Crop Resistance to Nematodes and Insects in Bolivian Hillside Production

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Executive Summary

The two nematode pests of potato in the *Alti Plano* and *Valles* of Bolivia are *Globodera* spp (potato cyst-nematode) and *Nacobbus aberrans* (Rosary nematode or false root-knot nematodes). They have been ranked by Bolivian scientists and socio-economists as 3rd and 1st equal respectively of pests and diseases of potato in Bolivia. *Globodera* can be controlled by rotation but most of the 400,000 small farmers in Bolivia lack the land area to accommodate the 5-6 years break between potatoes that this approach requires. Such infrequent cropping does not harvest sufficient potatoes to provide the rural poor's calorie need from this crop. There is no currently effective method of controlling *Nacobbus* when host weeds prevail in the field between potato crops.

This work has shown unequivocally that cystatins can provide partial resistance against both nematodes. In contrast, no natural resistance is effective against both nematodes. Efficacy was shown for *Globodera* in 2 containment and 2 UK field trials. Furthermore transgenic resistance was obtained using more than one cystatin and with more than one cultivar. Transformation was achieved of both *S tuberosum tuberosum* and a hybrid of this subspecies with *S. tuberosum andigena*. The latter subspecies has proven difficult to regenerate and transgenic lines have yet to be generated using it. This work and further development of constructs is being continued within R7294.

Bolivia has limited experience with the transgenic crops. This project has made a significant contribution to addressing biosafety issues in that country. It established standard operating procedures for work with GM potato. An incinerator was built and a glasshouse altered to improve its value as a containment facility. The project helped define Bolivian governmental approaches to the subject and was the catalyst for the DFID/CIP conference on GM potato for developing world needs in Manchester in June 2000. Field experiments without transgenic material demonstrated that male sterile potato can set seed when growing at least 20m from a cultivar that donated fertile pollen. This transfer did not happen in a subsequent year at another locality probably because of a lack of *Bombus* bees at that site. The biosafety hazard of gene introgression was addressed by transforming a male sterile cultivar (Revolution) and by making constructs and by generating male sterile potato plants by molecular means. The will be screened for male sterility in R7294. We treat as the primary priority the need to address by technology any biosafety hazard that can be identified. Work outside of the project has indicated the inherent food safety of the technology approaches we have developed.

It is our strongly held opinion that the anti-nematode transgenic technology should only be implemented within an IPM scheme. The level of resistance is already sufficient to prevent damage with the 3-4 year rotation that prevails in Bolivia. Further enhancement of the approach will be the main output from R7294.

The project also investigated the potential of some anti-insect potato plants developed by Pestax for which PSP has a royalty-free licence. The work built on some equivocal findings at the International Potato Centre (CIP). The lines were shown in this project and that at CIP to have little efficacy against potato tuber moth. However two lines expressing soybean Kunitiz trypsin inhibitor and the anti-nematode plants expressing a cystatin did show efficacy against the Andean weevil, *Rhigopsidius tucumanus*. The effect was on fecundity of females after they fed on tubers expressing the putative anti-weevil proteins. In addition there was some evidence for either reduced viability or readiness to hatch of those eggs that were laid. Overall the best line provided 80% reduction in the fecundity of the weevil and c90% control when the reduced egg hatch was also considered. These findings are to be confirmed by feeding adults on leaves of the transgenic plants in a small supplementary grant.

This project has identified promising anti-nematode technology of potential benefit to Bolivia and many other developing countries. This technology has already been donated to DFID. It also indicates that Andean weevils on potato in Bolivia and neighbouring countries could be controlled transgenically.

Background

Bolivia has the lowest GNP in South America and is the core geographical focus country for DFID in continental S. America (RNRRS, 1995-2005). IFAD of UN estimates 97% of the Bolivian rural population lives in extreme poverty, a proportion that compares unfavourably with even the poorest African States (Lindert & Verkoren, 1994). The highland regions of *Alti Plano* (high plain) and the *valles* (hillsides) are populated with small holding growers with freeholds created by land tenure reform in 1952.

Potato is the principal staple food for Bolivians. It provides 25% of agricultural consumption by Bolivian households costing 3-13% of total household expenditure depending upon social class. It provides 40-50% of total calories consumed by rural, highland households. Its planted area is about 11% of the total agricultural acreage involving over 400,000 small-farm families. The national yield was 603k metric tonnes in 1992 and potato is the number one ranked legally grown, crop. It and dried beans are the only crops showing an increase in production as population and income grow. Its production in 1970-72 outranked that for vegetables, maize, wheat, rice and other tuber crops combined! The two principal growing areas are the Departments of La Paz and Cochabamba in the *Alti plano* and *valles* regions respectively. They each have c25% of the national acreage. The subsistence growers in these area have mean, annual potato acreage's of 0.23 and 0.41 ha/family respectively. Differences in altitude, rainfall and irrigation ensure a continuous availability of either new or old (stored >45 days) potatoes at market.

This project is set within a context of clear development constraints. They are:

1. There are virtually no export opportunities for potato from Bolivia.
   - *Therefore crop improvement is aimed at benefiting the internal market in Bolivia.*
2. The high level of current consumption provides no opportunity for stimulating consumption per capita.
   - *Therefore increased production will destabilise current market values of surplus, cash income from potatoes for the rural poor.*
3. Improvements must emphasise agricultural improvements such as production costs or crop diversity and nutritional quality. The benefits of improved nematode control will be:
   - *Reduce acreage commitment to current potato production levels.*
   - *Reduce fertiliser needs to meet current yields*
   - *Free land from inefficient traditional IPM strategies such as fallow*
   - *Stimulate production of legumes etc. to enhance protein nutrition beyond the calorie security provided by potatoes.*

Project Purpose

Plant genes conferring resistance to nematode pests identified and incorporated into adapted genetic backgrounds of potato.

Evidence of demand: The technical staff of PROINPA rank *Naccobus* and potato viruses as first equal with PCN third in rank order of pests and diseases of potato in Bolivia. This provides the most reliable estimate available of the high importance of nematodes to potato cropping in this country. Only 22% of potato growers receive any technical assistance and approximately 36% are illiterate; only 16% of the Bolivian population receives secondary education. Nematodes do not provide clear symptoms at damaging densities. Extension advice is often not available and advisory leaflets cannot be read by many of the 400,000 small, family growers. They risk considerable losses from nematodes. Rotation and fallow are traditional cropping practises that ensure a reduction in nematode damage. They are widely practised. For Bolivia as a whole, a total of 34% of all growers had practised a three year fallow before growing potatoes in 1989-90 and they had forgone 45% of cropping opportunities in the three years prior to cropping by choosing fallow; 99% of all growers used fallowing and/or crop rotation. These practices are not solely for nematode control although they probably do indicate a traditional response to the nematode from observation that losses accrue from too frequent cropping.

Nematodes, and other cropping constraints, result in government estimates of the potato yield in Cochabamba to be 5 tonnes/ha. This is c20% of that possible in Bolivia and only 10% of that achievable in UK. Nematodes impose losses and probably enforce on traditional potato growing 2x the necessary area per year for potato production in small holdings. An objective of this work is to free land for crops offering improved nutrition for the rural poor.

Discussion between the applicant and Dr J. Franco (nematologist) and Dr G. Thiele (socio-economist) at PROINPA in October 1996 established the needs and benefits of the project.

Research Activities

Outputs

1. One or more cultivars resistant to *Nacobbus spp*.
2. One or more cultivars resistant to *Globodera spp*.
3. Establishment of guidelines for transgenic potato release.
4. Trial of nematode resistance under glasshouse and in the field .
5. Improvement of constructs to give optimisation of defences against nematodes.
6. Transgenic cultivars of potato produced against insects and tested under containment conditions

Summary of activities

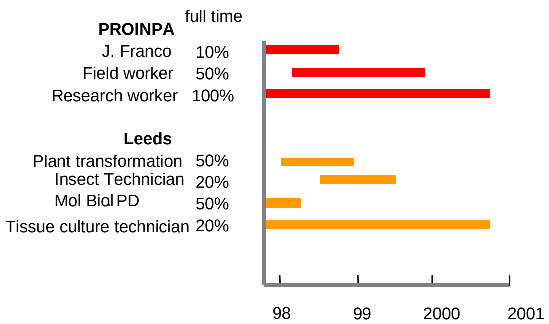


Fig 1: Summary of research effort

The grant award was £140,168. This was divided into 60% for CPS and 40% to PROINPA This project did not fund a high level of research activity at Leeds in 1999 or 2000 other than production and maintenance of transgenic plants (Fig 1).

1: Transgenic Resistance to Nacobbus

1.1 Development of constructs

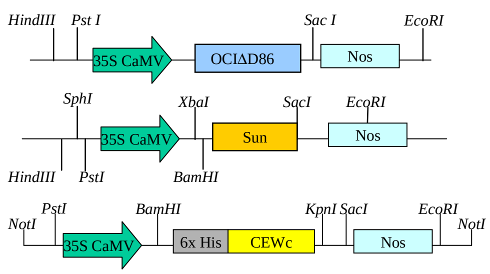


Fig 2: Constructs developed to containment and field trial in potato against nematodes.

Constructs were developed using cauliflower mosaic 35S promoter providing constitutive expression. It controlled expression of a protein engineered rice cystatin from rice (OcIΔD86), sunflower (Sun) and chicken egg white (CEWc). The latter will be phased out of the programme. It was used initially as a standard because of its known efficacy. All constructs contained a nos terminator (see Fig 2). All constructs were developed in pBi101.

1.2: Transformation of cultivars

Transgenic cv Desiree, Sante and Maris Piper were produced expressing Oc-IΔD86 (potato codon usage version), sunflower cystatin and chicken egg-white cystatin (CEWc) all under control of CaMV35S. Over 350 independent transformation lines were developed.

Sub-species & Cultivar	Transformed	% area	Rank
S. tuberosum andigena			
Waycha	No	14	1
Imilla Negra	NA	9	4
Imilla Blanca	NA		
Runa Toralapa		4	10
S. tuberosum tuberosum			
Desiree	Yes	10	2
hybrids			
Maria Huanca	Yes	?	?
Gendarme	Yes	3	12
Revolution	Yes	1.5	16

Table 1: Potato cultivars initially selected for preliminary attempts at transformation. *S. tuberosum andigena* has rarely been transformed. Values for Bolivian potato acreage due to cultivar and corresponding rank are from G. Thiele.

Selection of resistant lines

See 2.3 below.

2: Transgenic Resistance to *Globodera* spp

2.1: Development of constructs

The hybrids Maria Huanca, Gendarme and Revolution have been transformed and western positive lines obtained for Maria Huanca. The *S. tuberosum andigena* cultivar Waycha is not currently regenerating well enough for effective selection of transformed lines. Further work is continuing on its regeneration within R7294. Our ability to transform another cultivar of this subspecies (cv Gendarme) should facilitate the selection of conditions needed for regeneration a range of cultivars of this form of the potato.

2.2: Transformation of cultivars
See Table 1.

2.3: Selection of resistant lines
About 200 transgenic lines of these plants were confirmed to be expressing the cystatin by western blotting. The plants were also confirmed as positive by northern analysis. The lien chosen for field trial were those judged as highest expressers by both procedures.

3: Guidelines for transgenic release

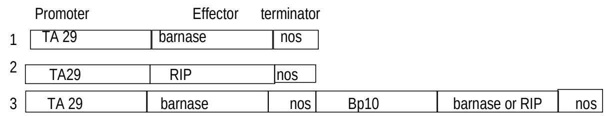
3.1: Biosafety Issues
Visit 1 to PROINPA, and Min. Agriculture Bolivia: Standard Operating Systems (SOPs) were developed at the meeting and by e-mail exchange for: a) safe use of a containment glasshouse at Toralapa with transgenic potatoes and b) disposal of transgenic plants by incineration. The operation of the incinerator and the improvements to the glasshouse were reviewed during the visit. Scientific progress and experiments for the coming year were also reviewed (see below). Dr S. Cowgill (CPS, Leeds) also spent 2 weeks ahead of this meeting at PROINPA gaining background on biosafety risks related to insects as non-target species if nematode resistance is introduced.
The visit ended with involvement in a biosafety conference organised by the Ministry of Agriculture in La Paz. This involved scientists from a range of S. American countries and was aimed at defining best practise for introduction and monitoring of GM crops.

Fig 3. Improvements to the glasshouse at Toralapa before use as a containment house for potatoes in this project.

Visit 2 to PROINPA, Cochabamba, Bolivia: The grant holder arranged a visit with Drs John Witcombe and Jill Lenne (DFID CPP) plus Dr S. Cowgill (CPS) to scope the biosafety issues and to define opportunities for linkages to PSP and CPP programmes. Key meetings were with PROINPA staff, members of the National Biosafety committee (see below) and Dr W. Collins, Research Director, CIP.
Biosafety meeting at Manchester June 2000: A key output of visit 2 to Bolivia was decision to hold the meeting in Manchester. Its scope was defined at the Bolivian meeting. The outputs from this project on GM opportunities and biosafety issues with regard to uptake of GM potato in developing opportunities has now been published at that meeting and elsewhere (see outputs).

Visit 3 to PROINPA, Cochabamba, Bolivia and CIP Peru: This meeting discussed a wide range of biosafety issues and the linkage of the current project and that about to start (EU INCO) with H.J. Atkinson as its co-ordinator. The EU project will examine risks such as gene flow from potato and effects of nematode resistant potatoes on non-target invertebrates

3.2: GM consent
National Bolivian Biosafety committee provided consent for a limited field trial in April 2000. However certain NGOs opposed this decision. Their agitation at Tiraque near Toralapa concerned the Director of PROINPA (Dr A. Gandarillas). He gave a public assurance not to field trial in 2000. Currently the intention is to containment trial in 2001 with a subsequent field trial if this is publicly accepted. This issue is now being addressed within



R7294.

3.3: Male sterile potatoes
Fig 4. Constructs for producing transgenic, male sterile potatoes.
This work is to address the hazard of introgression of transgenes into wild *solanaceae* via pollen. Male sterile constructs have been developed that involve a tapetum specific promoter (TA29) This regulates the expression. of either an RNAse (barnase) which is known to kill plant cells or a ribosomal inactivating protein (RIP) originally from maize kernels.
Fifty transformed Desiree lines of TA29/barnase/nos and a similar number of TA29/RIP/nos are in tissue culture for transfer to glasshouse in December 2000. The extent to which the pollen of these lines have reduced viability will be assessed in the first half of 2001 in R7294. More transgenic lines are available if necessary.
The construct TA29f/Bp/Barnase/nos is now in the final stages of development within R7294.

3.4: Pollen dispersal studies
A field experiment was conducted with a central block of *S. tuberosum andigena* cv Waycha (produces red flowers and many berries) surrounded by 20 concentric rows spaced at 1m intervals of cv Revolution (white flowers and no berries). Four blocks of Revolution were placed further away from the main site to confirm low berry numbers for this cultivar in replicated checks. Lack of berries is due to male sterility and is common for *S. tuberosum tuberosum* but not cultivars of the *andigena* sub-species. The experiment showed berries due to pollen transfer from Waycha at all distances up to 20m. Data for spread of pollen from a male fertile to a male sterile *S. tuberosum andigena* in Bolivia provided a preliminary value of relative risk from 10% cross-pollination at 1m to 10^{5} at 100m (Fig 8) and 10^{45} at 1km. This estimates the "worst case scenario" for 4 reasons : (1) cross-pollination within the same sub-species is the most fertile event (2)

the male sterile plants offer no competing pollen (3) the density of surrounding crop plants is higher than for any weed and (4) the mean distance is closer than for weeds that are restricted to field margins.

A second experiment on pollen flow from a male fertile (cv Waycha) to a male sterile potato (cv Revolution) was completed in 1999-2000. It occupied a larger area than in 1999, with linear rows. It was at an isolated position near Candelaria with a prevailing wind funnelled along the valley from the East. No berries were recorded on cv Revolution. This suggests that wind is an ineffective method of cross-pollination for potato in Bolivia. The change of site and emphasis of the experiment was decided unilaterally by PROINPA. They have therefore agreed to repeat the 1998 experiment with the 1999 design at Toralapa in growing season 2000-01.

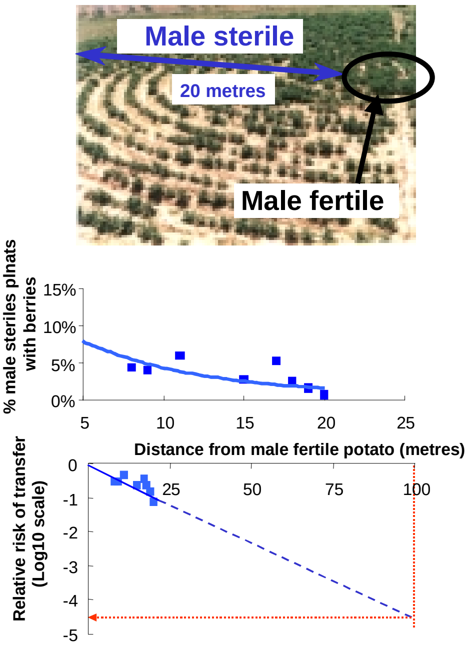


Fig 5 : The incidence of gene transfer to a male sterile potato with distance from the source at Toralapa and preliminary extrapolated relative risk suggesting a 10^5 reduction at 100m

Fig 6: The pollen flow experiment in progress at Candellaria in growing season 1999-2000.

4: Trial of nematode resistance under glasshouse and in the field

4.1: Challenge of transgenics with *Nacobbus*

A significant level of resistance was conferred on cv Desiree against *Nacobbus aberrans* by a cystatin. The levels of resistance were less than detected for Potato cyst nematode (see below). The plants were not grown under ideal conditions. The level of expression in more than one trial with different nematodes does seem to be influenced by plant stress. This may relate to the continued need for the plant to make the protective protein. Future work will need to determine the extent that plant stress under field conditions influences the levels of plant protection the defence confers.

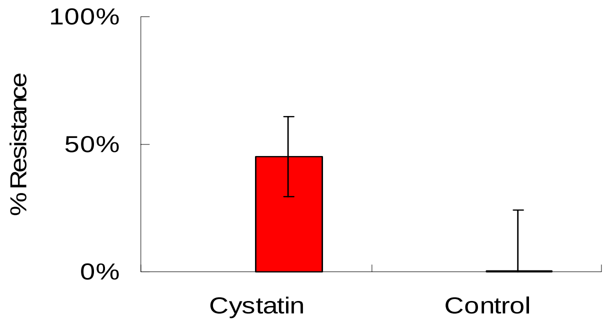


Fig 7: Partial resistance of cv Desiree expressing CWEC to *Nacobbus aberrans* from Bolivia

4.2: Containment Trial in UK against *G. pallida*: They were carried out in concert with the field trials. Tubers were planted into 2.8kg pots containing soil infested with 10 eggs /g soil *G. pallida*. In addition to the 4 transgenic lines tested in field, a further two lines, 6/10 and 6/16 were tested in containment that provided high expression levels in western blots but were not sufficiently advanced to be placed in the field.
The containment trials provided very similar levels of resistance for 3 of the four lines used in the field trial. The line D6/4 provided high levels of resistance in the field than containment. One of the additional lines (D6/16) gave similar levels of resistance to that of the two better lines used in the field (D6/2 and D6/7). They expressed c0. 4 % tsp as CEWc Six lines were grown in containment four of which were also transplanted to initiate a field trial.

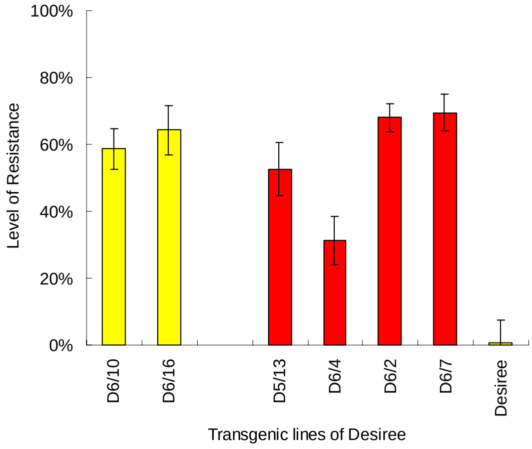


Fig 8: 1998: Level of resistance for 4 transgenic lines of Desiree also used in the field (in red) and two additional lines (in yellow) and an untransformed, susceptible cultivar Desiree. Resistance is measured described in Fig.1.2.

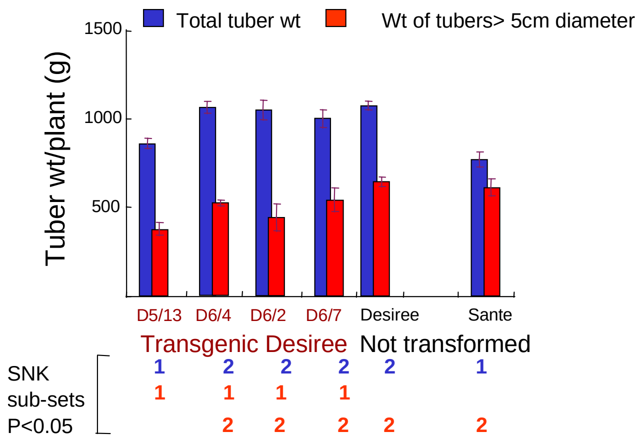


Fig. 9: 1998: Total tuber yield and tubers of marketable size (> 5cm diameter) recovered from field trial challenge of 4 transgenic lines of cv Desiree, an untransformed Desiree and the partially resistance cultivar cv Sante. The population of G. pallida was 7.5 eggs/g soil and so no loss of yield due to nematodes was expected.

4.3: Containment trial in Bolivia:

This experiment was completed with authority but before the regulations were widened to include containment trials. DTER does not cover such work in UK. The results show a level of resistance that is somewhat lower than that obtained in Leeds by about 10%. The most likely explanation of this effect from results at Leeds is that the potatoes in Bolivia were stressed by the high temperatures of the glasshouse which is often >30C. This will remain a problem for future containment trials under glass as those available in Bolivia lack the capacity to cool.

4.4: Field trials in UK:

We conducted field trials last year at University of Leeds Field Station, Headley Hall Farm, Tadcaster (DTER consent for 5 years from 1998 No. 98/R31/1). The 1998 field trial involved four lines of Desiree potato transformed with the construct *CaMV35s/CEWc*. They were the only lines developed in time for planting that year. These lines expressed PIs to ca. 0.4 % tsp. Plants were planted in random block design. The initial population of *G. pallida* was assessed as being 7.5 eggs /g soil. The plants were put in the field on the 20th June and the haulms removed on September 15th prior to the tubers being harvested.

Potatoes were collected riddled and weighed in different size groups. The total weight of tubers and the size of the larger, saleable sized tubers with a diameter of > 5cm are shown in Fig 1.1. Three of the four transgenic lines (D6/2, D6/4 & D6/7) had similar total and larger tuber yields to untransformed Desiree. The resistant cultivar Sante had a smaller yield of total but not larger tubers.

Soil samples were collected from the base of the plants around the tubers. The soil was dried at ca. 33 ^{o}C over a 2 wk period. The soil samples from 9 plants in a set were mixed thoroughly to ensure homogeneity using a sample divider. The samples were weighed into 100 g aliquots. At this point samples were encoded, and duplicate cyst and egg counts were carried out blind. Cysts were then extracted from these aliquots using a Fenwick can fitted with an elutriating tube. The cysts were counted and soaked for 1 wk. to allow the eggs to hydrate. Eggs were then counted. Duplicate samples were analysed. The two values for three samples differed and so a third population estimated was obtained in each case to obtain two values in close agreement.

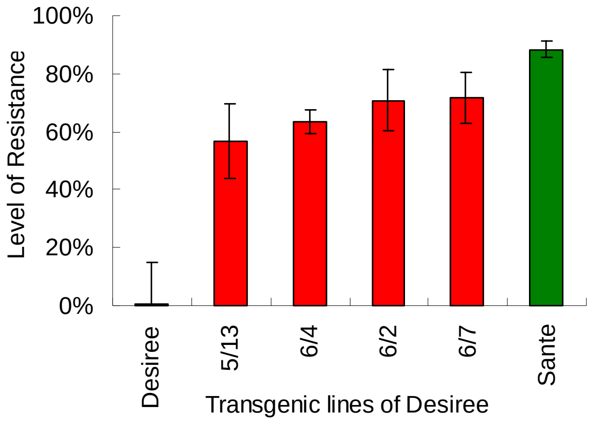


Fig. 10 1998 Level of resistance for 4 transgenic lines of Desiree, cv Sante with natural resistance to *G. pallida* and an untransformed, susceptible cultivar

Desiree. Resistance is measured as a reduction in numbers of eggs/ g soil post-harvest relative to that for the untransformed cv Desiree.

The final population for the untransformed Desiree was c22x above that of 7.5 eggs/g at planting. This demonstrates that the trial enabled high multiplication of *G. pallida* to occur on the susceptible cv Desiree. The levels of resistance were similar for the 4 transgenic lines. The highest level of resistance was 72 ± 9%. The value for Sante was 85 ±3%. This high value for cv Sante reflects the lack of virulence of the population of *G. pallida* at the field site. This is to be expected as cv Sante has not been grown on the University farm.

Southern analysis on the potato field trial lines showed that the D6/2 had two copies of the CEWC gene while D6/7 had only a single copy of CEWC inserted into the genome. Digests were carried out using *Bam* HI, *Hin*d III and *Xba* I, none of which fragment the *cewc* gene which was used as a probe (stringency 65 ^{o}C, 0.2 x SSC, 0.1 % SDS). Northern analysis was also conducted on the potato field trial lines out. A single size transcript is apparent in all the lines tested. There is no correlation between insert number as determined by Southern blot analysis and transcript abundance. Western blots followed by image analysis also fail to detect differences in CEWC expression levels among the four lines.

In both the field and containment, the principal effect was reduction in cyst number rather than cyst size. This was unexpected and may indicate CaMV35S provided a patchy level of cystatin ensuring some sites on the root systems where females can develop normally. If so, a change in promoter may raise the overall level of resistance. This possibility will be explored in containment in 1999.

In the 1999 field trial, the cv Desiree expressing either a sunflower cystatin or the modified rice cystatin OcIΔD86 (potato codon usage form) showed a higher levels of resistance than the natural resistance of cv Sante to a field population of c10 viable eggs/g soil. Also a cystatin showed significant additive resistance when expressed in cv Sante.

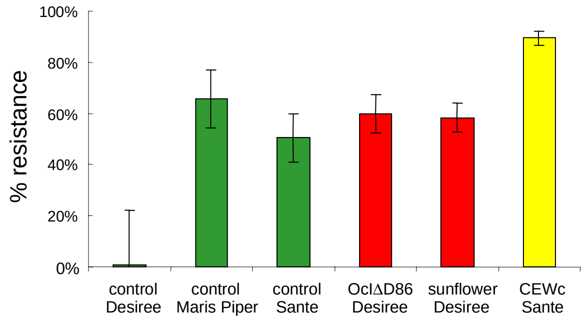


Fig 11. Level of resistance for 2 transgenic lines of Desiree expressing to plant cystatins (OcIΔD86 and sunflower) and one of cv Sante with chicken egg white cystatin (CEWC). In the latter case this provides additive resistance with its partial, natural resistance to *G. pallida.* The field population was a mixture of *G. rostochiensis* and *G. pallida*. Cv Maris Piper is fully resistant to UK populations of *G. rostochiensis.* See Fig 10 for further details.

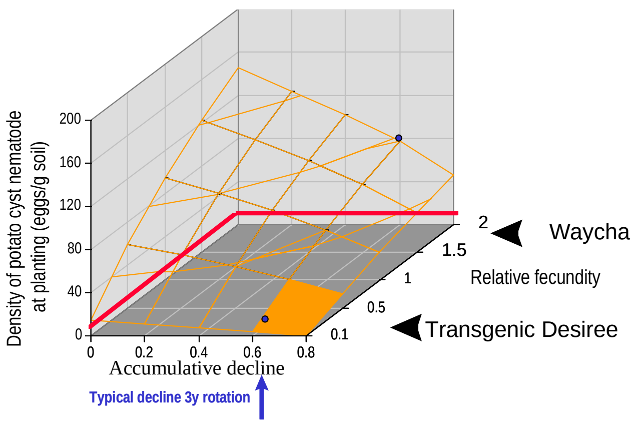


Fig 12: Relationship between the accumulative decline of *Globodera* when other crops are grown, the relative fecundity of females provided by different cultivars and eventual egg population at planting that will occur. The damage threshold is shown as a red line and the two blue symbols the likely outcome when cv Waycha and transgenic Desiree provide the host within a three year rotation with other crops.

Fig 13: *Rhigopsidius tucumanus* (Andean weevil). *Left*: adult feeding on a potato leaf. *Right:* grub feeding within potato tuber.

5: Refined development of constructs

This aspect of the project was transferred to R7294**.**

6: Transgenic cultivars with efficacy against nematodes and insects

A limited number of lines of interest were identified from those listed in a CIP report to PSP. Five of these were provided in tissue culture and 4 were successfully established free of contamination (see Table 1). Three transgenes are involved: an α-amylase inhibitor from wheat (AAI), a lectin from snowdrop (GNA) and a kunitz trypsin inhibitor from soybean (SKTi). A number of lines were selected as putative positive or negative (control lines) based on ranking lines in a final report provided to PSP by CIP for work done in collaboration with Pestax. Our understanding is that only the lines we hold still exist. They were transferred to CPS glasshouses and their number increased before testing efficacy against the insects.

Notation	Code	Level of Resistance 1= susceptible		Mean	Rank	Supplied by Pestax & established Leeds
INTERESTING LINES						
WAAI/GNA	PWG-70	0.43	0.52	0.47	5=	√
WAAI/SKTI	PWK-35	0.46	0.48	0.47	5=	√
"	PWK-50	0.29	0.06	0.17	1	√
"	PWK-58	0.36	0.35	0.35	3	X
SKTI	KTI-20	0.36	0.39	0.37	4	√
	KTI-25	0.14	0.48	0.31	2	√
NEGATIVE CONTROLS						X
WAAI/GNA	PWG-17					√
WAAI/SKTI	PWK-36					√
SKTI	KTI-34					X

Key

WAAI	Wheat alpha-amylase inhibitor
GNA	Snowdrop lectin
SKTI	Soybean Kunitz trypsin inhibitor

Table 3. CIP data for two experiments with a total of over 80 lines were ranked to identify those of potential efficacy. The level of resistance was based on numbers of weevils maturing in tuber expressing anti-insect proteins relative to controls. WAAI, wheat α-amylase inhibitor, SKTi, soybean Kunitz trypsin inhibitor, GNA snowdrop lectin.

Colonies of the potato moth and a potato weevil have been established in CPS under MAFF licence.
We found little effect on a potato tuber moth but the normal rate of progress of the weevil through its life cycle was slightly reduced by SKTi and possibly a line expressing this gene and AAI but not by a line of GNA +AAI. This suggests the active gene is SKTi.

We have shown that larvae of *Rhigopsidius tucumanus* fed on tubers expressing certain transgenes suppress both fecundity (Fig 14) and pupal emergence rates (Fig. 16). The egg production per week can be expected to follow a standard curve suggest as a Gompertz growth curve. Expressing the data on this basis allows the rate of egg laying and a maximum egg production to be estimated**.** This is providing for representative lines in Fig 15.

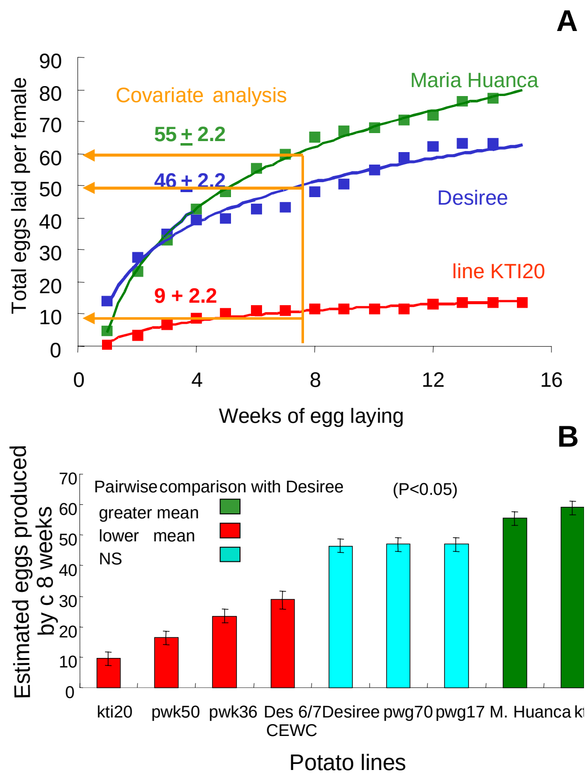


Fig 14. Egg production by *Rhigopsidius tucumanus* after feeding as adults on potato tubers of various transgenic lines. **A** Accumulative egg production for representative lines. **B.** Eggs produced after 8 weeks on the various potato lines.

A secondary effect was observed in addition to the main effect on egg production. It was observed that proportion of eggs laid that hatched from which weevils emerged was also delayed (Fig 16). This may be merely a delay or mortality. However the additive effect for SKTi was sufficient to suggest that the overall, effect of the transgene was 2x that indicated in Fig 14.

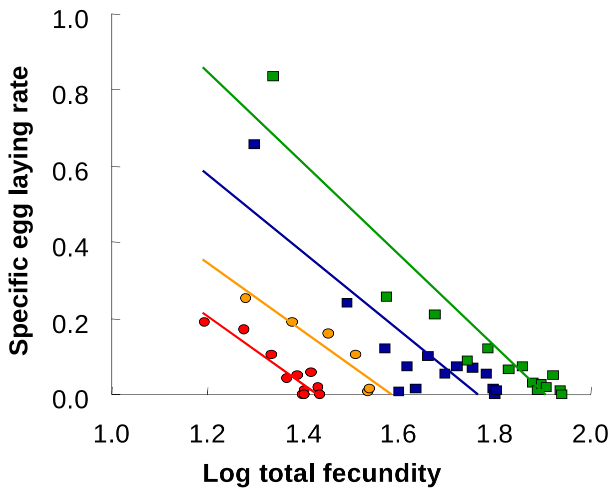


Fig.15: Accumulative egg deposition by an Andean weevil, *Rhigopsidius tucumanus*, after larvae had fed on control cultivars or Desiree expressing transgenes. Adults were fed on control leaves. The data has been summarised as Gompertz curves to provide estimates of final egg production. Wildtype cv Maria Huanca (☐) and Desiree (■), cv Desiree expressing 2 different transgenes.(○, ●)

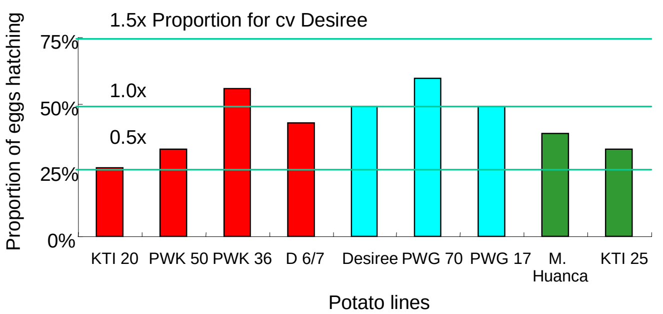


Fig 16. Proportion of eggs of *Rhigopsidius tucumanus* from which larvae emerged when the parental females had feed when larvae on of potato tubers expressing different transgenes.

7: Outputs

1 Atkinson, H.J. (1998) A Robin Hood approach to transferring appropriate plant biotechnology to the developing world *Science and Public Affairs* Winter 1998, 27-29.

2 Atkinson, H.J. (1999) Genetically Modified Crops and Future Food Security in the Developing World. p118-120 In: *Paths to Prosperty: Science in the Commonwealth 1999-2000.* Kensington Publications 240pp.

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4 Atkinson, H.J. and Green, J. (2000) The case in favour of transgenic, nematode resistant potatoes for Bolivia. In DFID PSP +CPP/CIP Conference: Biosafety of GM Potato in the Developing World, Manchester, June 2000.

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6 Atkinson, H.J. Urwin, P.E., Lilley, C.J. & McPherson, M.J. (1998) Engineered resistance to plant nematodes In: The Physiology and Biochemistry of free-living and plant parasitic nematodes. CABI St Albans, UK. (ed. Perry, R.N. & Wright, D.J., (CABI , St Albans, UK,).

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8 Urwin, P.E., Green, J. and Atkinson, H.J. (2000) Resistance to *Globodera* spp. in transgenic *Solanum tuberosum* cv. Désirée that express proteinase inhibitors *Aspects of Applied Biology* **59**: 27-32 2000

9 Urwin P.E., Troth,K., Zubko, E.I. and Atkinson, H.J. (2000) Effective transgenic resistance to *Globodera pallida* in potato field trials. *Molecular Breeding*. *In press.*

10 Atkinson, H.J. Holz, R.A., Riga E., Main, G. Oros, R and Franco, J. (2001) An algorithm for optimizing rotational control of *Globodera rostochiensis* on potato crops in Bolivia. *Journal of Nematology, accepted for publication*

8: Contribution of Outputs

Sought outputs

1. One or more cultivars resistant to *Nacobbus* spp
2. One or more cultivars resistant to *Globodera* spp.
3. Establishment of guidelines for transgenic potato release.
4. Trial of nematode resistance under glasshouse and in the field.
5. Improvement of constructs to give optimisation of defences against nematodes.
6. Transgenic cultivars of potato produced against insects and tested under containment conditions

The key outputs defined at the onset of the proposal have been achieved. However issues surrounding uptake of these new potato plants requires further consideration before use can be promoted.

There is general reservation about GM crops and particular concern about transgenic plants in areas of biodiversity of that plant species. Bolivia is an area of biodiversity for potato and its relatives. Further work is in progress in R7294 and other related projects to develop the plants for safe uptake. Given the considerable potential of the technology for nematode problems world-wide, it is essential that progress towards availability to growers consider all discernible hazards. The new crop should offer no unacceptable risks. The range of work in progress to meet these needs before uptake is summarised below.

Food safety is being addressed in related work. Work on toxicology is about to be completed (SERAD funding) and work done by others for MAFF (BIBRA) has demonstrated that the cystatins we use are not an allergenic risk

Environmental biosafety has also been addressed. Work in the UK (SERAD) has defined that many plant associates of green tissues of potato are not at risk from the technology. Work is about to start on environmental consequences for the soil environment (MAFF). Potato associates of potato in Bolivia have been defined in our work for CPP. R7294 is developing molecular means of preventing gene flow from transgenic potato via pollen. An EU INCO project (co-ordinator H.J. Atkinson) has just started to address environmental issues in Bolivia particularly the risk from the transgene and its widespread uptake to both non-target invertebrates including Bumble bees and soil organisms. The risk of transgene escape via hybrids of potato and wild relatives formed with pollen of the latter is also to be studied. The prospect of a phenotypic marker to allow growers to detected transgenic plants is being considered within R7294.

We advocate the general standard that any trait being considered should offer no risk to humans and pose no greater risks to the environment than other, current practices of farmers. A benefit for resource poor farmers should be demonstrated and the trait should be offered at no additional cost to them. No, or minimal changes, to normal agricultural practices should be required. We consider that the nematode resistance we are developing for potato can meet these exacting standards (see outputs 3 &4). The benefit in Bolivia is a smaller national acreage committed each year to potato, and an opportunity for increased production of more nutritious food crops by the poor on the land that is made available. Additional benefits to the rural economy would be considerable in terms of better nutrition, increased production, and increased employment opportunities. We hope to continue adaptive uptake of these potatoes in collaboration with PROINPA and CIP. The technology has considerable global potential for a range of nematode pests including *Globodera*, *Nacobbus* and *Meloidogyne* on potato crops of poor farmers.