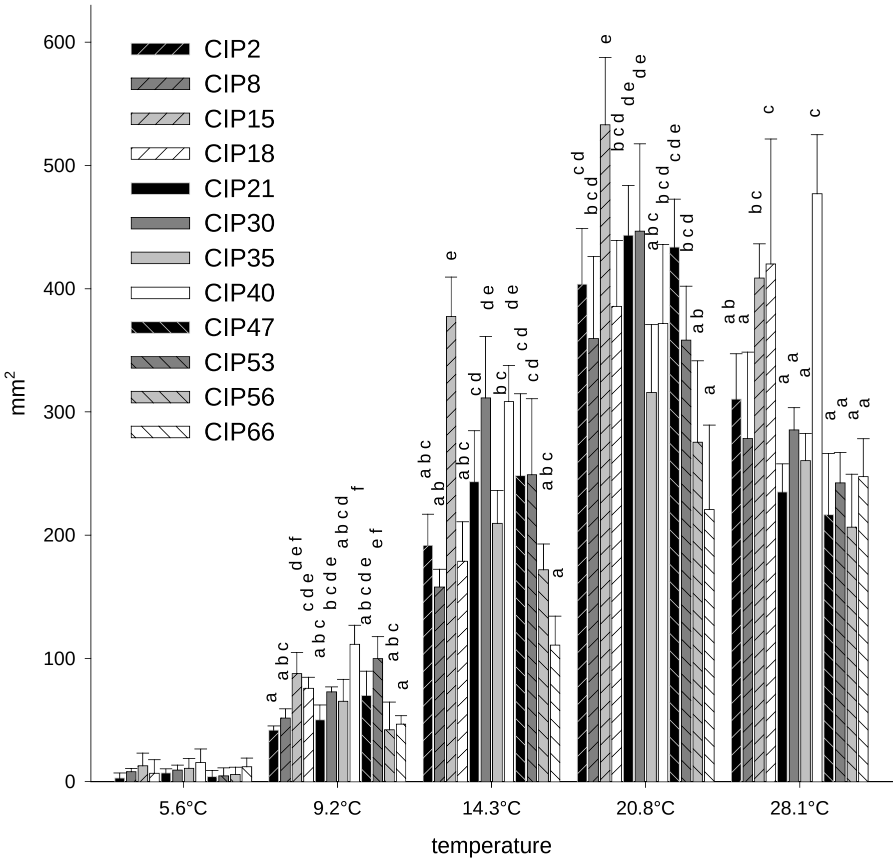

Figure 1: Growth in mm2 after 10 days at different temperatures of 12 *B. bassiana* isolates from the collection of entomopathogens at the International Potato Center. Different letters over columns of the same temperature indicate significant difference (Tukey, $p < 0.05$). At 5.6° C no significant differences were detected.

Results

Fungus growth

Most *B. bassiana* isolates tested showed increasing growth with increasing temperatures up to 20° C. For most isolates growth rates were maximal at 20.1° C and were reduced at higher temperatures except for the isolates CIP18, CIP40 and CIP66 whose maximum growth performance was at 28° C (Figure 1). At 9.2° C, the isolates with the best growing performance were CIP15, CIP40 and CIP53. At 5.6° C CIP40 showed the strongest growth, though not significantly different from the other isolates. Calculating the relative growth at 5.6° C as compared to the growth at 20.1° C, CIP35, CIP40 and CIP66 were least influenced by the low temperatures. For these isolates, growth at the low temperature was 3.4%, 4.2% and 5.4% respectively of the growth at 20.1° C. For all other isolates values at 5.6° C varied between 0.7 and 2.4%. At 9.2° C, the same isolates were the best performing isolates with 20.6%, 29.7% respectively 21.1% growth relatively to the growth at 20.1° C. Additionally CIP53 showed good performance at 9.2° C with 27.9% relative growth as compared to 20.1° C, but this isolate performed very weak at 5.6° C with only 1.3% relative growth. For all other isolates relative growth at 9.2° C was less then 20% of the growth at 20.1° C.

For the second experiment on growth, the isolates CIP40 and CIP66 have been selected due to their good performance at low temperatures. Additionally CIP56, which had shown the highest pathogenicity in previous bioassays, was included (Figure 2). At low temperatures, CIP56 grew always significantly less than the other two isolates. At 14.1° C CIP66 grew significantly more then CIP40, but at 13.1° C no significant difference was detected between these two isolates (due to contaminations, only one replicate of CIP40 could be evaluated). At 4.2° C CIP40 was the only isolates were mycelium growth could be observed. Growing performance at 13.1° C as compared to 19.7° C was 36%, 31% and 16% for CIP40, CIP56 and CIP66.

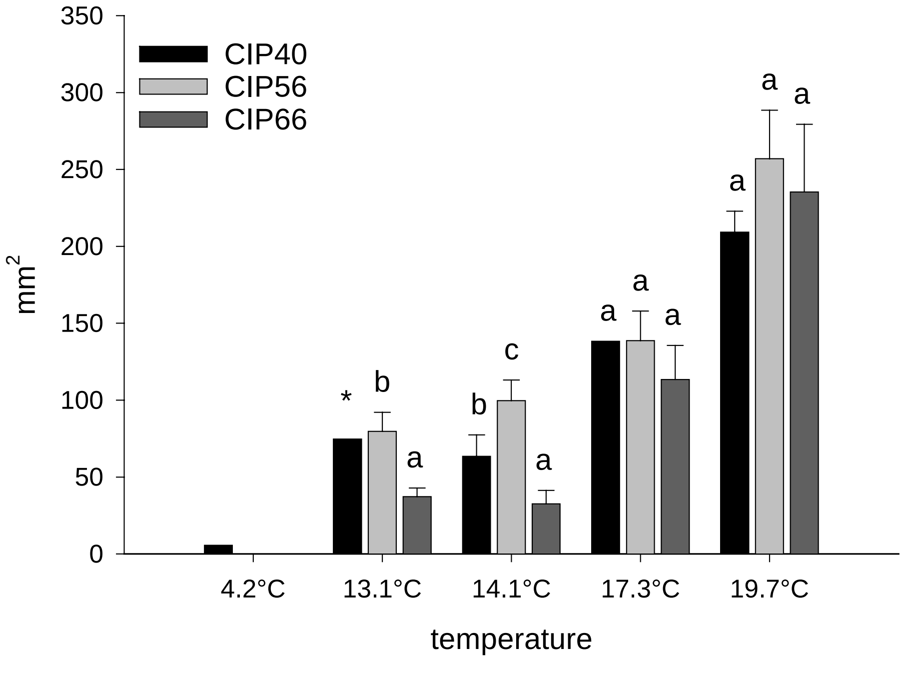

Figure 2: Growth in mm^2 of 3 *B. bassiana* isolates at different temperatures. Different letters over columns of the same temperature indicate significant difference (Tukey, $p < 0.05$). *Due to contaminations only one plate could be evaluated.

Pathogenicity

Both isolates were highly viable with germination rates of 97.2% respectively 98.9%. A very strong influence of temperature on the pathogenicity of the tested *B. bassiana* isolates against *P. suturicallus* has been found (Figure 3 and Figure 4). For both isolates the LC_{50} value is much higher at decreasing temperatures (Table 1) and the slopes of the regression lines is steeper at high temperatures. The potency indicates that at 19° C and at 15° C the isolate CIP40 is 1600 respectively 160 time more pathogenic than at 13° C. Isolate CIP56 is even stronger influenced by low temperature being at 19° C 2000 time more pathogenic than at 13° C. Similar results are found for the time series (Table 2) with CIP40 leading at 19° C and 15° C 2.5 respectively 1.4 times quicker to 50% mortality than at 13° C. For CIP56 the LT_{50} value is again stronger influenced by low temperatures.

Table 1: Medium lethal concentration (LC_{50}) of *P. suturicallus* inoculated with *B. bassiana* isolates CIP40 and CIP56 at 3 different temperatures.

temp. (°C)	n	slope (± SE)	LC_{50} (conidia/ml)	(95% fiducial limits)	χ^2	rel. potency	(95% confid. limits of pot.)
				CIP40			
13	30	0.55 (± 0.11)	6.7×10^6	$2.0 \times 10^6 - 5.5 \times 10^7$	1.53	1	
15	30	0.68 (± 0.10)	3.4×10^4	$1.0 \times 10^2 - 6.8 \times 10^5$	8.52*	1.6×10^2	$1.4 \times 10^1 - 1.0 \times 10^4$
19	30	1.16 (± 0.22)	8.3×10^3	$3.3 \times 10^3 - 1.7 \times 10^4$	2.33	1.6×10^3	$6.1 \times 10^1 - 5.1 \times 10^5$
				CIP56			
13	30	0.50 (± 0.14)	1.1×10^8	$1.5 \times 10^7 - 6.0 \times 10^{10}$	4.06	1	
15	30	0.66 (± 0.11)	3.1×10^5	$1.2 \times 10^5 - 7.9 \times 10^5$	2.23	1.6×10^2	$9.4 \times 10^0 - 2.6 \times 10^4$
19	30	2.10 (± 0.79)	6.3×10^4	$1.0 \times 10^4 - 1.1 \times 10^5$	4.15	2.0×10^3	$5.4 \times 10^1 - 8.0 \times 10^6$

The relative potency is given for each isolate relatively to the lowest tested temperature.

*an heterogeneity factor was applied for the calculation of the fiducial limits of the LC_{50}.

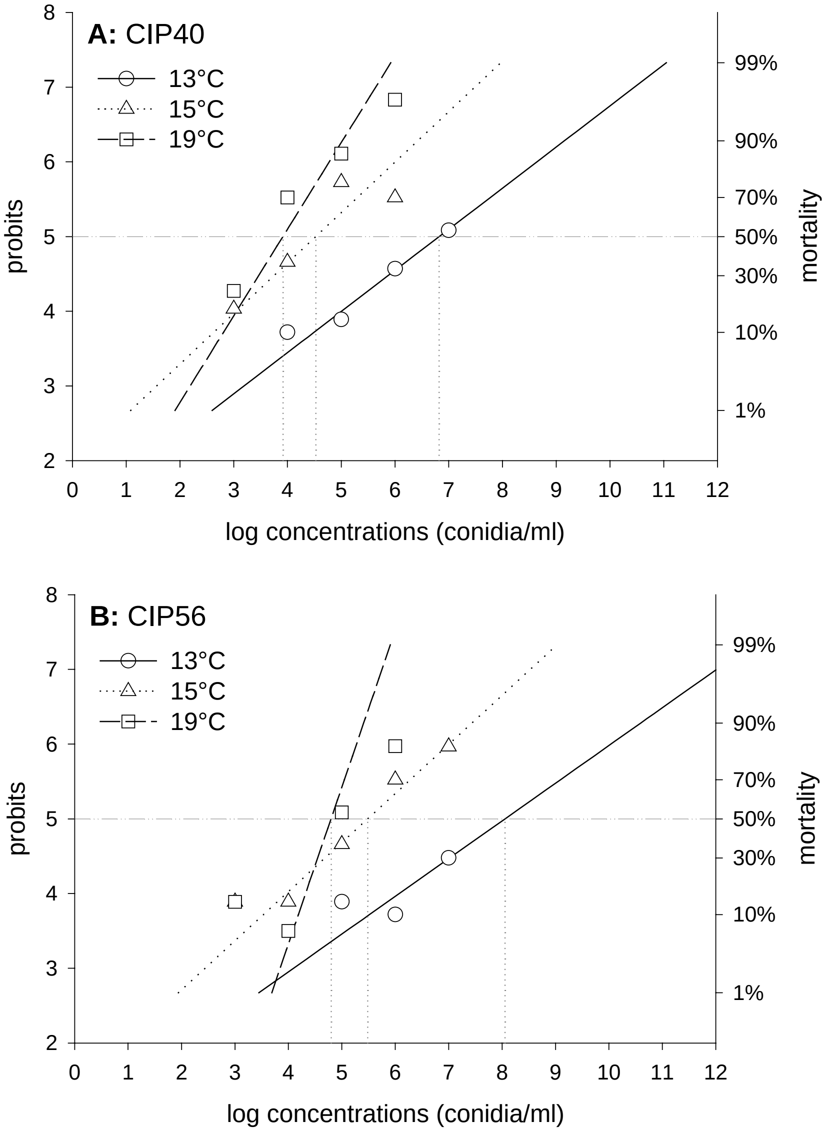

Figure 3: Concentration dependent mortality of *P. suturicallus* mature larvae by *B. bassiana* isolates CIP40 (A) and CIP56 (B) at 3 different temperatures after 20 days. On the left y-axes, mortality rates for the corresponding probit values are shown. Reference lines show 50% mortality and drop lines show LC_{50} values.

Discussion

Consistently with other studies, the optimum temperature for growth of all *B. bassiana* isolates was around 20° C or above (Fargues et al. 1997, Quesada-Moraga et al. 2006). All isolates showed a significantly reduced growth at low temperatures. CIP40 was the only one that germinated and grew at temperatures below 5° C. Additionally it also performed best at 28° C. Beside of being considered the isolate best adapted to low

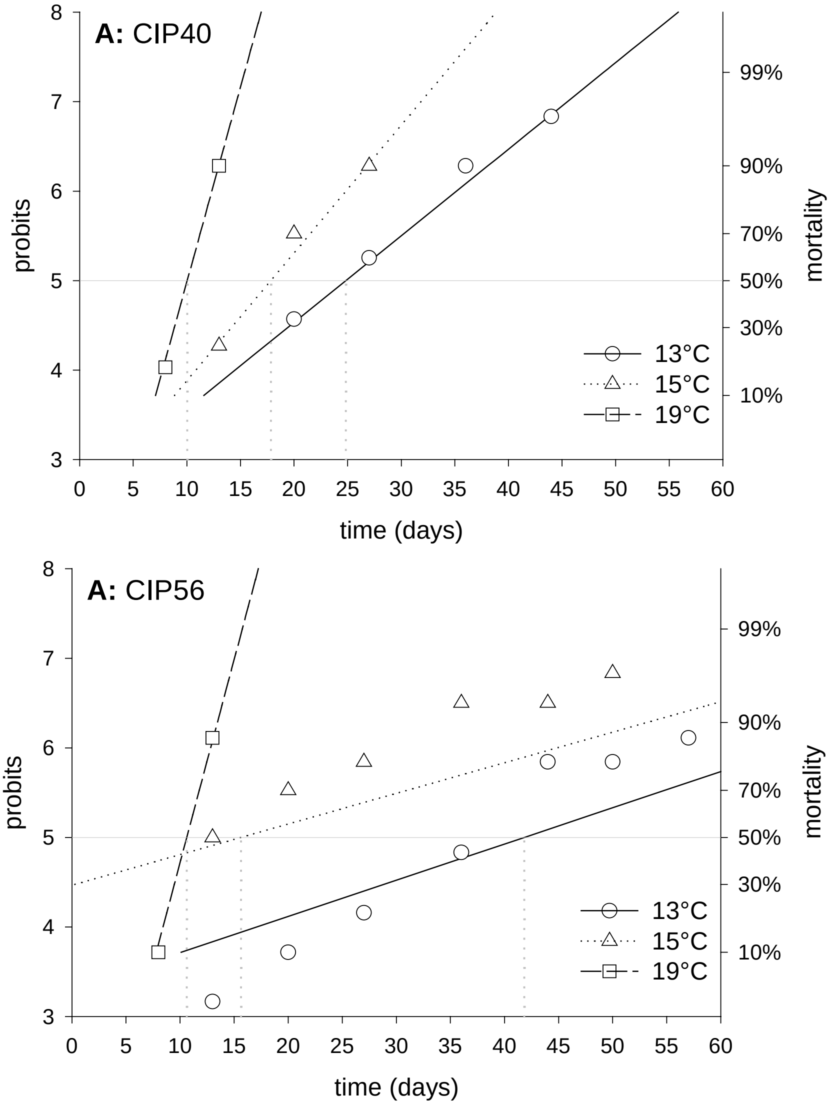

Figure 4: Time dependent mortality of *P. suturicallus* mature larvae by *B. bassiana* isolates C40 (A) and C56 (B) at 3 different temperatures and a concentration of 1 x 10^6 conidia/ml. On the left y-axes, mortality rates for the corresponding probit values are shown. Reference lines show 50% mortality and drop lines show LC_{50} values.

temperatures it seems to be the isolate best adapted to the high temperature variations found in the Andes with high day temperatures and low night temperatures. Mean temperatures measured at 5 cm above ground within a potato field during the cropping season 2002-2003 (December to April) in an area with high incidence of *P. suturicallus* at 3670 meter altitude were 9.9° C. Average day maximum and minimum temperatures were 19.1° C and 4.9° C but extreme temperatures varied from –0.9° C to 33.9° C (see chap. 3). This data reflect the situation at the soil surface, while deeper in the soil the amplitude of temperature fluctuation becomes less pronounced around the mean temperature.

Table 2: Medium lethal time (LT_{50}) of *P. suturicallus* inoculated with *B. bassiana* isolates CIP40 and CIP56 at 3 different temperatures.

temp. (°C)	n	slope (± SE)	LT_{50} (days)	(95% fiducial limits)	χ^2	rel potency	(95% confid. limits of pot.)
				CIP40			
13	30	0.097 (± 0.015)	24.8	(20.7 – 28.9)	7.40	1	
15	30	0.143 (± 0.022)	17.8	(15.1 – 20.7)	2.99	1.4	(1.1 – 1.7)
19	30	0.434 (± 0.068)	10.0	(9.0 – 11.1)	0.53	2.5	(2.0 – 2.9)
				CIP56			
13	30	0.040 (± 0.007)	41.8	(5.3 – 86.9)	22.07*	1	
15	30	0.034 (± 0.007)	15.6	--	62.72*	2.7	(0.6 – 4.8)
19	30	0.453 (± 0.071)	10.6	(9.6 – 11.7)	0.01	3.9	(3.0 – 4.9)

The relative potency is given for each isolate relatively to the lowest tested temperature.

*an heterogeneity factor was applied for the calculation of the fiducial limits of the LT_{50}.

It has been suggested (Davidson et al. 2003), that fungal growth rates follow the Sharp-DeMichel model with a central, linearly rising part, a reduction in growth rate at high temperatures and the curve flatting out in the lower part (Sharpe et al. 1977, Schoolfield et al. 1981). The flattening at the lower part is due to an effective increase of the growth rate as compared to estimations based on a linear regression for the same temperatures. The here presented data have too few data points to adapt the thermodynamic model of Sharp-DeMichele. But the distribution seems to follow the shape of the model. This would mean, that at fluctuating temperatures including low temperatures within the low part of such a model, growth rates would be higher as estimated for at the corresponding mean temperature. This of cause is only true as long as there is still any growth. If temperatures fall below the lower limit for growth, growth rate will be lower than for the corresponding mean temperatures.

All isolates had been isolated from APW cadavers collected in this environment but, as Fargues et al. (1997) showed for *B. bassiana* and De Croos and Bidochka (1999) for the entomopathogenic fungus *Metarhizium anisopliae*, the temperature adaptation of an isolate can not be deduced by its origin of collection. Yeo et al. (2003) come to the same conclusion that the selection of a potential biopesticide should not be based on the origin of collection but on the evaluation of its temperature tolerance.

In the same way as growth, the pathogenicity of the fungus is affected by the temperature. Similar rates of reduction in the pathogenicity of different entomopathogenic fungi were found by other authors (Soares et al. 1983, Moorhouse et al. 1994, Yeo et al. 2003). In our data, time to death was closely related to growth. For the different *B. bassiana* isolates, LT_{50} values are 2.5 and 3.9 times higher at 13° C than at 19° C and growth is 2.8 and 3.2 times less at 13.1° C as compared to 19.7° C. This shows, that the difference in the growth rate at different temperatures is a good indicator for the influence of temperature on the median lethal time at a given concentration. On the other side, for a given time lapse to reach the same mortality at low temperature the concentration had to be risen impracticable high. But also the LT_{50} values at low temperatures may be too high to reach an acceptable mortality before females oviposited a significant number of eggs (see chap. 3 for female fecundity curves).

Optimum temperatures for the development and reproduction of *P. suturicallus* have been shown to be around 13° C at constant temperatures under laboratory conditions (see chap. 3) and below 11° C mean temperature under field conditions (see chap. 5). This means that the optimum temperatures for both organisms, the pathogen and its host, differ by almost 10° C. Additionally, by its behaviour, the adult avoids high temperatures. Feeding on the plant takes place during the night, while it hides during the day at humid places below soil clods (Alcázar and Cisneros 1999), where the cold due to evaporation may damp extreme day temperatures.

Still it has been observed, that APW populations are regularly infested with *B. bassiana* see chap. 6). In the field cadavers of pre-pupae and adults with sporulating *B. bassiana* are easily found (personal observations). Depending on soil humidity conditions, pre-pupae remain for several months inactively in the soil (see chap.4), which may give the fungus enough time to develop. The same may be said about adults due to their long lifespan. It is difficult or impossible to determine the age of a perished weevil in the field.

Giving the results, we can not expect, that the tested isolates will perform acceptably under field conditions. Their growth capacity and pathogenicity are too low at temperatures found typically in the field. If *B. bassiana* is to be further investigated for the biological control of APW, a rigorous selection for isolates with high pathogenicity or good growing performance at low temperature as found in the field is required. For practical reasons it is recommended to start a selection process with growing and/or germination experiments at different temperatures. This allows a larger number of isolates to be tested compared to bioassays that would require a large and good working APW rearing.

Acknowledgements

We thank Marcelo Trebejo and Melisa Vargas of the International Potato Center (CIP) in Lima (Peru) for maintaining the APW rearing and for helping in the evaluation of the experiments. We also owe gratitude to Kerstin Jung and Dietrich Stephan from the Federal Biological Research Centre for Agriculture and Forestry (BBA) in Darmstadt (Germany) for advices concerning entomopathogenic fungi. The presented experiments were carried out at CIP as part of a project financed by the Government of the Grand-Duchy of Luxembourg.

References cited

Alcalá C., P. and J. Alcázar S. (**1976**). Biología y comportamiento de *Premnotrypes suturicallus* Kuschel (Col.: Curculionidae). Revista Peruana de Entomología **19**(1): 49-52.

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

Cole, D. C., F. Carpio, J. A. Julian and N. León (**1998**). Health impacts of pesticide use in Carchi farm populations. In: Economic, environmental, and health tradeoffs in agriculture: Pesticides and the sustainability of Andean potato porduction. C. C. Crissman, J. M. Antle and S. M. Capalbo (Eds). Dordrecht (The Netherlands), Kluwer Academic Publishers: pp. 209-230.

Davidson, G., K. Phelps, K. D. Sunderland, J. K. Pell, B. V. Ball, K. E. Shaw and D. Chandler (**2003**). Study of temperature-growth interactions of entomopathogenic fungi with potential for control of *Varroa destructor* (Acari: Mesostigmata) using a nonlinear model of poikilotherm development. Journal of Applied Microbiology **94**: 816-825.

De Croos, J. N. A. and M. J. Bidochka (**1999**). Effects of low temperature on growth parameters in the entomopathogenic fungus *Metarhizium anisopliae*. Canadian Journal of Microbiology **45**(12): 1055-1061.

Doberski, J. W. (**1981**). Comparative laboratory studies on three fungal pathogens of the elm bark beetle *Scolytus scolytus*: Effect of temperature and humidity on infection by *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus*. Journal of Invertebrate Pathology **37**(2): 195-200.

Dorschner, K. W., M.-G. G. Feng and C. R. Baird (**1991**). Virulence of an aphid-derived isolate of *Beauveria bassiana* (Fungi: Hyphomycetes) to the hop aphid, *Phorodon humuli* (Homoptera: Aphididae). Environmental Entomology **20**(2): 690-693.

Fargues, J., M. S. Goettel, N. Smits, A. Ouedraogo and M. Rougier (**1997**). Effect of temperature on vegetative growth of Beauveria bassiana isolates from different origins. Mycologia **89**(3): 383-392.

Ferron, P. (**1967**). Étude en laboratoire des conditions écologiques favorisant le développement de la mycose a *Beauveria tenella* du ver blanc. Entomophaga **12**(3): 257-293.

Finney, D. J. (**1971**). Probit analysis. Cambridge (UK), Cambridge University Press. 333pp.

Goettel, M. S., G. D. Inglis and S. P. Wraight (**2000**). Fungi. In: Field manual of techniques in invertebrate pathology. L. A. Lacey and H. K. Kaya (Eds). Dordrecht (The Netherlands), Kluwer Academic Publishers: pp. 255-282.

Hywel-Jones, N. L. and A. T. Gillespie (**1990**). Effect of temperature on spore germination in *Metarhizium anisopliae* and *Beauveria bassiana*. Mycological Research **94**(3): 389-392.

Inglis, G. D., D. L. Johnson and M. S. Goettel (**1997**). Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vuillemin against grasshoppers. The Canadian Entomologist **129**(1): 171-186.

James, R. R., B. A. Croft, B. T. Shaffer and B. Lighthart (**1998**). Impact of temperature and humidity on the host-pathogen interaction between *Beauveria bassiana* and a Coccinellid. Biological Control **27**(6): 1506-1513.

Leland, J. E., M. R. McGuire, J. A. Grace, S. T. Jaronski, M. Ulloa, Y.-H. Park and R. D. Plattner (**2005**). Strain selection of a fungal entomopathogen, *Beauveria bassiana*, for control of plant bugs (*Lygus* spp.) (Heteroptera : Miridae). Biological Control **35**(2): 104-114.

Martin, P. A. W., R. F. W. Schroeder, T. J. Poprawski, J. J. Lipa, E. Hausvater and V. Rasocha (**2000**). Temperature effects on the susceptibility of the Colorado potato beetle (Coleoptera: Chrysomelidae) to *Beauveria bassiana* (Balsamo) Vuillemin in Poland, the Czech Republic and the Unided States. Journal of Entomological Science **35**(3): 251-258.

Moorhouse, E. R., A. T. Gillespie and A. K. Charnley (**1994**). The influence of temperature on the susceptibility of vine weevil, *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae), larvae to *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). Annals of applied Biology **124**(2): 185-193.

Ortiz, O., J. Alcázar, W. Catalán, W. Villano, V. Cerna, H. Fano and T. S. Walker (**1996**). Economic impact of IPM practices on the Andean potato weevil in Peru. In: Case studies of the economic impact of CIP - related technology. T. S. Walker and C. C. Crissman (Eds). Lima, International Potato Center: pp. 95-110.

Quesada-Moraga, E., E. A. A. Maranhao, P. Valverde-García and C. Santiago-Álvarez (**2006**). Selection of *Beauveria bassiana* isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* on the basis of their virulence, thermal requirements, and toxicogenic activity. Biological Control **36**(3): 274-287.

Schoolfield, R. M., P. J. H. Sharpe and C. E. Magnuson (**1981**). Non-linear regression of biological temperature-dependent rate models based on absolut reaction-rate theory. Journal of Theoretical Biology **88**(4): 719-731.

Sharpe, P. J. H., G. L. Curry, D. W. DeMichele and C. L. Cole (**1977**). Distribution model of organism development times. Journal of Theoretical Biology **66**: 21-38.

Soares, G. G., M. Marchal and P. Ferron (**1983**). Susceptibility of *Otiorhynchus sulcatus* (Coleoptera: Curculionidae) larvae to *Metarhizium anisopliae* and *Metarhizium flavoviride* (Deuteromycotina: Hyphomycetes) at two different temperatures. Environmental Entomology **12**(6): 1886-1890.

Throne, J. E., D. K. Weaver, V. Chew and J. E. Baker (**1995**). Probit analysis of correlated data: Multiple observations over time at one concentration. Journal of Economic Entomology **88**(5): 1510-1512.

Yeo, H., J. K. Pell, P. G. Alderson, S. J. Clark and B. J. Pye (**2003**). Laboratory evaluation of temperature effects on the germination and growth of entomopathogenic fungi and on their pathogenicity to two aphid species. Pest Management Science **59**(2): 156-165.

Attachment of *Beauveria bassiana* conidia to neonate larvae of the Andean potato weevil

Magnus Kühne, Jesús Alcázar, Kerstin Jung, Dietrich Stephan and Stefan Vidal

Abstract

In bioassays, neonate larvae of the Andean potato weevil *Premnotrypes suturicallus* Kuschel (Coleoptera: Curculionidae) were 10,000 times less susceptible to the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. when feeding in potato tubers as when kept without tubers (medium lethal concentrations were 2.3 x 10^9 and 2.3 x 10^5 conidia/ml). With three different methods we demonstrated, that neonates loose conidia when moving through soil or feeding in tubers. Larvae were inoculated with a suspension of 1 x 10^8 conidia/ml of the fungus and placed in soil, in potato tubers or in empty containers. After 24 h they were washed and the suspension applied to selective medium for the determination of the number of colony forming units (CFU). On larvae kept in soil or in tuber few (in many cases no) CFU were detected, while on larvae kept in empty containers a high number of CFU was found, similar to larvae washed immediately after inoculation of the fungus. With the same treatment of the larvae, the loss of the conidia was confirmed with scanning electron microscopy and fluorescence microscopy. We conclude, that the high activity of neonate *P. suturicallus* in close contact to the substrate allows them to cast off the conidia. This makes them a difficult or impossible target for biological control with *B. bassiana*.

Keywords: *Premnotrypes suturicallus*, entomopathogenic fungi, scanning electron microscopy, fluorescence microscopy, colony forming units, conidial attachment.

Introduction

The entomopathogenic fungus *Beauveria* spp. has been used successfully for controlling pre-pupae of the Andean potato weevil (APW) *Premnotrypes* spp. (Coleoptera: Curculionidae) in storage areas (Alcázar and Cisneros 1997). Due to several limitations in this form of application (Winters and Fano 1997), ways of application in the potato field are currently investigated.

In general, the life cycle of APW is well adapted to the potato cropping seasons. Eggs of *P. suturicallus* are placed into plant debris around the plant stem. After hatching, neonates dig into the soil and bore into the potato tubers, inside which they remain until they finish development. Mature larvae leave the tuber for pupating in the soil (Alcázar and Cisneros 1999). Possible life stages that could be targeted for a control in the field are adults before they migrate into the potato field or within the field to avoid or reduce oviposition, eggs, neonate larvae before they bore into the potato tuber or pre-pupae and pupae in the soil.

Due to inconsistent results in applications of the fungus in the field, directed against adult weevils (see chap.6 and several other unpublished studies cited in chap. 1), other stages in the lifecycle of the weevil are investigated as possible targets. The proposal followed in the present study is controlling neonate larvae.

Preliminary trials had shown, that the mortality of neonate larvae of *P. suturicallus* Kuschel inoculated with *B. bassiana* (Bals.) Vuill. was very low when moving through soil and feeding in potato tubers. The same was true if the soil in which the tuber was placed was inoculated. But on the other side, after completing their development, mature larvae leaving the tuber for pupating in the soil were to a high percentage infected by *B. bassiana* (Kühne, unpublished data). Possible causes therefore may be, that larvae get liberated from conidia through moulting to the second larval stage (Vandenberg et al. 1998), that larvae loose the conidia through their activity in the soil and the potato tuber (Quintela and McCoy 1998b) or that the soil (or potato tuber) may have fungistatic effects on *B. bassiana* (Groden and Lockwood 1991, Chuankung et al. 2004). In the presented studies we are focusing on the second point to get an insight into the ecology of the interactions between the pathogen and the neonate larva.

Methods

Origin of insect material and entomopathogens

Neonate larvae of *P. suturicallus* for the experiments were taken from a permanent laboratory colony kept at the International Potato Center (CIP) in Lima. Insects were reared as described in chap. 3 at approximately 18° C producing approximately three generations per year. Rearing has been started with adults collected on the eastern slopes of the Rio Mantaro valley in the highlands of central Peruvian and was refreshed yearly with new introductions from the same area. Larvae were maximum 24 h old when used for the bioassays.

In all experiments, the *Beauveria bassiana* isolate CIP40 from the collection of entomopathogens of CIP was used. It was isolated from a cadaver of a *P. suturicallus* adult collected in the Rio Mantaro valley. It had shown high pathogenicity against this pest and a good performance at low temperatures (see chap. 7). The isolate was stored at 4 to 8° C on potato-dextrose-agar (PDA) and was passed regularly through APW prepupae for reactivation.

Common preparations for all experiments

The isolate was grown on phyto-yeast-medium (PYM: 2% glucose, 0.5% soy peptone, 0.5% yeast extract and 1.8% agar) for 15 to 21 days at 20° C until full sporulation. Conidia were than washed from the medium with 10 ml of 0.1% Tween 80 and the suspension was placed for 3 min into an ultrasonic bath to disrupt conidia clumps. To determine the conidia concentration, an improved Neubauer haemocytometer was used. Dilutions were made with 0.1% Tween 80. For each experiment, the quality of the conidia suspension was assessed determining the germination rate of the conidia. Therefore, 100 µl of the suspension with approximately 1×10^6 conidia/ml were applied to PYM medium and incubated for 24 h at 20° C. After 24 h a few droplets of lactophenol cotton blue were placed on each plate to stop germination and 100 conidia were counted for germination and non-germination in each of 5 fields per plate. For the inoculation, all neonate larvae for one conidia concentration were placed in the centre of a towel paper and 0.5 ml of the conidia suspension were dropped with a pipette over the larvae. The excess of liquid was absorbed by the towel paper. The larvae were manipulated with an air-dried, alcohol sterilized brush, using for each treatment a separate brush.

Pathogenicity

In a first bioassay, larvae were inoculated with 7 concentrations of 5.5×10^3 to 5.5×10^9 conidia/ml. Larvae were placed individually into small containers (approximately 3 x 3 x 2.5 cm) together with a small tuber (variety Peruanita). To assure, that neonates can bore into the tubers, one side of the tuber was cropped and the tuber placed with this exposed side downward into the container. Evaluation in form of a retrieval rate took place after 1 month. At this time larvae had developed to 3rd or 4th larval stage and could easily be recovered cutting open the tubers.

In a second bioassay, larvae were inoculated with 6 concentrations of 6.2×10^2 to 6.2×10^7 conidia/ml. Larvae were placed individually into microcentrifuge caps without addition of alimentation. Evaluation took place after 7 days in form of a mortality rate.

In both bioassays, 40 larvae were treated for each treatment. For the control, the same number of larvae was treated with 0.1% Tween 80 only. The experiments were conducted at 15° C. The data were analysed with probit analyses (Finney 1971) using the statistical package SPSS 10.0.7.

Conidial adherence

A suspension with 1 x 10^8 conidia/ml was prepared. After inoculation, for each treatment 15 larvae were distributed into 2 containers. Larvae were either placed in empty containers, or in containers containing a small tuber as described above for the bioassays, or in containers containing sterilized soil with 13% water content. After 24 h, 10 larvae (the additional larvae had been treated to assure that 10 larvae may be recovered) of each treatment were collected and, using individually sterilized brushes, placed individually into microcentrifuge caps containing 0.5 ml of 0.1% Tween 80. The caps were placed for 30 s on a vortex mixer and dilutions (10x and 100x) were prepared. Two controls were prepared, a non-treated control with larvae being inoculated only with 0.1% Tween 80 before they were washed and a treated control with larvae being washed immediately after inoculation with the conidia suspension. For each larva and dilution (0x, 10x and 100x) 100 µl were applied to two agar plates with selective medium (PYM + antibiotics: 2% glucose, 0.5% soy peptone, 0.5% yeast extract, 1.8% agar, 0.01% streptomycin and 0.005% Tetracycline) and distributed homogenously using a cell spreader. The agar plates were incubated for 2 weeks at 20° C before the number of colony forming units (CFU) was assessed. In case of doubts, colonies were observed under the light microscope for proper identification. For the evaluation, plates of those dilutions presenting between 5 and 50 CFU (if present) were considered.

In a repetition of the experiment, larvae were placed individually into containers containing a tuber, into microcentrifuge cap containing sterilized soil or into empty caps. After removing the larvae, the caps with the soil respectively the empty caps were washed with 1 ml of 0.1% Tween 80. For the determination of CFU, 3 agar plates were inoculated.

For the examination with the scanning electron microscope (SEM), the same treatments as above were installed. After 24 h, instead of being washed, 5 larvae of each treatment were fixed in 4% glutaraldehyde placing them overnight into the refrigerator. Secondary fixation was made in 1% osmium tetroxide in 0.1 M phosphate buffer during 45 min. Specimen were rinsed in the buffer, dehydrated in a 30 – 96% alcohol series, dried at critical point in CO_2 and coated with gold. At three randomly chosen section of the larvae, pictures were taken at 700x magnification and numbers of conidia per picture were counted.

Staining of the *B. bassiana* conidia for the examinations with the fluorescence microscope, was made as described by Quintela and McCoy (1998a). The conidia, suspended in 0.1% Tween 80, were washed in 0.05 M carbonate-bicarbonate buffer (pH 9.2), mixed, centrifuged for 5 min and the supernatant decanted. The conidia were then stained with 1 mg of fluorescein isothiocyanate (FITC) in 1 ml of carbonate-bicarbonate buffer during 1 h at room temperature. Stained conidia were washed five times by centrifugation to remove the excess stain. A viability of the conidia of over 97% was confirmed by determining the germination rate as described above. The same conidia concentration as for the previous experiments was prepared and the same treatments were installed. After 24 h (or immediately after inoculation for the control treatments) larvae were placed into a freezer at –20° C to stop any development of the larvae and the fungus. The larvae were observed at 400x magnification using an Olympus BH-2 microscope with an U-MWB cube (excitation filter BP450-480 nm, dichroic mirror DM500 nm and barrier filter BA515 nm). Pictures were taken from the head capsule and 2 more, randomly chosen places of 7 larvae per treatment and the total number of conidia on these pictures was counted.

Results

Pathogenicity

In all experiments, the viability of the conidia was high with germination rates over 95%. The pathogenicity of *B. bassiana* against neonate larvae of *P. suturicallus* was very low in the experiment with the neonate larvae feeding in the potato tuber while it was high when larvae were kept without alimentation (Figure 1). No control mortality was observed in the second case, indicating, that the lack of alimentation did not cause mortality. The LC_{50} for the larvae feeding in the tuber (2.3×10^9 conidia/ml) was 10,000 times higher as for those not feeding (2.3×10^5 conidia/ml), which means, that larvae that feed in tubers are very little susceptible to infections with *B. bassiana*. Additionally, the slope of the regression was significantly steeper in the second case (1.66 ± 0.22) than in the first case (0.58 ± 0.15), which means, that an augmentation of the conidia concentration has a stronger effect in the second case.

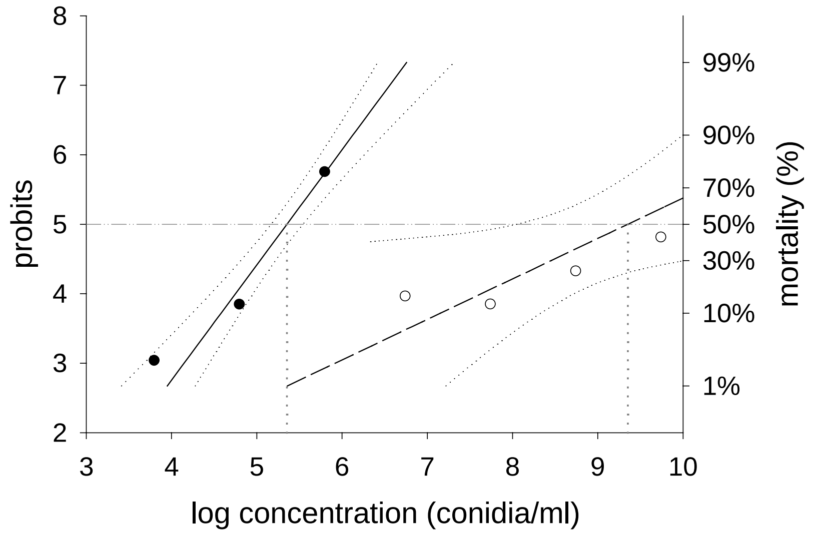

Figure 1: Concentration dependent mortality of *P. suturicallus* neonate larvae after inoculation with *B. bassiana*. Dotes are observed mortalities corrected by Abbott formula, lines are probit regressions; ●—● mortality rate of neonates kept for 7 days without potato tuber (χ = 4.416; P = 0.353), ○--○ retrieval rate of neonates feeding for 1 month in tubers (χ = 10.471; P = 0.063, a heterogeneity factor was applied), dotted lines represent the corresponding confidence limits. On the left y-axes, mortality rates for the corresponding probit values are shown. The reference line shows 50% mortality and the drop lines show LC_{50} values.

Conidial adherence

Washing the larvae, no CFU were observed for the larvae of the non-treated control. In the treated control, around 10000 CFU per larva were found in both trials (average 13600, median 14200 for trial 1 and average10100, median 7900 for trial 2) (Figure 2). From the larvae kept in an empty container, in the first trial in average 9600 (median 8000) CFU and in the second trial (kept in an microcentrifuge cap) in average 626 (median 167) CFU were found. In the second trial, due to the high variance, this was not significantly different from the other treatments (Games-Howell, $p < 0.05$), though it was still markedly higher. From larvae feeding in potato tuber or moving through soil only few CFU were recovered. Several larvae (up to 70% in the case of larvae feeding in tubers in trial 2) were completely free of CFU. In the second trial, washing the empty microcentrifuge cap respectively the soil after removing the larva, in average 340 (median 267) CFU and 4040 (median 3670) CFU were recovered.

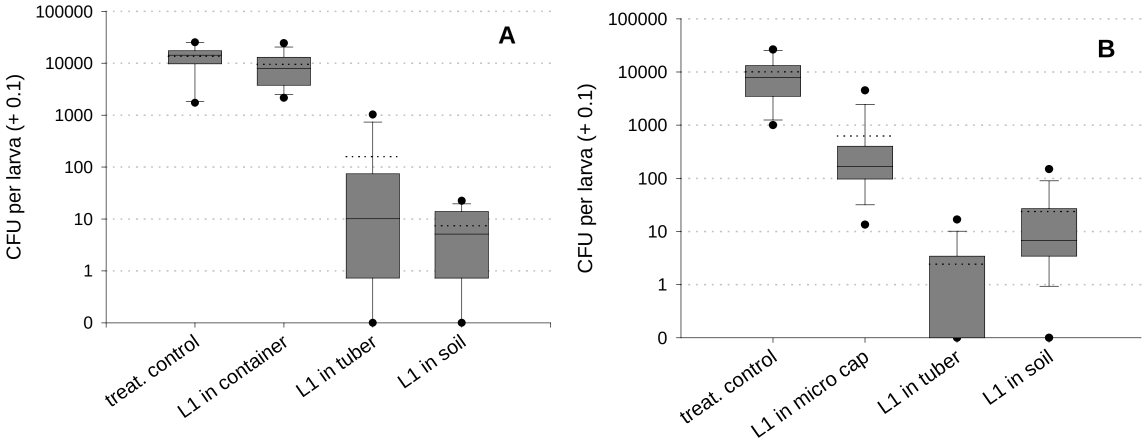

Figure 2: Colony forming units (CFU) of *B. bassiana* recovered from neonate *P. suturicallus* after different treatments (A: trial 1; B: trial 2). Boxes indicate interquartil range, whiskers 10 and 90 percentiles, dots 5 and 95% percentiles, solid lines within the boxes the medians and dotted lines the means. To allow the representation of zero values on the logarithmic scale, 0.1 was added to each data point.

Examinations with the scanning electron microscope as well as with the fluorescence microscope revealed very few conidia on neonate larvae that fed in the tuber or moved through soil (Figure 3 and Figure 4). Larvae kept in an empty microcentrifuge cap had a significant higher load of conidia, not significantly different from the treated control. The variance of the treated control in the fluorescence microscope study was extremely high blurring the difference with the treatment of larvae moving through soil.

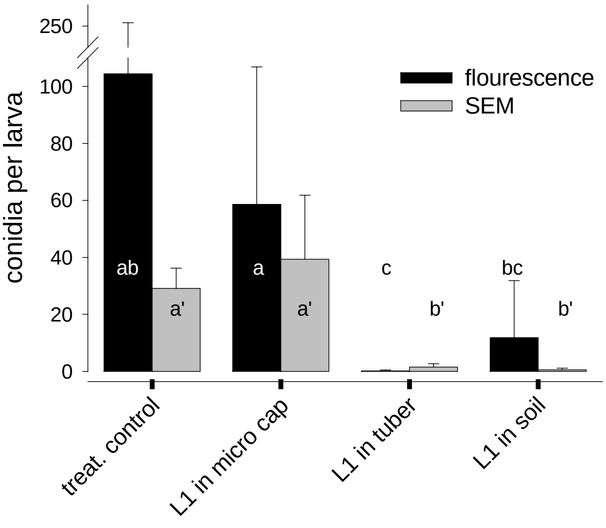

Figure 3: *B. bassiana* conidia counted on neonate *P. suturicallus* with the fluorescence microscope and the scanning electron microscope (SEM). Error bars indicate standard deviations. Bars of the same microscope study with the same letters are not significantly different (Games-Howell, $p < 0.5$).

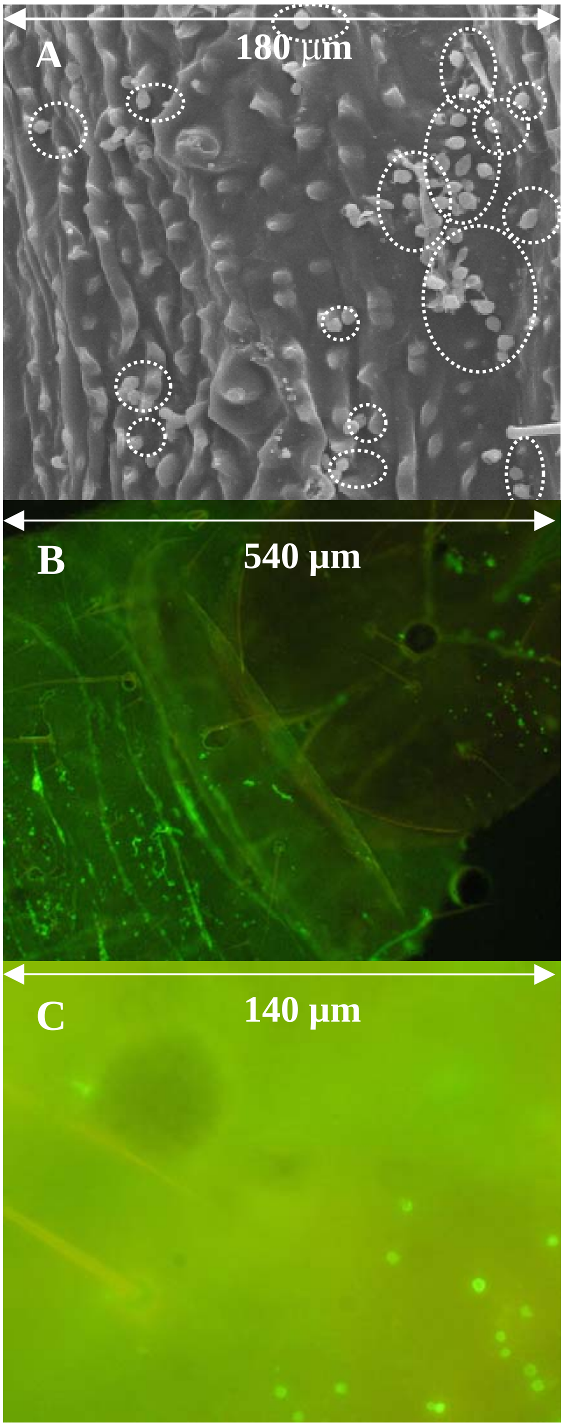

Figure 4: Scanning electron microscope picture (A: 700 x) and fluorescence microscope pictures (B: 100 x; C: 400 x) of neonate *P. suturicallus* with *B. bassiana* conidia attached.

Discussion

Neonate larvae of *P. suturicallus* feeding in potato tubers are little susceptible to infections by the entomopathogenic fungus *B. bassiana* while larvae kept in empty containers without alimentation are highly affected by the fungus. No control mortality was observed in the second case. Still, it cannot be ruled out, the lack of alimentation might have caused physiological stress for the larvae making them more susceptible to fungal infections. The high number of CFU per larva found after 24 h on larvae kept in an empty container compared to a low number or absence of conidia on larvae moving through soil or feeding in potato tubers shows that other factors than starving stress must play a role.

It might be objected, that the different environmental conditions in the treatments may also affect the capability of the fungus to adhere to the larval cuticle and hence the difference in CFU may be due to the conidia being more or less easily washed off the larvae. The studies with the scanning electron microscope and the fluorescence microscope showed however, that this is not the case. It was possible to quantify the difference of conidia on the larvae by SEM though, fluorescence microscopy is the preferable method for quantitative analyses.

We suggest, that the activity of the neonate larvae leads to the lost of conidia from their cuticle. This seems to be favoured by close contact to soil particles or potato tuber, as larvae moving in an empty container did not or to a lesser extent lose the conidia. Quintela and McCoy (1998a) found similar results when they reduced the mobility of larvae exposing them to sublethal doses of the insecticide imidacloprid. Bourcias et al. (1996) describe how the reduced activity caused by imidacloprid disrupts the grooming behaviour of termites and increases thereby the susceptibility to fungal infections. Observations in preliminary studies (Kühne, not published) had shown, that mature larvae get infected with *B. bassiana* when passing through soil inoculated with the fungus, while neonate larvae passing through the same soil were very little infected. This may be due to the fact that mature larvae are preparing for pupation and hence are less active.

Our observations lead us to discard the possibility that the low susceptibility of neonate larvae could be due to avoiding infection through moulting as described by Vandenberg et al. (1998). This was supported by preliminary studies that had shown, that at 15° C the moulting to the second larval stage takes place after 10 to 12 days (Kühne, not published data), a time lapse in which an infection would already have led to death. Equally, fungistatic effects of the soil can be discarded, as the tested soil has been autoclaved. Fungistasis is normally supposed to be based upon antibiosis by other soil microorganisms (de Boer et al. 2003) which should have been eliminated by sterilization. In the case of the potato tuber, a fungistatic effect can not be discarded. Solanin has been reported to have some inhibitory effects on *B. bassiana* (Costa and Gaugler 1989).

B. bassiana, as several other entomopathogenic deuteromycetes produces dry, hydrophobic conidia covered by a layer of rodlet fascicles. Attachment of conidia to insect cuticle is passive and non-specific. The principal binding force of the conidial wall to the insect cuticle is hydrophobicity and to a lesser extent electrostatic interactions. The presence of cuticular spines is thought to facilitates attachment of conidia while they were more easily removed from smooth parts (Boucias et al. 1988, Boucias and Pendland 1991, Hajek 1997). The use of surfactants or detergents (Tween 80 being a detergent), is commonly used for inoculum preparation and formulation of the hydrophobic conidia of entomopathogenic fungus (Goettel et al. 2000), but might have an influence on the adhesion of conidia to the cuticle (Boucias et al. 1988, Holder and Keyhani 2005).

In the case of *P. suturicallus* neonate, the cuticle is neither covered by spines nor is it smooth (Figure 4A). In the light of the fact, that the fungus depends on the capability of its conidia to stick to the cuticle of its host, it is not clear whether larvae void actively their cuticle of conidia as proposed by Quintela and McCoy (1998a) and shown for the social behaviour of termites (Boucias et al. 1996), whether it is a passive shedding when moving through substrate or whether the first instars cuticle of *P. suturicallus* is a non-favourable substrate for the conidia. The ability of conidia to bind to the cuticle may also vary with the age and life stage of the host (Boucias and Pendland 1991). Presumably, as stated by Quintela and McCoy (1998a) hosts will get in contact with fungus conidia and depending on the environmental conditions, on soil and on host stage and fitness, the balance between conidia attachment to the cuticle and the host voiding these conidia will incline into different directions.

For a successful infection of the host, we should expect the movement of the conidia being from the soil towards the larval cuticle. To reach this, the right host life stage should be targeted and/or the microhabitat conditions manipulated to improve adhesion of conidia to the host. In the case of neonate larvae of *P. suturicallus*, they lose inoculated conidia from their cuticle through their activity in soil and potato tuber. This makes them little susceptible for infections by *B. bassiana* and makes them difficult or impossible to target for the control with this biological control agent.

Acknowledgements

Especial thanks to Octavio Zegarra of the International Potato Center (CIP) in Lima (Peru) for his assistance in the preparation of the larvae for SEM observations, to Melisa Vargas of CIP for helping in the evaluation of the trials and to Manuel Castello from the Universidad National Mayor de San Marcos in Lima for operating the SEM. The research was done at CIP and was made possible through funding by the Government of the Grand-Duchy of Luxembourg.

References cited

Alcázar, J. and F. Cisneros (**1997**). Integrated Management for Andean potato weevil in Pilot Units. In: Program Report 1995-1996. B. Hardy and B. Smith (Eds). Lima, International Potato Center: pp. 169-176.

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

Boucias, D. G. and J. C. Pendland (**1991**). Attachment of mycopathogens to cuticle. The initial event of mycoses in arthropod hosts. In: The fungal spore and diseases initiation in plants and animals. G. T. Cole and H. C. Hoch (Eds). New York (USA), Plenum: pp. 101-128.

Boucias, D. G., J. C. Pendland and J. P. Latge (**1988**). Nonspecific factors involved in attachment of entomopathogenic Deuteromycetes to host insect cuticle. Applied and Environmental Microbiology **54**(7): 1795-1805.

Boucias, D. G., C. Stokes, G. Storey and J. C. Pendland (**1996**). The effect of imidacloprid on the termite *Reticulitermes flavipes* and its interaction with the entomopathogenic fungus *Beauveria bassiana*. Pflanzenschutz-Nachrichten Bayer **49**(2): 105-150.

Chuankung, X., M. Minghe, Z. Leming and Z. Keqin (**2004**). Soil volatile fungistasis and volatile fungistatic compounds. Soil Biology & Biochemistry **36**(12): 1997-2004.

Costa, S. D. and R. Gaugler (**1989**). Sensitivity of *Beauveria bassiana* to solanine and tomatine: Plant defensive chemicals inhibit an insect Pathogen. Journal of Chemical Ecology **15**(2): 697-706.

de Boer, W., P. Verheggen, P. J. A. Klein Gunnewiek, G. A. Kowalchuk and J. A. van Veen (**2003**). Microbial community composition affects soil fungistasis. Applied and Environmental Microbiology **69**(2): 835-844.

Finney, D. J. (**1971**). Probit analysis. Cambridge (UK), Cambridge University Press. 333pp.

Goettel, M. S., G. D. Inglis and S. P. Wraight (**2000**). Fungi. In: Field manual of techniques in invertebrate pathology. L. A. Lacey and H. K. Kaya (Eds). Dordrecht (The Netherlands), Kluwer Academic Publishers: pp. 255-282.

Groden, E. and J. L. Lockwood (**1991**). Effects of soil fungistasis on *Beauveria bassiana* and its relationship to disease incidence in the Colorado Potato Beetle, *Leptinotarsa decemlineata*, in Michigan and Rhode Island soils. Journal of Invertebrate Pathology **57**(1): 7-16.

Hajek, A. E. (**1997**). Ecology of terrestrial fungal entomopathogens. In: Advances in microbial ecology. J. G. Jones (Ed.). New York, Plenum. Vol. 15: pp. 193-249.

Holder, D. J. and N. O. Keyhani (**2005**). Adhesion of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana* to substrata. Applied and Environmental Microbiology **71**(9): 5260-5266.

Quintela, E. D. and C. W. McCoy (**1998a**). Conidial attachment of *Metarhizium anisopliae* and *Beauveria bassiana* to the larval cuticle of *Diaprepes abbreviatus*

(Coleoptera: Curculionidae) treated with Imidacloprid. Journal of Invertebrate Pathology **72**(3): 220-230.

Quintela, E. D. and C. W. McCoy (**1998b**). Synergistic effect of imidacloprid and two entomopathogenic fungi on the behavior and survival of larvae of *Diaprepes abbreviatus* (Coleoptera : Curculionidae) in soil. Journal of Economic Entomology **91**(1): 110-122.

Vandenberg, J. D., M. Ramos and J. A. Altre (**1998**). Dose-response and age- and temperature-related susceptibility of the diamondback moth (Lepidoptera : Plutellidae) to two isolates of *Beauveria bassiana* (Hyphomycetes : Moniliaceae). Environmental Entomology **27**(4): 1017-1021.

Winters, P. and H. Fano (**1997**). The economics of biological control in Peruvian potato production. Working Paper. Lima, International Potato Center**:** 33.

Conclusions

This research project on the Andean potato weevil (APW) aimed at adapting and improving integrated control strategies for this important potato pest that causes regularly high losses especially to the poor farmers.

A shortcoming working with APW relates to the problem that although many studies do exist on a local level few have been published. Most of the knowledge on this insect is found in bachelor or master thesis, in reports or in summaries of national conferences. Some data are published in national entomological journals of the Andean countries and only few specialized papers have been published in internationally reviewed journals. To utilize this knowledge, we reviewed the information available and commented on it.

The knowledge of the life history of a pest is crucial with regard to the development of an effective control strategy. The most prominent parameters for the understanding of the life history of an insect are the development time and rate and the survival rate of each life stage as well as female fecundity, which again is linked to female longevity. These parameters have been studied for the Andean potato weevil *Premnotrypes suturicallus* with regard to three main environmental factors (temperature, humidity - relative humidity of the air as well as soil humidity - and altitude). The thermodynamic model by Sharpe and DeMichele (1977) was adapted to the data of the temperature dependent development time and will allow predicting occurrence of the different life stages under diverse temperatures conditions. Time to hatching of the eggs after the appearance of adults in the field can now be estimated as well as the development time of larvae until they abandon the tubers.

Moisture as a key factor for development has been studied in detail only for a few insects (Tauber et al. 1998). We proved soil humidity to be a key factor determining the duration of the life cycle of the weevil. Pupation as well as adult emergence is depending on soil humidity higher than 8% water content. Additionally, results of field experiments showed that dispersion of weevils towards altitudes below 2400 meter above sea level (corresponding to 16.7° C) can be ruled out. The ecological data for *P. suturicallus* presented here, also allows understanding the present and possible future distribution patterns of the weevil and formulating reasonable agro-sanitary precautions.

From an ecological point of view, having a low optimal temperature of 12 to 14° C for survival and fecundity and a maximum temperature for development of 25° C and for fecundity of less than 20° C, *P. suturicallus* can be regarded a cold adapted insect. This is a cold adaptation due to low optimal temperatures rather than due to freeze tolerance . This weevil is therefore well adapted to the climatic conditions in which potatoes are grown in the highlands of the Andes. The temperature limitations also cast into doubt the status as a quarantine pest, established by the European Plant Protection Organization (OEPP/EPPO 1984). In fact, as revealed by the first descriptions of APW species from potato shipments to the United States of America (Pierce 1914, 1918), this pest probably has already been transported to temperate zone without being able to establish itself. A facultative dormancy during the stages of pre-pupae and of adults before emergence allows the weevil to respond to different humidity and dryness conditions, an important parameter in the highly divers ecosystem patchwork of the Andes. Being flightless, the adults depend on the presence of their host plants in proximity of their emergence sites and in absence of rain, potatoes too, do not sprout.

We have been unable to implement the main goal of the research project, the development of a biopesticide based upon the entomopathogenic fungus *Beauveria bassiana*. Prevailing low temperatures in the higher altitudes of the Andes are a main constrain for the use for this pathogen. The difference in optimum temperatures for both organisms (more than 20° C for the fungus and 12 to 14° C for the weevil) clearly points to the incompatibility of the fungus as an effective control agent. At temperatures comparable to those found in the field, pathogenicity of the tested isolated was too low to impact the weevil larvae. For further studies with entomopathogenic fungi, it would be crucial to use isolates showing high activity at low temperatures. In this respect, in vitro growth experiments can be used as a good and easily performed selection method. On the other hand we have been able to show that control of first instar larvae in advance of boring into the tuber is impossible. The high mobility of the larvae allows them to strip of attaching conidia from their cuticle.

In future studies on *P. suturicallus*, the influence of soil humidity on pupation and emergence should be investigated in more detail under field conditions together with soil humidity measurements. So far it is not clear, how changes in humidity may influence the dormancy. Such knowledge could allow making estimations on the emergence time of the weevil and adapting the pest and crop managements to avoid the pest. Another important field of research would be the understanding of the dispersion capacity of the flightless weevil within a given field and within the entire cropping system. How far can adults migrate from their emergence sites to new potato fields? How important are the different pupation sites (e.g. fields or storing devices) as potential infestation sources? How are population dynamics of the weevil influenced by socio-cultural conditions and by rotation systems? If they are in proximity of potato fields, storage areas may be potential infestation sources. However, storage systems greatly vary between and within regions. Farmers may store their potatoes in a special room, at a roofed space along the house wall or on wooden boards below the roof of the house; stores may be in the house or close to the field or the storage site may be changed after several weeks; special communitarian storage units may exist. Can reduced APW incidences, as reported by Ewell et al. (1994) for sectorial fallow rotation systems (Orlove and Godoy 1986) be quantified? In these cases, the importance of these systems for the agricultural production in the Andes and their influence on other factors in potato production (e.g. other insect pests, late blight, but especially soil born diseases like the nematodes *Globodera palida* and *G. rostochiensis* and the bacterial wilt *Ralstonia solanacearum*) should be evaluated to appreciate its potential for an integrated crop management. In such a land use management it is important to be sure that no alternative host plants exist, as stated by Alcázar and Cisneros (1999). However, spontaneous potato plants on former potato fields and wild potato species are almost ubiquitously present in the Andes.

It is also important to consider the different species of *Premnotrypes* and *Rhigopsidius*, forming the APW complex. This is especially true for the *Premnotrypes* species, because they are often treated as if their only differences would be their morphological traits. However, attempting to rear *P. latithorax* in the laboratory under the same conditions as *P. suturicallus* failed, because females did not oviposit. The studies so far published did not identify any ecological differences between the weevil species, apart form *P. vorax* passing through 5 larval stages and *P. pusillus* feeding only superficially on the tubers. It could be hypothesized, that the *Premnotrypes* species evolved in adaptation to separate ecological niches. Alternatively the speciation could have been conditioned by geographic separation due to high mountain ranges. Understanding the actual distribution patterns of the species also has phytosanitarian implications for potato transports between different regions. Hence, the ecology of the different species of APW, especially the dominant species *P. vorax*, *P. latithorax* and *P solaniperda*, should be studied in more detail and should be compared directly with the data for *P. suturicallus* elaborated in this study.

References cited

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

Ewell, P. T., K. O. Fuglie and K. V. Raman (**1994**). Farmers' perspectives on potato pest management in developing countries: Interdisciplinary research at the International Potato Center (CIP). In: Advances in potato pest biology and management. G. W. Zehnder, M. L. Powelson, R. K. Jansson and K. V. Raman (Eds). St. Paul (MN, USA), APS PRESS: pp. 597-615.

OEPP/EPPO (**1984**). EPPO data sheet on quaratine organisms No. 143. EPPO Bulletin **14**(1): 55-60.

Orlove, B. S. and R. Godoy (**1986**). Sectoral fallowing systems in the central Andes. Journal of Ethnobiology **6**(1): 169-204.

Pierce, W. D. (**1914**). New potato weevils from Andean South America. Journal of Agricultural Research **1**(4): 347-352.

Pierce, W. D. (**1918**). Weevils which affect Irish potato, sweet potato, and yam. Journal of Agricultural Research **12**(9): 601-612.

Sharpe, P. J. H. and D. W. DeMichele (**1977**). Reaction kinetics of poikilotherm development. Journal of Theoretical Biology **64**(4): 649-670.

Tauber, M. J., C. A. Tauber, J. P. Nyrop and M. G. Villani (**1998**). Moisture, a vital but neglected factor in the seasonal ecology: hypotheses and tests of mechanisms. Environmental Entomology **27**(3): 523-530.

Zusammenfassung

Der Anden Kartoffelrüssler *Premnotrypes suturicallus* – Ökologie und Interaktion mit dem entomopathogenen Pilz *Beauveria bassiana*

Der Anden Kartoffelrüssler (Andean potato weevil - APW) ist ein Komplex aus 14 Arten der Gattungen *Premnotrypes* and *Rhigopsidius*. Er ist einer der wichtigsten Kartoffelschädlinge in den Anden von Venezuela bis Nordwest Argentinien oberhalb 2800 Meter. Die Larven fressen in der Kartoffelknolle und verursachen erhebliche Schäden. In einer Befragung von über 600 Bauern aus ganz Peru, wurden durchschnittliche Schäden von 23% durch APW angegeben. Zur Zeit beschränkt sich die Bekämpfung fast ausschließlich auf die Applikation der Insektizide Metamidophos und Carbofuran. Die vorliegenden Studien wurden an der im zentralem Hochland Perus dominierenden Art *Premnotrypes suturicallus* durchgeführt.

Sowohl temperaturabhängige Entwicklungs- und Mortalitätsraten aller Lebensstadien als auch die Langlebigkeit und Fruchtbarkeit der Adulten wurden bei konstanten Temperaturen unter Laborbedingungen bestimmt. Das nicht-lineare, thermodynamische Model von Sharp und DeMichele wurde an die Daten angepasst. Die Überlebensraten aller Lebensstadien von APW sowie die Fruchtbarkeit der Weibchen waren maximal bei Temperaturen zwischen 11° C and 15° C. Vor allem die Eiablage war stark beeinträchtigt bei Temperaturen über 20° C. Für die im Boden lebenden Stadien wurde zusätzlich der Einfluss der Bodenfeuchtigkeit auf Entwicklung und Mortalität bestimmt. Bei ungefähr 8% Wasser Gehalt des Bodens wurde ein Schwellenwert beobachtet unterhalb dessen die Entwicklung der Präpuppen und der Schlupf der Adulten deutlich verzögert wurden. Zum Überstehen der Trockenzeit kann das Vorhandensein einer fakultativen Dormanz bei Präpuppen angenommen werden. Unter Feldbedingungen wurde die Populationsentwicklung entlang eines Höhengradienten von Meereshöhe bis 4100 Meter Höhe untersucht. Dabei überlebten nur wenige Individuen auf 2400 Metern (entsprechend 16.7° C) was diese Höhe zur unteren Verbreitungsgrenze von *P. suturicallus* macht. Diese Untersuchungen bestätigten die Kälteanpassung des Rüsslers wie sie schon unter Laborbedingungen gefunden wurde.

Im Labor an Präpuppen und Adulten Rüsslern durchgeführte Bioassays mit dem entomopathogenen Pilz *Beauveria bassiana* zeigten gute Wirkungen, konnten jedoch in Käfig- und Feldversuchen nicht bestätigt werden. Anschließende Versuche zeigten, dass die mittlere letale Konzentration (LC_{50}) bei 13° C etwa 1600 mal höher (beziehungsweise die Pathogenität niedriger) war als bei 19° C. Das bedeutet, dass unter für den Rüssler optimalen Umweltbedingungen, die Leistungsfähigkeit des Pilzes erheblich reduziert war. Bei weiteren Versuchen wurden die Möglichkeiten der Bekämpfung im Boden von frisch geschlüpften Larven mit *B. bassiana* untersucht. Für Larven welche in Kartoffelknollen gefressen haben waren die LC_{50} Werte 10,000 mal höher als für Larven welche in einem leeren Behälter gehalten wurden. Durch Bestimmung der Kolonie bildenden Einheiten (colony forming units – CFU) auf der Larvenkutikula, durch Rasterelektronenmikroskopie und durch Fluoreszenz Mikroskopie wurde der Verlust der meisten (in vielen Fällen aller) Konidien von der Kutikula der frisch geschlüpften Larven belegt. Diese Resultate zeigen, dass Umweltbedingungen und Verhalten der Larven eine erfolgreiche Anwendung von *B. bassiana* zur Bekämpfung von APW fast unmöglich machen.

Resumen

El gorgojo de los Andes *Premnotrypes suturicallus* – Ecología y interacción con el hongo entomopatógeno *Beauveria bassiana*

El gorgojo de los Andes (Andean potato weevil - APW) es un complejo de 14 especies de los géneros *Premnotrypes* y *Rhigopsidius*. Es uno de los más importantes insectos plaga de la papa en los Andes desde Venezuela hasta el noroeste de Argentina por enzima de los 2800 metros. Las larvas comen dentro del tubérculo de la papa causando graves daños. En una encuesta con más de 600 agricultores de todo el Perú, se reportó un daño promedio de 23% causado por APW. Actualmente el control se base principalmente en la aplicación de las insecticidas Metamidophos y Carbofuran. El presente estudio fue llevado a cabo con *Premnotrypes suturicallus*, la especie dominante de la sierra central del Perú.

Tazas de desarrollo y de mortalidad de todos los estados de vida como también la longevidad y fecundidad de los adultos fueron determinadas en función de la temperatura bajo condiciones de laboratorio con temperaturas constantes. El modelo non-linear termodinámico de Sharp y DeMichele fue adaptado a los datos. La supervivencia de todos los estados de APW y la fecundidad fueron máxima a temperaturas entre 11° C y 15° C. Especialmente la oviposición fue gravemente afectada por temperaturas por enzima de 20° C. Adicionalmente la influencia de la humedad de suelo en el desarrollo y en la mortalidad fueron determinados para los estados ocurriendo en el suelo. Se observó un umbral de aproximadamente 8% de contenido de agua del suelo por de abajo del cual el desarrollo de las pre-pupas y la emergencia de los adultos fueron notablemente retardados. Una dormancia facultativa podría ser sugerida para pre-pupas para superar la estación seca. Bajo condiciones de campo, el desarrollo de la populación fue investigado a lo largo de un gradiente de altura desde el nivel del mar hasta los 4100 metros. Solo pocos individuos sobrevivieron a 2400 metros (correspondiente a 16.7° C), haciendo de esta altura el limite de distribución inferior de *P. suturicallus*. Estos estudios de campo confirman la adaptación del gorgojo al frío como lo fue encontrado bajo condiciones de laboratorio.

Bioensayos mostrando una buena patogenicidad del hongo entomopatógeno *Beauveria bassiana* contra pre-pupas y gorgojos adultos en el laboratorio no han podido ser confirmado en ensayos en jaulas y en campo abierto. Más experimentos mostraban que la concentración letal media (LC_{50}) fue 1600 veces más alto (respectivamente la patogenicidad más bajo) a 13° C que a 19° C. Esto significa que bajo las condiciones optimas del gorgojo, la eficiencia del hongo puede ser reducida substancialmente. Investigaciones adicionales apuntaban a estudiar las posibilidades de controlar larvas recién nacidas en el suelo con *B. bassiana*. Los valores de LC_{50} para larvas comiendo en el tubérculo fueron 10,000 veces más alto que para larvas que fueron mantenido en un contenedor vacío. Determinando las unidades formadoras de colonias en la cutícula de las larvas, a través del microscopio electrónico de barrido y a través de microscopia de fluorescencia, se comprobó la perdida de la mayoría (en muchos casos de todos) de las conidias de la cutícula de las larvas recién nacidas moviéndose a través del suelo o comiendo en el tubérculo de la papa. Estos resultados demuestran que las condiciones ambiental y el comportamiento de las larvas vuelven el uso exitoso de *B. bassiana* para el control de APW casi imposible.

Acknowledgments / *Agradecimientos* / DANKSAGUNGEN

I thank Prof. Dr. Stefan Vidal for accepting the supervision of this dissertation and Prof. Dr. Hans-Michael Poehling from the university of Hannover for acting as second reviewer of this work.

I thank Dr. Aziz Lagnaoui, now at the World Bank for letting me the workplace at the International Potato Center (CIP) in Lima.

Best thanks to Dr. Kerstin Jung and Dr. Dietrich Stephan from BBA in Darmstadt for their help and advices and for their patience.

Special thank to Marcelo Trebejo from CIP, the soul of the laboratory in Lima, to Adan Vega from the CIP station in Huancyo for his invaluable help in everything concerning field experiments, to Carlos Torpoco formerly at the CIP station in San Ramon who also helped in conducting field experiments and to Melisa Vargas formerly at CIP for her help in the laboratory.

> *Especialmente estoy agradeciendo a Marcelo Trebejo del CIP-Lima, la alma del laboratorio de Lima, Adán Vega del CIP-Huancayo por su ayuda incansable en todo alrededor de los ensayos de campo, a Carlos Torpoco, antes del CIP-San Ramón, quien ayudo también en algunos experimentos de campo y a Melisa Vagas, antes del CIP-Lima, por su ayuda en el laboratorio*

I thank Jesús Alcázar from CIP for many discussions on everything around the Andean potato weevil and who, if anywhere out there should be a potato weevil, for sure he will find him.

> *Agradezco a Jesús Alcázar del CIP-Lima por muchos discusiones fructíferas alrededor del gorgojo de los Andes y quien, si hay algún gorgojo por allá afuera, el lo encuentra con seguridad.*

I also thank the whole rest of the team of entomology of CIP for supporting me always with good humour.

> *También agradezco a todo el equipo de entomología del CIP-Lima por su apoyo y por acompañando mi trabajo siempre con buen humor.*

All the work was possible due to the support of the many people from the workshops, logistics and administration at CIP-Lima and CIP-Huancayo.

> *Todo el trabajo solo fue posible gracias al apoyo de toda la gente de los talleres, de logística y de administración del CIP-Lima y CIP-Huancayo.*

Of the different people who made the field work easier I like to mention Mr. Donato from La Libertad (Concepcíon) on whose land we could work and Mrs. Donato who invited us several times for lunch during hard field work and Mrs Hirminia Simon and Mr. Javier Garcías from Huasahuasi for their help and advice for everything in and around Huasahuasi.

> *De toda la gente que hicieron el trabajo de campo más agradable, quiero mencionar al Sr. Donato de La Libertad (Concepción) quién nos permitió trabajar en su terreno, a su esposa por invitar nos varias veces a almorzar durante el duro trabajo de campo y a la Sra. Hirminia Simon y el Sr. Javier Garcías de Huasahuasi por su ayuda a avisos en todo en y alrededor de Huasahuasi.*

I also thank the team of agroentomology of the university of Göttingen for the nice working surrounding.

> ICH DANKE AUCH DEM TEAM DER AGROENTOMOLOGIE DER UNIVERSITÄT GÖTTINGEN FÜR EINE ANGENEHME ARBEITSATMOSPHÄRE.

The whole project was only possible because of the confidence and financial support by the Government of the Grand-Duchy of Luxembourg.

And of course, due to the love of my wife Rocío.

Curriculum Vitae

Persönliche Daten:

Magnus Kühne

geboren am 22.10. 1969 in Frankfurt am Main

Kontakt: magnus.kuhne@gmx.net

Ausbildung:

2001-2007: Promotion in Agrarwissenschaften an der Georg-August-Universität Göttingen.

Dissertation: „The Andean potato weevil *Premnotrypes suturicallus* - Ecology and interactions with the entomopathogenic fungus *Beauveria bassiana*” angefertigt in der Abteilung Agrarentomologie

2000-2001: Aufbaustudium Erwachsenenbildung im Fernstudium an der Technischen Universität Kaiserslautern

1992-1998: Studium der Biologie an der Johannes-Gutenberg-Universität Mainz

Diplomarbeit: „Empfindlichkeit der Rübenmotte gegen *Bacillus thuringiensis*“ angefertigt an der Biologischen Bundesanstalt für Land- und Forstwirtschaft (BBA) in Darmstadt

1996: Auslandssemester an der Universidad de los Andes in Bogotá (Kolumbien) mit Kontaktstipendium

1990: Abitur am Athénée de Luxembourg in Luxemburg

Praktika und Berufliche Tätigkeiten:

2001-2005: Wissenschaftlicher Mitarbeiter am International Potato Center in Lima (Peru)

2001: Praktikum an der Biologischen Bundesanstalt für Land- und Forstwirtschaft (BBA) in Darmstadt

2000: mehrmonatige Tätigkeit als pädagogischer Assistent am Lycée technique in Esch sur Alzette (Luxemburg)

1999-2000: halbjähriges Praktikum am International Potato Center in Lima (Peru) finanziert durch die Gesellschaft für technische Zusammenarbeit (GTZ)

1999: kurzzeitige Tätigkeit am Musée d’Histoire Naturel in Luxemburg

1991: mehrmonatige Tätigkeiten im Garten- und Landschaftsbau in Maintal bei Frankfurt am Main.

Göttingen, Juli 2007