DFID CROP PROTECTION PROGRAMME
FINAL TECHNICAL REPORT
Nacobbus aberrans and weeds in Bolivian potato fields: Environmental consequences of current and future nematode control options
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1: Executive Summary

Nacobbus aberrans and *Globodera* species (PCN) occur on 91% of the potato fields of Bolivia and provide national potato losses of 20-30%. *N. aberrans* has been ranked by PROINPA as the most severe biotic stress on potato in Bolivia. Its population dynamics are not well understood in contrast to those of *Globodera* spp because the latter has global pest status. A main objective of the research was to determine the role of weed species, density and management practices in maintaining *Nacobbus* populations at levels capable of reducing potato yields. A second aspect of the work was to collect preliminary data on non-target invertebrates potentially at risk from deployment of nematode resistant transgenic potatoes. This underpins the need to ensure transgenic resistance developed at CPS does not present environmental risks before its use by subsistence farmers in Bolivia can be contemplated.

Three factors were identified that allow *Nacobbus* to persist in potato fields: 1) We confirmed in collaboration with R7325 that two weed hosts *Spergula arvensis* and *Brassica campestris* are sufficiently prevalent and abundant in fields to favour persistence of *Nacobbus*. 2) Volunteer potato densities are also likely to be important in many fields in maintaining the nematode between potato crops. 3) Work by PROINPA suggests that seed potatoes are a common source of introduction of the nematode to fields. The results also suggest that if a potato crop is grown, *Spergula* supports early establishment of the parasitic stages of the nematode. Its close proximity to potato roots, the lack of host preference by the nematode and the increasing relative density of potato relative to *Spergula* roots are all important. They favour the increasing presence of *Nacobbus* at damaging levels on potato as the crop establishes.

The work identified a sub-set of non-target organisms for which the deployment of transgenic nematode resistant potatoes offers the greatest potential hazard. However bioassays showed that expression levels that provide enhanced nematode resistance do not necessarily pose a risk to many such insects that feed on aerial plant parts. The intention is to deploy only transgenic plants with expression restricted to root systems. This provides a second basis for protection for non-target aerial invertebrates.

The effect of transgenic plants on soil mesofauna has been compared with the effect of an alternative nematode control option, the oxime carbamate nematicide aldicarb. Such compounds are used in Bolivia for insect control and are already widely used in Peru to control nematodes on potato. They are very harmful to non-target organisms. This contrasts to the lack of risk from transgenic nematode resistance. Existing management practices such as manuring and cultivar choice also impact populations of soil microarthropods. This work underpins a rational basis for considering the relative environmental impact of different nematode control options.

Two types of work are required. Integrated management procedures must be developed that prevent *Nacobbus* from damaging potato crops. A project is required to establish the contribution that transgenic resistance can make to effective IPM of *Nacobbus*, *Globodera* and *Meloidogyne* spp on potato in Bolivia.

2: Background

Virus, fungal and insect pests all reduce yields in small holders potato cropping in hillside production systems in Bolivia. However, the largest biotic stress to potato yields is nematodes. *Nacobbus aberrans* and *Globodera* species (PCN) occur on 91% of the potato fields of Bolivia and provide mean national losses of 20-30%. Damage caused by PCN depends on the ability of the pest to persist as encysted eggs between potato crops. The economic importance of the animal in Europe has resulted in a good understanding of its persistence. In contrast *Nacobbus* is not well studied and its population dynamics differ from PCN. It depends mainly on a wide host range to enable it to maintain populations that can damage potato. There are different races of the nematode with certain crops such as *Phaseolus vulgaris* being a differential host. However weeds in the potato crop and in the field at other times in the rotation cycle are probably important in maintaining populations at a level that can damage potato.

It is likely that weeds must be present at a high density to provide damaging populations of *N. aberrans*. For PCN it is known that densities of volunteer potatoes of 4 or 16 plants/m2 are sufficient to build sub-economic densities of this species but not those that can damage the next potato crops. Standard population models for PCN can be modified to demonstrate the effect of host density. A similar relationship needed to be defined for *Nacobbus* not just for volunteer potatoes but its weed hosts and any other crop plants grown in Bolivia with host status.

Previous collaboration between PROINPA and the Centre for Plant Sciences had established that different potato cultivars grown in Bolivia provide different host efficiencies for PCN. Parallel work with *Nacobbus* needed to be carried out to identify cvs that support reduced reproduction of both nematodes.

Potatoes have been developed through PSRP R6830 to express cysteine proteinase inhibitors (cystatins). The defence confers resistance to a wide range of nematodes including all those attacking potatoes in Bolivia. Before deployment of these plants can be considered their potential environmental impact must be assessed. Some data for plants with anti-insect properties have been interpreted as indicating that the plants are potentially harmful to non-target species or other trophic levels such as predatory species. Others have shown that hymenopteran parasites benefit relative to the use of pesticides and that some other insects are not harmed. An environmental impact assessment has been completed for cystatin-expressing potatoes in the UK. The need was to develop this work to provide a reliable oversight of the risk to non-target organisms before deployment of the transgenic plants in Bolivia is contemplated.

PROINA identified the demand for new research on nematode pests of potato; their surveys ranked nematodes as the most important pest of potato. Nematodes probably enforce on tradition potato growing 2x the necessary area per year for potato production in small holdings.

3: Project Purpose

PROINPA rank *Nacobbus* and *Globodera* spp (potato cyst-nematode) first and third in rank order of pests and diseases in Bolivia. Only 22% of potato growers receive extension advice. 45% of potato growers have been reported to forego potato cropping opportunities because of nematodes and rotations are sufficiently short that losses are certainly occurring. The potato yields in Cochabamba are 10% of that achievable in UK and 20% of the maximum possible in Bolivia. PROINPA surveys establish that the density and incidence of nematodes requires 2x the acreage that should be needed to be allocated to potato in Bolivia currently. The purpose of the project was two fold:

- To examine the effect of weed species, density and management practices on the presence of *Nacobbus* populations at levels capable of reducing potato yields
- To collect preliminary data on non-target invertebrates relevant to the safe and effective deployment of nematode resistant transgenic potatoes.

The linking theme of this work is the current lack of effective IPM scheme for *Nacobbus* that are consistent with the intensity of potato production required by subsistence agricultural in Bolivia. One part of the work aims to understand the main factors that ensure *Nacobbus* is commonly at densities that damage potatoes The second part provided background on the environmental biosafety of transgenic resistance we have developed that can control both *Globodera* and *Nacobbus* within an IPM scheme.

4: Research Activities

4.1 *Nacobbus* and weeds in potato fields

***Initial review of work priorities*:** A visit was completed to PROINPA in November 1999 to determine which aspects of the biology of *Nacobbus* have received insufficient study. It was decided that knowledge of volunteers and of infestation of seed potatoes with *Nacobbus* was adequate for current needs. In contrast too little is known about the interaction between *Nacobbus*, and its host weeds and potato.

Surveys: The project also linked with R7325 from which the prevalence of weeds in Bolivian potato fields was estimated. A survey of volunteer densities was not carried out within R7325 or as a separate activity in this project.

Pot experiments: *Spergula arvensis,* was a known common weed host of *Nacobbus*. Therefore pot experiments were carried out to determine aspects of host/nematode interactions. In addition field trial was conducted to measure yield losses of different cultivars under challenge from *Nacobbus*.

The timing of the start of the work proved to be too late in the growing season and *Nacobbus* seems to have entered diapause. In addition the glasshouse temperatures proved to be too low for reproduction of *Nacobbus*. Therefore experiments listed below were repeated in the latter part of 2000.

This need was defined in a meeting held in late September at PROINPA to review progress. The data from the repeated experiments will not be analysed until Spring 2001. A supplementary report will be issued. The experiments are as follows.

1. Detection of prelevance of *Nacobbus* in potato fields. This involved a bioassay based on growing a potato root system in a polythene bag with the soil sample.
2. Determining difference in host ranges for common weeds for 3 distinct biotypes of *N. aberrans*.
3. Measurement of egg production on host plants at different soil inoculum levels of eggs to determine the *N. aberrans* multiplication rate.
4. In addition, the investigators have decided to supplement the work in two ways at their own expense. We are adding more data on *S. arvensis* and volunteer potato densities in farmers' fields.

A main aim is to provide a simple algorithm for identifying fields whose weed populations predispose potato to damage from *Nacobbus* and to assess the *Nacobbus* problem. This will be attempted when all the experiments are completed.

4.2 Environmental consequences of expression of cystatins in potato

Surveys of non-target organisms: Samples of the invertebrate fauna of potatoes were taken during a survey in January 2000 conducted under R7325. Farmers' fields were selected for study using criteria defined by members of the R7325 survey team. At each location 5 randomly selected plants were visually examined for insects; insects were collected and preserved in alcohol in a labelled tube. If sufficient time was available 5 samples of rhizosphere soil were collected using a 3cm corer. Because the survey could only provide 'snap-shot' of the fauna present at a particular point in the growing season additional data were collated from PROINPA reports to provide a summary of the temporal variation in the composition of the insect associates of potato.

Histochemical localisation of cysteine proteinases: Representatives of the commonly occurring non-target groups were studied in more detail in UK, to determine which, if any possessed cysteine proteinase activity in their digestive tract. These groups were assumed to be at more risk from transgenic plants expressing cysteine proteinase inhibitors (cystatins) than groups that use other classes of proteinases in digestion. Histochemical techniques were used to characterise and localise cysteine proteinase activity in sections of non-target organisms. Briefly, cryosections of invertebrates were incubated with a synthetic substrate, N-CBZ-L-Ala-L-Arg-Arg-4-methoxy-2-naphthylamide and coupling agent in incubation buffer for one hour. The presence of cysteine proteinase activity is indicated by the deposition of fluorescent crystals. Examples from three different invertebrate families are shown below. Using this technique four groups were selected for further study, Cicadellidae, Aphididae, Collembola and Acari. The results of these studies are discussed below.

4.3 Potential impact of transgenic nematode resistance on non-target organisms

Cicadellidae: This group of hemipterans commonly called leafhoppers have a range of feeding behaviours that include consumption of mesophyll cell contents or xylem and phloem contents. The palearctic species, *Eupteryx aurata*, was used as a test species and the effect of consumption of transgenic tissue on insect fitness was studied under controlled conditions. <24h old nymphs were clip caged onto transgenic plants expressing a modified version of the rice cystatin, Oc-I. The establishment of nymphs on the plants was recorded and survival was recorded daily until adulthood.

Aphididae: Aphids are phloem feeders and their survival is affected when they are confined on transgenic plants in which expression of a lectin is driven by the CaMV35s promoter. The plants currently under study in our laboratory also use this promoter. As a result we were interested in testing whether populations of aphids were adversely when exposed to plants with the CaMV35s-Oc-IΔD86 construct.

Collembola and Acari: Springtails and mites are important components of the soil mesofauna. Preliminary histochemical studies had identified cysteine proteinase activity in representatives of both groups. Collembola feed on a wide variety of food types including root hairs, lateral roots mycorrhizae and fungal hyphae. Members of some acarine orders are predatory while others feed on fungal hyphae. Because of difficulties in rearing individual species of soil Collembola and Acari it was decided to study these groups only under field conditions. The experimental design is described below.

Field experiment: This was carried out in the UK to test for effects on populations of *Myzus persicae* and *Macrosiphum euphorbiae*. The experimental design comprised a randomised block with 4 replicates of three treatments. The treatments were a transgenic line (D9/31) expressing the CaMV35s-Oc-IΔD86 construct, control Desiree and control Desiree treated with Aldicarb. Aldicarb was chosen because it represents an alternative method of PCN control that is available to farmers in Bolivia. Aldicarb was incorporated into the soil at the recommended field rate immediately after transplanting. There were 32 plants in each plot arranged on 4 ridges. The plots were separated by non-transgenic guard rows of cv Maris Piper.

Soil samples: In addition to regular counts of aphid numbers soil cores were taken from the rhizosphere to a depth of 10 cm using a 3cm ∅ core. Three samples were taken 31, 56 and 80 days after transplanting, respectively. Invertebrates were extracted from the samples using a modified Tullgren apparatus. The number of mites and Collembola in each sample was recorded and expressed as numbers per 100g dry weight of soil.

4.4 Environmental consequences of existing management practices

The current study represents a preliminary investigation of the effect of transgenic-nematode resistant potatoes on potential non-target organisms. In the future we anticipate that these plants will use root specific promoters to drive expression of effector proteins. In this case the main focus of interest will be the effects on non-target soil organisms. As part of the current study we have investigated additional factors which currently influence the abundance and composition of microarthropods in the potato rhizosphere. These include the effect of potato genotype and farmers' management practices on microarthropods.

Potato genotype: Seven potato genotypes were sown in a randomised block design with 3 replicates in January 2000. The potato rhizosphere was sampled at regular intervals during the growing season using a 3cm corer. Soil microarthropods were extracted from 100g subsamples using a Tullgren apparatus and preserved in alcohol. At the end of the growing season plants were harvested and the number of *Nacobbus* nodules per gm root was calculated.

Farmers' management practices: During the surveys conducted under R7325 soil cores from the potato rhizosphere were collected and the soil mesofauna extracted using Tullgren funnels. Survey data referring to farmers' management practices were collected by members of project R7325. Differences in counts of microarthropods between provinces, rotation types, length of last fallow, chemical fertiliser level and manuring level were investigated with ANOVA.

5: Outputs

5.1 *Nacobbus* and weeds in potato fields

***Weed Survey* (R7325):** Previous work at PROINPA established *N. aberrans* occurs in 74% of fields. The survey established that *S. arvensis* was the most prevalent weed in the survey. It was detected in 79 of 119 fields. A second host for *N. aberrans*, *Brassica campestris* was the second most prevalent weed (78 of 199 fields). The combined frequency of either of these weeds at a density of <1% was 58 (49%) of the fields surveyed. *S.arvensis* was extremely abundant in some fields (Fig1). It is evident that these two host weeds are sufficiently common to suggest they are a major factor in the ability of *Nacobbus* to persist in many fields. It remains to be established that a host weed widely distributed through a field even at a density of 1% the field area is sufficient to ensure an economic density of *N. aberrans* can occur.

Fig1: *Spergula arvensis* (left with galls of *N. aberrans* (insert) and a high density of this plant in a field at Toralapa.

Volunteer potatoes as a host between potato crops. Previous work by PROINPA had examined the density of potato volunteers in years when potato was not cropped in the two localities of Toralapa and Candelaria. Potato commonly occurs at density that would ensure persistence of *Nacobbus* between potato crops.

Seed potatoes as a transport host: A second important factor is infestation or re-infestation of a field by *N. aberrans* associated with seed tubers or soil adhering to them of potato. PROINPA has surveyed prevalence of *N. aberrans* on different cultivars from many sources in the informal production system were examined. It was detected on 29% of tubers intended for seeds from fields infested with *N. aberrans* In 55.7% of cases this resulted in the spread of *N. aberrans* when the seed potatoes were sown. Clearly this is a second factor that leads to infestation of fields and possibly economic damage to that crop when at high density. After introduction to fields with host weeds it is likely to persist between potato crops.

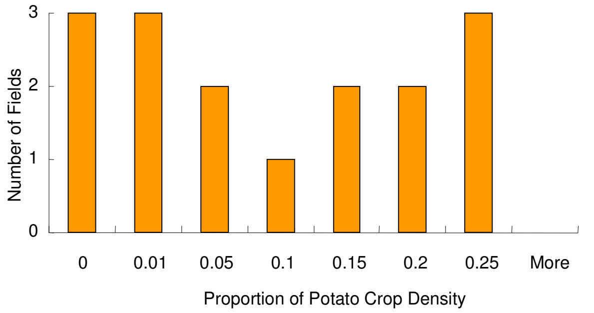


Fig 2: Density of volunteer potatoes relative to sowing density for the species as a crop in 16 fields in two localities, Toralapa and Candelaria.

Spergula *as a host for* Nacobbus*:* This weed is capable of growing at high density (Fig1) with a maximum root density occuring at 30 plants/ 2 Kg pot (Fig 3).

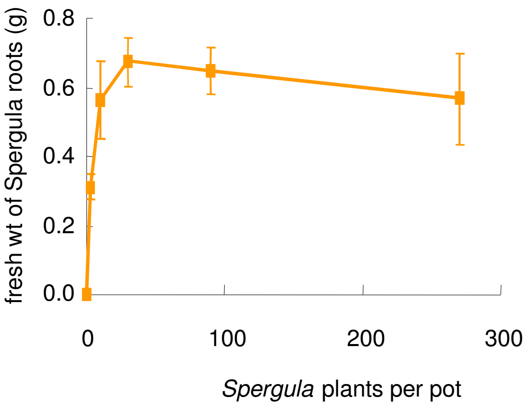


Fig 3: Changes in fresh weight of *Spergula* with density of this plant in 2Kg pots.

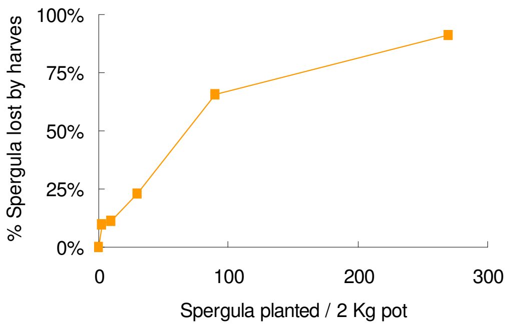


Fig 4: Proportion of *Spergula* sown lost at harvest at different sowing rates per 2Kg /pot.

Spergula at high densities in pots showed severe competition and many plants died under such circumstances. (Fig 4). Therefore subsequent assays were limited to densities of less than 30 plants/2Kg. Pot.

When different densities of *Spergula* were challenged with soil carrying a natural infestation of *N. aberrans* the density of galls produced reached a minimum at 10 and 22 *Spergula* per pot. This is reflects that these two densities provide similar root densities. A high density of plants does not benefit this nematode population. In general it is likely that root density provided by these small plants define the extent of intraspecific competition by the parasites rather than the density of individual plants.

Nacobbus *and potato:* *Spergula* is an annual that emerges early and before potato. It is harvested as a forage crop. It often grows with its roots within the rhizosphere of the establishing potato crop plants (Fig. 5).

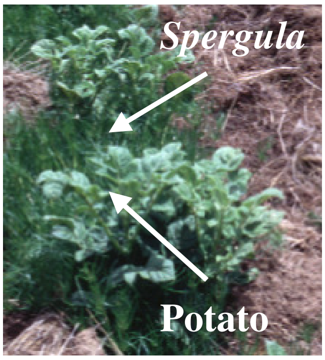


Fig 5: *Spergula* growing in close proximity to emerging potato crop plants in a field near Candelaria

Therefore one potato plant was grown in the same pot containing different densities of *Spergula*. The galls obtained were expressed per unit fresh weight of roots of each host and plotted as a ratio against the density of *Spergula* at different together in pots (Fig. 6).

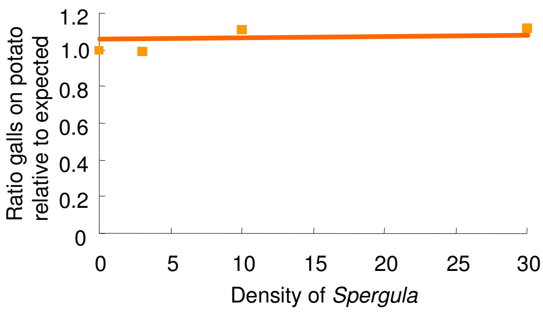


Fig 6: The ratio of *Nacobbus* galls / g root on a potato plant relative to *Spergula* when both are grown together at different densities of the latter.

The results establish that *Nacobbus* shows no preference for either host. *N. aberrans* re-invades roots before becoming an adult female. Therefore during the second invasion period of first generation and both invasion phases of subsequent generations, distribution of the parasites on potato or *Spergula* will be related to relative root density at that time. Given the large size of potato and the annual nature of *Spergula* this will favour establishment of the parasite on potato as the crop establishes.

A randomised field trial was set up in a field at Toralapa with a *Nacobbus* infestation. The trial compared galling levels by the parasite for different susceptible cultivars. Differences in galling occurred and this can be correlated with yield loss (Fig. 7). This suggests that management of *Nacobbus* must in future ensure that the galling is restricted to < c8% to prevent losses due this nematode. This result emphases the high pest status of *Nacobbus* with losses occurring if the animal builds to a density of ca 10% of the root system. It also provides some indication that cultivars vary in their relative host efficiency as judged by galling. This requires substantiation by further experimentation. It would be of particular interest if any cultivars favoured by subsistence farmers showed relatively low galling. The cultivar Gendarme does provides partial resistance to *Nacobbus* but take-up by growers has been limited.

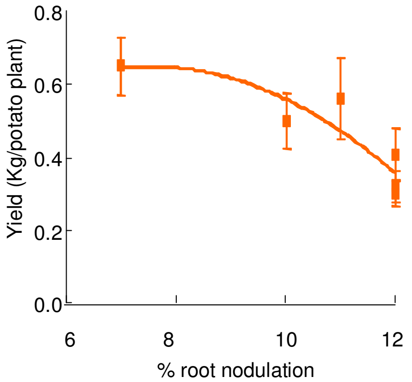


Fig 7: Correlation between nodulation levels on different potato cultivars and their yield.

Modelling the weed host densities and* Nacobbus *densities: All the data required to adapt a standard population model for *Globodera* to *Nacobbus* were not collected during the one-year grant because of apparent problems with the diapause of *Nacobbus* (see section 4.1). Therefore the example below is illustrative and will be amended when the data collection is completed.

If the model is broadly correct, it suggests that even comparatively low non-host prevalence of a potato crop density over much of a field is sufficient to maintain *Nacobbus* at densities that can threaten a potato crop in the next year. Densities of this order of *Spergula* or *B. campestris* occurred in 49% of fields and volunteer potato potatoes are also common at this density. If this conclusion is correct it establishes a basis on which *Nacobbus* can perpetuate in the potato fields of Bolivia.

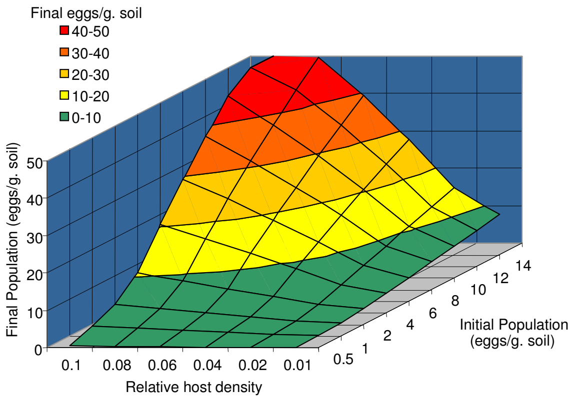


Fig 8: Predicted relationship between the initial and final population of *Nacobbus* at a locality. where volunteer potatoes or a host weed (e.g. *Spergula*) at a root density of 1-10% of a potato crop

5.2 Environmental consequences of expression of cystatins in potato

Survey of non-target organisms: The prevalence and relative abundance of different insect groups recorded from potato during the survey are shown in Fig 9 and 10.

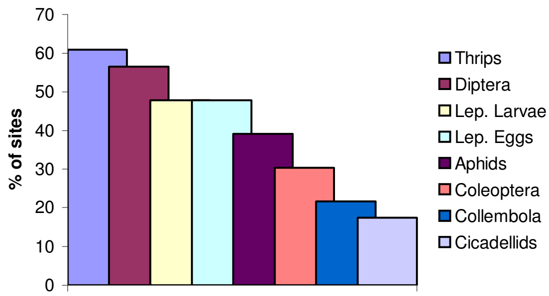


Fig. 9. Prevalence of insect groups on potato.

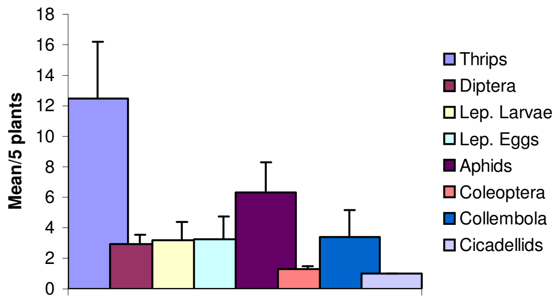


Fig. 10. Mean abundance of representatives of different insect groups on potato

Data from 23 locations were pooled for analysis. Thysanoptera (thrips) and Diptera (flies) were both abundant and widespread. Where aphids occurred they were present at greater densities than many other insect

groups. Collembola were recorded from the base of the potato crop and may represent a combination of phytophagous and litter-dwelling species. Although dipterans were recorded from the greatest number of sites the collected specimens were not identified to genus because it was assumed they were not feeding directly on potato tissue.
Data available from PROINPA reports showed that the insect group which shows the greatest variation in density during the potato growing season is the Coleoptera (Fig. 11). This was due to large fluctuations in the density of *Epitrix* species. Other Coleoptera recorded from potato are *Diabrotica* spp., *Eurysacca* sp. and unidentified Scarabs.

The differences in the abundance of Diptera between sampling dates may be attributed to differing climatic conditions. Similarly thrips were not abundant although they were widespread during the period of sampling in the present study. The surveys may have underestimated the importance of some insects, such as andean potato weevils, which are nocturnal and therefore were not found on potato foliage during the daytime.

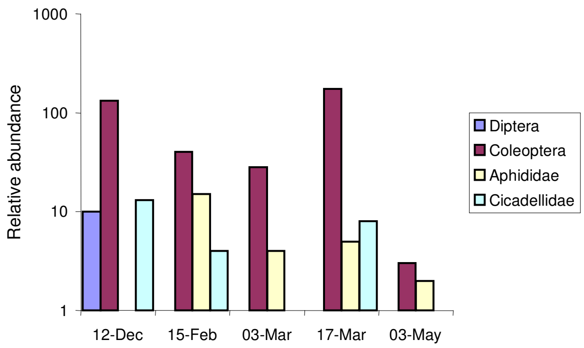
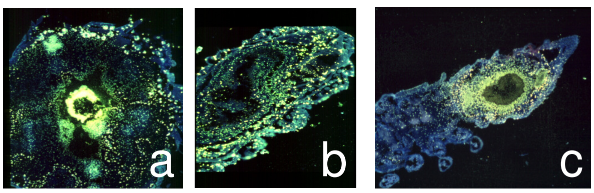


Fig. 11. Temporal variation in the abundance of insect groups at Cazorla (Mizque-Cochabamba)

Histochemical characterisation and localisation of proteinase activity in insect sections: Members of three insect groups showed positive reactions with the synthetic substrate N-CBZ-L-Ala-L-Arg-Arg-4-methoxy-2-naphthylamide. These were the Aphididae larvae of the Andean potato weevil, *Rhigopsidius tucumanus* and Cicadellidae (Fig. 12).



Figs. 12 a, b & c. Cross sections through a) An aphid nymph, b) *R. tucumanus* larvae and c) a Cicadellid nymph showing the presence of cysteine proteinase activity at higher levels than other tissues (fluorescent, yellow crystals).

5.3 Potential impact of transgenic nematode resistance on non-target organisms

Cicadellidae: Establishment of the nymphs on transgenic and control plants exceeded 90%. The survival of nymphs and the time to the final moult were also similar on the two plant types (Table 1). However, the adult sex ratio differed between the plant types. The ratio of males:females was 1:1 on the control line but 1:3 on the transgenic line. The biology of many cicadellids has not been well studied and currently it is not possible to predict how changes in the sex ratio will influence the population dynamics of the insects.

Line	% Establish-ment	Days to adulthood	% Survival
Oc-IΔD86	96.3%	17.91 ± 1.5	87.13 ± 14
Control	92.6%	18.32 ± 1.1	91.75 ± 15

Table 1: The performance of cicadellid nymphs clip-caged onto transgenic or control plants.

Aphididae: The highest aphid numbers occurred during the first 32 days after transplanting. After this time aphid numbers declined and the data were not included in the analysis. Throughout the sampling period aphid density on the potatoes in aldicarb-treated plots did not exceed 1 aphid/ 5 plants. During the same period peak densities on the transgenic and wild type Desiree plants were 26 aphids/ 5 plants and 35 aphids/5 plants respectively. The final aphid loading on the transgenic and control lines was similar and significantly greater than that in the aldicarb-treated plots (Fig. 5)

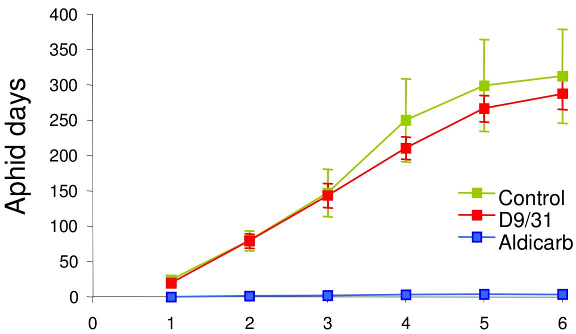


Fig. 13. Aphid loading on transgenic and control Desiree and control Desiree treated with Aldicarb during a field trial in UK.

Collembola and Acari: Mites were the most abundant microarthropods extracted from soil in the replicate plots. Significantly more mites were recovered on the final sampling date (15 September). When data from all sampling dates pooled there was a significant difference in mite density between the treatments. Consistently more mites were recorded in the control plots than the Aldicarb-treated plots. Mite densities in the transgenic plots were similar to those in control and Aldicarb-treated plots. Densities of Collembola were low and were not analysed.

5.4 Environmental consequences of existing management practices

Potato genotype: There were consistent differences in the density of mites in the rhizosphere of the different potato genotypes during the growing season (Fig. 14).

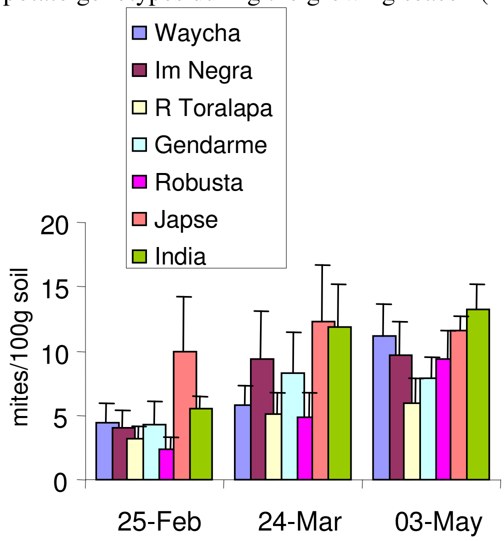


Fig. 14. The density of mites in the rhizosphere of 7 potato genotypes on three sampling dates during the growing season.

India and Jaspe consistently supported higher numbers of mites/ 100g rhizopshere soil than genotypes such as Runa Toralapa and Robusta. The numbers of Collembola appeared to be less influenced by potato genotype (Fig. 15). Higher numbers of Collembola were recovered from soil at the end of the growing period (May); at this time Waycha supported densities of Collembola which were ca 3x those on the genotypes Gendarme or Runa Toralapa.

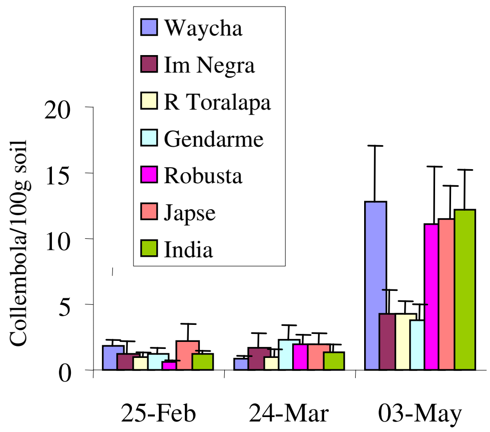


Fig. 15: The density of Collembola in the rhizosphere of 7 potato genotypes on three sampling dates during the growing season.

Gendarme plants showed a lower level of root nodulation at the end of the growing season compared to the other genotypes (Fig. 16).

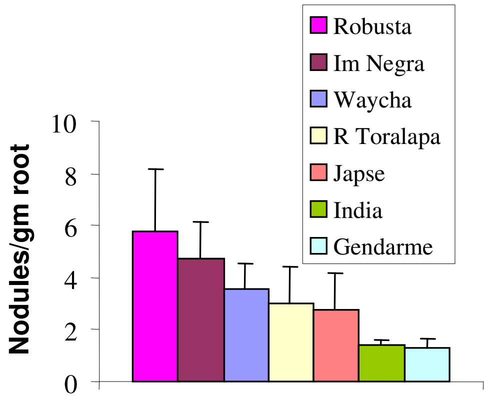


Fig. 16. Mean number of nodules caused by *Nacobbus aberrans* /g root tissue at harvest for 7 potato genotypes grown in a field trial at Toralapa.

Farmers' management practices: The number of mites/gm soil was strongly influenced by the type and amount of fertiliser used by farmers. Higher densities of mites occurred where farmers used manure rather than inorganic fertiliser and more mites were recorded where farmers applied greater amounts of manure. No significant correlations between quantitative environmental variables and the counts of microarthropods were found during the study, although positive correlations of Collembola and enchytraeids approached significance at 5% level.

6. Contribution of Outputs

6.1 We have identified *S. arvensis* and *Brassica campestris* as important hosts for *Nacobbus* and have successfully collaborated with R7325 to identify the conditions and management practices that favour the proliferation of *S. arvensis*. The weed is used as a forage crop and harvested from potato fields. The weed establishes early in the growing season and so supports early establishment of the parasitic stages of the nematode. Its close proximity to potato roots, the lack of preference for the two host plants and the increasing relative density of potato relative to *Spergula* roots all favour establishment of damaging populations on potato as the potato crop establishes.

6.2 We provide evidence to suggest three factors are of particular importance in maintaining damaging populations of *Nacobbus aberrans* in Bolivian fields:

- The weeds *Spergula arvensis* and *Brassica campestris* occur in a sufficient proportion of fields at an adequate density to play a major role in maintaining damaging populations of *Nacobbus.*
- Volunteer potato densities are also likely to be important in many fields in maintain the nematode at densities that can threaten subsequent potato crops.

- Work by PROINPA suggests that seed potatoes are a common source of introduction of the nematode to fields and may sometimes caused the infested potato seed to produce a damaged crop.
- The relationship between host density and levels of nodulation on potato roots is currently being studied. This new information will facilitate the development of management strategies to minimise *Nacobbus* damage in farmers' fields.

6.3 We have identified a sub-set of non-target organisms for which the deployment of transgenic nematode resistant potatoes offers the greatest potential hazard. However bioassays have shown that the levels of expression that provide enhanced nematode resistance do not necessarily pose a hazard to this sub-set of these insects that feed on aerial plant parts. The levels of expression are not similar in all green tissues. The intention to deploy only transgenic plants with expression restricted to root systems provides a second level of protection for non-target aerial invertebrates.

6.4 The effect of transgenic plants on soil mesofauna has been compared with the effect of an alternative nematode control option, the oxime carbamate nematicide aldicarb. Such compounds are used in Bolivia for insect control and very widely used in Peru to control *Globodera* spp. In addition the impact of currently used management practices on soil mesofauna has also been studied. The work shows that aldicarb applied to soil to control nematodes has a profound effect on non-target invertebrates. Other agronomic practises also have consequence. It provides a basis for the development of further more detailed analyses on a rational basis. Such work underpins decisions by farmers, institutions and government about the relative environmental impact of different nematode control options.

7. Further research indicated

7.1 Key aspects of the biology of *Nacobbus* require further definition.

- Current bioassays do not provide an economic threshold at potato planting. This has value in developing an algorithm for pest management.
- Definition of cropping practices that help prevent the nematode from maintaining densities that damage potato.
- Differences in host range of the known biotypes
- Development of weed management practices that help suppress *Nacobbus* populations such as intercropping
- Development of alternative forage plants to *Spergula* without adding cost to growers. alternative forage plants.

7.2 Estimating the control "gap" possible with IPM schemes that growers are likely to adopt.

7.3 Establishing if GM approaches to IPM strategy and bridge the control "gap".

7.4 Further work on the environmental safety of the transgenic approach should concentrate on soil organisms.

8. Uptake pathways

- The work on IPM of *Nacobbus* should be developed as collaboration involving PROINPA and CPS and others such as those with interest in weed control. The nematode is only a problem in the Alto Plano and valles Bolivia and the Alto Plano of Peru where this abuts that of Bolivia. Hence PROINPA is an appropriate developing world institute. CPS now has c10 person years experience of collaborating with PROINPA and have done much to progress understanding of nematode damage to potato crops in Bolivia.
- The opportunities for transgenic control of *Globodera* and *Nacobbus* should be developed involving PROINPA, CPS and possibly CIP. Sufficient progress has been made to show that the approach could be deployed effectively within an IPM scheme for control of both *Globodera* spp and *Nacobbus*. Work to date has shown the approach is environmentally safe and further work on this continues (EU and MAFF funding). The approach has also been shown to be biosafe from a food standpoint. We agree with the view expressed in a recent meeting of DFID environmental protection department that this technology represents the best opportunity for DFIFD to support a biosafe GM approach with a clear poverty focus. We believe is work should be funded by a collaboration between CPP and PSP programmes of DFID.

8. Publications

This grant has been for a 1-year period only. The main findings will be submitted for publication within 12 months. Some of our views developed in part in this work are in press in two reviews. They concern the relevance of transgenic potato for nematode control in Bolivia is summarised in a review in press.

1. Atkinson, H.J., Green, J. Cowgill, S. Urwin, P., Franco, J. and Witcombe, J. (2001). Developing a paradigm for safe adoption of GM crops with a poverty focus: a specific example of nematode resistance for potato in Bolivia. In Conference proceedings "*Sustainable agriculture in the new millennium – the impact of biotechnology on developing countries*" Brussels, May 28-31, 2000. In Press, FOE, Europe.
2. Atkinson, H.J., Green, J. Cowgill, S, and Levesley, L. (2001). The case for GM crops with a poverty focus, *Trends in Biotechnology*, *In press*.

These views were also expressed in an invited contribution to DFID GM policy:

1. Atkinson, H.J. (2000). Developing a paradigm for safe adoption of GM crops with an poverty focus: as specific example of nematode resistance for potato in Bolivia.
   Consultation of Environmental Protection Dept. of DFID with research programme managers, project leaders and advisers on 19th December 2000 at 94 Victoria St, London.

Our other work relevant to the scope of this report is:

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.
3. 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.
4. 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,).
5. Cowgill, S.E. (2000). The effect of transgenic potatoes on non-target organisms. In DFID PSP +CPP/CIP Conference: Biosafety of GM Potato in the Developing World, Manchester, June 2000
6. Urwin, P.E., Lilley, C.J., Atkinson, H.J. (1998) Nematode control by genetically modified crops (Ed. Dale, M.F.B. et al) In, *Aspects of Applied Biology* 52: Production and Protection of sugar beet and potatoes. pp. 255-262
   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
7. 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.*
8. 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*