CROP PROTECTION PROGRAMME

INTEGRATED MANAGEMENT OF MAJOR INSECT PESTS OF POTATOES IN HILLSIDE SYSTEMS IN THE COCHABAMBA REGION OF BOLIVIA

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FINAL TECHNICAL REPORT

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EXECUTIVE SUMMARY

Andean potato *(Solanum tuberosum ssp. andigena)* is the principal staple food for Bolivians. It covers 25 % of calorific needs of Bolivian households and represents from 3 – 13 % of total household expenditure, increasing at lower income levels. It provides 30 – 50 % of total calories consumed by rural highland households. It is grown nationally by around 400,000 small-farm-families on around 130,000 hectares:

Potatoes are commonly grown in hillside production systems where yields are low due to a complex of biotic factors such as weeds, nematodes, pests and diseases, as well as poor soils and erosion. Within this complex, insect pests have a substantial impact on the capacity of farmers to secure a livelihood. The two most important insect pests, and those specifically targeted in this proposal, are the Andean potato weevils (APW) *Premnotrypes* spp. and *Rhigopsidius piercei (tucumanus),* (limited to the south of Bolivia) and the potato tuber moths (PTM), *Phthorimaea operculella* and *Symmetrischema tangolias.*

The Purpose of the project was to develop strategies to reduce the impact of pests and stabilise yields and cultivation practices of crops in Hillsides systems, for the benefit of poor people. The project aimed to contribute to this Purpose by producing new methods for control of the major insect pests of potato in Bolivia based on natural pathogens and attractants, and to disseminate these for use by poor farmers in IPM programmes already promoted by PROINPA and CIP.

Project outputs were to develop, produce and promote a new liquid formulation of the *P. opercullela* granulosis virus; to identify, develop and promote a new viral control agent for *S. tangolias*; to develop pheromones and host plant attractants for *P. latithorax* and *R. piercei* and to develop and promote use strategies; and to promote and assess uptake of IPM technologies.

Project activities involved development of new liquid formulations of the PoGV at NRI and evaluation of these in potato stores in Bolivia in comparison with a powder formulation, Matapol, previously developed by PROINPA. CIP and PROINPA carried out collections of *S. tangolias* cadavers in Peru and Bolivia, and these were examined for the presence of a specific entomopathogenic virus by electron microscopy and ELISA techniques. Laboratory and field trapping bioassays to assess responses of Andean potato weevils to volatiles from conspecific weevils and host plants were developed. Volatiles were collected from weevils and host plants in Bolivia and analysed by gas chromatography (GC) linked to mass spectrometry and GC linked to electroantennographic (EAG) recording from weevil antennae at NRI. Dispensers for synthetic attractants were developed and their performance assessed in the laboratory. Traps and lures for weevils were evaluated in farmers' fields. A baseline study was carried out of farmers' perceptions of pest and disease problems in three communities, and training days were carried out in these communities on IPM. Local staff were given on-the-job training and a member of PROINPA staff visited UK for training in virus identification and pheromone research.

Among the project outputs, a new liquid formulation of the PoGV was developed at NRI and evaluated at various doses in storage situations in Bolivia. High levels of infection of the pest insects were achieved, but there was little reduction in damage, probably because the virus is too slow-acting. The effectiveness of the Matapol formulation was shown to be due, at least in part, to the kaolin base. Over 10,000 samples of potentially infected larvae of *S. tangolias* were collected and these are still being processed at CIP, although to date no virus specific for *S. tangolias* has been found. In work on attractants for the Andean potato weevil species *P. latithorax*, *P. suturicallus* and *R. piercei,* no evidence was found for intraspecific, pheromonal communication in any of the species. However, strong attraction of weevils to volatiles from potato leaves was observed. The 44 main components in the potato volatile collections were identified and two were found to elicit EAG responses from weevils. Dispensing systems for these were developed and they were evaluated in laboratory and field bioassays. In the laboratory the synthetic compounds tended to be repellent, probably because the dose was too high, but in the field they were as attractive as potato leaves. The project carried out a base-line survey of farmers' perceptions of pests and diseases of potatoes in three communities. Training days were carried out in these communities on the different IPM components with emphasis on biological control of APW and PTM and on the rational use of pesticides. Work was also started on education of children in rural schools in IPM of potato pests using specially designed textbooks. PROINPA held a course on "Pest Diagnosis" for scientists and technicians in Bolivia and neighbouring countries. A PROINPA scientist also received training in identification of pheromones and molecular techniques for identification of entomopathogenic viruses at NRI.

The project outputs have contributed towards the purpose and goal. Although liquid formulations of the PoGV have been developed, they are not as effective as the powder formulations with the current strain of virus. However, the results have highlighted the hitherto unrecognised importance of the kaolin in the powder formulations and possibilities for improving this formulation further with a faster-acting strain of the PoGV. A specific virus for *S. tangolias* has not yet been found. Collection protocols and approaches to analysis of the samples have been improved and refined, and, as this species is now the dominant species of potato tuberworm moth in many regions of Bolivia and other S. American countries, the finding of a specific virus against this species will be a significant contribution to achieving the project purpose. Research in this project has proved conclusively that the Andean potato weevil species, *P. latithorax*, *P. suturicallus* and *R. piercei* do not use intraspecific pheromones, contrary to previous reports with related species. They do respond to volatiles from potato leaves and two components of the volatiles which elicit EAG responses from the weevils have been identified. These have been shown to attract weevils to traps in farmers' fields, and convenient locally-made traps have been developed. The base-line survey of farmers' perceptions of pests and diseases, training days in IPM and development of a curriculum on IPM for children in rural schools contribute directly to the project purpose and goal. Capacity building and training of local staff also contributed directly to the purpose and goal.

Further work is required to optimise the attractiveness of the traps for Andean potato weevils and to explore with farmers the use of these for reducing weevil damage. PROINPA has already produced and distributed the PoGV powder formulation "Matapol" on a semi-commercial scale through a commercial subsidiary BioTop, and it is anticipated that a similar approach could be taken for production and distribution of traps and lures for weevils. In order to enhance further the dissemination outputs of this project, the materials developed for education of children in rural schools need to be disseminated and validated by the pupils. It is also important that local organisations in the Andean Region have materials and expertise required to continue development and adaptation of the outputs of this project on improved methods for control of Andean potato weevils and potato tubermoths into an Integrated Pest Management Strategy.

BACKGROUND

Andean potato *(Solanum tuberosum ssp. andigena)* is the principal staple food for Bolivians. It covers 25 % of calorific needs of Bolivian households and represents from 3 – 13 % of total household expenditure, increasing at lower income levels. It provides 30 – 50 % of total calories consumed by rural highland households. It is grown nationally by around 400,000 small-farm-families on around 130,000 hectares: Cochabamba is one of the most important potato producing departments and represents between 20 and 25% of this total. Most potato farmers are poor, few crop more than one hectare per year, and average yields are around 6 tonnes/ha.

For many farmers potatoes are not only their food staple but also their principal cash crop. Potatoes are commonly grown in hillside production systems where yields are low due to a complex of biotic factors such as weeds, nematodes, pests and diseases, as well as poor soils and erosion. Within this complex, potato insect pests have a substantial impact on the capacity of farmers to secure a livelihood. The two most important insect pests, and those specifically targeted in this proposal, are the Andean potato weevils (APW) *Premnotrypes* spp. and *Rhigopsidius piercei (tucumanus),* (limited to the south of Bolivia) and the potato tuber moths (PTM), *Phthorimaea operculella* and *Symmetrischema tangolias.* APW affects 5,000 ha in Cochabamba (33,000 ha nationally) and PTM affect about 10,000 ha in Cochabamba (35,000 ha nationally). Surveys in infected areas have found that the Andean weevil damages between 27 and 49 % of tubers, which represents an economic loss of the order of 240 to 460 $US per hectare per year. Losses from potato tuber moth are estimated at 300 to 500 $US per hectare per year. Besides the economic losses, current farmer control practices, which depend upon the use of highly toxic pesticides applied with little or no protective equipment, cause substantial adverse impacts on health and the environment, as has been confirmed in other potato growing areas of the Andes.

Farmers are not usually familiar with insect life cycles and ecology. In many areas farmers will cite either APW or PTM as their principal problem in the potato crop. They attempt to control APW using two or three applications of toxic pesticides (Parathion, Metamidophos, Carbaril, Malathion, Dimethoato and Nuvacron) but, given their limitations of knowledge about the biology and behaviour of the pest, spraying is untimely and dosages are inappropriate so that control is not very effective. In the case of PTM, some farmers dust potatoes in store with Phosforates: though dusting can be effective as a control measure but at great risk to health. Many farmers store potatoes in their houses and so cannot use conventional pesticides

PROINPA in partnership with CIP and other collaborators developed and commercialised the granulosis virus product "Matapol" for the PTM species *P. operculella.* This shows an efficient control higher than 90 %, allowing farmers to save 50% in the cost of seed protection. This work has demonstrated the enthusiasm of farmers to adopt new approaches that are effective and safe, but the current powder formulation of Matapol is only suitable for treating small quantities of tubers and there is a perceived need to develop a new liquid EC formulation. Furthermore, Matapol is not effective against the more aggressive PTM, *S. tangolias*, which has now spread to all the main potato-growing areas. Hence it is necessary to develop new biological control agents for this pest.

Similarly, a pilot IPM programme against APW has been implemented in La Paz by PROINPA with support from CIP. Over 200 farmers were educated in IPM and offered a menu of different control components. This programme has reduced pest damage by a third, which represents benefits of 100 $US/ha. While the IPM pilot programme for APW has met with some success, many farmers remain dependent on the use of chemical pesticides, and there is an urgent need to find simple, low-cost alternatives that could be included in the IPM menu to enhance its probability of adoption on a wide scale.

Demand for the specific outputs of this project was identified during a CPP Programme Development visit by Dr Lenné to PROINPA in November-December 1999. In 1996 PROINPA carried out a ranking exercise in which all their research programs where evaluated following five basic criteria: geographic distribution of the problem, amount (in percentage) of affected producers, economic importance of the limiting factor, relative importance of the analysed region. Regarding pests, out of twelve PTM ranked fourth and APW ranked seventh. This ranking effort allowed PROINPA to acknowledge the opinion of field experts and organisations involved in the potato chain. It also allowed us to confirm the accuracy of our research paths.

Since submission of the original project proposal, the Fundación para el Desarrollo Tecnologico Agropecuario - Altiplano (FDTA-Altiplano) of the Sistema Boliviano de Tecnologia Agropecuaria (SIBTA) conducted a survey during 2002 to identify agriculturally related problems. From 336 responses, 144 (43%) were related to the potato crop, and 46 (32%) were specifically related to pest control. Consequently the FDTA-Altiplano is keen to develop technologies which can be used to respond to this demand, providing they are effective, cheap and readily available. Further work on the traps for weevils was included in a proposal submitted to the DFID Facilitating Innovative Technology (FIT) programme in January 2004, and the proposal was accompanied by written support from the FDTA-Altiplano. However, the proposal was considered unsuitable for funding under this programme.

In May 2004, the DFID INNOVA project carried out an assessment of the demand and priorities for improvement of all aspects of potato production and marketing as expressed by smallholders in three different climatic zones of the Municipality of Umala in the Bolivian Altiplano. Although other constraints differed in the three zones, all three zones gave highest priority to reducing damage caused by potato tubermoths and weevils.

PROJECT PURPOSE

The Purpose of the project was to develop strategies to reduce the impact of pests and stabilise yields and cultivation practices of crops in Hillsides systems, for the benefit of poor people.

The project aimed to contribute to this Purpose by producing new methods for control of the major insect pests of potato in Bolivia based on natural pathogens and attractants. These would be developed for use by poor farmers in IPM programmes already promoted by PROINPA and CIP.

Achievement of these objectives would help poor farmers reduce losses due to these pests in a cost-effective, sustainable way, and minimise use of conventional chemical insecticides. As potatoes are the principal staple food for Bolivians and also a major cash crop, these developments would impact directly on livelihoods by reducing poverty and improving food security.

RESEARCH ACTIVITIES

A NEW LIQUID FORMULATION OF THE *P. OPERCULLELA* GRANULOSIS VIRUS DEVELOPED, PRODUCED AND PROMOTED

Development of a new liquid formulation of PoGV

Using PoGV supplied by PROINPA a number of different liquid formulations were prepared. The PoGV was purified using sucrose gradient purification at NRI then the virus counted and standardised prior to formulation.

The test formulations included

1. Aqueous suspension
2. Wettable powder
3. ULV formulation

In addition a PROINPA type formulation was also prepared as a control. Initially each formulation was made up at three concentrations of virus equivalent to application rates of $3.20x10^8$, $3.20x10^9$ and $3.20x10^{10}$ viral occlusion bodies (OB) per Kg. These concentrations equate to application rates equal to existing Matapol, one 10 times lower and one 10 times higher. All details off the techniques are given in the tests protocol (Appendix 1, 3) and project report (Appendix 2).

The aqueous suspension and wettable powder formulations were chosen as PROINPA already had the facilities to produce these formulations so that their adoption as standard technologies would be easy. Water-based sprayers are also the most common systems used by local farmers so adoption was again straightforward. The ULV formulation required wet milling techniques not available at PROINPA but ULV oil formulations would be preferred by large processors as the good coverage and low water content would make this system of application better and cheaper for large volumes of potatoes.

Evaluation of new formulations

In order to test the new formulations a new bioassay system for liquid formulations was developed at PROINPA by the NRI/PROINPA team. Full details are included in the Appendices 1, 2 and 3. In trials both spraying and dipping with the new aqueous formulations were evaluated. While spraying was a technology used by some farmers the poorest farmers were more likely to use dipping for seed potatoes as they lacked access to sprayers. ULV formulations were developed specifically for large scale farmers and potato processors who could afford sprayers so only spraying was evaluated.

Formulations were tested on potatoes at Toralapa research station 70 km outside Cochabamba. To check the new formulations against existing methods also included in the trial were the existing clay formulation of Matapol plus containing PoGV and Bt and a blank clay formulation without either virus or Bt. Full details of the trial technique are given in Appendix 1.

The initial trials showed that the virus concentrations in the liquids were too low so later a new set of formulations were prepared with much higher virus concentrations x30 ($9.6x10^{10}$ OBs/Kg) and x100 ($3.2\ x10^{11}$ OB/Kg) that in the original Matapol. Two series of trials were set up to test these high potency formulations and details of these are given in Appendix 3.

After the first series of high potency trials a second series was set up but by then the widespread appearance of *S. tangolias* at the research station made conduct of formulation trials using PoGV virus alone impossible. When trials were set up to 90% of damage by PTM in controls was due to *S. tangolias* and without a virus active against *S. tangolias* this could not be prevented. In addition the appearance of high levels of microsporidean parasites in the stock culture made it more difficult to produce the much larger amounts of PoGV needed for high potency formulations. Both problems were still unsolved when the project ended.

As no final acceptable liquid formulation was identified the planned trials on its physical and storage properties were not carried out.

Production of new formulations

As no effective liquid formulation was developed for the reasons discussed in outputs below no local production was carried out at PROINPA.

A NEW VIRAL CONTROL AGENT FOR *S. TANGOLIAS* IDENTIFIED, DEVELOPED AND PROMOTED

Collection and identification of new viral agents for *S. tangolias*

Larvae of *S. tangolias* were collected from several stores in different zones of Bolivia and Peru during November 2001 to August 2004. The sampled sites are indicated in Appendix 4. The collection was performed with the assistance of NRI in Bolivia and with the assistance of CIP in Peru. Collected samples were sent to CIP, Lima, Peru, where larvae of *S. tangolias* were separated from the samples and individually screened for presence of entomopathogens. The screening process, which included macroscopic sighting of diseases and symptoms of infections; light microscopy, transmission electron microscopy (TEM) and double antibody sandwich Enzyme-linked inmunosorbant assay (DAS-ELISA), is described in detail in Appendix 4.

For molecular characterization of the new granulovirus isolates and identification of genomic variation (polymorphisms) compared to the existing granulovirus strains infecting *P. operculella* (*Po*GV), DNA from virus progenies produced in *P. operculella* larvae were digested with restriction enzymes and examined by electrophoresis (Appendix 4).

Evaluation of new viral agents

No prospective new virus isolate could be tested to determine LC_{50} and relative potencies because all isolates failed to infect *S. tangolias*.

Development of mass production of new viral agent

Because no virus specific to *S. tangolias* could be identified this activity was not carried out.

Joint formulation of new viral agent with PoGV

As no final acceptable liquid formulation was identified the planned trials on its physical and storage properties were not carried out.

PHEROMONES AND HOST PLANT ATTRACTANTS FOR *P. LATITHORAX* AND *R. PIERCEI* DEVELOPED AND USE STRATEGIES DEVELOPED AND PROMOTED

Determination of roles of pheromones and host plant attractants

Laboratory bioassays in Bolivia (Appendix 5)

Laboratory bioassays were carried out at the PROINPA laboratory at Toralapa, Bolivia, during the three cropping seasons of 2001 – 02, 2002 – 03 and 2003 – 04.

For bioassays in 2001 – 02, adults of *Rhigopsidius piercei* were collected from inside stored potato tubers and were assumed to be virgin. There are no obviously sexually dimorphic characteristics, and sexing was done arbitrarily on size taking males as smaller than females. Adults of *Premnotrypes latithorax* were obtained from the field either on plants or in traps and were assumed to be mated. This species can be reliably sexed as adults. In the subsequent two seasons adults of both species were obtained from cultures maintained on potato tubers in the laboratory under ambient temperature and humidity conditions. Adults were sexed soon after emergence and kept in single sex groups. Thus they were virgins when tested.

The basic experimental testing unit was a dual-choice pitfall bioassay chamber. This was formed by a petri-dish through which two holes were punched. Eppendorf tubes whose ends had been cut off were fitted into these holes and the tubes were then inserted into glass sample tubes. This arrangement allowed weevils to fall into the glass tubes but prevented them returning. An array of up to 18 bioassay chambers was formed by fixing the glass tubes of each within a sheet of polystyrene. In each petri-dish a piece of filter-paper was placed covering the entire surface to make it easier for the insects to move around. Several smaller pieces of paper were put into each petri-dish to permit the weevils to hide from the light.

The experiment consisted of placing one or more test weevils in the centre of the petri-dish in between the holes above each of the two glass tubes. Data were collected up to three times a day (bioassays were run during the approximate periods 8.00 – 13.00, 13.00 – 17.00 and 17.00 – 8.00), although in later bioassays only the overnight period was used as this was when weevils were generally more responsive. Where more than one test period per day was employed, the same individual weevils were normally used. Numbers in each treatment tube, together with any non-responders, were counted and recorded at the end of each bioassay period.

Three sets of bioassays were carried out using live weevils and/or potato leaves as baits. Four sets of bioassays were subsequently done with synthetic compounds. Contingency tables and χ^2 tests were used to check for significant differences between treatments with equal distribution as the null hypothesis. With some of the bioassays in 2003 – 04 the level of replication was such as to allow analysis of variance to be carried out (using arc-sine transformed proportions as the data to be analysed). Non-responders were not included in either of these analyses. Results are summarised as the Response Index (RI) = (number in Treatment 1 - number in Treatment 2)/Total number tested.

Laboratory bioassays in Peru (Appendix 6)

Bioassays at CIP, Lima, Peru, were carried out on virgin *P. suturicallus*. A three-choice pitfall bioassay was used consisting of a box (50 x 40 x 40 cm) with three holes in the base leading to 250-ml glass conical flasks. Test materials were placed in the flasks, the weevils

(20) released in the centre of the box and the box sealed for 24 hr before results were recorded.

In 2003, experiments were carried out to compare attractiveness of conspecific weevils of the same or opposite sex, attractiveness of potato leaves, comparison of potato and maize leaves and effect of moisture. More recently comparisons have been run between leaves from different commercial potato varieties, different native varieties and different wild varieties.

Experiments were also carried out with (*Z*)-3-hexenol and (*E*)-2-hexenal dispensed from polyethylene vials, supplied by NRI. Both *P. suturicallus* and *P. latithorax* were used in these tests.

Results were analyzed with SAS system by analysis of variance and Waller–Duncan test.

Field trapping experiments in Bolivia (Appendix 7)

Trapping experiments were conducted during each of the 2001–02, 2002–03 and 2003–04 cropping seasons.

Traps were pitfall traps or sticky stake traps. The pitfall traps were made from white, plastic flowerpots (12 cm diameter, 9 cm deep, 6 openings 3.2 × 1.5 cm) to which a green, corex lid was attached by elastic string. The trap was set into a small hole in the ground so that the bottom edges of the openings were level with the soil allowing insects to crawl in. The leaves and weevils acting as baits were retained inside small plastic containers (old photographic film holders, 3 cm diameter × 4.5 cm) which had been adapted with fine plastic mesh, to allow airflow through. These bait holders were attached under the centre of the trap-lid.

The sticky stake traps consisted of wooden stakes, 1 × 2 cm in cross-section, driven into the soil so that they stood 30 cm high. Beginning 5 cm above soil a 20 cm length of the stake was coated with sticky insect glue; at the top of the stake a small plate was used to hold a small plastic container containing the adult weevils or potato leaves that acted as bait.

Two field trapping experiments to catch *P. latithorax* were conducted from 22 January – 27 February 2002 in farmers' fields a few hundred metres from the research station at Toralapa. Traps were positioned 10 m apart along the edge of potato field facing a potato store. This location was used in the expectation that weevils moving into the field from the store would encounter the traps. Each of the experiments was set out to a randomized complete-block (RCB) design with three replicates. Traps were checked at least once a week when the numbers of male and female *P. latithorax*, and of other invertebrates were recorded separately. At the same time the bait leaves and weevils were replaced.

In 2002 – 03 a further experiment at Toralapa also compared numbers of male and female *P. latithorax*, and of other invertebrates, caught in pitfall traps. Traps were baited with fresh potato leaves alone, two virgin adult male or female *P. latithorax* (each with 1 – 2 fresh leaves for food) or left un-baited. The design of the pitfall trap was the same as for the previous season except that the number of trap openings was reduced from six to five. The experiment ran from 4 December 2002 – 27 March 2003. Six replicate blocks were set out to a RCB design, three each in two fields (one belonging to a farmer) a short distance from the research station. Individual traps were positioned 5 m apart along the edges of fields, and the spacing between blocks was 10 m. Traps from one field had to be redeployed to another in early January following vandalism. Traps were checked approximately two times per week.

A similar experiment was also carried out with "white flowerpot" pitfall traps in respect of *R. piercei* catches in farmers' fields at Yampara, near Sucre from 12 January – 29 March. The experiment differed in that the virgin adult *R. piercei* were not supplied with leaves as food. This was because traps were only checked weekly, so leaves would not have been fresh, and because this species is better able to survive periods with feeding. Initially, traps containing the same treatments were grouped together, rather than being arranged in a randomised block design, but this was corrected on 4 February.

The data analysed were total catches of each sex in each trap over the respective trapping period. One-way analysis of variance was used to examine the data for treatment effects. Blocking effects were accounted for in the models except in the case of the experiment with *R. piercei* at Sucre in 2002 – 03 (when traps were not initially set up with a blocked design). Data were not transformed.

Isolation and identification of attractants

Collection of volatiles (Appendix 8)

In 2002, *P. latithorax* adults were collected during January-February from farmers' stores previously used for potatoes. *R. piercei* adults were dissected from infested tubers. Later in 2002, adult *P. latithorax* were collected during October from the soil in an area used to store potatoes. *P. latithorax* could be sexed and were kept separately after collection. *R. piercei* could not be sexed without dissection and were separated on the basis that females are bigger than males.

All volatile collections were made in the PROINPA laboratory at Toralapa, Bolivia, using equipment previously developed at NRI. For initial collections, two weevils of the same sex and species were placed in a glass vessel with potato leaves. Charcoal-filtered air was drawn over the insects and volatiles trapped on a filter containing the adsorbent, Porapak Q. Collections were run for 1-2 days after which the filters were wrapped in aluminium foil and stored in a refrigerator before mailing or carrying to NRI. Four such collection devices were used simultaneously. Collections were made from potato leaves only in 2002. During November 2003, similar collections were made from 10 weevils of the same sex and species on potato leaves. In all, 28 collections were made from each sex of each species and 8 collections from potato leaves only.

Gas chromatography-mass spectrometry (GC-MS) (Appendix 8)

At NRI the filters were washed with dichloromethane (3 x 0.5 ml) to remove trapped volatiles, and the resulting extracts were stored at –20°C. GC-MS analyses were carried out with a fused silica capillary column coated with polar ZBWax linked directly to a Finnigan Ion Trap Detector 700 operated in electron impact mode. Kovats retention indices (KI) were calculated relative to the GC retention times of normal hydrocarbons. Mass spectra were compared with those in the NBS/NIH/EPA library, the ITD terpenoid library and a library generated from samples analysed previously at NRI.

Gas chromatography-electroantennography(GC-EAG) (Appendix 9)

EAG preparations were made by embedding the weevil ventral side up in a plasticine block. The head was held in place with an entomological pin and the antennae spread out over the surface of the plasticine and held in place by fine wire staples. Glass microelectrodes were pulled just prior to use and filled with saline. The recording electrode was inserted into the club of one antenna through a hole made with a microknife. The reference electrode was inserted into soft tissue between the head and thorax.

EAG responses to volatile collections were measured by applying the sample (1 µl) to the inside wall of a glass Pasteur pipette. Solvent was removed by a pulse of nitrogen (200 ml/min for 3 sec). The pipette tip was then positioned 1 cm above the EAG preparation and a similar pulse of nitrogen used to blow volatiles from the sample over the preparation. Three replicates of each treatment were done at random at intervals of two minutes on three insects.

GC-EAG analyses were carried out using methods and equipment developed at NRI. GC columns were polar CPWax52CB or non-polar CPSil5CB with oven temperature programmed at 50°C for 2 min then at 6°C/min to 240°C. The GC column outlet was split with half going to the flame ionisation detector and half going to a small glass reservoir in the GC oven, the contents of which were blown over the EAG preparation at 15-second intervals.

Field evaluation of trapping systems

Trap design experiments (Appendix 7)

During 2002 – 03 a trap design comparison was carried out in experimental fields within the Toralapa station. This was between with the hitherto standard "white flowerpot" trap and three other designs of pitfall trap made from various plastic containers – a black flowerpot, a plastic bucket and plastic bottle. All traps were baited with fresh leaves.

In parallel with this experiment some laboratory observations on the ability of weevils to escape from each of the four trap designs were carried out, in order to provide additional information on their relative efficiency. Ten weevils were placed in one trap of each of the different designs overnight, in the laboratory, and the respective numbers retained and escaped were recorded. This procedure was done once for *R. piercei* and three times with *P. latithorax*.

Development of dispensers for (Z)-3-hexenol and (E)-2-hexenal (Appendix 10)

Two components in volatile collections from APW on potato leaves were shown to elicit responses from both *P. latithorax* and *R. piercei* in linked GC-EAG analyses. These were identified as (*Z*)-3-hexenol and (*E*)-2-hexenal. In order to investigate the behavioural activity of the synthetic compounds in laboratory and field, suitable dispensers were required that provided ideally a constant, known release rate over periods of several weeks.

Dispensers investigated were polyethylene vials sealed with a glue-gun, and polyethylene sachets sealed with a heat sealer. In some experiments a cotton wool plug was placed in the vial occupying approximately half the volume. Dispensers were loaded with 100 µl of the chemical.

Duplicate dispensers were maintained in a laboratory wind-tunnel (27°C, 8 kph windspeed) and release rates determined by periodic weighing and by trapping volatiles released and quantitative assay by gas chromatography (GC) (Appendix 10).

Trapping experiment with synthetic host plant volatiles (Appendix 7)

(*Z*)-3-Hexenol and (*E*)-2-hexenal were evaluated as attractants for *P. latithorax* in a trial carried out in farmers' fields close to the Toralapa station during 3 December 2003 – 10 March 2004. Eight treatments were deployed in a RCB design with five replications. The two synthetic compounds were used individually or together, dispensed from polyethylene vials or white sachets, plus traps baited with potato leaves and control (blank) traps. Vials and sachets contained 100 µl of one or both compounds (*i.e.* lures dispensing both compounds initially contained 200 µl of material).

It was originally intended that a similar experiment should be conducted near to Sucre to trap *R. piercei*. Unfortunately, scarce and very late rains prevented almost all potato planting in the region and hence no suitable site for the experiment could be found.

UPTAKE OF POTATO IPM TECHNOLOGIES PROMOTED AND ASSESSED

Baseline evaluation of potato production by smallholder farmers and constraints

For this activity three communities were selected in Bolivia – Cebada Jichana, Lope Mendoza Alto and Candelaria. The methodology was defined and initiated in one community. In addition, focus groups (case studies) were identified within groups of farmers differentiated by gender in order to gather qualitative information about the management of the APW and PTM. Baseline data on potato pests and their control were collected and reported from a total of three farmer communities. In each community 30 farmers were interviewed. The baseline study was completed in 2003 and results are presented in Appendix 11.

Promotion of IPM and investigation of acceptability of biological control agents

Results of the baseline study showed that Andean Potato Weevils (APW) and Potato Tuber Moths (PTM) were the most important potato pests. Consequently and by request of farmers a series of training events were planned on each issue and dates were agreed with farmers (Appendix 12).

Assessment of project impact and future developments

Training activities within the rural schools were conducted in four communities (Huajllapujru, Cebada Jichana, Caña Kota y Boqueron k' asa), near Toralapa Centre (Appendix 13).

Capacity building and training

NRI staff provided on-the-job training for PROINPA staff during field visits.

As an add-on to the project Luis Crespo of PROINPA visited NRI for a month and was trained in techniques for the identification, evaluation and enumeration of insect viruses. These included baculovirus counting, bioassay techniques, microscopic techniques for identifying BVs, molecular techniques for strain identification (including restriction endonuclease analysis and PCR) and BV purification. This training was to further trials work assist in the search for a new *S. tangolias* virus and to build PROINPA insect pathology and molecular biology capacity. He also assisted in work on pheromone identification and formulation including GC-EAG and GC-MS work and assessment of formulations (Appendix 14).

OUTPUTS

A NEW LIQUID FORMULATION OF THE *P. OPERCULLELA* GRANULOSIS VIRUS DEVELOPED, PRODUCED AND PROMOTED

The output of developing and testing new liquid formulations of POGV were achieved but as the liquids proved in trials to be less effective than existing powder formulations no production and promotion was undertaken.

New formulation of PoGV developed produced and promoted

The liquid formulations were evaluated as described in potato storage trials against the existing Kaolin formulation "Matapol Plus" formulation and a control insecticide MVP based upon *Bacillus thuringiensis*. The results are presented as tuber damage scores in Figure 1, pest numbers Figure 2 and percent PoGV infection in Figure 3.

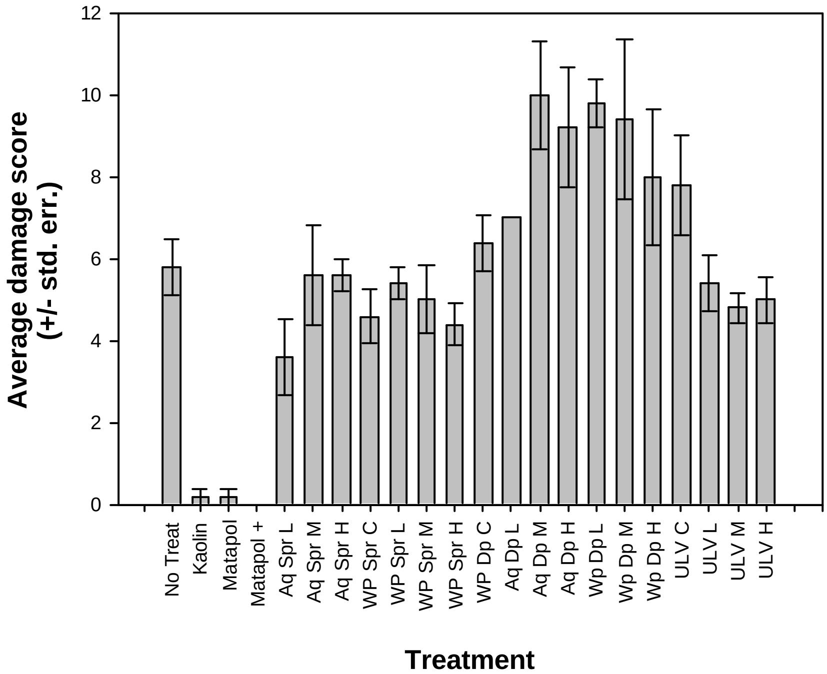


FIG 1. Average damage scores on potato tubers from formulation trials May-June 2002 (C, L, M, H = Control (Zero), Low, medium and high rate PoGV, No treat = control no spray or dip, Aq. Spr = aqueous suspension sprayed, WP Spr, = wettable powder sprayed, Aq Dp = Aqueous dip, WP Dp = Wettable powder Dipped, ULV= Ultra low volume spray).

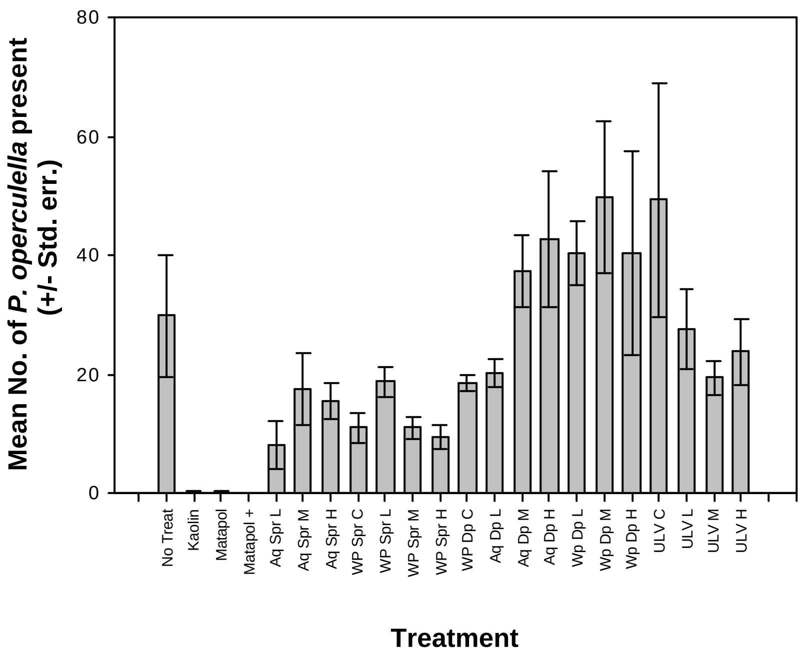


FIG 2. Average numbers of *P. operculella* larvae in different treatments from formulation trials May-June 2002 (C, L, M, H = Control (Zero), Low, medium and high rate PoGV, No treat = control, Aq. Spr = aqueous suspension sprayed, WP Spr, = wettable powder sprayed, Aq Dp = Aqueous dip, WP Dp = Wettable powder Dipped, ULV= Ultra low volume spray)

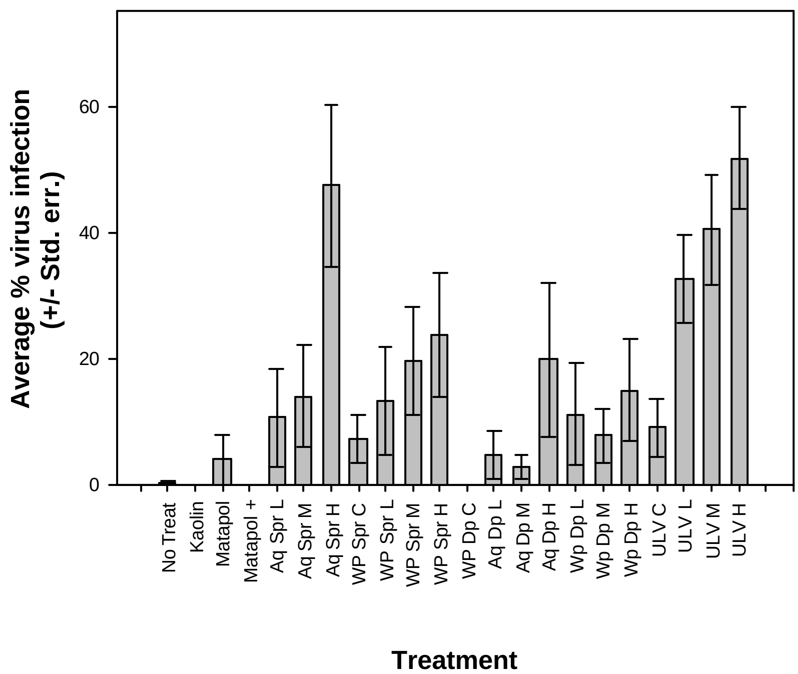


FIG 3. Average infection rates in *P. operculella* in tubers from formulation trials May-June 2002 (C, L, M, H = Control (Zero) Low, medium and high rate PoGV, No treat = control, Aq. Spr = aqueous suspension sprayed, WP Spr, = wettable powder sprayed, Aq Dp = Aqueous dip, WP Dp = Wettable powder Dipped, ULV= Ultra low volume spray).

The damage results in Figure 1 clearly showed that none of the new formulations gave damage control equal to the existing clay formulation Matapol plus or even the blank Kaolin (clay only) formulation. Aqueous and wettable powder dips indeed seemed to produce damage levels higher than in the undipped control.

The pattern of insect numbers in different treatments (Figure 2) mirrored that of the damage scores and indeed showed that none of the new liquid formulations equalled the clay formulations for controlling *P. operculella* numbers in stored tubers. Overall, the dip treatments had highest levels of PTM infestation levels of all treatments with levels that were significantly higher than any water or oil-based spray treatment (1-way ANOVA, $P < 0.001$, df = 21, F= 4.26).

The infection data in Figure 3 gives insights into what was happening. It shows that there was a concentration related response in the WP sprayed, aqueous sprayed and ULV sprayed treatments in that the highest infection rates occurred in the treatments with the highest concentration of PoGV. In both aqueous and ULV sprayed treatments infection rates were above 50% in the highest PoGV virus concentration. Dipping treatments produced no consistent concentration related effected. The above trial was repeated twice at Toralapa but gave results not substantially different to the first trial whose results were shown above.

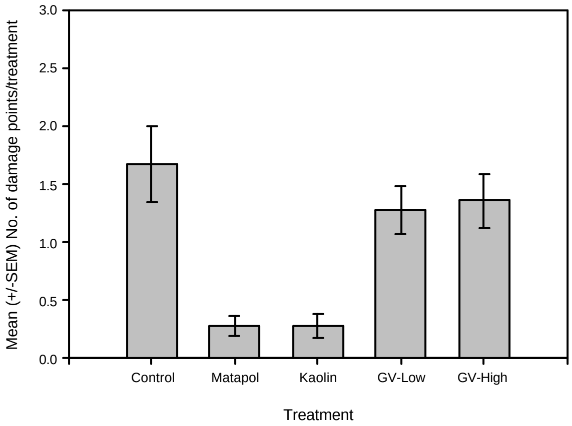
What the trials did show most interestingly was that much of the control effect of Matapol is due to the action of the kaolin and that a move to a liquid formulation will require much higher levels of PTM GV (x30-x100) than in the existing Kaolin formulations. Diatomaceous earths such as Kaolin have long been known to have entomopathic properties and are widely used as stored grain protectants. These were originally believed to act by abrading the cuticle of insects but currently the theory is that adsorption of cuticular lipids leading to dehydration is a more important factor. Thus the efficacy of Kaolin alone against PTM larvae is not surprising. It may be that some cuticular abrasion speeds up entry of POGV and subsequent infectivity but this is probably a minor factor as PoGV normally requires entry to insect midgets to release active virus particles from their protective OB.

Following these early series of trials the trials were repeated with high potency liquid POGV formulations. As the supply of PoGV available for these high potency formulations was limited only aqueous suspensions were tested as part of this second series of trials to ascertain if application rates for liquid formulations could be optimised. Full details of these trials are in Appendix 2.

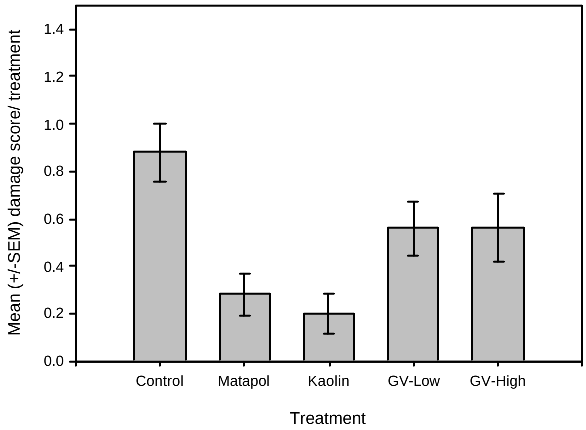
The results in Figure 4 show that even with these higher potency formulations having 30 to 100 times higher concentration of PoGV the level of entry points in tubers, tuber damage and *P. operculella* numbers are not reduced to that found in Matapol plus treated potatoes or even those treated with Kaolin alone.

The infection data shows that >80% of larvae present in the potatoes are in fact infected with PoGV using the new high potency liquid formulations. Thus the liquid formulations do succeed in infecting the majority of larvae at these concentrations. It must therefore be concluded that the virus used alone without the synergy of the clay formulation cannot prevent larvae causing tuber damage in stores.

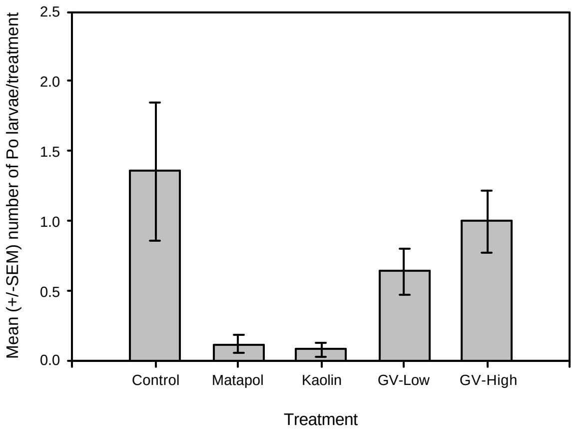
Formulation trial, assessment 1. Effect of treatment on the number of entry points on tubers made by *P. operculella* larvae.



Formulation trial, assessment 1. Effect of treatment on damage caused by *P. operculella* larvae.



Formulation trial, assessment 1. Effect of treatment on number of *P. operculella* larvae present.



Formulation trial, assessment 1. Effect of treatment on % GV-infection in *P. operculella* larvae

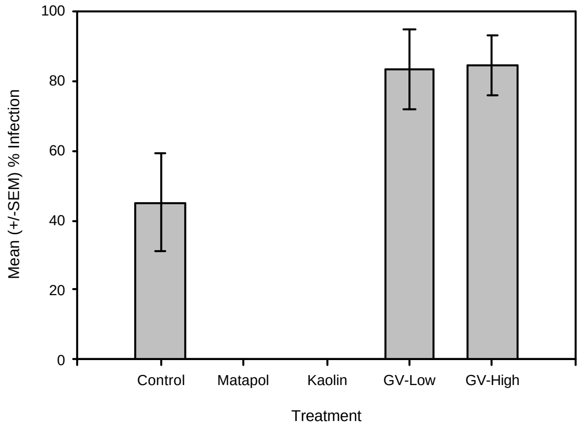


FIG 4. Results from evaluation trials on high potency PoGV formulations showing entry points, made by *P. operculella,* damage caused numbers of larvae present and percentage infection by PoGV (Control = unsprayed, Matapol = treated with Matapol plus powder, Kaolin = treated with blank powder without PoGV or Bt, GV low = GV high =

The evidence gathered overall in this work could be taken to indicate that the Matapol formulation containing PoGV or Bt has little beneficial effect on level of PTM larvae present or the amount of damage sustained over a treatment of the base material alone (Kaolin).

The probable explanation for the inability of high potency liquid formulations to fail to control insect damage despite producing a high degree of infection is that the PoGV applied as a spray is too slow acting to protect potato tubers any more effectively than an untreated control. It is apparent that the PoGV is highly pathogenic and infects a high percentage (>85% on average) of PTM larvae at an application rate of $9.6x10^{10}$ OBs/Kg of tubers. However, it would appear that the infection develops in larvae only once they are inside the tubers and does not prevent entry of larvae into the tubers. By the time larvae have succumbed to infection and died they are large and have already caused major damage. It may indeed be that once entry points are created by larvae much of the subsequent damage is due to secondary infections by fungi that become established at the site of entry so that even the early death from infection might not prevent unacceptable damage.

It was suspected prior to the project that PoGV might be very slow to act at the low ambient temperatures (<10 °C) experienced in the Altiplano. The PoGV isolate was one originally isolated from Tunisia (Arx and Gebhardt 1990) and much of the research to study it in field and laboratory had been at relatively high ambient temperatures of 20-30°C (Kroschel et al 1996). At these temperatures the virus seems to act within 14 days but its lethal time at much lower temperatures is not reported. It may be therefore that while this virus works well at these higher temperatures it is simply too slow at the lower temperatures to kill or incapacitate the larvae before unacceptable damage is done to the tubers. It is possible that identification of a faster acting strain of PoGV might make a liquid formulation viable.

More firm evidence from trials would have been desirable but all the indications are that application of existing strains of PoGV via a liquid spray is not a viable option for control of PTM in potato stores.

A NEW VIRAL CONTROL AGENT FOR *S. TANGOLIAS* IDENTIFIED, DEVELOPED AND PROMOTED;

Collection and identification of new viral agents for *S. tangolias* (Appendix 4)

A total of 10,435 larvae were separated from the samples sent to CIP (Table 1).

TABLE 1. TOTAL NUMBERS OF INDIVIDUAL *S. TANGOLIAS* LARVAE COLLECTED DURING THE PERIOD 2001 TO 2004.

COUNTRY	No. LARVAE
Bolivia	383
Peru	10,052
Total	10,435

Of these, 383 larvae showed macroscopically abnormalities or symptoms of infections (bacteria, fungus, protozoa). However, it was not possible to detect baculoviruses in these samples through observations with light microscopy (dark-field optic, 400x, all 10435 samples were screened). For more reliable detection of baculovirus incidence all larval samples were subjected to a baculovirus specific DAS-ELISA. This test revealed baculovirus infection in 29 larvae. TEM confirmed the presence of granulovirus in 23 DAS-ELISA positive larvae and additionally granulovirus incidence in a further 23 DAS-ELISA negative larvae (Table 2 and details of samples in Appendix 4). However, the presence of granulovirus could be classified as low.

TABLE 2. POSITIVE RESULTS FROM EXAMINATION OF *S. TANGOLIAS* CADAVERS BY DOUBLE-ANTIBODY SANDWICH ENZYME-LINKED IMMUNOADSORBENT ASSAY (DAS-ELISA) AND TRANSMISSION ELECTRON MICROSCOPY (TEM)

ASSAY	No. POSITIVE
DAS-ELISA only	6
TEM only	23
DAS-ELISA and TEM	23
TOTAL	52

Attempts were made to multiply the viruses present in these samples in the original host *S. tangolias* and in the close related common potato tuber moth species *P. operculella* (both Gelechiidae) to verify specificity of the viruses. However, the *in vivo* multiplication of all virus samples tested failed in *S. tangolias* larvae but successfully multiplied in larvae of *P. operculella.* None of the infested *S. tangolias* larvae became infected, while infection rates in *P. operculella* ranged from 2 to 20.5%. Consequently, the virus found in *S. tangolias* larvae may be one and the same as *Po*GV, which is very virulent against *P. operculella*, causing only chronic infections in *S. tangolias* larvae in certain circumstances. Virus progenies of the new isolates obtained from multiplication in *P. operculella* were purified by ultra-centrifugation and quantified. All efforts to infect *S. tangolias* using very high concentrations of the new purified virus were not successful.

For molecular characterization of the new granulovirus isolates and identification of genomic variation (polymorphisms) compared to the existing granulovirus strains infecting *P. operculella* (*Po*GV), DNA from virus progenies produced in *P. operculella* larvae were digested with restriction enzymes and transmitted to electrophoresis (the amount of virus from original *S. tangolias* larvae were to low for virus purification and DNA extraction was not possible). The restriction endonuclease analysis (REN) demonstrated no differences between new virus isolates and *Po*GV according to the fragment length pattern (see Appendix 4: electrophoresis profiles). This result strengthened the theory that the new virus identified in *S. tangolias* is *Po*GV. Most virus-positive samples were from Huancayo in Peru, where generally both PTM species prevail and occur together in potato stores. Since *Po*GV is naturally abundant in that zone and the two species occur frequently together in potato stores it is possible that *Po*GV particles could be consumed by *S. tangolias* larvae and be present in their gut. These virus particles could be detected with the technique we used, suggesting the possibility that these particles had not passed from the midgut through the peritrophic membrane and did not cause infection of midgut cells.

Evaluation of new viral agents

As described in Appendix 4, of the samples collected during the year 2002, baculovirus incidence was observed in 13 samples (Peru 2, Bolivia 11) by DAS-ELISA and in further 23 samples (Peru 1, Bolivia 22) by observations of transmission electron microscopy (carried out during 2003). These 36 samples failed to multiply in *S. tangolias* larvae, but successfully multiplied in *P. operculella* (of each sample 0.1 ml homogenate are still remaining stored at -20°C). This leads to the assumption that the virus observed in *S. tangolias* may be more specific to *P. operculella* and may be the same *Po*GV. Virus progenies from these 36 samples multiplied in *P. operculella* larvae were purified (ultra centrifugation on a sucrose gradient) and virus-DNA extracted. These probes will be submitted to REN analysis for genetic comparison (DNA fragment profiles) against a *Po*GV standard.

The 28 virus-positive samples collected during 2003 were processed in the same manner as described above. Further efforts were made to multiply granulovirus in the target insect *S. tangolias*.

During 2003, a total of 3817 larvae were collected from different regions within Peru (Junin, Cusco, Huaraz, Huanuco, Cajamarca) and transmitted individually to a granulovirus sensitive DAS-ELISA. 28 samples resulted granulovirus-positive, however, the positive results could not be confirmed by observations with the light microscope using dark-field optics, which probably is because of very low virus concentrations in the homogenates. All samples send from Bolivia (6) demonstrated negative results (DAS-ELISA).

PHEROMONES AND HOST PLANT ATTRACTANTS FOR *P. LATITHORAX* AND *R. PIERCEI* DEVELOPED AND USE STRATEGIES DEVELOPED AND PROMOTED

Determination of roles of pheromones and host plant attractants

Laboratory bioassays in Bolivia (Appendix 5)

During February-March 2002 and October-November 2002, two-choice pitfall bioassay experiments were carried out to investigate attraction of single weevils of either sex to weevils of the same or different sex with leaves relative to leaves alone, to weevils alone relative to an empty tube or to leaves alone relative to an empty tube.

For the first set of bioassays with *P. latithorax* the percentage of responding weevils varied from 72 – 99%, but was greatest for the overnight period. Generally, the majority of treatment pairs produced positive values of RI, indicating attraction to conspecifics over leaves or blank tubes. However, only a few of these results were statistically significant and there was little consistency with respect to attraction to the same or opposite sex (Appendix 5). One consistent result, across test periods, was of attraction to potato leaves relative to weevils. This was particularly marked during the overnight period and statistically significant in two out of four cases. Similar results were obtained during the second set of experiments (Table 3).

TABLE 3. TWO-CHOICE, PIT-FALL BIOASSAYS WITH *P. LATITHORAX* (TORALAPA, OCTOBER 2002; 11 REPS)

#	TEST[1]	TRT1[1]	TRT2	daytime RI[2]	daytime P[3]	overnight RI[2]	overnight P[3]
1	F	M+leaf	leaf	-0.09	NS	-0.45	NS
2	M	M+leaf	leaf	0.27	NS	0.00	NS
3	M	F+leaf	leaf	-0.18	NS	0.00	NS
4	F	F+leaf	leaf	0.36	NS	0.36	NS
5	F	M	blank	0.18	NS	0.09	NS
6	M	M	blank	0.18	NS	0.27	NS
7	M	F	blank	0.09	NS	0.27	NS
8	F	F	blank	0.09	NS	0.00	NS
9	F	leaf	blank	-0.18	NS	0.64	<0.05
10	M	leaf	blank	0.18	NS	0.73	<0.01
responsiveness				51%		77%	

[1] F=female, M=male
[2] Positive value = treatment 1 preferred; negative = treatment 2 preferred.
[3] P value indicates where distribution of responses differed from equality (?^{2}, 2 × 2 goodness of fit, 1 d.f.) at the 5% level (NS = P >0.05).

For *R. piercei*, response levels were generally lower and no consistently significant results were observed. In the first set of experiments, Response Indices (RI) were uniformly low in all cases, indicating little preference for either of the two treatments. In the second set of experiments with *R. piercei* there were a few significant results (Table 4). In two cases these suggested attraction to the opposite sex, but this was only seen during the day and not the night and was negated by the apparent attraction of males to other males in one treatment pair. There was no obvious attraction to potato leaves.

TABLE 4. TWO-CHOICE, PIT-FALL BIOASSAYS WITH *R. PIERCEI* (TORALAPA, OCTOBER 2002; 10 REPS)

#	TEST[1]	TRT1	TRT2	daytime RI[2]	daytime P[3]	overnight RI[2]	overnight P[3]
1	F	M+/leaf	leaf	0.60	< 0.05	0.20	NS
2	M	M+leaf	leaf	-0.30	NS	0.60	< 0.05
3	M	F+leaf	leaf	0.30	NS	-0.10	NS
4	F	F+leaf	leaf	0.10	NS	0.20	NS
5	F	M	blank	0.00	NS	-0.20	NS
6	M	M	blank	0.40	NS	0.20	NS
7	M	F	blank	0.60	< 0.05	0.60	NS
8	F	F	blank	0.40	NS	-0.70	< 0.05
9	F	leaf	blank	-0.50	NS	0.00	NS
10	M	leaf	blank	0.30	NS	-0.20	NS
responsiveness				65%		68%	

[1] F=female, M=male
[2] Positive value = treatment 1 preferred; negative = treatment 2 preferred.
[3] P value indicates where distribution of responses differed from equality (?^{2}, 2 × 2 goodness of fit, 1 d.f.) at the 5% level (NS = P >0.05).

Bioassays carried out during February–March 2003 were intended to check the finding of the earlier bioassays of apparent attraction of weevils – at least for *P. latithorax* - to fresh potato leaves, and to confirm that this was not merely due to moisture in the leaves. Attraction of either sex to combinations of leaves, moist filter paper or an empty tube was tested. For *P. latithorax*, leaves were confirmed as more attractive than blank tubes, although not all results were statistically significant (Table 5). Leaves were also clearly more attractive than damp paper for males. The same was true for females, although this result was not significant. Paper was not significantly more attractive than blank tubes for either sex, suggesting that the attraction to leaves was not due to humidity.

TABLE 5. TWO-CHOICE, PIT-FALL BIOASSAYS WITH *P. LATITHORAX* (TORALAPA, FEBRUARY 2003; 15-18 REPS)

			am		pm		overnight	
Test[1]	TRT1	TRT2	RI[2]	P[3]	RI[2]	P[3]	RI[2]	P[3]
F	leaf	blank	0.47	NS	0.60	<0.01	0.50	<0.05
F	paper	blank	0.13	NS	0.20	NS	0.28	NS
F	leaf	paper	0.13	NS	0.20	NS	0.44	NS
responsiveness			91%		64%		96%	
M	leaf	blank	0.53	<0.05	0.33	NS	0.50	<0.05
M	paper	blank	0.00	NS	0.33	NS	0.33	NS
M	leaf	paper	0.53	<0.05	0.00	NS	0.50	<0.05
responsiveness			84%		67%		96%	

[1] F=female, M=male
[2] Positive value = treatment 1 preferred; negative = treatment 2 preferred.
[3] P value indicates where distribution of responses differed from equality (?^{2}, 2 × 2 goodness of fit, 1 d.f.) at the 5% level (NS = P >0.05).

For the bioassays with *R. piercei*, response rates were again lower than for *P. latithorax* (Table 6). Males showed significant attraction to leaves, relative to blanks and paper, but only during the morning test period. Female *Rhigopsidius* evinced no significant results.

TABLE 6. TWO-CHOICE, PIT-FALL BIOASSAYS WITH *R. PIERCEI* (TORALAPA, FEBRUARY 2003; 18-24 REPS)

			am		pm		overnight	
Test[1]	TRT1	TRT2	RI[2]	P[3]	RI[2]	P[3]	RI[2]	P[3]
F	leaf	blank	0.17	NS	-0.04	NS	0.14	NS
F	paper	blank	-0.17	NS	-0.17	NS	0.00	NS
F	leaf	paper	0.28	NS	0.13	NS	0.10	NS
responsiveness			57%		58%		68%	
M	leaf	blank	0.44	< 0.05	0.21	NS	-0.14	NS
M	paper	blank	-0.17	NS	-0.13	NS	0.05	NS
M	leaf	paper	0.39	< 0.05	0.00	NS	0.10	NS
responsiveness			44%		69%		57%	

[1] F=female, M=male
[2] Positive value = treatment 1 preferred; negative = treatment 2 preferred.
[3] P value indicates where distribution of responses differed from equality (?^{2}, 2 × 2 goodness of fit, 1 d.f.) at the 5% level (NS = P >0.05).

Following indications of the attraction to leaves, bioassays carried out in November 2003 – February 2004 were designed to examine responses to the two components of potato leaf volatiles found to elicit EAG responses from the weevils, (*Z*)-3-hexenol and (*E*)-2-hexenal. These were tested individually, dispensed from polyethylene vials or from filter paper.

When dispensed from polyethylene vials both compounds were repellent to both sexes of both *P. latithorax* and *R. piercei* This was significant in several cases, particularly in overnight tests. There was also evidence that given a choice between the two compounds, most weevils preferred (*Z*)-3-hexenol (Table 7).

When the same compounds were presented as 0.2 mg doses on small filter-paper pieces, no significant repellency or attraction was noted for either species or any treatment pair except for a repellent effect seen in respect of (*E*)-2-hexenal with *P. latithorax* females (Table 7).

Identical bioassays with serial five-fold dilutions of the compounds (i.e. 0.04 mg) also produced mostly insignificant results, although at this lower dose there was significant attraction to (*E*)-2-hexenal for *R. piercei* females. Strangely, for *P. latithorax* both sexes showed repellency to both compounds presented against blanks, but a preference for (*E)*-2-hexenal against (*Z*)-3-hexenol. However, only the (*Z*)-3-hexenol *vs*. (*E*)-2-hexenal and (*E*)-2-hexenal *vs.* blank results for males were statistically significant (Table 7).

When the two synthetic compounds were presented together in the same 0.04 mg doses, individually, and compared with blank tubes the combination proved significantly repellent to *R. piercei* males and females. Male *R. piercei* preferred potato leaves to the binary blend, while although both sexes preferred leaves to blank tubes neither result was significant. None of the corresponding results for *P. latithorax* were statistically significant (Table 8).

During the course of the bioassays *P. latithorax* weevils generally showed higher levels of responsiveness (*i.e.* made a clear choice between the treatment pairs) during overnight test periods. On the other hand, *R. piercei* weevils showed no preferred activity period. Accordingly, overnight was the only period used to test both species during bioassays conducted after November 2003.

TABLE 7. TWO-CHOICE, PIT-FALL BIOASSAYS WITH *P. LATITHORAX* AGAINST SYNTHETIC COMPOUNDS (TORALAPA, NOVEMBER 2003 – FEBRUARY 2004; OVERNIGHT)

			Vials (n=6)		0.2 mg (n=18)		0.04 mg (n=18)	
TEST[1]	TRT 1[2]	TRT 2[2]	RI[3]	P[4]	RI[3]	P[4]	RI[3]	P[4]
5 F	Z3	E2	0.37	< 0.05	0.10	NS	-0.18	NS
5 F	E2	blank	-0.47	< 0.01	-0.19	< 0.01	-0.23	NS
5 F	Z3	blank	-0.13	NS	-0.11	NS	-0.17	NS
	responsiveness		86%		87%		95%	
5 M	Z3	E2	-0.10	NS	0.12	NS	-0.32	< 0.01
5 M	E2	blank	-0.43	< 0.01	-0.08	NS	-0.17	< 0.01
5 M	Z3	blank	-0.30	NS	-0.03	NS	-0.17	NS
	responsiveness		83%		80%		94%	

[1] F=female, M=male. [2] Z3 = (*Z*)-3-hexenol; E2 = (*E*)-2-hexenal
[3] Positive value = treatment 1 preferred; negative = treatment 2 preferred.
[4] P value indicates where distribution of responses differed from equality ($?^2$, 2 × 2 goodness of fit, 1 d.f.) at the 5% level (NS = P >0.05).

TABLE 8. TWO-CHOICE, PIT-FALL BIOASSAYS WITH *R. PIERCEI* AGAINST SYNTHETIC COMPOUNDS (TORALAPA, NOVEMBER 2003 – FEBRUARY 2004)

			Vials (n=6)				0.2 mg (n=18)		0.04mg(n=18)	
			overnight		am		overnight		overnight	
TEST[1]	TRT1[2]	TRT2[2]	RI[3]	P[4]	RI[3]	P[4]	RI[3]	P[4]	RI[3]	P[4]
5 F	Z3	E2	0.17	NS	0.06	NS	0.13	NS	0.13	NS
5 F	E2	blank	-0.57	<0.01	-0.03	NS	-0.10	NS	0.21	<0.05
5 F	Z3	blank	-0.37	<0.05	-0.04	NS	-0.20	NS	-0.13	NS
	responsiveness		63%		57%		78%		75%	
5 M	Z3	E2	0.37	<0.05	0.09	NS	0.13	NS	-0.10	NS
5 M	E2	blank	-0.53	<0.01	0.09	NS	-0.13	NS	0.08	NS
5 M	Z3	blank	-0.37	<0.01	0.10	NS	0.07	NS	-0.11	NS
	responsiveness		58%		56%		73%		66%	

[1] F=female, M=male
[2] Z3 = (*Z*)-3-hexenol; E2 = (*E*)-2-hexenal
[3] Positive value = treatment 1 preferred; negative = treatment 2 preferred.
[4] P value indicates where distribution of responses differed from equality (?^{2}, 2 × 2 goodness of fit, 1 d.f.) at the 5% level (NS = P >0.05).

Laboratory bioassays in Peru (Appendix 6)

Three-choice pitfall bioassays with *P. suturicallus* were used to investigate attraction between conspecific weevils of either sex. These tests showed no significant intra-specific attraction, i.e. no evidence for the existence of any sex or aggregation pheromone produced by either sex (Table 9).

TABLE 9. MEAN NUMBERS (± SE) OF *P. SUTURICALLUS* CAPTURED IN PIT-FALL TRAPS BAITED WITH VIRGIN MALES OR FEMALES (LA MOLINA, 2003; 20 INSECTS; 9 REPS)

	TEST INSECTS/MEANS (± SE)	
TREATMENTS	FEMALES	MALES
Males (5)	3.33 (± 0.52)	4.00 (± 0.56)
Females (5)	4.77 (± 0.76)	3.55 (± 0.73)
Blank	6.22 (± 1.02)	3.77 (± 0.54)
Responsiveness	73%	65%

In a comparison of attraction of males to female weevils with leaves or to females and leaves separated so that the weevils could not eat the leaves, the males showed a significant preference (P < 0.05, 1-way ANOVA followed by Waller-Duncan test) for the separated females and leaves (Table 10). This was attributed to the fact that the leaves were all eaten in the females + leaves treatment.

TABLE 10. MEAN NUMBERS (± SE) OF *P. SUTURICALLUS* MALES CAPTURED IN PIT-FALL TRAPS BAITED WITH VIRGIN FEMALES AND LEAVES (LA MOLINA, 2003; 20 INSECTS; 12 REPS; MEANS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT P<0.05)

TREATMENT	TEST INSECTS/MEANS (± SE) MALES
5 females + leaves (separated)	12.58 (± 0.63) a
5 females + leaves	3.33 (± 0.51) b
Blank	0.83 (± 0.43) c
Responsiveness	84%

Comparison of potato leaves and maize leaves showed that the former were significantly more attractive to both male and female *P. suturicallus,* although the maize leaves showed significant attraction relative to the blank (P < 0.05, 1-way ANOVA followed by Waller-Duncan test) (Table 11).

TABLE 11. MEAN NUMBERS (± SE) OF *P. SUTURICALLUS* CAPTURED IN PIT-FALL TRAPS BAITED WITH POTATO OR MAIZE LEAVES (LA MOLINA, 2003; 20 INSECTS; 12 REPS; MEANS WITHIN COLUMNS FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT P<0.05)

TREATMENTS	TEST INSECTS/MEANS (± SE)	
	FEMALES	MALES
Potato leaves	14.17 (± 0.72) a	14.83 (± 0.57) a
Maize leaves	3.75 (± 0.60) b	3.25 (± 0.58) b
Blank	1.17 (± 0.56) c	0.50 (± 0.30) c
Responsiveness	91%	91%

Bioassays comparing potato leaves and moistened cotton wool confirmed that the attractiveness of the leaves was not due simply to moisture, although the moist cotton wool was significantly more attractive than the blank(P < 0.05, 1-way ANOVA followed by Waller-Duncan test). A preliminary experiment was done to confirm that cotton wool itself was not attractive to the weevils (Table 12). In these and subsequent experiments, 10 male and 10 female insects were tested together.

TABLE12. MEAN NUMBERS (± SE) OF *P. SUTURICALLUS* MALES CAPTURED IN PIT-FALL TRAPS BAITED WITH POTATO LEAVES OR COTTON WOOL BEFORE AND AFTER MOISTENING WITH WATER (LA MOLINA, 2003; 10 MALE/10 FEMALE INSECTS; 12 REPS; MEANS WITHIN EACH TREATMENT GROUP FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT P<0.05)

TREATMENT	TEST INSECTS/MEANS (± SE) FEMALES/MALES
Leaves + cotton wool	15.44 (± 0.83) a
Cotton wool	1.67 (± 0.52) b
Blank	1.11 (± 0.66) b
Responsiveness	93%
Leaves + moistened cotton wool	13.24 (± 0.59) a
Moistened cotton wool	5.95 (± 0.59) b
Blank	0.14 (± 0.18) c
Responsiveness	96%

Subsequent bioassays were done to compare attraction of *P. suturicallus* by leaves of three different commercial potato varieties, three different varieties of native potato and three different wild varieties. Differences were apparent as shown in Table 13.

TABLE 13. MEAN NUMBERS (± SE) OF *P. SUTURICALLUS* MALES CAPTURED IN PIT-FALL TRAPS BAITED WITH LEAVES FROM DIFFERENT VARIETIES OF POTATO (LA MOLINA, 2004; 10 MALE/10 FEMALE INSECTS; 18 REPS; MEANS WITHIN A TREATMENT GROUP FOLLOWED BY DIFFERENT LETTERS ARE SIGNIFICANTLY DIFFERENT P<0.05)

TREATMENT	TEST INSECTS/MEANS (± SE) FEMALES/MALES
Commercial Varieties	
Yungay	5.72 (± 0.49) b
Perricholi	3.56 (± 0.41) c
Amarilis	8.06 (± 0.50) a
Responsiveness	87%
Native Varieties	
Peruanita	8.11 (± 0.53) a
Huayro	6.39 (± 0.50) a
Amarilla	3.61 (± 0.42) b
Responsiveness	91%
Wild Varieties	
OCH-1132	7.39 (± 0.47) a
OCH-1426	5.00 (± 0.53) b
OCH-1439	6.44 (± 0.53) ab
Responsiveness	94%

Bioassays were also carried out at CIP to investigate the attractiveness of (*Z*)-3-hexenol and (*E*)-2—hexenal dispensed from polyethylene vials to both *P. suturicallus* and *P. latithorax* (Table 14). At these release rates, both compounds proved highly repellent to both species, although this experiment further confirmed the attractiveness of potato leaves to both species. When either synthetic lure was tested in comparison with leaves, the overall response was good and most of the weevils were attracted to the leaves. When the two synthetic compounds were compared, the overall response dropped markedly and the few weevils that did respond mostly ended up in the blank tube.

TABLE 14. MEAN NUMBERS (± SE) OF WEEVILS CAPTURED IN PIT-FALL TRAPS BAITED WITH SYNTHETIC COMPOUNDS DISPENSED FROM POLYETHYLENE VIALS (LA MOLINA, 2003; 10 MALE/10FEMALE INSECTS; 3 REPS)

TREATMENTS	TEST INSECTS/MEANS (± SE)	
	P. suturicallus	*P. latithorax*
(*E*)-2-hexenal	0.33 (± 0.62)	0.00 (± 0.00)
Potato leaves	15.67 (± 1.53)	17.67 (± 0.88)
Blank	0.00 (± 0.00)	0.00 (± 0.00)
Responsiveness	80%	88%
(*Z*)-3-hexenol	1.00 (± 0.82)	0.00 (± 0.00)
Potato leaves	13.33 (± 0.88)	17.67 (± 0.88)
Blank	1.67 (± 1.18)	0.00 (± 0.00)
Responsiveness	80%	88%
(*E*)-2-hexenal	2.33 (± 1.18)	0.00 (± 0.00)
(*Z*)-3-hexenol	0.33 (± 0.62)	0.00 (± 0.00)
Blank	5.00 (± 1.33)	1.67 (± 1.01)
Responsiveness	39%	8%

Field trapping tests in Bolivia (Appendix 7)

During January-February 2002 and December 2002 – March 2003, trapping trials for *P. latithorax* were carried out at Toralapa. Pitfall traps were baited with two female weevils with potato leaves, two males with leaves, potato leaves only and unbaited. No significant treatment differences were noted in any of the individual experiments (P > 0.05 for treatment effects, F-ratio, ANOVA) (e.g. Figure 5).

As might be expected with pitfall traps large numbers of "other" invertebrates, mainly spiders and a variety of non-target beetle species, were trapped in all experiments. No weevils at all were trapped with the sticky stake traps during early 2002 and these were not investigated further. A similar result was obtained with trapping trials for *R. piercei* carried out at Sucre in 2003. In this experiment the weevils were not supplied with leaves as food (Figure 6).

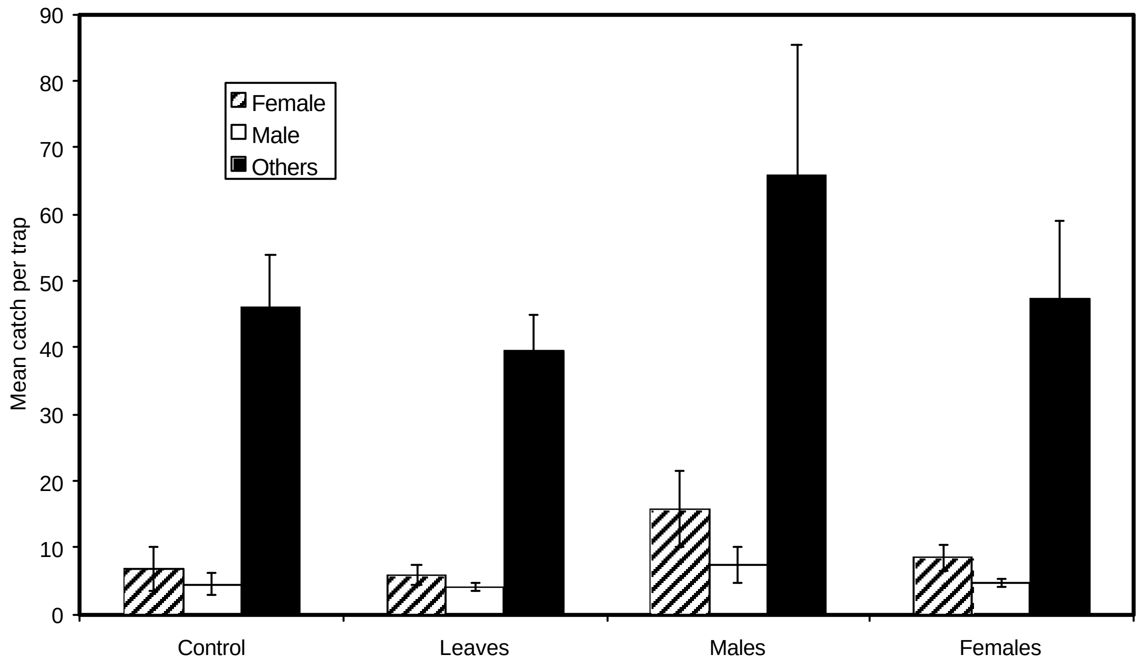


FIG. 5. Mean total catch (± S.E.) per trap of *P. latithorax* and "other" invertebrates in white flowerpot pitfall traps, un-baited or baited with fresh potato leaves, 2 male or 2 female *P. latithorax* weevils, at Toralapa, 4 December 2002 – 27 March 2003 (6 reps).

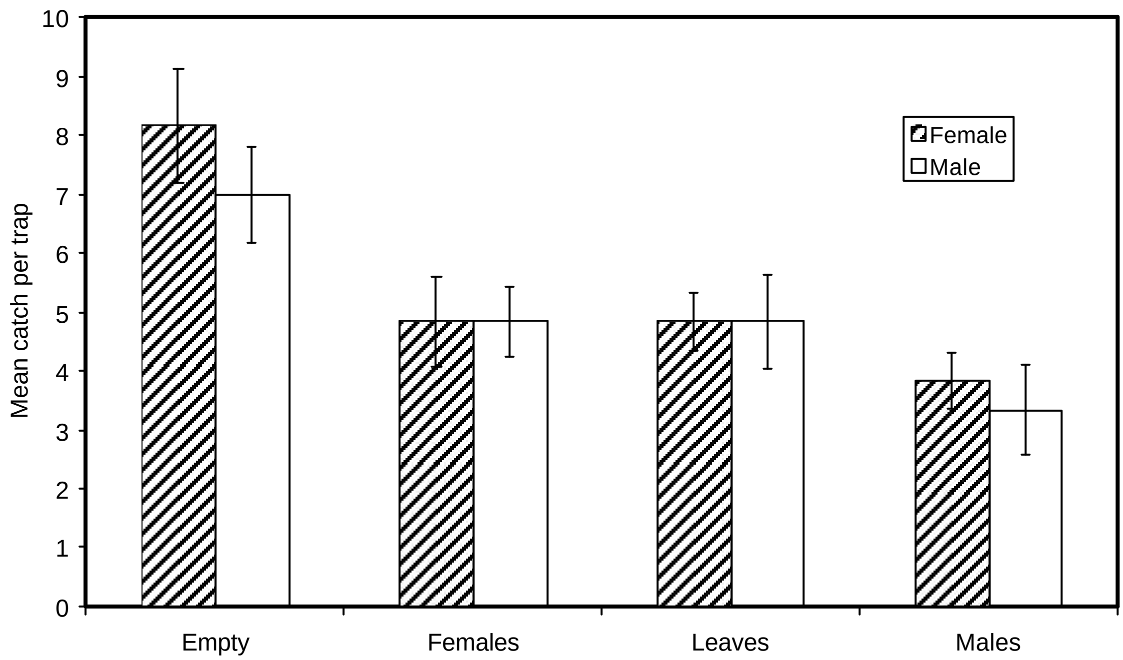


FIG. 6. Mean total catch per trap (± S.E.) of *R. piercei* in white flowerpot pitfall traps, un-baited or baited with fresh potato leaves, 2 male or 2 female *R. piercei* weevils, at Yampara (Sucre), 12 January - 29 March 2003 (6 reps).

Isolation and identification of attractants

Gas chromatography-mass spectrometry (GC-MS) (Appendix 8)

In all, 28 volatile samples were collected from adults of each sex of each of the two species, *P. latithorax* and *R. piercei*, on potato leaves and eight samples of volatiles from potato leaves only. By keeping the sexes separate after collection, every effort was made to use virgin insects. However, although sexing of *P. latithorax* was reasonably reliable, accurate sexing of *R. piercei* was not possible. In most collections, only two insects were used. Although this might have reduced the total amount of any pheromone collected, this approach reduced the chances of mixing the sexes and also reduced any possible physical or chemical interference between individuals. In the final set of collections, ten insects were used per sample in an attempt to increase the amount of any pheromone present.

All 120 samples were analysed individually by GC-MS and then combined by sex and species for each batch of collections. The most significant 44 compounds were identified by comparison of their mass spectra with those in the three libraries available and in some cases also by comparison of GC retention times and mass spectra with those of authentic materials.

Although there was considerable variation in absolute and relative amounts of the different components, no obvious and consistent differences in presence or absence of compounds could be detected by visual observation between any of the collections, i.e. between those from the two sexes or those from the two species or from potato leaves only. As representative, Figure 7 shows GC-MS analyses of collections from 10 individuals of each sex from each species (full details in Appendix 8). The identified compounds are listed in Table 15 in order of retention time on the polar GC column, along with their KI's, and the corresponding numbers indicated on the GC-MS chromatograms in Appendix 8.

The major components identified were ß-caryophyllene, α-humulene, Germacrene D, caryophyllene oxide, globulol, a hydroxygermacradiene, farnesol and oplapanone.

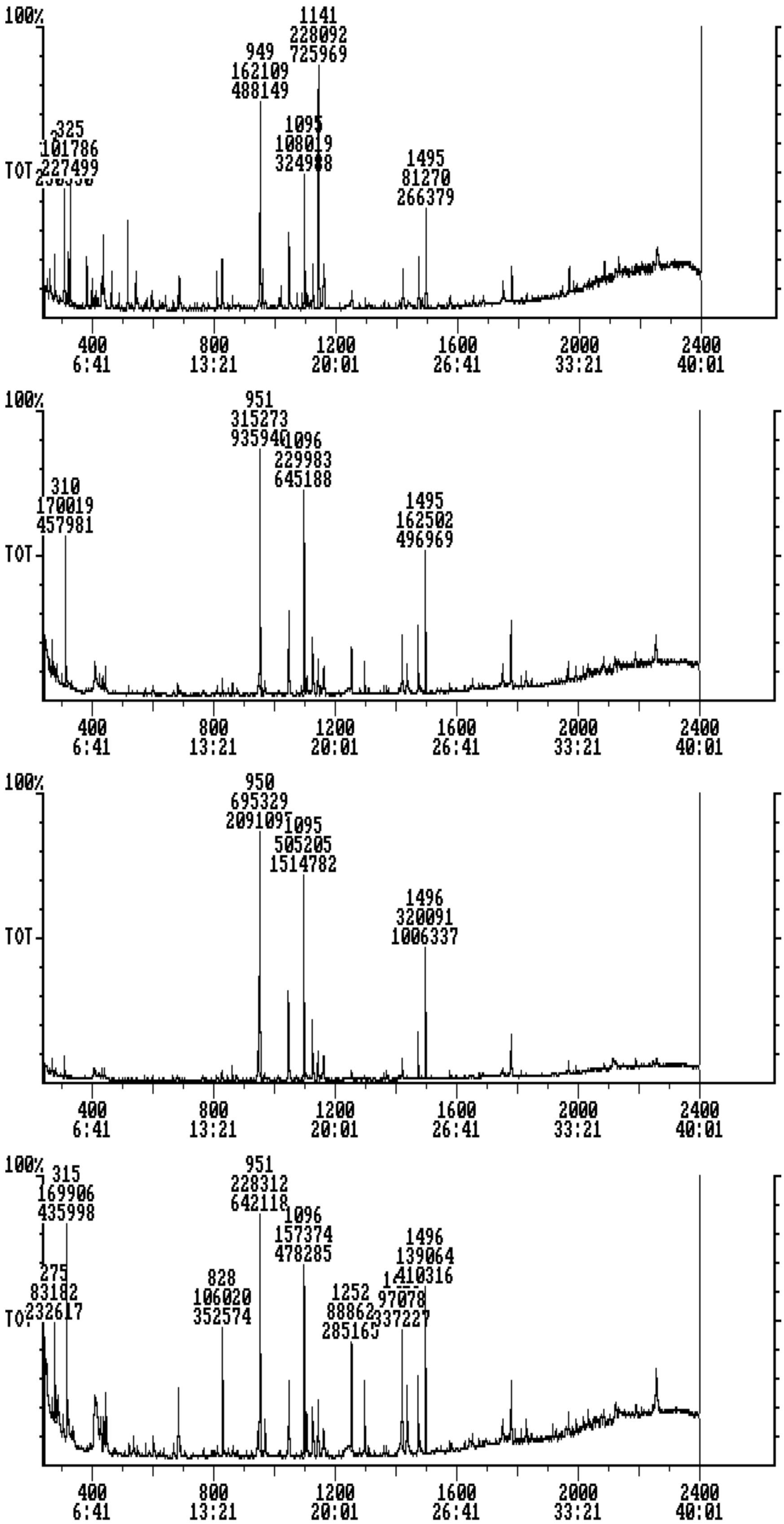


FIG. 7. GC-MS Analyses on polar GC column of volatiles from 10 weevils with potato leaves for 2 days (from top: *P. latithorax* females; *P. latithorax* males; *R. piercei* females; *R. piercei* males)

TABLE 15. COMPOUNDS INDENTIFIED IN GC-MS ANALYSES OF VOLATILES FROM ANDEAN POTATO WEEVILS, *P. LATITHORAX* AND *R. PIERCEI*, AND POTATO LEAVES

No.	SCAN	KI	COMPOUND[1,2]
1	275	1102	undecane*
2	303	1124	unknown
3	318	1136	*dimethylbenzene*
4	325	1141	*dimethylbenzene*
5	380	1185	*dimethylbenzene*
6	428	1220	(*E*)-2-hexenal*
7	435	1225	*trimethyl or methyl,ethylbenzene*
8	461	1244	*trimethyl or methyl,ethylbenzene*
9	485	1260	*trimethyl or methyl,ethylbenzene*
10	514	1281	*trimethyl or methyl,ethylbenzene*
11	542	1300	tridecane
12	570	1320	(*Z*)-3-hexenyl acetate*
13	665	1387	(*Z*)-3-hexenol*
14	679	1396	nonanal
15	685	1401	tetradecane*
16	809	1491	α-copaene
17	825	1503	pentadecane/decanal
18	859	1527	cyperene
19	862	1529	benzaldehyde*
20	949	1593	ß-caryophyllene*
21	960	1601	hexadecane*
22	1020	1649	pulegone
23	1045	1669	α-humulene
24	1070	1688	?-muurolene
25	1089	1704	heptadecane
26	1095	1708	Germacrene D*
27	1122	1730	bergamotene
28	1141	1745	naphthalene*
29	1160	1760	d-cadinene
30	1252	1833	*1-(dimethylphenyl)-ethanone*
31	1295	1868	*1-(dimethylphenyl)-ethanonel*
32	1308	1878	benzyl alcohol*
33	1418	1982	caryophyllene oxide*
34	1419	1983	*1-(dimethylphenyl)-ethanol*
35	1435	1997	*1-(dimethylphenyl)-ethanol*
36	1470	2030	globulol
37	1482	2041	limonene oxide
38	1495	2053	hydroxygermacradiene
39	1572	2127	spathulenol
40	1649	2200	docosane

TABLE 15 (CONT.)

No.	SCAN	KI	COMPOUND[1,2]
41	1682	2231	α-cadinol
42	1749	2300	tricosane
43	1778	2323	farnesol
44	1965	2501	oplopanone

[1] compounds in italics are known impurities from the Porapak adsorbent
[2] * denotes comparison of GC retention time and MS with those of authentic material

Gas chromatography-electroantennography(GC-EAG) (Appendix 9)

When EAG responses of *R. piercei* weevils to volatile collections were measured, there were no significant difference in response to collections from potato leaves only, male weevils on potato leaves and female weevils on potato leaves. All were significantly greater than responses to the solvent ($P < 0.01$, *t*-test) (Figure 8).

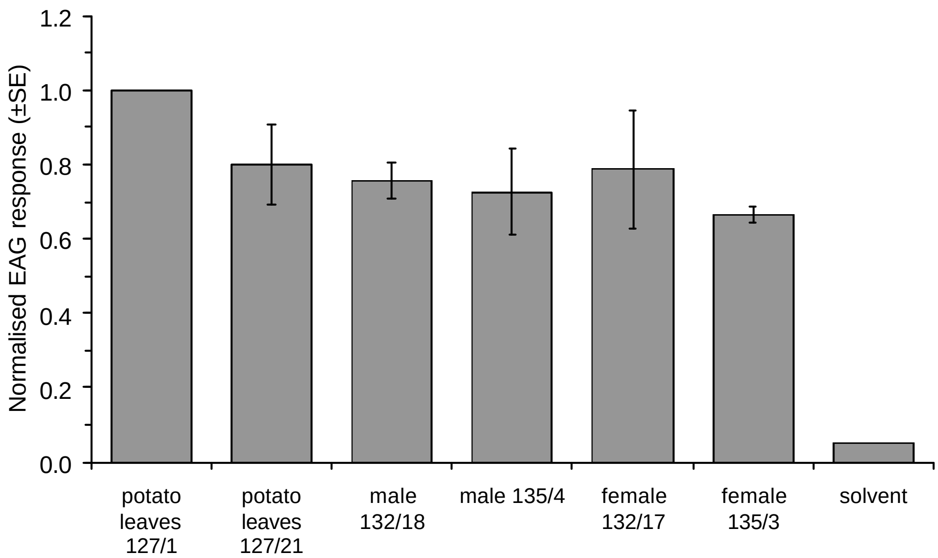


FIG. 8. EAG responses (+ SE) of *R. piercei* to volatile collections (means of three tests on each of three insects; normalised to response to volatiles from potato leaves 2002/127/01).

Volatile collections from male and female *R. piercei* and from potato leaves only were analysed by GC-EAG with nominal male and female EAG preparations on both polar and non-polar GC columns. Preparations were noisy with significant baseline drift. The only consistent EAG responses observed in all analyses on the polar GC column were at approximately 13 min and a smaller response at approximately 9 min. These retention times were equivalent to KI's of 1369 and 1212 respectively (Appendix 9).

In analyses on the non-polar column, a single response at approximately 7 min was observed with a possible response at approximately 5.5 min (Figure 9). These retention times were equivalent to KI's of approximately 850 and 790 respectively.

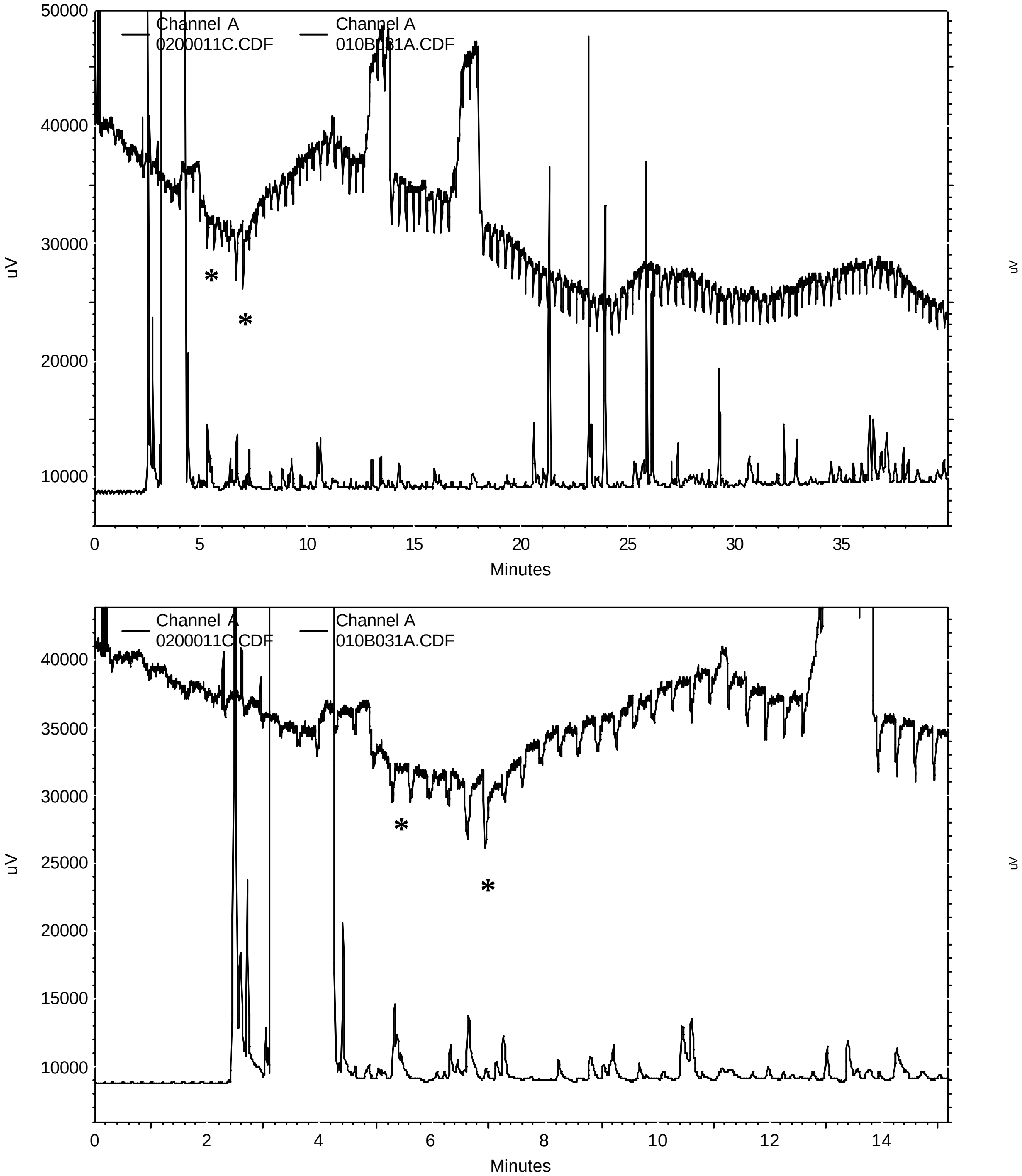


FIG. 9. GC-EAG analysis of volatiles from female *R. piercei* (2002/132/17) on non-polar column with nominal male *R. piercei* EAG preparation.

Examination of the corresponding GC-MS analyses carried out on a similar polar column indicated that the component responsible for the main EAG response at 13 min was (*Z*)-3-hexenol. Comparison of the retention indices of all but one of the hexenol isomers confirmed that the (*Z*)-3- isomer gave the closest fit on both polar and non-polar GC columns (Appendix 9).

Subsequent examination of the GC-MS traces indicated that the minor EAG response might be due to (*E*)-2-hexenal, and this was confirmed by comparison of retention times of the natural and synthetic compounds (Appendix 9).

Synthetic (*Z*)-3-hexenol elicited an EAG response from *R. piercei* in linked GC-EAG analyses (Figure 10). The (*E*)-3- isomer also elicited an EAG response and the presence of this may have been responsible for the double response often observed to the (*Z*)-3-hexenol.

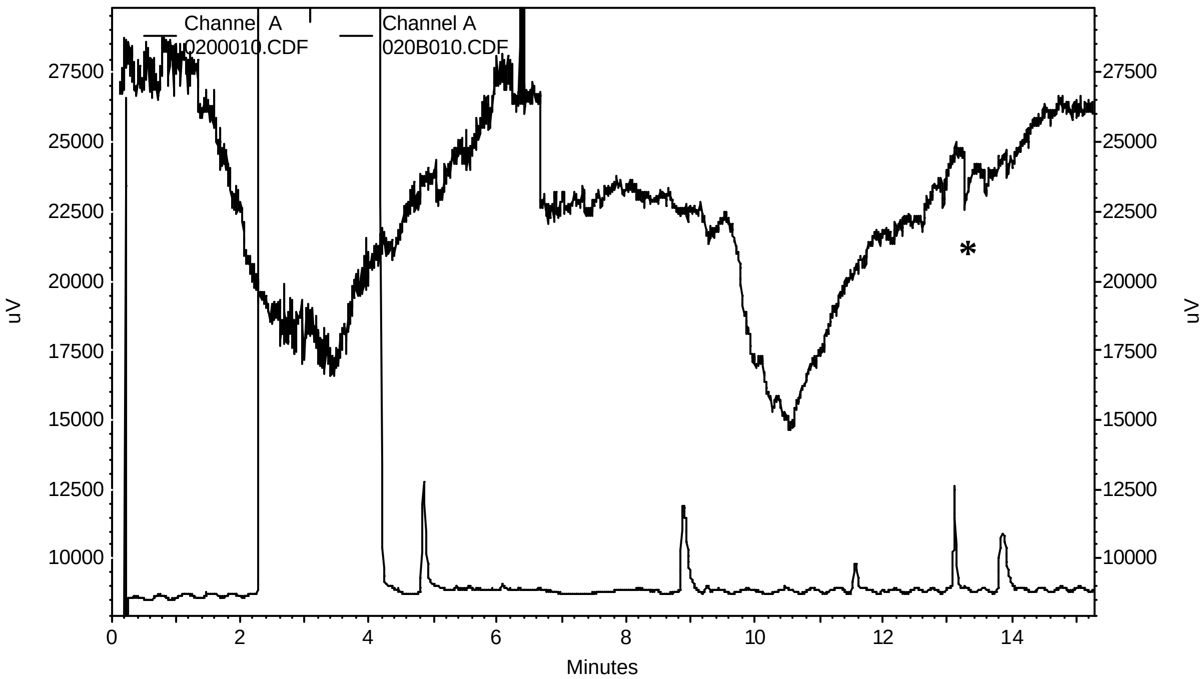
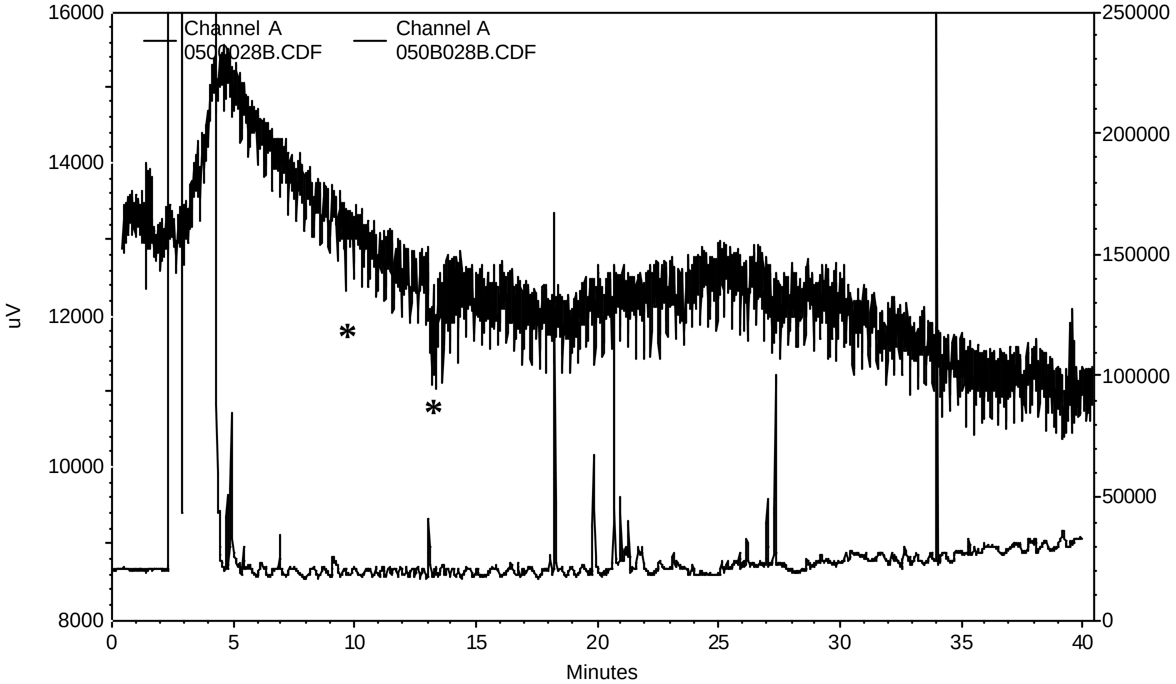


FIG. 10. GC-EAG analysis of synthetic (*Z*)-3-hexenol and hydrocarbon on polar column with nominal male *R. piercei* EAG preparation.

GC-EAG analysis of volatiles from female or male *P. latithorax* weevils using a *P. latithorax* female EAG preparation gave the same result as found with *R. piercei* (e.g. Figure 11). A major EAG response was observed to a component at 13.1 min and a minor response to a component at approximately 9.2 min. These correlated with peaks assigned to (*Z*)-3-hexenol and (*E*)-2-hexenal respectively in GC-MS analyses. As with *R. piercei*, the female *P. latithorax* gave an EAG response to both (*E*)- and (*Z*)-3-hexenol (Appendix 9).



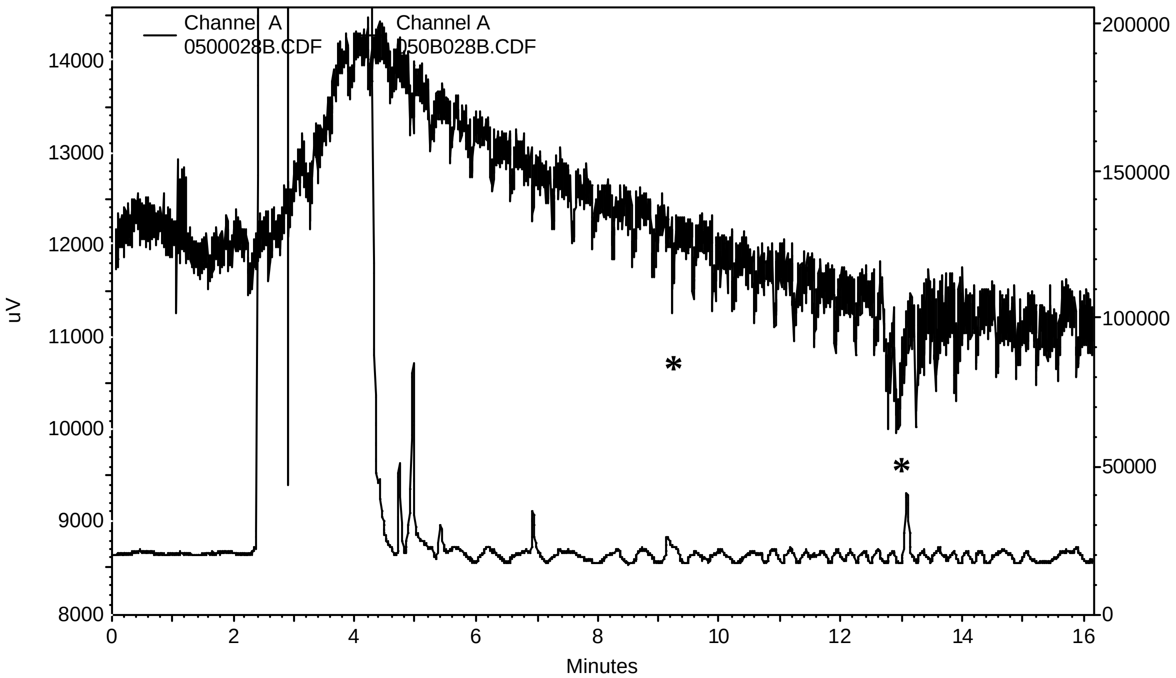


FIG. 11. GC-EAG analysis of volatiles from male *P. latithorax* weevils on polar column with female *P. latithorax* EAG preparation.

Field evaluation of trapping systems

Trap design experiments (Appendix 7)

The trapping experiment to compare different locally-made designs commenced at a relatively late stage of the season, hence overall catches mostly did not exceed 1 male or female *P. latithorax* per trap. Catches were highest in the plastic bottle traps (1 litre) but no significant treatment differences were noted ($P > 0.05$ for treatment effects, F-ratio, ANOVA – separate analyses for each sex) (Figure 12).

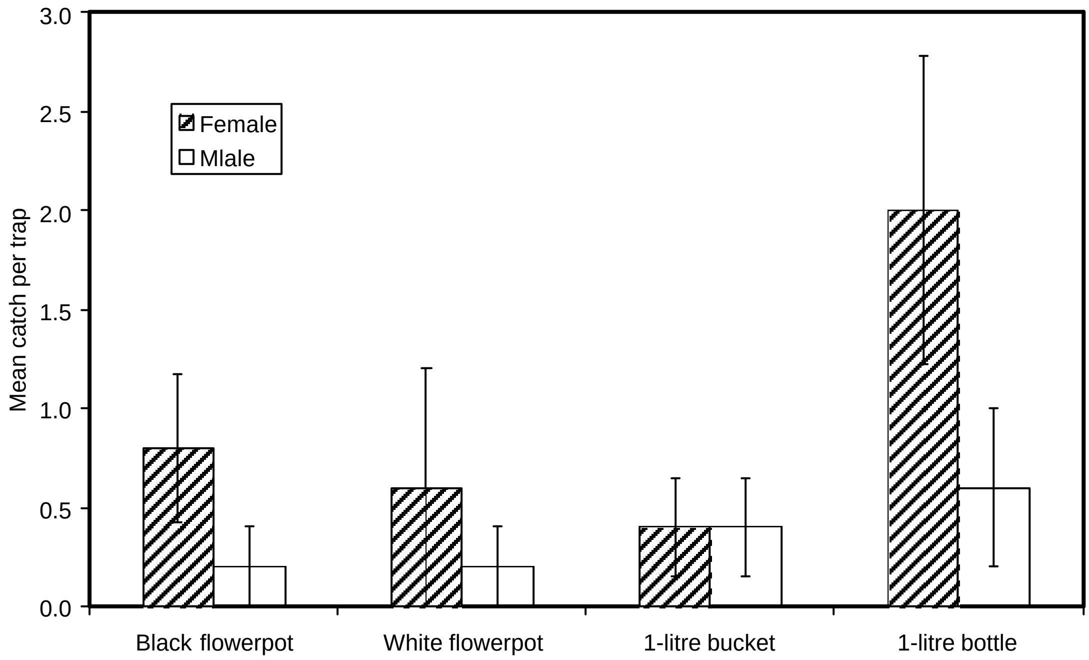


FIG. 12. Mean total catch (± S.E.) per trap of *P. latithorax* in different designs of pitfall traps baited with 1 – 2 fresh potato leaves at Toralapa, 11 February - 19 March 2003 (5 reps).

The plastic bottle traps were the deepest or highest of the four designs. This hampered the retrieval of trapped weevils, and was considered by PROINPA staff to be a practical disadvantage. Against this the screw-top nature of the bottle obviated the need for an elastic cord to retain the lid, as was the case with the two flowerpot designs. In addition, there was some evidence from the laboratory test of the retention efficiency that the bottle trap was most effective at preventing escape of *P. latithorax* weevils (Appendix 7). No *R. piercei* escaped from any of the trap four designs in the laboratory test.

Development of dispensers for (Z)-3-hexenol and (E)-2-hexenal (Appendix 10)

Release from the polyethylene sachets was constant and rapid (Figure 13). (*E*)-2-Hexenal was all released within 2 days and (*Z*)-3-hexenol within 20 days at 27°C.

Release from the polyethylene vials was much slower and did not start for 2-3 days while the material penetrated through the vial wall. (*Z*)-3-hexenol was released at constant rate over the 48 days of the experiment. Release of (*E*)-2-hexenal was also constant, but rates from different vials seemed to vary much more than has been found with other compounds. The experiment was repeated with the same result (Appendix 10).

It is difficult to seal the polyethylene vials completely reliably. In other work, leakage has been prevented by inserting cotton wool into the vial which absorbs the liquid material but does not affect the release rate (e.g. with 1-octen-3-ol). This was found to be the case with (*Z*)-3-hexenol (Figure 14) but not with (*E*)-2-hexenal which was essentially not released at all from vials with cotton wool (Figure 15).

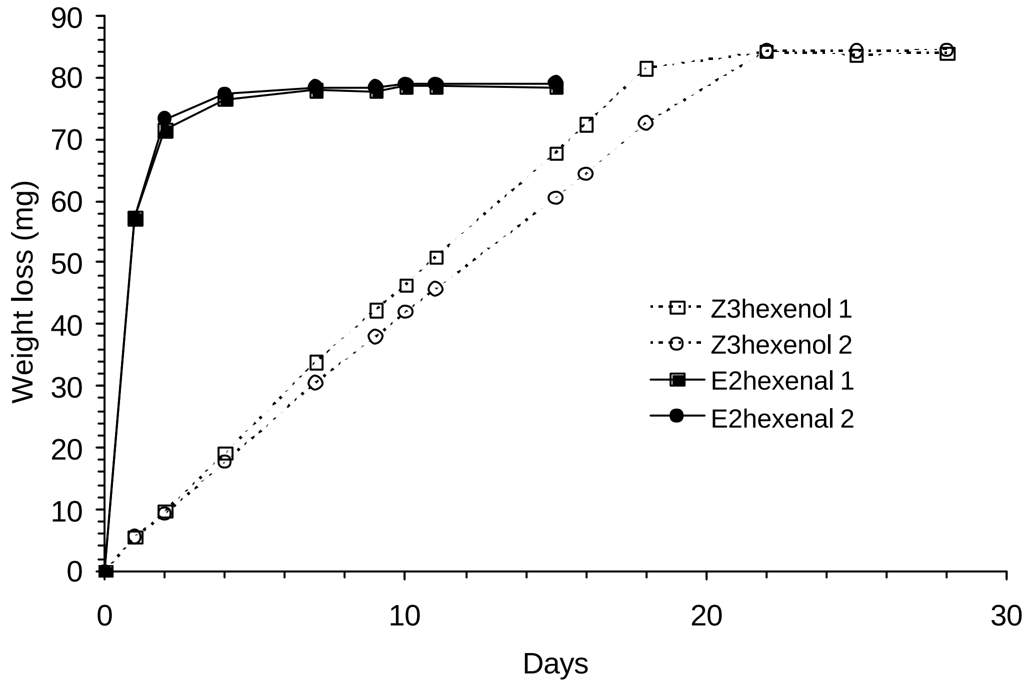


FIG. 13. Release of (*E*)-2-hexenal and (*Z*)-3-hexenol from polyethylene sachets in laboratory windtunnel.

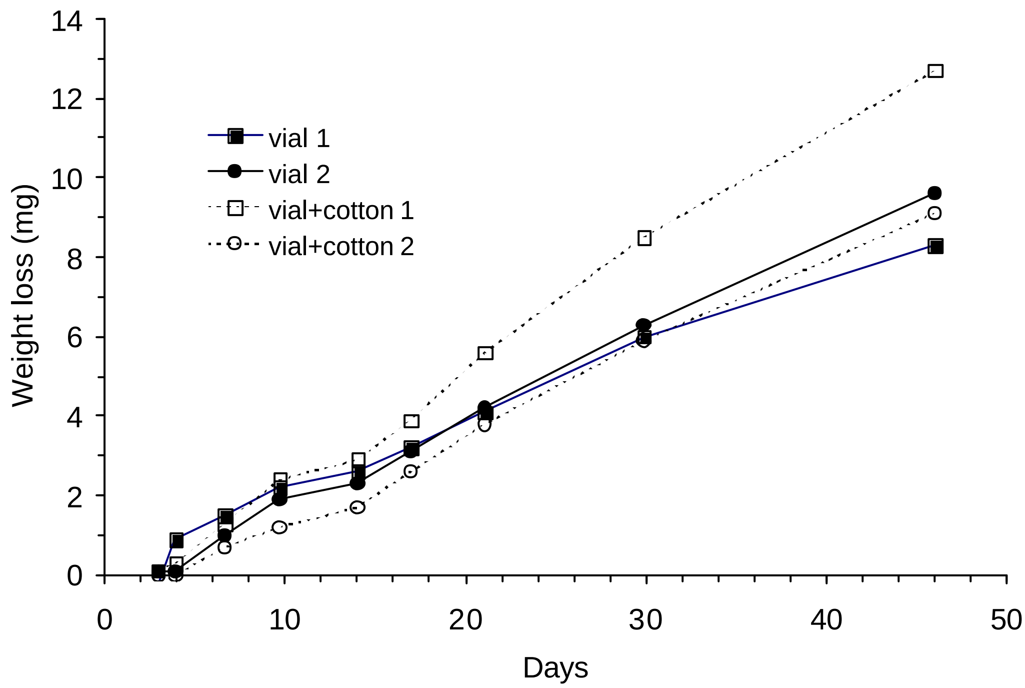


FIG. 14. Release of (*Z*)-3-hexenol from polyethylene vials with and without cotton wool in the laboratory windtunnel.

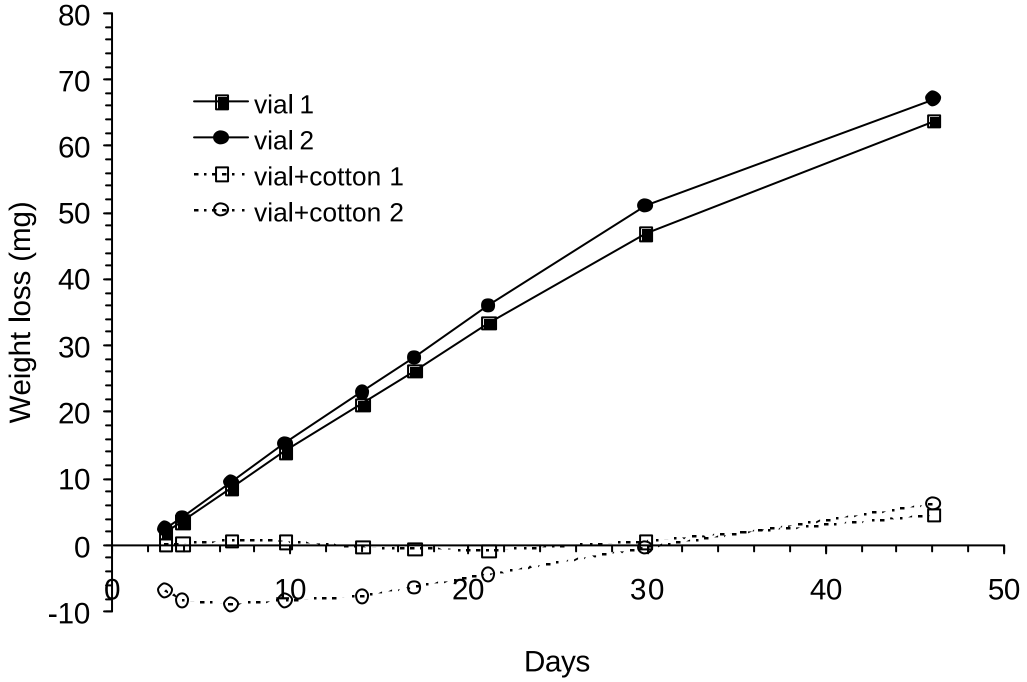


FIG. 15. Release of (*E*)-2-hexenal from polyethylene vials with and without cotton wool in the laboratory windtunnel.

Release rates measured by trapping of volatiles and GC analysis were in reasonable agreement with those measured by weight loss (Table 13).

TABLE 16. SUMMARY OF MEASUREMENTS OF RELEASE RATES OF (*E*)-2-HEXENAL AND (*Z*)-3-HEXENOL FROM POLYETHYLENE VIALS AND POLYETHYLENE SACHETS IN THE LABORATORY WINDTUNNEL MEASURED BY WEIGHT LOSS OR TRAPPING OF VOLATILES.

	Weight loss (mg/day)			Entrainment (mg/day)	
	sachet	vial	vial/cotton	sachet	vial
(*Z*)-3-hexenol	4.28	0.19	0.24	3.97	0.17
(*E*)-2-hexenal	36.20	1.42	0.12		1.31

Trapping experiment with synthetic host plant volatiles (Appendix 7)

Trapping trials against *P. latithorax* were carried out during December 2003 – March 2004 in farmers' fields with the proposed synthetic attractants, (*Z*)-3-hexenol and (*E*)-2-hexenal. The compounds alone or combined, dispensed from vials or sachets were compared with fresh potato leaves and an unbaited trap. Mean catches in this experiment were somewhat higher than previous seasons, averaging around 20 males and 20 females per trap for most of the treatments, including fresh leaves. The blank control treatment produced less than half this figure but, due to some large variability in catches between replicate blocks, this difference was not statistically significant (P > 0.05, F-ration, ANOVA, separate analyses for each sex). Results are summarised in Figure 16.

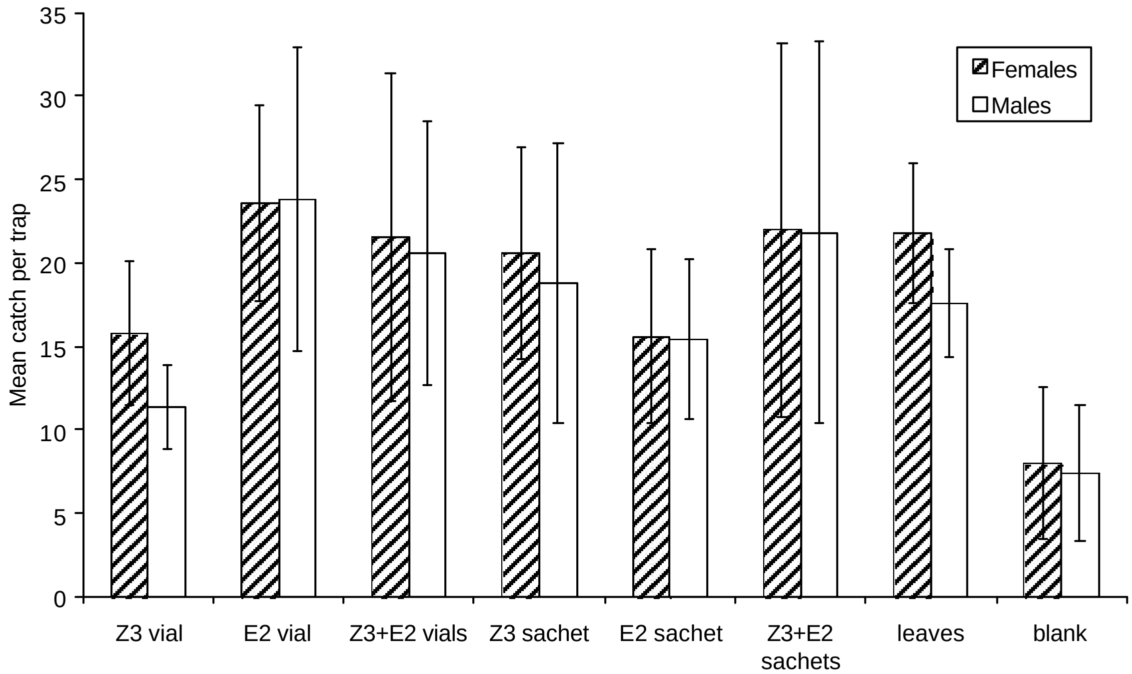


FIG. 16. Mean total catch (± S.E.) per trap of *Premnotrypes* in "white flowerpot" pitfall traps baited with synthetic host volatile lures at Toralapa, 3 December 2003 – 10 March 2004 (5 reps).

UPTAKE OF POTATO IPM TECHNOLOGIES PROMOTED AND ASSESSED

Baseline evaluation of potato production by smallholder farmers and constraints

Baseline data was collected in three communities - Candelaria, Cebada Jichana and Lope Mendoza. The study was completed in 2003 and the full report is at Appendix 11.

Socio-economic aspects

In the three communities selected for this study there were approximately equal numbers of females and males, 47% and 53% respectively. The majority of the population was between 12 and 25 years old and typically there were four people per household.

In these communities, migration was at a very low level in Cebado Jichana and Candelaria (10%) and slightly higher (approx 20%) in Lope Mendoza. In general it was the men who migrate and typically for less then one month.

Regarding the level of education in the three communities, the majority had received education at basic or primary level followed by intermediate level. Only in the Lope Mendoza community was it observed that 5% of the population had received no formal education at all.

Productivity aspects

For more than 90% of the population in each of the three communities the principal source of income was agriculture, particularly potato cultivation. Potato was the main crop both for food and as a source of income. The most widely cultivated variety was Waycha: this is indigenous with long growth cycle and the most marketable. This is followed by the Runa Toralapa variety, also for its marketability, and by several other native varieties for local consumption, especially in Candelaria. The majority of farmers use their own seed or renew occasionally by buying seed from institutions such as PROINPA and SEPA, or at local markets.

In Cebada, Jichana and Lope Mendoza, 70-90% of the farmers have access to irrigation which allows them to cultivate potatoes two or three times per year. In Candelaria only about 5% have access to irrigation, but this does not prevent them from producing potatoes two or three times per year as in the other two communities because it is in zone with sufficient rainfall.

As for pests and diseases in potato cultivation, blight is highlighted as most important, and more recently insects, particularly Andean potato weevils and potato tubermoth. Blight is a problem throughout the life cycle and causes severe losses if not controlled.

The insects are equally damaging. The farmer notices damage due to weevils during growth and at harvest. In contrast, the potato tubermoth is most important in stores and in seed potatoes, and hence ranks in fourth or fifth place.

Knowledge of pests

Investigation of the knowledge of farmers about the life cycle of Andean potato weevils showed that a high percentage (85-95%) recognised the larval stage, a lower percentage (50-74%) recognised the adult, very few, if any, had seen the eggs or recognised the pupa, and 10-43% of the farmers questioned related the larva and adult. Similarly, very few of the farmers were clear on the origin of the populations of weevils which infested their potato plots.

The situation was similar with potato tubermoth where 50-95% recognised the adult, 81-95% recognised the larva and only 43-65% related the larva and adult. Also the majority of farmers did not know where the tubermoths in their stores came from.

These data provide an understanding of the lack of knowledge of the farmers concerning the biology and habits of the pests, knowledge which is essential for implementation of IPM for control of these pests.

The damage caused by APW is greater in Cabado Jichana and Lope Mendoza alto, particularly at the time of sowing and harvesting during January-May, due mainly to the life cycle of the pest. In Candelaria the damage caused by this pest is less than in the other two communities due to the topography which makes movement of the pest difficult.

The damage caused by this pest is reflected directly in the quality of the potato at harvest and this has a great influence on the price in the market. The price for batches of potatoes with a small amount of weevil damage can be reduced by 20% or more, and batches with more severe damage cannot be sold. This causes a large economic loss for the producer.

The major damage by potato tubermoth occurs in stores to the stored seed potatoes, with losses between 22-85%. This also results in serious economic loss for the farmer.

Faced with these problems, the farmer looks for means to control these pests and diseases and to reduce the damage caused. For this they resort to pesticides, the most common being Karate, Perfectthion and Tamaron. The cost per hectare can be between 45-250 bolivianos, depending upon the product used and the number of applications. It should be mentioned that in the majority of cases the application of pesticides by the farmers is badly organised, at the wrong time and without knowledge of the biology and habits of the pest. As a result, the control achieved by application of pesticides is not high in the majority of cases.

The situation is similar with potato tubermoth where some of those questioned use Karate for control in the stores.

In the majority of cases the application of pesticides is carried out by the men. In a few cases (up to 10%) application of pesticide is carried out by the women. Most often when the plots are near the house this is the responsibility of the women and when the plots are far from the home the men are responsible.

When questioned about natural enemies, it is apparent that the farmers associate the term with predators such as chickens and other birds, and, in a few cases, ants. Only one farmer in Candelaria mentioned use of MATAPOL-PLUS (product supplied by PROINPA) as a biological control agent for PTM.

Knowledge and use of IPM technologies

A very small group of farmers know and apply several of the components of IPM strategies against weevils and tubermoth, and not all of them apply this knowledge. This was observed among those farmers who at some time have had contact with technical staff from institutions which worked or are working in their region. In contrast, the majority of farmers know about and use pesticides for control of pests and diseases which they have learned from institutions, from their neighbours or from pesticide agents in the local markets.

Environmental and health aspects

This section takes account of the direct effect of pesticides on the people who use them, the effects on the families and the effect on the community. In the communities of Cebada Jichana and Candelaria cases of poisoning by pesticides were reported, in one case involving the farmer who applied it and in another a member of his family.

Under this aspect it was found that in the three communities a very small percentage (< 10%) was familiar with the categories of pesticides and the colours of the wrappers which distinguish them. The same was true for the procedures for handling and storage of the pesticides.

Promotion of IPM and investigation of acceptability of biological control agents

The above findings lead us to believe that efforts at training in IPM strategies must be directed at both men and women to have impact on the community.

Given that 63% of the population is young (up to 25 years old), it is important to direct training at this group. Moreover, imparting knowledge to this group is much easier and with a high potential for success for the future of local agriculture.

In order to maximise this impact and to reach all the family, projects on training in IPM strategies should be introduced into the educational system, considering the numbers of young people who attend courses at fifth basic and intermediate levels who can become intermediates and agents of change for educating all the family.

The results obtained from this study lead us to plan training events on different themes, including:

- Biology and habits of APW;
- IPM strategies against APW;
- Biology and habits of PTM;
- IPM strategies against PTM;
- Proper use of pesticides;
- Biological control in IPM strategies

Under proper use of pesticides, it was necessary to assume that the knowledge of the farmers is very basic and the majority have no knowledge at all, so it was necessary to introduce this theme at a very basic level.

The training will need to be planned with different goals and methods, depending upon the group concerned (formal manuals, outline manuals to support educational improvement, support manuals for rural trainers, etc.). It is also necessary to produce support material as publicity leaflets relating to the training themes directed at the particular groups with which we are going to work.

In the three communities where we carried out the base line studies, Candelaria, Cebada Jichana and Lope Mendoza, eight training days were carried out on the different IPM components with emphasis on biological control of APW and PTM and on the rational use of pesticides. The above activities were attended by 168 men and 64 women.

Each session of farmer training was supported by set of slides and videos; in certain occasions live samples of APW and PTM were used (Appendix 12).

To support future farmers' training several leaflets on the biology and integrated management of APW and PTM were prepared. For the same reason and to provide technical material to field technicians a technical potato pest hand book was published (Calderon et al., 2004a, 2004b).

Assessment of project impact and future developments

Training activities within the rural schools were conducted in four communities (Huajllapujru, Cebada Jichana, Caña Kota and Boqueron k' asa), near Toralapa Centre, and for that purpose a teachers guide and the student notebook was prepared for technical personnel of the project and a group of six selected teachers. Considering the base-line results, four issues were inserted in this programme: the potato, the Andean potato weevil, the potato tuber moth and the safe use of pesticides. All these materials were prepared for students from fifth and sixth grade, since these students already work in their family plots (Appendix 13). Currently, this material has been validated with 131 students in order to improve it and have a final version for using in others rural schools where these issues are considered a problem.

Capacity building and training

NRI staff provided on-the-job training for PROINPA staff during field visits.

Luis Crespo of PROINPA visited NRI for a month and was trained in techniques for the identification, evaluation and enumeration of insect viruses. These included baculovirus counting, bioassay techniques, microscopic techniques for identifying BVs, molecular techniques for strain identification (including restriction endonuclease analysis and PCR) and BV purification. This training was to further trials work assist in the search for a new

S. tangolias virus and to build PROINPA insect pathology and molecular biology capacity. He also assisted in work on pheromone identification and formulation including GC-EAG and GC-MS work and assessment of formulations (Appendix 14).

CONTRIBUTION OF OUTPUTS

CONTRIBUTION OF OUTPUTS

A new liquid formulation of the *P. opercullela* granulosis virus developed, produced and promoted

Although liquid formulations of the PoGV have been developed, these formulations with the existing strains of PoGV are not effective crop protection agents for potato stores and the kaolin-based formulations currently in use cannot be replaced by liquid-only formulations. The research output has been achieved, although not contributed directly to the project purpose. However, the results have highlighted the hitherto unrecognised importance of the kaolin in the powder formulations and possibilities for improving this formulation further with a faster-acting strain of the PoGV.

A new viral control agent for *S. tangolias* identified, developed and promoted

A virus specific for *S. tangolias* has not yet been found. Collection protocols and approaches to analysis of the samples have been improved and refined, and work is continuing on the samples collected during this project. As this species is now the dominant species of potato tuberworm moth in many regions of Bolivia and other S. American countries, the finding of a specific virus against this species would be a significant contribution to achieving the project purpose.

Pheromones and host plant attractants for *P. latithorax* and *R. piercei* developed and use strategies developed and promoted

Research in this project has proved conclusively that the Andean potato weevil species, *P. latithorax*, *P. suturicallus* and *R. piercei* do not use intraspecific pheromones, contrary to previous reports with related species. They do respond to volatiles from potato leaves and two components of the volatiles which elicit EAG responses from the weevils have been identified. These have been shown to attract weevils to traps in farmers' fields, and convenient locally-made traps have been developed. Despite the initial setback of not finding pheromones for the weevils, considerable progress has been made in identifying attractive components of the host plants. In order to contribute fully to the project purpose further work is required to optimise the attractiveness and validate the use of the traps for controlling the pests with farmers.

Uptake of potato IPM technologies promoted and assessed.

A base-line survey of farmers' perceptions of pests and diseases of potatoes in three communities was carried out. Resulting from this, training days were carried out in these communities on the different IPM components with emphasis on biological control of APW and PTM and on the rational use of pesticides. This output has been achieved and contributes directly to the project purpose and goal. Work was also started on education of children in rural schools in IPM of potato pests using specially designed textbooks. Children and women are often responsible for potato cultivation and the schools provide an untapped opportunity for introducing large numbers of children to the concepts of IPM at an early age and facilitate a way to reach and be more convincing with parents.

The project also provided capacity building in the collaborating organisations. During the project local research staff at PROINPA and CIP were trained in molecular techniques for virus identification and various aspects of the identification, evaluation and use of semochemicals.

FUTURE WORK

Pheromones and host plant attractants for *P. latithorax* and *R. piercei* developed and use strategies developed and promoted

Further work is required to optimise the attractiveness of the traps for Andean potato weevils and to explore with farmers the use of these for reducing weevil damage. Various trapping systems have been used by farmers previously, so the approach is not new but needs optimising and verifying.

Traps developed during the project are made from locally-available plastic containers. The chemicals are readily-available from commercial suppliers and the dispensing systems relatively easy to source and produce. PROINPA has already produced and distributed the PoGV powder formulation "Matapol" on a semi-commercial scale through a commercial subsidiary BioTop, and it is anticipated that a similar approach could be taken for production and distribution of traps and lures for weevils. Alternatively, other commercial companies in Bolivia might be involved.

Uptake of potato IPM technologies promoted and assessed

In order to enhance further the dissemination outputs of this project, the materials developed for education of children in rural schools need to be disseminated and validated by the pupils.

The aims of the project were achieved, but it is also important that local organisations in the Andean Region have materials and expertise required to continue development and adaptation of the outputs of this project on improved methods for control of Andean potato weevils and potato tubermoths into an Integrated Pest Management Strategy.

Future proposals

PROINPA has already submitted a proposal for funding under the SIBTA Proyectos de Innovacion Tecnologica Applicada (PITA) scheme. This is in direct response to demand expressed in an exercise by the DFID INNOVA project from small-scale farmers in Umala and entitled "Improvement of the production and quality of cultivation of potato in the Municipality of Umala by integrated crop management with emphasis on control of pests". This will focus on potato tubermoths and weevils and take up and develop outputs of the present project in a farmer participatory research programme.

PROINPA has also submitted a proposal to the McKnight Foundation in response for their request for projects on improving food-security in the Andes. This would assist in further development of the weevil traps in farmer participatory research programmes in Bolivia. A pre-proposal was accepted for development as a full proposal which has just been submitted.

A request has also been submitted to the DFID CPP for additional funding to carry out aspects of the above recommendations for future work.

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