Appendix 5: Final Technical Report: R7886
Crop Resistance to Andean Weevil in Bolivian hillside potato Production

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Executive Summary

This project has completed the evaluation the potential of anti-feedant genes, available to PSP on a royalty-free basis, for the control of Andean potato weevils (APW). These insects are a severe problem for Bolivian and Peruvian subsistence potato growers at altitudes above 2,800m. Tuber damage by APW influences both nutritional and commercial value of tubers. Previous work in Peru has established that levels of c50%, 25-50% and 1-25% cause loss in market values of potatoes of 67%, 50% and 17% respectively. There has been much effort in both by scientists in both USA and Australia at developing cultural control practices. They are infrequently adopted by farmers because they are time consuming, or require materials that are not affordable. One option growers favour in Peru is chemical control and there is farmer pressure for this approach within Bolivia. Several pilot studies have shown tuber infestation levels of 30-50% can be reduced to c15% by Integrated Pest Management (IPM). The aim of this work is to assess the potential of new traits for APW resistance to be used within IPM for full control of weevils.

In total three sets of experiments funded by DFID now suggest that Kunitz Soybean Trypsin inhibitor has adverse effects on an Andean weevil. Scientists at the International Potato Centre (CIP) reported the initial finding but PSP sought their verification by a second lab. Lines identified as promising at CIP expressed wheat α-amylase inhibitor plus soybean Kunitz trypsin inhibitor (SKTi) or just the proteinase inhibitor alone had efficacy. A second group of experiments in Centre for Plant Sciences at Leeds as an adjunct to another grant (R6830) confirmed efficacy for SKTi.

The results have now been extended in small grant of £7.5k. They establish that SKTi reduces the fecundity of female weevils. Females that consume SKTi producing 50% less eggs than females fed control potatoes. The effect is restricted to feeding as larvae. On the two previous occasions the effect was 65% (at CIP) and 80% (at CPS, Leeds).

Overall it is clear that SKTi expressing potatoes provides an important resistance trait of value within an integrated management of weevil pests of potato for subsistence cropping systems in the Andean region. Such potatoes do not require modifications to farmers' existing management practices and could be provided at no additional cost and may provide environmental benefits through a reduction in insecticide use.

This approach to weevil control may also have potential for control of weevils on other crops such as banana.

Background

Fig 1: *Rhigopsidius tucumanus* (an andean potato weevil) adult feeding on a potato leaf (Left) and a larvae and feeding damage within a potato tuber (right).

All APW species migrate into potato crops as adults (only *P. muriceus* can fly) and feed nocturnally on leaf margins. They lay eggs close to tubers and larvae develop within tubers. Pupae of *Premnotrypes* spp and *P. muriceus* overwinter in soil whereas those of *R. tucumanus* remain in tubers. The tuber damage APW causes influences both the nutritional and commercial value of tubers. Key IPM tactics include: (1) *grower education* (knowledge of life cycle) (2) *disease escape* (both sowing and harvest dates), (3) *cultural control* (tillage to expose pupae, lupin barriers around potato crops), (4) *biological control* (nocturnal manual picking of adults from plant, chickens to glean pupae and the fungal pathogen *Beauveria*, to create a soil epizootic among larvae emerging from tubers) (5) *physical control* (ditch traps around stores and potato field) and (6) *chemical control* (organophosphate, carbamates and chlorinated hydrocarbons). Unfortunately attempts to implement new aspects to IPM are failing and growers are choosing to rely on insecticides (CIP and PROINPA reports).

Scientists at the International Potato Centre (CIP) reported antifeedant lines had value against weevils but not potato tuber moth but PSP sought their verification by a second lab. Lines identified as promising at CIP expressed wheat α-amylase inhibitor plus soybean Kunitz trypsin inhibitor (SKTi) or just the proteinase inhibitor alone had efficacy. A second group of experiments in Centre for Plant Sciences at Leeds as an adjunct to another grant (R6830) confirmed efficacy for SKTi. We previously showed that one gene (soybean Kunitz trypsin inhibitor; SKTi) has anti-weevil activity when expressed in potato tubers. Females of the Andean Potato Weevil, *Rhigopsidius tucumanus*, which had fed on SKTi-expressing tubers, as larvae were less fecund than females that had fed on control Desiree tubers as larvae. The effect was most pronounced when larvae fed on tubers expressing only SKTi, although SKTi in combination with an α-amylase inhibitor (PWK lines) also reduced female fertility (Fig. 2). Some lines were included in the experiments that were not reported in earlier work at CIP to be effective including ones expressing both an α-amylase inhibitor and snowdrop lectin (PWG lines).

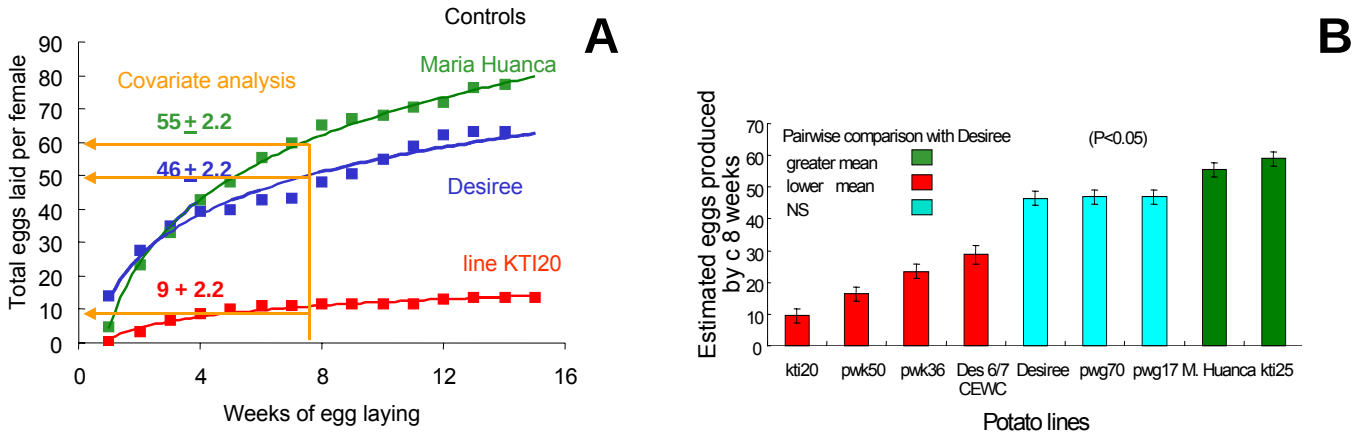

Fig.2 A. Representative curves of accumulative eggs laid per female showing the time point of comparison used in covariant analysis. SKTi4 20 is expressing SKTi. **B**. The estimated egg production at 8 weeks for different lines as determined using co-variant analysis which subdivided the lines into three sets. Each mean is compared with that for untransformed cv Desiree.

Project Purpose

Plant genes conferring resistance to pests identified and incorporated into adapted genetic backgrounds of target crops. This project completes evaluation of the potential of soybean typsin inhibitor for control of Andean potato weevils.

Research Activities

In this small project we have continued work with the lines shown in our previous work (Fig 2) to be most active against the weevil (SKTi 4 20, PWK6 36, PWK6 50). The new experiments have now determined the level of control that is provided when both larvae and adults of *R. tucumanus* feed on tissues expressing SKTi or SKTi and α-amylase. Adults were collected in Bolivia and sent to UK. Their occurrence is seasonal and their late arrival in Leeds ensured the experiment we were not completed until the end of 2001. This report is therefore necessarily late by three months.

1 2 3 4 5

Fig 3: Western blot of Desiree for SKTi recovered from leaves. **1**, SKTi 4-20; **2**, SKTi 4-25; **3**, PWK 6-36; **4**, PWK 6-50 and **5**, Wildtype Desiree. Expression levels were confirmed to be at 0.2-0.3% tsp and 20.1k Mr by standards (not shown).

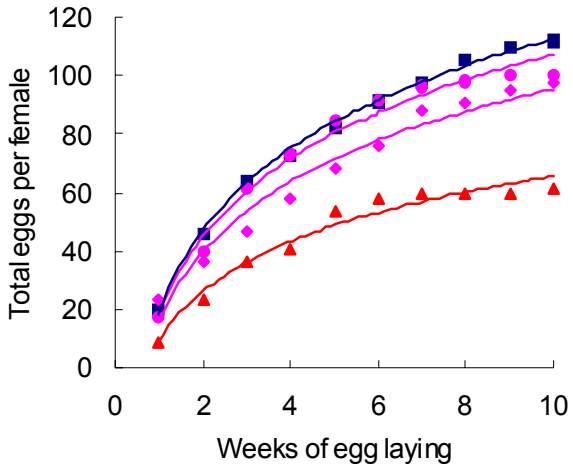

Fig. 4. Total egg production per female on control (■) or transgenic Desiree lines expressing SKTi 4 20 (▲) or SKTi and α amylase inhibitor (PWK 36 (◆) & PWK 50(●).

Larvae were reared on tubers of one of four lines (Desiree WT, SKTi 4 20, PWK6 36, PWK6 50). Western blot confirmed that all three lines used expressed SKTi at c0.2-0.3% tsp (Fig 3). When adults emerged they were fed leaves corresponding to the line on which they were reared as larvae. The results from this study confirm our previous findings that the best SKTi line from our earlier work (SKTi 4 20) is the most effective line in reducing weevil fecundity. After 8 weeks of egg laying females on this line had produced ~50 eggs whereas those on the other two transgenic lines and the WT Desiree had produced ~90, 97 and 100 eggs respectively (Fig. 2).

Egg production per female was used to calculate the level of resistance for each line. Fecundity of females feeding on SKTi 4 20 was reduced by 50% relative to that of control females (Table 1).

Line	Effectors	Mean total egg production (± SEM)	% resistance
Wildtype Desiree	None	99.5 ± 59.2	0
PWK 6 36	SKTi + α amylase genes	96.7 ± 43.0	3
PWK 6 50	SKTi + α amylase genes	80.7 ± 50.4	19
SKTi 4 20	SKTi	50.0 ± 20.6	50

Table 1: Egg production per pair when fed on either control or transgenic Desiree tubers and leaves.

We have used Gompertz curves to estimate total fecundity per female and establish a severe decline in egg laying rate (Fig. 5). This suggesting that most eggs had been laid when the experiment was terminated to complete this report.

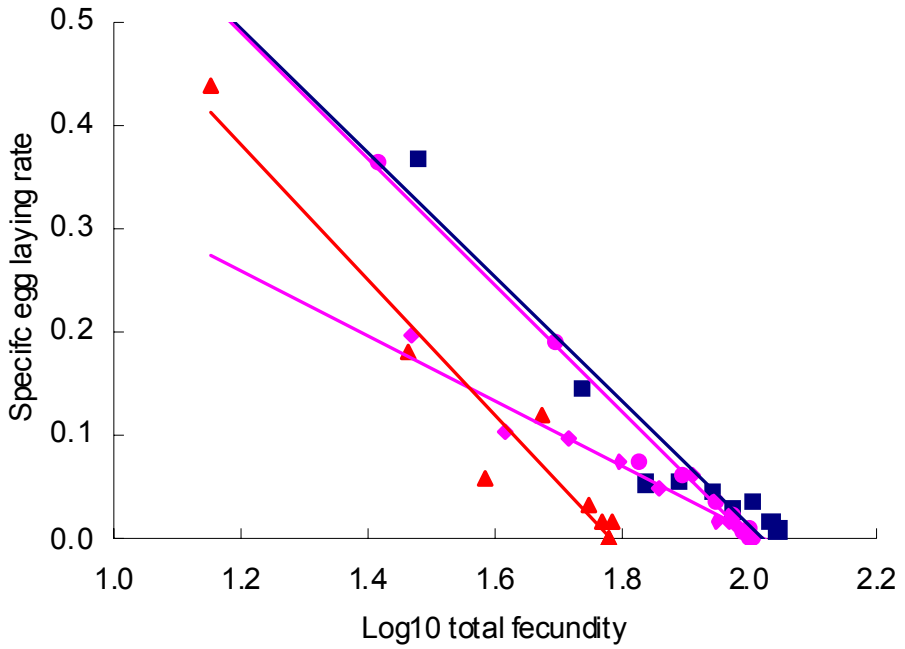

Fig. 5. Estimated total fecundity (Gompertz curve) when fed on either control (■) or transgenic Desiree expressing SKTi 4-20 (▲) or SKTi and α amylase inhibitor (PWK 36 (◆) & PWK 50(●).

Females on lines other than PWK 6 36 showed parallel decline in new eggs laid as they approached their maximum fecundity. The difference is that line SKTi 4 20 showed a lower egg-laying rate throughout the egg laying process. PWK 6 36 differed in also showing a lower initial egg laying rate but it declined less quickly as initial egg number accumulated. The curves were used to calculate the estimated total fecundity per female and the overall predicted reduction in fecundity due to consumption of transgenic tissue. The maximum reduction was predicted for females fed SKTi 4-20, they were predicted to be 58% less fecund than females fed untransformed Desiree. One aspect of the results is difficult to resolve. At the time of western blots (Fig 3) all three transgenic lines used in the experiments plus SKTi 4 25 used earlier showed similar expression levels of SKTi. However only one line SKTi 4 20 consistently shows good levels of resistance. This may reflect difference in levels of expression in tubers where larvae occur. More work would be required to resolve this issue.

Comparison of Figs. 2a and 4 suggests that exposure to proteinase inhibitors during the larval stage rather than adults is important for to suppress the fecundity of *R. tucumanus*. Consumption of green tissue expressing transgenes by adults seems not provide additional levels of resistance to the weevil. The results might indicate that the larvae accumulate much of the protein required for egg laying. Another possibility is a change in digestive proteinase class important to the two stages. Trypsin-like activity has been detected in several weevil species, such as rice weevil, sweet potato weevil and the citrus weevil. Other curculionids have complex proteolytic systems with proteinases of different mechanistic classes, including cysteine proteinases. Feeding weevil larvae with trypsin inhibitors has previously been shown to retard growth and development as reported in this work. The apparent lack of effect on adult weevils may be due to a different complement of digestive enzymes in the different life stages. Another possibility is that the adults

circumvent the effect of PIs by physiological changes, such as production of inhibitor insensitive proteases.

Previous studies have shown that the effect of proteinase inhibitors (PIs) is most pronounced when combinations of PI are provided in the diet. In the present study the combination of a serine proteinase inhibitor with a wheat α-amylase inhibitor (PWK6 36, PWK6 50) failed to provide additional benefits over plants expressing SKTi only and may reflect the lack of importance of α-amylases in weevil digestion. Several weevil species have been shown to possess cysteine proteinase activity in their digestive tract and the expression of cystatins (cysteine proteinase inhibitors) in combination with serine PI's in transgenic plants may hold promise for increasing the levels of resistance to *R. tucumanus*.

Outputs

An assessment was completed of the potential of anti-feedant genes in transgenic potato against different weevil lifestages under containment conditions. The results establish that soybean trypsin inhibitor has potential fro control of Andean Potato weevils as one tactic within an integrated pest management schemes that also involves other approaches that growers will adopt. The findings will be submitted to an international scientific journal in 2002.

Contribution of Outputs

The outputs contribute towards DFID's development goals by providing an approach that can eliminate the current damage caused by Andean potato weevils when all the control options grows are willing to adopt are implemented. Implementation would also lessen the impact on resource poor farmers of pesticide purchase and the attendant risks and environmental harm of pesticide application.

The best performing line in the current study produces a 50-80% reduction in egg laying by female *R. tucumanus* in three different experiments. This suggests that SKTi has potential to contribute to IPM of weevil pests of potato. Successful IPM of a major constraint of potato production would effect poverty reduction and mitigate environmental problems resulting from the extensive use of insecticides.

Our previous work has shown that cystatins (cysteine proteinase inhibitors) available at Leeds do adversely affect *R. tucumanus* fecundity (Fig. 2; Des 6/7 CEWc). Plants expressing serine PI and cystatins are also available and could be tested against *R. tucumanus* as a development of the current work. Such plants may represent a more durable approach to APW management. The combination of PIs would lessen the consequences of the weevils demonstrating switches in proteinase activity that circumvent the effects of a single PI.

Now data collection is complete the work to be submitted for publication to an international scientific journal

a. What further market studies need to be done?

- Biosafety work to ensure the approach is safe and not a risk to the environment. This would need to be completed before further trials could be contemplated.
- The potential of the control of banana weevils by the approach requires evaluation

b. How the outputs will be made available to intended users?

- The technology has been donated to DFID.
- A first step would be to transform the potato cultivars grown by Bolivian farmers with the new traits.
- Once this had been achieved then the new cultivars could be distributed through seed producing charities (e.g. SEPA) or other organisations (e.g. PROINPA).

c. What further stages will be needed to develop, test and establish manufacture of a product?

- Field work is required to determine efficacy in the Central Andes within an IPM scheme that growers will adopt.
- A current constraint here is the release of GM plants into the field in Bolivia.
- IPM studies to determine the value of the contribution the plants provide to APW control within an integrated pest management system.

d. How and by whom, will the further stages be carried out and paid for.

- The work could transferred to CIP and later phases could be carried out by PROINPA and SEPA.