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Final Report

Improving management of white-fringed weevils in potatoes

Dr Geoffrey Allen
Tasmanian Institute of Agriculture (TIA) - University of Tas

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Project Leader's contact name and contact details and a list of other key personnel:

Geoff Allen, Tasmanian Institute of Agriculture (TIA), University of Tasmania, Private Bag 54, Hobart, Tasmania 7001

Tel: (03) 62336841; Email: Geoff.Allen@utas.edu.au

Paul Walker (TIA), Noel Davies (Central Science Laboratory, University of Tasmania), Paul Horne (IPM Technologies Pty Ltd), Scott Johnson (University of Western Sydney).

Purpose of the Report:

The white-fringed weevil (*Naupactus leucoloma*) is a major pest of potatoes in Australia. Grubs live in the soil where they cause devastating damage to the roots and tubers of crops. Increasingly, potato growers rely on the application of pre-plant insecticide sprays to insure against white-fringed weevil grub damage. However, the application of such sprays may be unwarranted if densities of white-fringed weevil grubs are too low to cause economic damage. To make informed decisions on the need to spray insecticides, it is necessary to accurately determine the density of white-fringed weevil grubs present in a paddock using a reliable sampling plan. Such a sampling plan has been developed for mainland potato crops but has not yet been tested for Tasmanian populations of white-fringed weevil nor has it been adequately extended to all Tasmanian potato growing districts. Currently, there is no alternative method for monitoring white-fringed weevil populations in crops other than sampling for grubs in the soil. Recent research has shown that the grubs of many subterranean insects, detect and orient towards specific volatile compounds released from their host-plants. This report details Project PT09027 which: A) Evaluated and extended the existing white-fringed weevil grub sampling plan to Tasmanian potato growers; B) Conducted novel research to determine how white-fringed weevil grubs detect the presence of host-plant roots in the soil.

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MEDIA SUMMARY

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Currently, there is no alternative method for monitoring white-fringed weevil populations in crops other than sampling for grubs in the soil. Recent research has shown that the grubs of many subterranean insects, such as weevils, do not find their host-plants through random searching for suitable roots in the soil. Rather, they detect and orient towards specific volatile compounds released from their host-plants.

Accordingly, this project had two objectives: A) The evaluation and extension of an existing white-fringed weevil grub sampling plan to Tasmanian potato growers; B) Conduct novel research to determine how white-fringed weevil grubs detect the presence of host-plant roots in the soil.

We found that the sampling plan developed for mainland potato growing regions was suitable for adoption in Tasmania. Sampling in winter months ensured that all white-fringed weevil grubs were large and readily visible in the soil. Several workshops for potato industry stakeholders were held to extend the sampling plan. The results of bioassays found strong evidence that white-fringed weevil grubs could detect the presence of lucerne roots in the soil but were unable to discriminate lucerne from the roots and tubers of other plants, including unfavourable hosts such as sorghum. The attraction of potato roots and tubers to white-fringed weevil grubs was weak. Chemical analysis of compounds emanating from the roots of lucerne and potato plants found volatile compounds but none appear likely to act as a significant attractant to white-fringed weevil grubs. There was considerable variation in the composition and amount of volatile compounds emanating from potato roots, both within and between potato varieties, and this may have been responsible for the inconsistencies observed among the bioassays that measured susceptibility of tubers to white-fringed weevil grubs.

Recommendations for further research are given. A literature review on whitefringed weevils is attached in the Appendices.

TECHNICAL SUMMARY

The white-fringed weevil (*Naupactus leucoloma*) is a major pest of potatoes in Australia. Larvae live in the soil where they can cause devastating damage to the roots and tubers of crops. To make informed decisions on the need to spray insecticides to control *N. leucoloma* larvae before planting potatoes, it is necessary to accurately determine the density of white-fringed weevil larvae present in a paddock using a reliable sampling plan. Such a sampling plan has been developed for mainland potato crops but has not yet been tested for Tasmanian populations of white-fringed weevil nor has it been adequately extended to all Tasmanian potato growing districts.

Currently, there is no alternative method for monitoring white-fringed weevil populations in crops other than sampling for larvae in the soil. Recent research has shown that the larvae of many subterranean insects, such as weevils, do not find their host-plants through random searching for suitable roots in the soil. Rather, they detect and orient towards specific volatile compounds released from their host-plants.

Accordingly, this project had two objectives: A) The evaluation and extension of an existing white-fringed weevil larval sampling plan to Tasmanian potato growers; B) Conduct novel research to determine how white-fringed weevil larvae detect the presence of host-plant roots in the soil.

Major outcomes and findings of the project were: i) the sampling plan developed for mainland potato growing regions was found to be suitable for adoption in Tasmania; ii) sampling in winter months ensured that the majority of white-fringed weevil larvae were present as instars 9-11th and readily visible in the soil; iii) several workshops for potato industry stakeholders were held to extend the sampling plan; iv) we found strong evidence that white-fringed weevil larvae could detect the presence of lucerne roots in the soil but were unable to discriminate lucerne from the roots and tubers of other plants, including unfavourable hosts such as sorghum; v) although chemical analysis of compounds emanating from the roots of lucerne found volatile compounds, none were likely to act as an attractant to *N. leucoloma* larvae; vi) there was some evidence that *N. leucoloma* may also be attracted to volatiles emanating from feeding conspecifics or damaged host plants; vii) respiratory CO_2 emissions from host-plant roots and/or feeding conspecifics may be involved in the attraction of *N. leucoloma* larvae and warrants further investigation; viii) the attraction of potato roots and tubers to *N. leucoloma* larvae was weak and potato varieties displayed considerable variability in susceptibility to larvae attack; ix) chemical analysis found substantial variation in the composition and amount of compounds emanating from potato roots, both within and between varieties; x) further investigation of this variation in the chemical composition of potato roots and tubers is needed to interpret possible mechanisms underpinning potato varietal susceptibility to *N. leucoloma* larval attack. A literature review on whitefringed weevils is attached in the Appendices.

INTRODUCTION

The white-fringed weevil, *Naupactus leucoloma*, is a serious pest of potatoes, lucerne and several other crops in Australia. Native to South America, *N. leucoloma* was first reported attacking lucerne in New South Wales in 1932. Since then it has spread to Victoria, South Australia, Queensland, Western Australia and most recently to Tasmania. The soil-dwelling larva is the damaging stage, attacking the roots and tubers of a wide variety of crop and ornamental plants. While adult *N. leucoloma* also feed on the leaves of a wide variety of crop plants, they do not cause economic damage. Control of *N. leucoloma* larvae is difficult due to their protected location in the soil. Damage is usually worse in crops planted immediately after long rotations of lucerne or pasture containing legumes, such as clover, under which high populations of *N. leucoloma* can build-up unnoticed. Consequently, it is essential that sampling plans for *N. leucoloma* larvae are conducted prior to planting susceptible crops in spring. A sampling plan has been developed for mainland potato crops but has not yet been tested for Tasmanian populations of white-fringed weevil where seasonal phenology may differ nor has it been adequately extended to all Tasmanian potato growing districts.

Currently, there is no alternative method for monitoring white-fringed weevil populations in crops other than sampling for larvae in the soil. However, recent research has shown that the larvae of many subterranean insects, such as weevils, do not find their host-plants through random searching for suitable roots in the soil. Rather, they detect and orient towards specific volatile compounds released from their host-plants. This exciting finding may offer a way to develop novel methods for monitoring and/or controlling white-fringed weevil larvae. By isolating and identifying the compounds which attract or deter white-fringed weevil larvae, it may be possible to develop a synthetic attractant/deterrent bait.

The objectives of this project were:

A) Further development of an efficient monitoring and spray decision system for *N. leucoloma*: extension of an existing sampling programme for *N. leucoloma* larvae (developed on the mainland) to Tasmanian potato growers through field days in order to prevent the application of unwarranted 'insurance' sprays at planting; further development of sampling plan to determine thresholds for spraying and expanding the knowledge of *N. leucoloma* biology in Tasmania. Extension of information through field days, in collaboration local agronomists, and through publications that where possible will be made freely available via AUSVEG, TIA and industry related web sites.

B) Conduct novel research to find volatile compounds in plant roots that could be used as deterrents or attractants for monitoring or controlling *N. leucoloma* larvae.

Accordingly, this report is presented in two sections to reflect the above objectives. Recommendations arising from both project objectives are presented together. A literature review on the biology and ecology of *N. leucoloma* was conducted at the start of the project and is attached in Appendix 1.

SECTION A: Extension of existing sampling plans for white-fringed weevil larvae to Tasmanian potato growers

Introduction

Although *N. leucoloma* was first recorded in New South Wales in 1932, it has only relatively recently reached Tasmania and become a major pest of potatoes. *Naupactus leucoloma* probably entered Tasmania in the mid-1980's but was not officially recognised until 1992 (McQuillan et al. 2007). Infestations were initially found around Scottsdale, Swansea and Ouse but *N. leucoloma* is now present in most agricultural areas of the state, including in the major potato growing region in Sassafras-Devonport-Ulverstone area, northern Tasmania, where it has had a major impact on production.

Once established in a field, *N. leucoloma* infestations are difficult to eradicate or suppress without long rotations of non-host crops or the use of expensive soil fumigants (Horne et al. 2002). Currently, many growers apply sprays of broad-spectrum insecticides at planting to 'insure' against *N. leucoloma* infestations in potato tubers without determining the presence of the pest beforehand. Such applications are not only costly but they are also wasteful if pests are not present at economically damaging levels and they may be harmful to beneficial soil organisms. Both the adults and larvae of *N. leucoloma* are notoriously difficult to control effectively with insecticides due to their natural tolerance to several toxins, patchy distribution, difficulty in reaching pests located in the soil and due to the low damage threshold of many crops (Barnes and De Barro 2009). Nematodes and fungi, developed as biological control agents for other weevil species, may be effective in controlling *N. leucoloma* but their efficacy is unknown and they are expensive to purchase. The new biofumigant, methyl isothiocyanate, can also result in variable control and is often not viable to use due to high costs ($A700/Ha) (Barnes and De Barro 2009).

A sampling plan for insect soil pests, including *N. leucoloma* larvae, in potato crops was devised in Western Australia by Matthiessen & Learmonth (1993) and further developed by Learmonth (2005). The method uses a spade to sample a 15 cm x 15 cm area of soil, to a depth of about 20 cm, thus each sample unit consisted of a surface area of 0.0225 m^2 (or 0.0045 m^3 volume of soil). In an average sized paddock, the recommended minimum number of samples to take is five clusters at nine evenly placed locations, so that a total surface area of 1.0125 m^2 is sampled.

The sampling plan relies on the *N. leucoloma* larvae being of a size visible to the unaided eye. Consequently, in south-eastern Australia sampling is best conducted during late winter as this is when most larvae are a large size (P. Horne, pers. comm.). Exceptions may occur if conditions over winter have been too cold and/or too dry for larval development. In pasture paddocks being rotated to potatoes, sampling is best done before the paddock is worked in preparation for planting as this can make larvae harder to find (Learmonth 2005).

The sampling plan has not yet been tested for Tasmanian populations of *N. leucoloma* nor has it been adequately extended in Tasmanian potato growing districts. Therefore, the objective of this part of the project was to apply the sampling method in Tasmanian potato growing districts to determine whether it was accurate in detecting the presence of *N. leucoloma* larvae in the soil. We

also gave presentations on the sampling plan at several field days to Tasmanian potato growers and consultants.

Material and methods

Paddocks sown to potatoes or pasture with legumes were sampled during 2011, 2012 and 2013, in order to assess the appropriateness of the sampling plan to Tasmania. We targeted potato paddocks with known infestations of *N. leucoloma* larvae in the past and pasture paddocks that were being rotated to potatoes in the following spring, where possible. Between February and July 2011, 10 paddocks in NW Tasmania (Sassafras-Devonport-Ulverstone area) were selected for sampling. In June 2012 and November 2012, 10 paddocks located in NW (as above), NE (Scottsdale-Herrick area) and eastern (Swansea area) Tasmania were selected for sampling. Follow-up sampling of those paddocks sampled in 2012 was conducted in March 2013 in order to relate larval numbers and economic damage where potatoes were planted. The clustered sampling method was adopted, based on the method of Learmonth (2005) and modified by P. Horne, whereby five soil samples (15 x 15 x 20 cm) from nine randomly selected locations were taken from each paddock. Each spade sample was visually searched to record the number of *N. leucoloma* larvae found.

Fig. 1. Sampling for *N. leucoloma* larvae in a pasture paddock in north-west Tasmania (photo courtesy of S. Jamieson).

In 2012, representative soil samples from each field sampled were transported back to the laboratory to search for the presence of early instar *N. leucoloma* larvae which may have been overlooked during visual searches. Soil samples from each site were processed by means of simultaneous vibration and washing separation (wet sieving: Fritsch Analysette 3 Pro) through sequential stacked sieves of apertures 2.0 mm, 1.0 mm and 355 µm. Samples took between 20-30+ minutes to process depending primarily on clay content. The final sieved sample (particles <1.0 mm and >355 µm) was washed back into the sample container and the organic component floated off with ~ 300 ml of +1.2 specific gravity (SG) sugar solution. The organic component was checked under a binocular microscope for the presence of *N. leucoloma* larvae.

Results

In 2011, *N. leucoloma* larvae were present in two potato fields and one pasture paddock, out of a total of 10 fields selected for sampling. In all three, low numbers of late-instar (9-11th) *N. leucoloma* larvae (< 1/spade sample) were found. In 2012, high larval numbers were found in four out of ten paddocks sampled (Table 1). In one pasture paddock sampled in NW Tasmania, large numbers of larvae of other weevil species were found but were readily discounted as *N. leucoloma* larvae based on their smaller size, smaller mandibles and lack of a retracted head capsule. In a pasture paddock in eastern Tasmania, low numbers of fly larvae and other weevil species were also found. Three of the pasture paddocks were subsequently sprayed with insecticide (fipronil or fenitrothion) for the control *N. leucoloma* larvae before planting potatoes in spring. No significant damage of tubers by *N. leucoloma* larvae was reported from these paddocks and sampling in March 2013 did not find any larvae, pupae or adult weevils. The other highly infested pasture paddock was not planted to potatoes but was partially sprayed with fenitrothion in September 2012 in order to reduce *N. leucoloma* larvae which were significantly damaging legumes. On resampling in March 2013, only very low numbers of adult weevils were found. Another pasture paddock sampled in spring 2012 which had moderate numbers of *N. leucoloma* larvae, was also sprayed with fipronil before planting. Again, no significant levels of *N. leucoloma* damage in potatoes was reported. In all other paddocks sampled in winter 2012, where no or very low numbers of *N. leucoloma* larvae were detected, no significant damage to potatoes was reported.

No early instar *N. leucoloma* larvae were found in any of the soil samples, despite washing the soil through sequential stacked sieves of apertures 2.0 mm, 1.0 mm and 355 µm. However, a number of other organisms (including worms, ants and other beetle larvae) were detected, confirming that the technique used was effective in the extraction of small invertebrates from sediment samples.

Table 1. Summary of number of *N. leucoloma* larvae found in Tasmanian pasture and potato fields sampled in 2012.

District sampled	Paddock history	Date sampled	Min/max no. larvae per m^2	Mean no. larvae/ sample	Risk	Action in following season	Tuber damage
NW Tasmania	Potatoes	June 2012	0/4	0.02	Low	No action	No damage reported
NW Tasmania	Potatoes	June 2012	0/0	0	Low	Not planted to potatoes	No damage reported
NW Tasmania	Pasture	Nov 2012	0/18	0.6	Medium	Pre-plant spray	Acceptable
NW Tasmania	Pasture	Nov 2012	0/27	1.2	High	Pre-plant spray and avoided planting in highly infested areas	Acceptable
NW Tasmania	Pasture	June 2012	0/0	0	Low	No action	No damage reported
NW Tasmania	Pasture/ potato	June 2012	0/0	0	Low	No action	No damage reported
NE Tasmania	Pasture	Nov 2012	0/49	1.6	High	Pre-plant spray	Acceptable
NE Tasmania	Pasture	Nov 2012	0/18	0.6	High	Delayed planting, sprayed pasture to reduce legume damage	Not planted
E Tasmania	Pasture	Nov 2012	0/9	0.1	Medium	Pre-spray after ripping	Low
E Tasmania	Pasture	Nov 2012	0/0	0	Low	Pre-spray after ripping	Low

Discussion

This project confirmed that sampling plans for *N. leucoloma* larvae developed on the mainland were suitable for adoption in Tasmanian potato growing regions. Sampling conducted during winter months in pasture fields, that were due to be prepared and planted to potatoes in the following season, confirmed that all *N. leucoloma* larvae present were in the late instar stage (9-11th instar). The absence of early instars, meant that any larvae present in the soil were readily visible, easy to count and identify. Therefore, the recommended timing of sampling for *N. leucoloma* larvae in Tasmania during winter is appropriate and should be adhered to. The accuracy of the sampling plan to reliably estimate *N. leucoloma* numbers in paddocks was affected by the risk of false positives (i.e. overestimation of the number of *N. leucoloma* present) when other weevil larvae species were present that were difficult to differentiate from *N. leucoloma* larvae. Conversely, false negatives (i.e. underestimation of the number of *N. leucoloma* present) could occur due to the patchy distribution of *N. leucoloma* larvae, particularly in large paddocks where infestations could be readily missed.

Several presentations were given to Tasmanian growers and consultants on the sampling method during the course of the project and publications were distributed (Tables 6 and 7). However, the impact of this extension on the incidence of 'calendar' and 'insurance' sprays of broad-spectrum insecticides against *N. leucoloma* is unknown. For confidentiality reasons, it was not possible to obtain data on the amount of insecticide applied for *N. leucoloma* control from chemical suppliers in order to compare the amount applied at the beginning and end of the project. Also, extension activities on *N. leucoloma* sampling plans started prior to the commencement of this project, due to lengthy delays in the finalisation of the contract. In 2009, Dr Iain Kirkwood (formerly Tasmanian Institute of Agriculture) organised two field days where Dr Paul Horne and Jessica Page (IPM Technologies Pty Ltd, Victoria) demonstrated the *N. leucoloma* larvae sampling method to growers and consultants. Consequently, from talks with several Tasmanian growers and consultants at the start of the project, it was apparent that there was already a level of awareness of the sampling plan and of cultural control practices which could reduce *N. leucoloma* damage on farms (such as sowing legume-free pasture during crop rotations). Conversely, there were perceived problems with the sampling plan and a reluctance by some consultants to adopt the practice due to the time taken to undertake sampling. The recommended number of samples needed for accurately detecting the presence of *N. leucoloma* larvae (a minimum of 45 spade samples) was considered by some agronomists too laborious for regular uptake. While these agronomists were prepared to conduct sampling for *N. leucoloma* larvae if requested by a grower, they more relied on their knowledge of the history of infestations in individual paddocks when making a decision to apply insecticides. There was also a reluctance to risk crop damage without the application of an ‘insurance’ insecticide spray prior to planting, particularly in the case of growers of seed potatoes, where the threshold for the presence of *N. leucoloma* larvae in the soil was very low (N = 1) due to the high economic penalty for damaged/infected potatoes. Accordingly, a reduction in the number of ‘insurance’ sprays applied to potato crops based on the adoption of existing *N. leucoloma* sampling plans, appears unrealistic unless an alternative sampling method is devised that is less laborious to apply.

SECTION B: Orientation of white-fringed weevil larvae to host-plant roots and tubers

Introduction

Currently, there is no chemical attractant which can be used to monitor or control *N. leucoloma*. Because all *N. leucoloma* populations consist of females (as they reproduce by parthenogenesis), this species does not utilise a sex pheromone. However, recent research has shown that the larvae of many subterranean insects, such as weevils, do not find their host-plants through random searching for suitable roots in the soil. Rather, they detect and orient towards specific volatile compounds released from their host-plants (Bais et al. 2006, Johnson et al. 2005, Johnson & Gregory 2006). At least 74 other compounds have been found to elicit behavioural responses in root-feeding insects, with the majority (> 80%) causing attraction (Johnson & Nielson 2012). This exciting finding offers a way to develop novel methods for monitoring and/or controlling soil-feeding insects such as *N. leucoloma*. By isolating and identifying the compounds which attract or deter root-feeding *N. leucoloma* larvae, it may then be possible to develop a synthetic attractant/deterrent bait, such as is successfully used against other subterranean pests (e.g. termites). An attractant could be used to monitor for the presence of damaging populations of *N. leucoloma* to replace the more laborious method of sampling soil for weevil larvae. An attractant could later be combined with a biopesticide (such as a nematode or fungi) to form a lethal bait ('lure and kill') which could then be distributed in the soil at planting. Alternatively, a deterrent could be used to reduce the amount of *N. leucoloma* feeding on potato roots and tubers.

Research on the attraction of *N. leucoloma* to the roots/tubers of host-plants has not been conducted. Although both the larval and adult stages of *N. leucoloma* are highly polyphagous, some host-plants are more favourable to survival and fecundity than others. Adults reared on legumes, particularly lucerne and clover, can lay over 1000 eggs while those reared on fibrous rooted plants, such as sorghum, lay less than 58 eggs (Ketchersid & Klingeman 2007; Ottens & Todd 1979; East 1977). Survival and fecundity is also likely to be affected by the quality of the larval diet but no studies to show this appear to have been conducted. Consequently, it is likely that there is strong selection pressure for *N. leucoloma* larvae to be able to effectively orientate towards the roots of favoured host plants in mixed stands of plants, such as in natural or cultivated pastures. Hardwick & Prestidge (1996) suggested that *N. leucoloma* larvae do actively select the roots white clover plants in New Zealand pastures. The roots of unfavourable host plants may also release compounds that act as a deterrent to *N. leucoloma* larvae. There is evidence that sorghum may be toxic to *N. leucoloma* larvae (Learmonth 2005), therefore it is possible that the roots may contain compounds that act as a repellent.

We undertook novel research to look for evidence of attraction/repulsion of *N. leucoloma* larvae to plant root/tuber volatiles. Expert advice was obtained from Dr Scott Johnson (formerly Scottish Crop Research Institute) who was hosted at the University of Tasmanian for two weeks in January/February 2011 in order to discuss methodologies with the project team members. Bioassays were conducted to determine the ability of *N. leucoloma* larvae to detect the presence of roots of a highly favourable host-plant (lucerne), compared to those of less favourable host-plants (potatoes, cabbage, carrots) and an unfavourable (sorghum) host-plant. The relative attraction/susceptibility of the tubers of five potato varieties to *N. leucoloma* attack was also compared. Volatile compounds emanating from the roots/tubers of lucerne and potato plants, that may play a role in the orientation of *N. leucoloma* larvae, were collected and analysed using gas chromatography.

Materials and methods

Laboratory cultures of *N. leucoloma*

A laboratory culture of *N. leucoloma* was established from adult weevils collected from potato crops in northern Tasmania (Devonport-Ulverstone region). The colony was supplemented with adults reared from late-instar larvae collected in pasture (Herrick region, northern Tasmania) and with collections of adults collected from lucerne (Tamworth region, New South Wales). To obtain eggs, adult weevils were held in cylindrical 500 mL plastic containers and provided with lucerne as food. The lucerne was inserted into a water container to keep fresh and changed as needed. The base of containers were lined with tissue paper and pieces of wooden sticks (paddle pop sticks) as a medium for egg laying. The tissue paper and wooden sticks were checked every 3-4 days for egg masses. Egg masses were placed in petri dishes (9 cm diam.) lined with filter paper (Whatman® No. 4), saturated with distilled water. Petri dishes were sealed with Parafilm® to retain moisture. Following the methods of Gross (1972a), the development of egg masses was manipulated to provide 1st instars for bioassays or cultures on an as need basis. Egg masses were kept in petri dishes at room temperature under excess moisture conditions (100% humidity) for approximately one week to allow partial embryo development. If larvae were required immediately, egg masses were re-watered to allow the full development of embryos. Hatching was delayed by allowing the petri dishes containing the partially developed embryos to dry out, and re-watering when larvae were required. Partially developed egg masses could be effectively stored at room temperature for up to three months, without a significant reduction in hatching rates, to provide 1st instar larvae for testing in bioassay chambers on an as need basis. Also, an extended supply of egg masses was attainable due to the longevity of adult females, some of which laid eggs for up to three months in captivity.

On hatching, 1st instar larvae (24-48 hours old) were transferred to 50 mL plastic pots partially filled with potting mix (Yates, passed through a 3 mm sieve and moistened with distilled water to 20-25% w/v), using a wet, fine hair brush. Three to five 1st instars were placed in each pot. Sections of carrot tubers (c. 5 mm thick) were provided as food for larvae and changed on an as need basis. Pots were placed in plastic containers (30 x 20 x 15 cm) with a tight fitting lid, lined with moistened paper towelling to maintain a high humidity. Larvae were held at room temperature until they developed to the instars required for testing in bioassays. By staggering hatching times, a continuous supply of larvae for bioassays was achieved. A proportion of the larvae were successfully reared to pupation and adulthood for culture maintenance.

While obtaining eggs and 1st instar larvae under laboratory conditions was not difficult, we experienced problems in culturing 1st instars larvae to mid-late instars and pre-pupae to the pupal stage. Primarily, this was due to the low survival rate of 1st instars (5-20%), thus requiring a large numbers of pots to be set-up in order to provide adequate numbers of larvae for bioassays. A high proportion (40-80%) of prepupae failed to pupate for unknown reasons (but see Literature Review). An improvement in 1st instar survival rates occurred when the initial soil moisture was reduced from 25% to 20%. Also, it was difficult to differentiate the final feeding larval instar (10th instar) from the non-feeding final instar (11th instar), affecting the ability to choose larvae that were suitable for bioassays.

Bioassays for testing attraction/repulsion of *N. leucoloma* larvae to plant roots and tubers

Two types of two-choice bioassay chambers were constructed based on the design of Johnson et al. (2004): a Y-tube design (Fig. 1A) and a linear tube design (Fig. 1B & C). Bioassay chambers were constructed from plastic vials (50 mL, height = 55 mm, diam. = 40 mm) with the bases removed to form an open cylinder. A Y-section was formed by two vials cut at a 45 ° angle and attached together using silicone sealant. Vial lids, with the centres removed, were placed over the cut end of vials and sealed using silicone. This allowed the assembly of several vials together to form modular bioassay units, which could be readily dismantled by unscrewing in order to determine the position of *N. leucoloma* larvae at the end of the experiment. The arms of bioassay units were attached to 500 mL pots, in which plant material for testing the response of *N. leucoloma* larva was grown, using a vial lid with the centre removed, inserted into a hole of corresponding diameter cut into the side and sealed with silicone (Fig. 1A, B). The arms of linear bioassay chambers were also connected to 50 mL plastic vials to test the response of *N. leucoloma* larvae to sections of plant tubers or to young seedlings. Fine gauze was placed over the ends of bioassay chambers to prevent *N. leucoloma* larvae from entering treatment pots and contacting plant roots.

Assembled bioassay units were placed in plastic boxes (54 x 39 x 30 cm) and covered with fine sand maintain *N. leucoloma* larvae in darkness (Fig. 4B). The soil in bioassay chambers were left to equilibrate for three days before introducing a single *N. leucoloma* larva via a pipette tube pushed into a hole in the centre (neutral zone). Early, mid and late instar larvae from laboratory cultures that had fed on carrot tubers in the last 24 hours were selected for bioassays and were starved for 1-2 days before placing in the bioassay units. Individual larvae were only used in a bioassay once. Larval instars were estimated based on larval weights and the data of Gough & Brown (1991).

Experiments were conducted under ambient temperature conditions. Initially, bioassays were run for 24 hours but due to the high number of larvae remaining in the neutral zone, this was extended to 72 hours to allow larvae more time to choose between treatments.

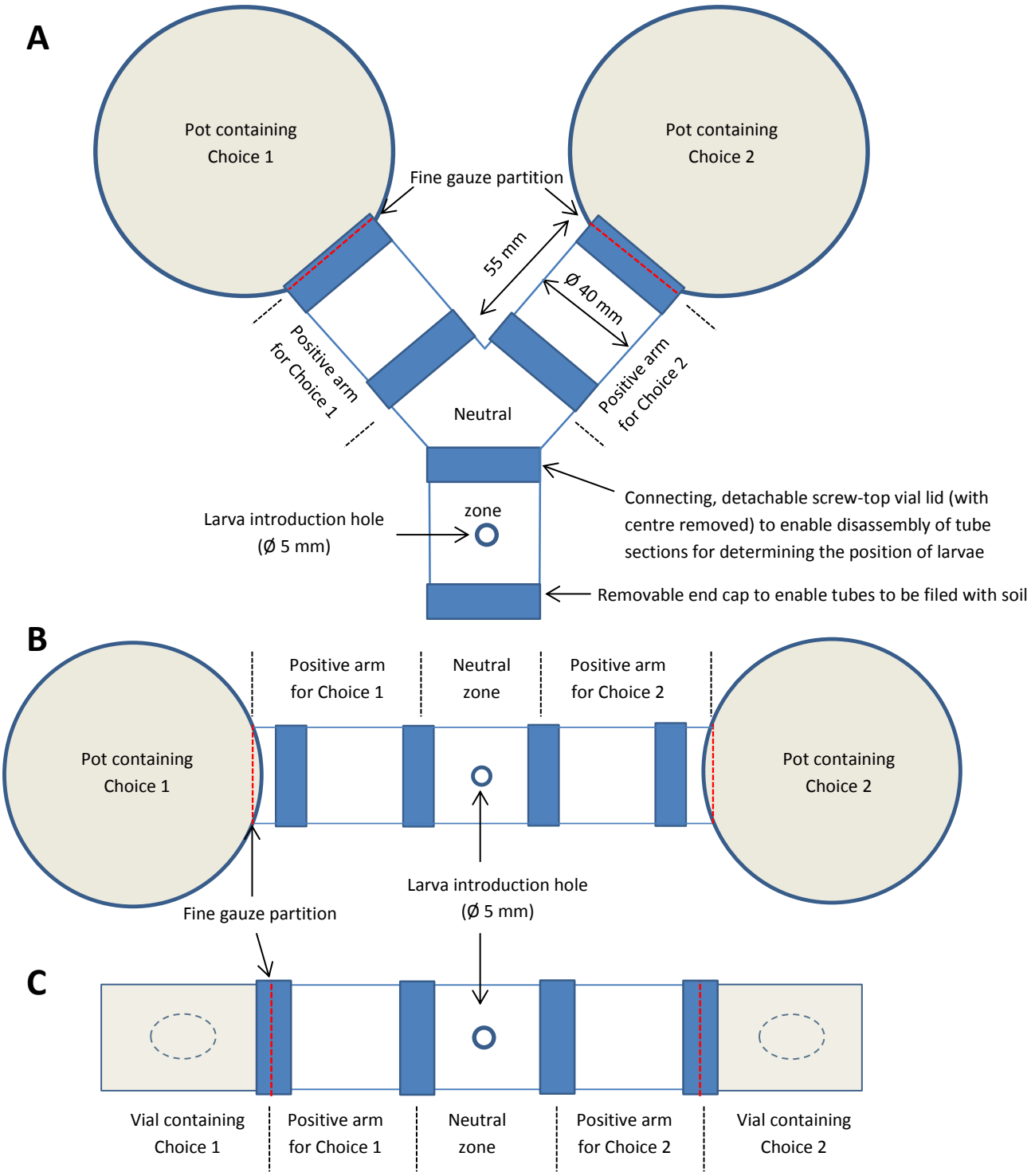

Fig. 2. Schematic of choice test bioassay units. (A) Y-tube bioassay unit attached to pots containing choice material (e.g. plants or tubers in soil, soil only); (B) linear tube bioassay unit attached to pots containing choice material (as in A); (C) linear tube bioassay unit attached to vials containing choice material (e.g. sections of plant tubers or young seedlings). Fine gauze partitions prevented larvae from contacting the roots or tubers of test plant material. Tubes were filled with soil and a single *N. leucoloma* larva was introduced through a hole at the end of the Y-tube or at the centre of the linear tube.

Fig. 3. Choice test using a Y-tube bioassay unit to compare the attraction of plant roots (potato plant vs. soil only pictured) to *N. leucoloma* larvae. Assembled units were placed in a plastic box and covered with fine sand to maintain the Y-tube in darkness during the bioassay.

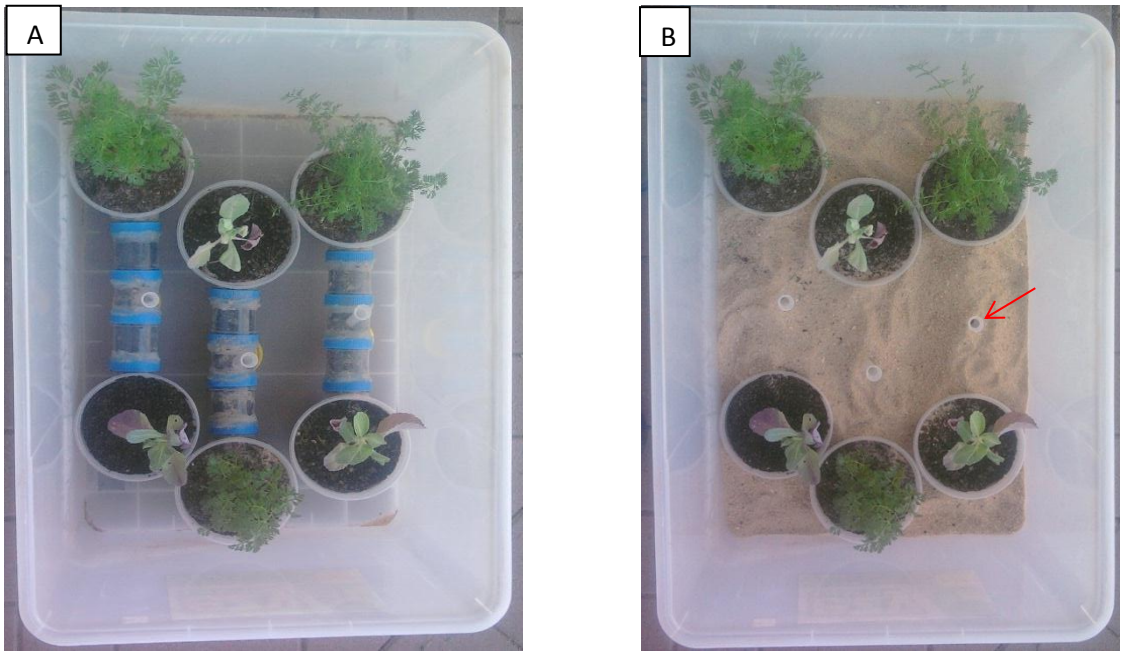

Fig. 4. Choice test using linear bioassay units to compare the attraction of plant roots (carrots plants vs. cabbage plants pictured) to *N. leucoloma* larvae. (A) Linear tubes loosely packed with sieved and moistened potting mix and connected to test plants grown in separate pots. (B) Assembled units covered with fine sand to keep the linear tubes in darkness during the bioassay.

Varietal susceptibility and attraction of potato tubers to *N. leucoloma* larvae

Choice and no-choice experiments were conducted to compare the susceptibility/attraction of five varieties of potato tubers (Nadine, Sebago, Mozart, Golden Delight and Red Delight) to *N. leucoloma* larval attack. Tubers of similar size and weight (~ 150 g) were selected for all experiments.

Choice tests: Plastic boxes (36 cm x 29 x 20 cm) were filled with sieved potting mix (20% water w/v) to a depth of ca 1 cm. One tuber of each potato variety was randomly placed concentrically on top of the potting mix. Tubers were covered with further potting mix and three late-instar *N. leucoloma* larvae were placed on top in the centre (Fig. 5). Ten replicate boxes were set-up and the experiment was repeated so that a total of 20 tubers of each variety were tested. After 14 days, the soil and larvae were removed to assess damage levels. The number of larval feeding holes in each tuber was recorded and the diameter and depth of feeding holes was measured.

A second choice experiment was set-up, using the same methods as above, to compare the attraction/susceptibility of tubers of potato varieties Nicola, Russet Burbank, McCain 1 Innovator, Dutch Cream and Moonlight.

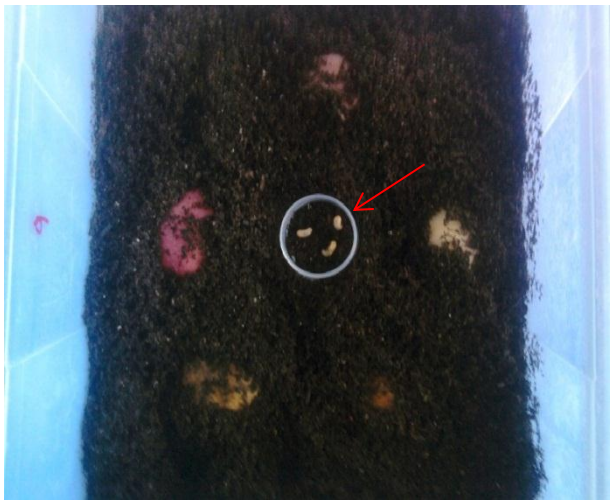

Fig. 5. Choice experiment to compare the susceptibility/attraction of five potato varieties (Nadine, Golden Delight, Red Delight, Mozart and Sebago) to *N. leucoloma* larvae. One tuber of each potato variety was randomly arranged concentrically in a plastic box and buried with sieved, moistened potting mix. Three late instar *N. leucoloma* larvae (arrow) were placed in the centre of each container and left to feed on tubers for 14 days. Ten boxes containing each tuber variety were set-up and the experiment was repeated so that 20 replicate choice tests were conducted. After 14 days, larvae were removed and levels of damage to tubers was recorded.

No-choice tests: As with the choice test, the susceptibility of tubers of five potato varieties (Nadine, Sebago, Mozart, Golden Delight and Red Delight) to *N. leucoloma* attack was compared. Late instar *N. leucoloma* larvae were placed individually into cylindrical plastic vials (7 cm x 2.5 cm diam.) filled with sieved, moistened potting mix (20% water w/v). The open end of the vial was then held against the epidermis of a tuber and secured in place using elastic bands, masking tape and staples (Fig. 6A).

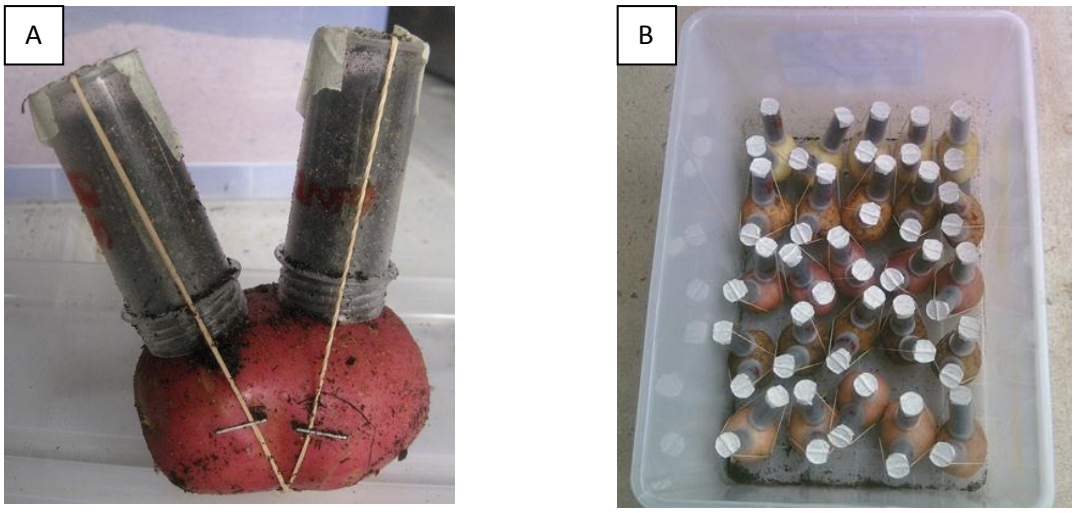

Fig. 6. No-choice experiment to compare the susceptibility of five potato varieties to *N. leucoloma* larvae: A) a late instar *N. leucoloma* larva was placed in a plastic vial containing sieved, moistened potting mix. The open end of the vial was secured to a tuber using rubber bands, masking tape and staples, to allow larva access to the epidermis. Two vials were placed on each of 10 tubers each variety (N = 20 larvae tested/variety). B) Tubers with vials attached were placed into a plastic box and buried with potting mix. After 7 days vials were removed to record levels of *N. leucoloma* damage to tubers.

In each experiment, five tubers of each potato variety with two larvae/tuber (i.e. 10 larvae/variety tested) were placed in a plastic box and buried with potting mix to maintain larvae in darkness.

The experiment was repeated so that a total of 20 tubers/variety were tested. After seven days, the soil and larvae were removed from the tubers to record the number attacked, the number of larval feeding holes in each tuber and their diameter and depth. The volume of potato consumed (mm^3) was estimated as in choice experiments. All *N. leucoloma* larvae were weighed at the start and end of the experiment to measure changes in weight.

Analysis of compounds emanating from plant roots

Closed-loop air entrainment method

Initially, volatile compounds emanating from the roots of potato and lucerne plants were sampled using a closed-loop air entrainment system. Certified disease free potato tubers (Russet Burbank and Innovator (Mc 1)) and lucerne seeds were planted into 25 cm plant pots filled with 3 litres of vermiculite and lined with a disposable polyester oven bags (Glad Products, 25 cm x 38 cm) (Fig. 7).

Potato root exudate analysis was conducted at tuber initiation. Lucerne root exudate analysis was conducted when canopy growth exceeded 30 cm. Six replicates were conducted for each plant type. Root volatiles were collected using a closed-loop air entrainment system. Root volatile samples were collected using a micro-diaphragm pump (KNF NMP015S 6V DC vacuum pump, Javac Pty, Melbourne) and Thermal Desorption Unit tubes (TDU; Markes International).

Volatiles from the root systems were collected by inserting two sections of 12 mm diameter Tygon® tubing into the Vermiculite, connected to the pump outlet (exhaust) and inlet (suction) to a depth of 20 cm and 10 cm respectively. The pump inlet and outlet tubes were spaced ca. 5 cm apart in the pots. Airflow was controlled by using a variable resistor connected to the pump's 12 V power source.

Fig. 7. Closed-loop air entrainment system used to collect plant root volatiles.

The pump was calibrated to provide a constant air flow of 100 mL/min using a bubble flowmeter (Grace Division, Melbourne). The air was filtered for impurities before entry into the vermiculite (pump outlet) using activated charcoal (BDH, product 330344Y) packed into a Pasteur pipette with the tip removed. Salinised glass wool (Alltech, Part no. 4037) was used to stop charcoal loss during sampling. The TDU tubes, filled with two absorbents (Tenax and Unicarb), were connected to the pumps inlet tube (suction). Samples from were collected for 24 hours to collect sufficient quantities of volatiles for Gas Chromatography-Mass Spectroscopy (GC-MS) analysis.

However, GC-MS data collected from root aerations using TDU tubes contained excessive artefacts from contaminants within the growing medium (Vermiculite). For this reason, this method was discontinued and an alternate method using Stir Bar Sorptive Extraction (SBSE) method was utilised.

Stir Bar Sorptive Extraction (SBSE) method

Certified disease free potato tubers (var. Russet Burbank and Innovator (Mc 1)) were planted to a depth of 5 cm and lucerne to a depth of 2.5 cm in plastic tubs (35 x 30 x 20 cm) containing 10 cm of compost. Both lucerne and potatoes were in planted in Gardeners Friend Organic Compost (product code 3385451) sieved to 2 mm. Plants were hand watered *ad libitum* and housed in a heated (20 ˚C) glasshouse at the Tasmanian Institute of Agriculture's glasshouse facility at the University of Tasmania, Sandy Bay. Potato root exudate analysis was conducted at tuber initiation. Lucerne root exudate analysis was conducted when canopy growth exceeded 30 cm.

Potato and lucerne plants that had reached their appropriate growth stage were removed from the soil, taking care to ensure minimal damage occurred to the root mass. The roots systems were

washed twice in tap water and twice in distilled water to remove as much soil from the root systems as possible. The root systems were then suspended in a 600 ml glass beaker containing 250 ml of ultra-pure water (Milli-Q, CPMQ004RE) by tying foam blocks to the plant stems using flagging tape (Fig. 8). Care was taken to ensure that only ca. 50% of the root mass was immersed in the water to prevent water logging symptoms from occurring to the plants.

Fig. 8. Stir Bar Sorptive Extraction (SBSE) method used to sample root volatiles. Potato (pictured) or lucerne plants were removed from pots and the roots thoroughly washed in water before suspending in a 600 ml beaker containing 250 ml of ultra-pure water. A foam block and flagging tape was used to ensure roots remained suspended in the water. A SBSE stir bar was then placed into the beaker and left to adsorb root exudates from the water for two days, after which compounds were desorbed for analysis by gas chromatography.

Root exudates were sampled utilising a Stir Bar Sorptive Extraction (SBSE) technique. This was achieved by placing a SBSE stir bar (Gerstel, Twister™ GC011222-001-00) into the beaker, which was left to adsorb any root exudates from the water for two days. The beakers were agitated three times daily to maximise exudate adsorption onto the stir bar. Water control samples were also conducted by placing a SBSE stir bar into a 600 ml beaker containing 250 ml of ultra-pure water.

After the adsorption phase was completed, volatiles collected by the stir bars were desorbed using a thermal desorption unit into a gas chromatograph (GC) for compound identification. SBSE stir bars were placed directly into silicosteel tubing with Silane-treated glass wool (Grace Davison, Part no. 4037) placed into either end of the tube to prevent stir bar movement during desorption. Each sample (n = 5) was then desorbed in a Markes International Inc. – Unity Thermal Desorption Unit QUI – 0002. Helium was used as the carrier gas at a flow of 20 ml/ minute with split desorption conducted at 270 ˚C for 10 minutes. The TDU trap was an inert sulphur trap (U-T6SUL) held at 25 ˚C. The trap was desorbed at 290 ˚C for 3 minutes.

Gas chromatography was conducted using a Varian CP-3800 with a 30:1 split ratio. The oven temperature was programmed from 40 ˚C (10 minute hold) to 290 ˚C at 8 ˚C per minute (2 minute hold). Carrier gas flow was helium at 1.2 mL/ minute using a constant flow mode. Mass spectroscopy was conducted using a Bruker 300-MS triple quadrupole mass spectrometer in electron ionisation

mode using 70 eV electrons. The MS was scanned from m/z 30 to 350 at 3 scans per second. Identities of major compounds were assigned based on mass spectra and Kovats retention indices relative to those reported in the NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/).

Data analysis

The results from Y-tube and linear tube bioassays were analysed using χ^2 tests on insect counts. Larvae which remained in the neutral zone of the bioassay units were excluded from analysis.

In choice and no-choice experiments, comparing the susceptibility of the tubers of five potato varieties to *N. leucoloma* attack, the results from the two replicate experiments were combined for analysis. The approximate volume of potato consumed (mm^3) was estimated by applying the formula for calculating the volume of a cylinder ($\pi*r^2*$length), which was similar to the shape of larval feeding holes. The number of tubers attacked, number of feeding holes, volume of potato eaten and changes in larval weights in each potato variety were compared using one-way ANOVAs.

Results

Bioassays for testing attraction/repulsion of *N. leucoloma* larvae to plant roots and tubers

Over 300 *N. leucoloma* larvae were used in Y-tube and linear bioassays to look for evidence of attraction/repulsion to plant roots and tubers (Table 2). Initial bioassays using early instar *N. leucoloma* larvae (2^{nd}-5^{th}) were found to be impractical due to the low recovery rates of larvae from soil in the bioassay arms, presumably due to high mortality rates. Also, searching for early instar larvae in the soil took considerable time, requiring the use of a microscope. Therefore, subsequent bioassays concentrated on the use of mid-late instar larvae (6^{th}-11^{th}) which had much higher survival rates and were more readily visible in the soil.

The only bioassay to give a highly significant ($P < 0.0001$) difference in the number of late-instar *N. leucoloma* larvae selecting one arm of a bioassay unit was in the choice test between lucerne plants and soil only: 16 (94%) of larvae were found in the arm of the Y-tube attached to the lucerne plant, no larvae were found in the arm attached to the soil pot, while one larva (6%) was found in the neutral zone (Fig. 9).

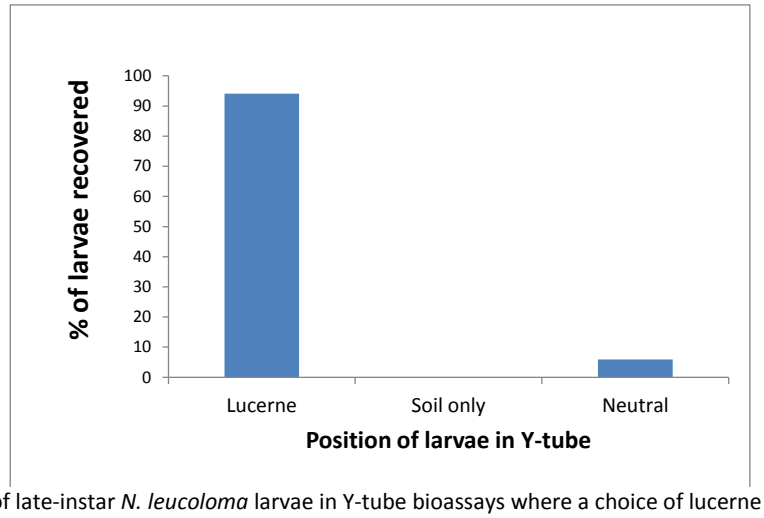

Fig. 9. Response of late-instar *N. leucoloma* larvae in Y-tube bioassays where a choice of lucerne plants vs. soil only was presented (N = 17 replicate bioassays).

When this bioassay was repeated using mid-instar *N. leucoloma* larvae, 11 out of 20 larvae (55%) remained in the neutral zone of the Y-tube, while seven larvae (35%) were found in the arm attached to the lucerne plant and two larvae (10%) were found in the arm attached to the soil pot. Discounting those larvae that remained in the neutral zone, 78% of larvae that made a choice were found in the lucerne arm compared to 22% in the soil arm. However, due to the low number of larvae making an active choice, this difference was not statistically significant ($P > 0.05$).

The only other bioassay to produce a significant ($P < 0.05$) difference in the number of *N. leucoloma* larvae selecting one arm of a bioassay unit was when late-instar larva were placed with sections of carrot and compared with soil only (Table 1). On dismantling, all sections of carrot had been damaged by *N. leucoloma* larvae during the bioassay period, except for two replicates (where the test larva had chosen the soil only arm and another remained in the neutral zone). A similar bioassay, where one arm contained a late-instar larva placed with a section of carrot and compared with the opposing arm containing carrot only, did not produce a significant result ($P > 0.05$) despite all larvae feeding on the carrot on which they were confined. Similar bioassays, conducted with a late-instar larva placed with sections of potato tubers (var. Nadine) and compared with potato or soil only, also did not produce a significant result ($P > 0.05$) (Table 1). However, these comparisons were made invalid as nearly all of the *N. leucoloma* larvae failed to feed during the bioassay period and a high proportion of test larvae remained in the neutral zone of the bioassay unit.

All other bioassays conducted did not detect any significant differences ($P > 0.05$) between the choices presented, again mainly because the majority of test *N. leucoloma* larvae remained in the neutral zone of the Y-tubes or linear tubes, resulting in the number of larvae making a choice between treatments being too low for statistical analysis. Larvae did not show any strong preference to any arm of the bioassay units when they were presented with a choice between lucerne plants and potato, cabbage or sorghum plants. Bioassays using sorghum plants, did not find that these plants had any deterrent effect on larvae. Also, we did not find any evidence of larval attraction/orientation towards potato plants grown from tubers (var. Kipfler and Nadine) or cut sections of un-rooted tubers (var. Nadine).

Table 2. Summary of Y-tube and linear bioassays to look for evidence of attraction/deterrence in *N. leucoloma* larvae towards plant roots or tubers.

Bioassay type	Larval instar used[1]	Comparison		Total N larvae tested	N larvae recovered	% larvae in bioassay zone[2]		
		Treatment 1	Treatment 2			Treatment 1	Treatment 2	Neutral
Y-tube	Mid	Lucerne	Soil	20	20	35.0	10.0	55.0
Y-tube	Mid	Lucerne	Cabbage (var. Hearty)	20	20	30.0	25.0	45.0
Y-tube	Late	Lucerne	Soil	20	17	**94.1**	**0**	5.9
Y-tube	Late	Lucerne	Potato plants (var. Kipfler)	9	9	44.4	33.3	22.2
Y-tube	Late	Lucerne	Potato plants (var. Nadine)	20	20	30.0	25.0	45.0
Y-tube	Late	Lucerne	Sorghum	17	17	29.4	11.8	58.8
Y-tube	Late	Lucerne	Cabbage	18	18	16.7	44.4	38.9
Y-tube	Late	Potatoes	Soil	19	18	5.6	22.2	72.2
Y-tube	Late	Cabbage	Soil	20	20	40.0	45.0	15.0
Linear	Early	Lucerne	Sorghum	14	11	27.3	18.2	54.5
Linear	Late	Lucerne	Sorghum	10	10	40.0	20.0	40.0
Linear	Mid-late	Carrot tuber	Soil	20	20	25.0	50.0	25.0
Linear	Mid-Late	Carrot tuber + larva	Soil	20	20	**65.0**	**20.0**	15.0
Linear	Mid-late	Carrot tuber + larva	Carrot tuber	19	19	21.1	47.4	31.6
Linear	Late	Carrot tuber + larva	Potato tuber + larva	20	20	20.0	35.0	45.0
Linear	Late	Carrot plants	Cabbage	20	20	20.0	50.0	30.0
Linear	Late	Potato tuber	Soil	20	19	15.8	15.8	68.4
Linear	Late	Potato tuber + larva	Potato tuber	10	8	25.0	12.5	62.5

[1] Early = 2^{nd}-5^{th} instars, mid = 6^{th}-8^{th} instars, late = 9^{th}-11^{th} instars.

[2] Figures in bold indicate a significant difference between Treatment 1 and 2 at $P < 0.05$.

Varietal susceptibility and attraction of potato tubers to *N. leucoloma* larvae

Choice tests

The number of tubers damaged by larvae was low: of the 20 replicate boxes set-up, each containing 5 tubers (one of each variety), 10 boxes had one tuber damaged, six had two tubers damaged, one had three tubers damaged and three had no tubers damaged (total number of tubers damaged = 25 out of 100). There was no significant difference in the number of tubers damaged by *N. leucoloma* ($F_{4,95} = 0.64$, $P > 0.05$) or the number of feeding holes between potato varieties ($F_{4,95} = 0.32$, $P > 0.05$) (Table 3). The total number of larval feeding holes was 40, ranging from one to three per tuber. The volume of potato consumed in damaged tubers was highest in Golden Delight and lowest in Sebago (Fig. 10) but, due to the variability of the data, there were no significant differences between varieties ($F_{4,32} = 0.06$, $P > 0.05$).

Table 3. Choice tests on the susceptibility of tubers of five potato varieties to *N. leucoloma* larval attack. One of each tuber variety was randomly arranged in a container (N = 20), in a concentric pattern, buried with sieved, moistened potting mix, and three late instar *N. leucoloma* larvae were added for 14 days. The same letters after numbers within columns indicate no significant difference between means ($P > 0.05$).

Potato variety	Mean no. tubers damaged (± SE)	Mean no. feeding holes (±SE)	Mean volume of potato consumed (mm^3) – all tubers (± SE)	Mean volume of potato consumed (mm^3) - damaged tubers only (± SE)
Golden Delight	0.20 ± 0.09 a	0.55 ± 0.32 a	72.7 ± 49.0 a	145.4 ± 46.6 a
Red Delight	0.15 ± 0.08 a	0.20 ± 0.12 a	20.5 ± 13.1 a	102.3 ± 40.2 a
Nadine	0.25 ± 0.10 a	0.40 ± 0.18 a	41.7 ± 66.5 a	139.2 ± 66.5 a
Mozart	0.30 ± 0.11 a	0.35 ± 0.13 a	35.9 ± 17.1 a	102.5 ± 40.2 a
Sebago	0.35 ± 0.11 a	0.50 ± 0.18 a	43.5 ± 15.5 a	86.9 ± 17.3 a

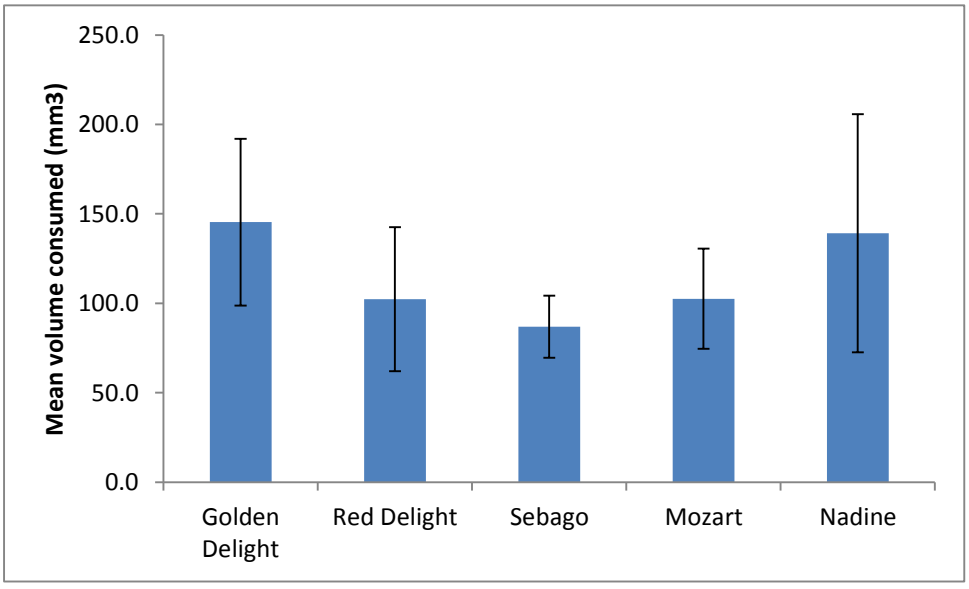

Fig. 10. Estimated mean (± SE) volume (mm^3) of potato eaten by larvae, for damaged tubers only, in choice tests comparing the susceptibility of tubers of five potato varieties to *N. leucoloma* larval attack. There was no significant difference between varieties ($P > 0.05$).

In a second choice experiment, to compare the attraction/susceptibility of tubers of potato varieties Nicola, Russet Burbank, Innovator (McCain 1), Dutch Cream and Moonlight, none of the tubers were damaged by *N. leucoloma* larvae.

No-choice tests

As in choice experiments, the number of *N. leucoloma* larvae attacking tubers in no-choice tests was low ranging from 30-50%, depending on variety (Table 4). On dismantling the bioassay to score damage, many of the larvae that fed on tubers were still inside holes (Fig. 11). There was no significant difference in the number of tubers damaged by *N. leucoloma* ($F_{4,94} = 0.54$, $P > 0.05$) or the number of feeding holes between potato varieties ($F_{4,94} = 0.25$, $P > 0.05$) (Table 3). The mean volume of potato consumed in damaged tubers only was significantly different between varieties ($F_{4,44} = 3.06$, $P < 0.05$) with Golden Delight tubers suffering less damage than Mozart and Nadine tubers but not between other varieties due to the large variation in damage levels (Fig. 12). Reflecting the amount of potato consumed, the mean percentage gain in the weight of *N. leucoloma* larvae was lowest in those feeding on Golden Delight and highest on Nadine (Fig 13 & 14), but due to the amount of variation in the data there was no significant difference between varieties ($F_{4,38} = 1.20$, $P > 0.05$).

Table 4. No-choice tests on the susceptibility of tubers of five potato varieties to *N. leucoloma* larval attack. Late instar larvae were confined to the epidermis of tubers of each variety (N = 20), for 7 days. The same letters after numbers within columns indicate no significant difference between means (P < 0.05).

Potato variety	Mean no. tubers damaged (± SE)	Mean no. feeding holes (±SE)	Mean volume of potato consumed (mm^3) – all tubers (± SE)	Mean volume of potato consumed (mm^3) - damaged tubers only (± SE)	Mean % change in weight of actively feeding larvae
Golden Delight	0.50 ± 0.11 a	0.60 ± 0.17 a	42.3 ± 13.4 a	70.5 ± 17.0 a	6.0 ± 3.4 a
Red Delight	0.30 ± 0.09 a	0.45 ± 0.16 a	30.2 ± 16.8 a	75.5 ± 37.8 ab	13.9 ± 4.2 a
Nadine	0.50 ± 0.10 a	0.50 ± 0.10 a	67.9 ± 21.1 a	135.8 ± 29.3 ab	20.1 ± 6.7 a
Mozart	0.45 ± 0.11 a	0.45 ± 0.11 a	59.3 ± 23.8 a	148.2 ± 44.3 b	14.1 ± 5.4 a
Sebago	0.47 ± 0.10 a	0.53 ± 0.12 a	53.7 ± 18.9 a	102.1 ± 29.1 b	16.7 ± 3.4 a

Fig. 11. *N. leucoloma* larva partially burrowed into a potato tuber (var. Mozart) in a no-choice experiment to compare the susceptibility of five varieties of potato tuber to larval attack.

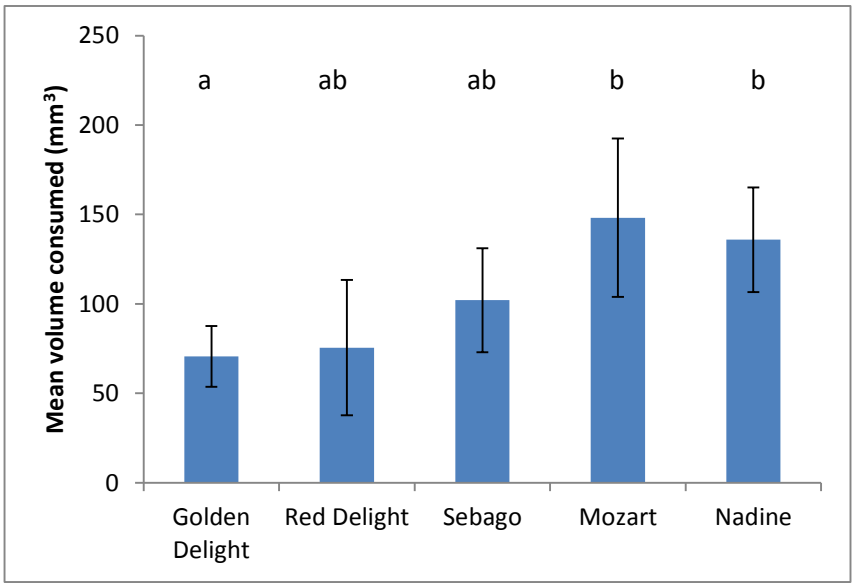

Fig. 12. Estimated mean (± SE) volume (mm^3) of potato eaten by larvae, for damaged tubers only, in no-choice tests comparing the susceptibility of tubers of five potato varieties to *N. leucoloma* larval attack. Bars with a different letter above were significantly different ($P < 0.05$).

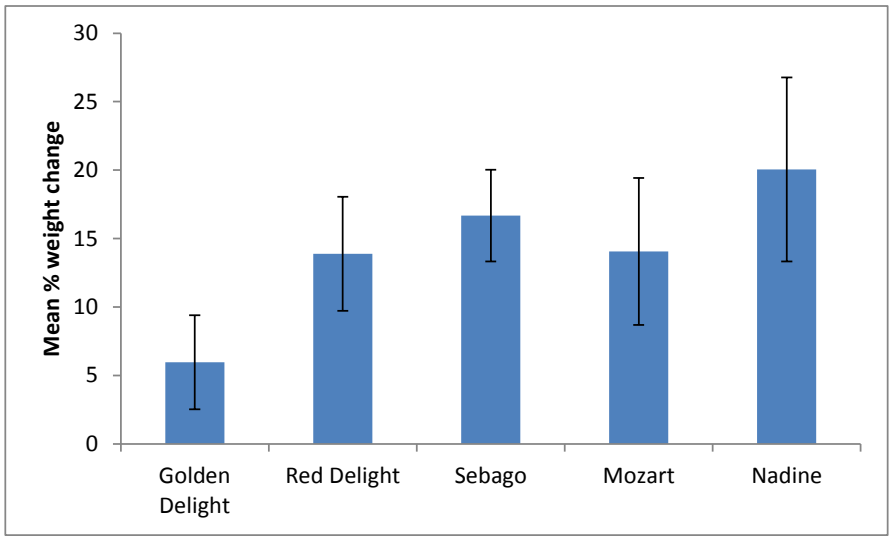

Fig. 13. Mean (± SE) percentage change in larval weight after feeding in no-choice tests comparing the susceptibility of tubers of five potato varieties to *N. leucoloma* larval attack (N = 20 larvae/variety). There was no significant difference between varieties ($P > 0.05$).

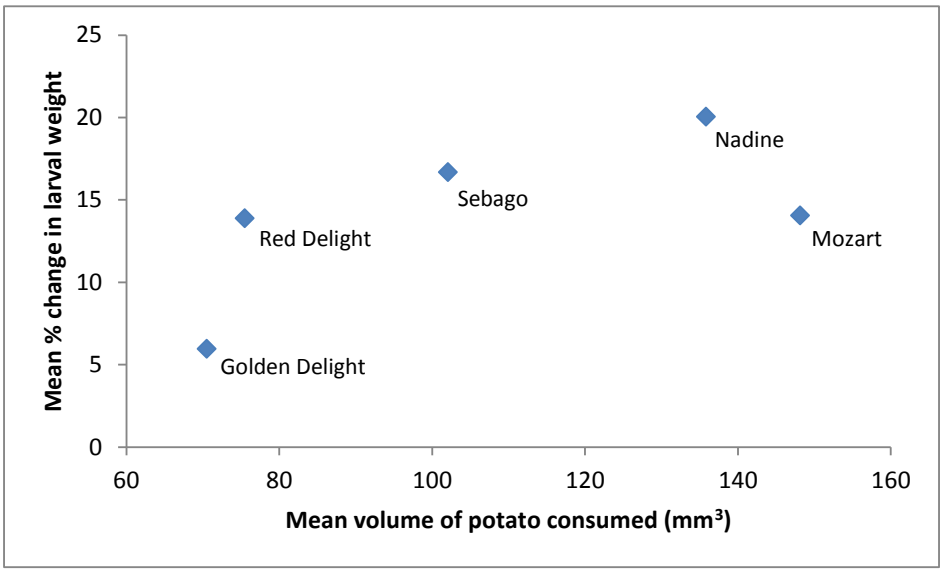

Fig. 14. Relationship between mean percentage change in larval weight and the estimated mean volume (mm^3) of potato eaten, in no-choice tests comparing the susceptibility of the tubers of five potato varieties to *N. leucoloma* larval attack.

Analysis of compounds emanating from plant roots

Over 20 compounds were detected in stir-bar collections from the roots of two potato varieties but only four compounds were detected from lucerne plants (Table 5). Despite the improvements observed with the use of the SBSE stir bars in comparison to the root aeration method, contaminants were still evident in most samples (Figs 15 & 16). Compounds such as the fatty acids Nonanoic, Decanoic and Dodecanoic acids, were most likely to have been of bacterial and/or fungal origin. In Russet Burbank, the pyrazine 2,5-diisobutylpyrazine and the lactones; γ-decalactone, γ-undecalactone and γ-dodecalactone, which have only ever been isolated from bacteria and fungi (Feron et al. 1996; Schulz & Dickschat 2007) were also observed, indicating the presence of other non-plant organisms in the rhizosphere, which contaminated the exudate samples.

Nevertheless, there were clear differences in potato root exudate chemistry, both within potato varieties between potato and lucerne (Fig 15). Within Innovator (Fig 16), root exudates contained the sesquiterpenes; solavetivone, phytuberin (peak 26) and the currently unidentified compound 27 (Table 4). Of these compounds, phytuberin and the unknown sesquiterpene were not observed in all Innovator samples. Similarly, several compounds were only isolated in Russet Burbank including the terpene α-copaene (peak 10) and the putatively identified β-himachalene (peak 17). The quantities of solavetivone appeared variable in concentration in all samples within both Innovator and Russet Burbank.

Numerous compounds were found to be common to both potato varieties (Table 4) including several short-chain alcohols and aldehydes some of which have been previously identified in potato tubers and potato foliage previously (Karlsson et al. 2009). Only one compound was identified in both potato and lucerne root exudates - the alcohol 2-ethylhexanol. Only one compound (peak number 18) was unique to lucerne but could not be readily identified.

Table 5. Compounds detected by GC-MS in stir bar samples from the roots of Russet Burbank and Innovator potato plants and lucerne (*Medicago sativa*) plants. Peak numbers correspond to GC-MS traces in Figs. 13 and 14.

Peak Number	Compound	Russet Burbank	Innovator	*Medicago sativa*
1	2-ethylhexanol	✓	✓	✓
2	2-nonenal	✓	✓	-
3	3-nonanol	✓	✓	-
4	2-decenal	✓	✓	-
5	Decanal	✓	✓	✓
6	Nonanoic acid	✓	-	✓
7	γ-decalactone	✓	✓	-
8	2,5-diisobutylpyrazine	✓	-	-
9	Dodecanal	✓	✓	-
10	α-copaene	✓	-	-
11	γ-undecalactone	✓	-	-
12	Dodedecanol	✓	-	-
13	Sesquiterpene A	-	✓	-
14	2-tetradecanol	✓	-	-
15	Dodecanoic acid	✓	✓	-
16	Unknown	✓	✓	-
17	β-himachalene	✓	-	-
18	Unknown	-	-	✓
19	Unknown lactone	✓	-	-
20	1-Tetradecanol	✓	-	-
21	γ-dodecalactone	✓	✓	-
22	Sesquiterpene B	✓	-	-
23	Tetradecanoic acid	✓	✓	-
24	Solaventivone	✓	✓	-
25	Sesquiterpene C	✓	-	-
26	Phytuberin	✓	✓	-
27	Sesquiterpene D	✓	✓	-

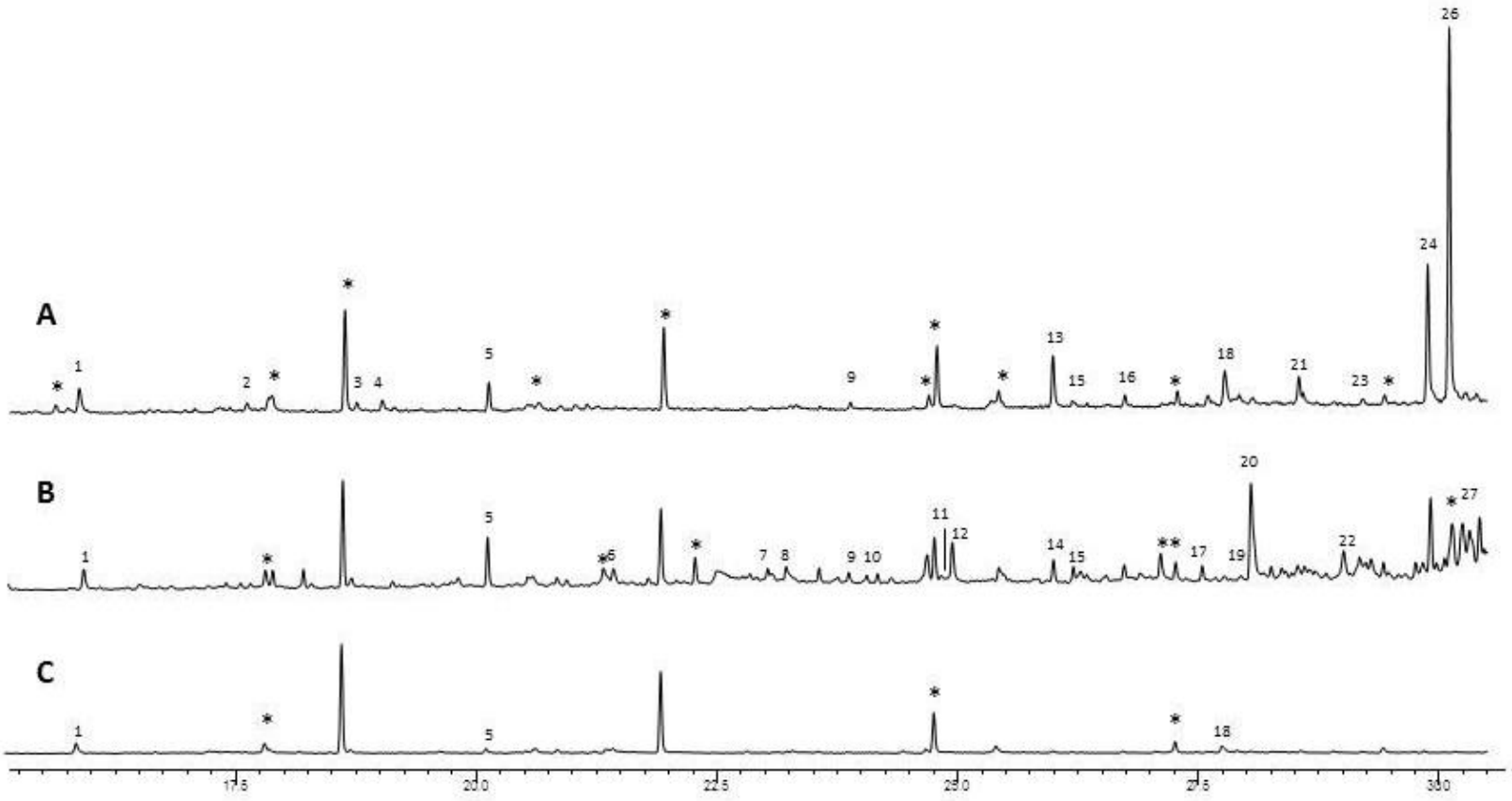

Fig. 15. Representative GC-MS chromatographs of compounds, collected by the Stir Bar method, emanating from the roots of A) Russet Burbank potato plants, B) Innovator potato plants and C) Lucerne plants. Numbers above peaks correspond to compounds listed in Table 4. Asterisks indicate contaminants.

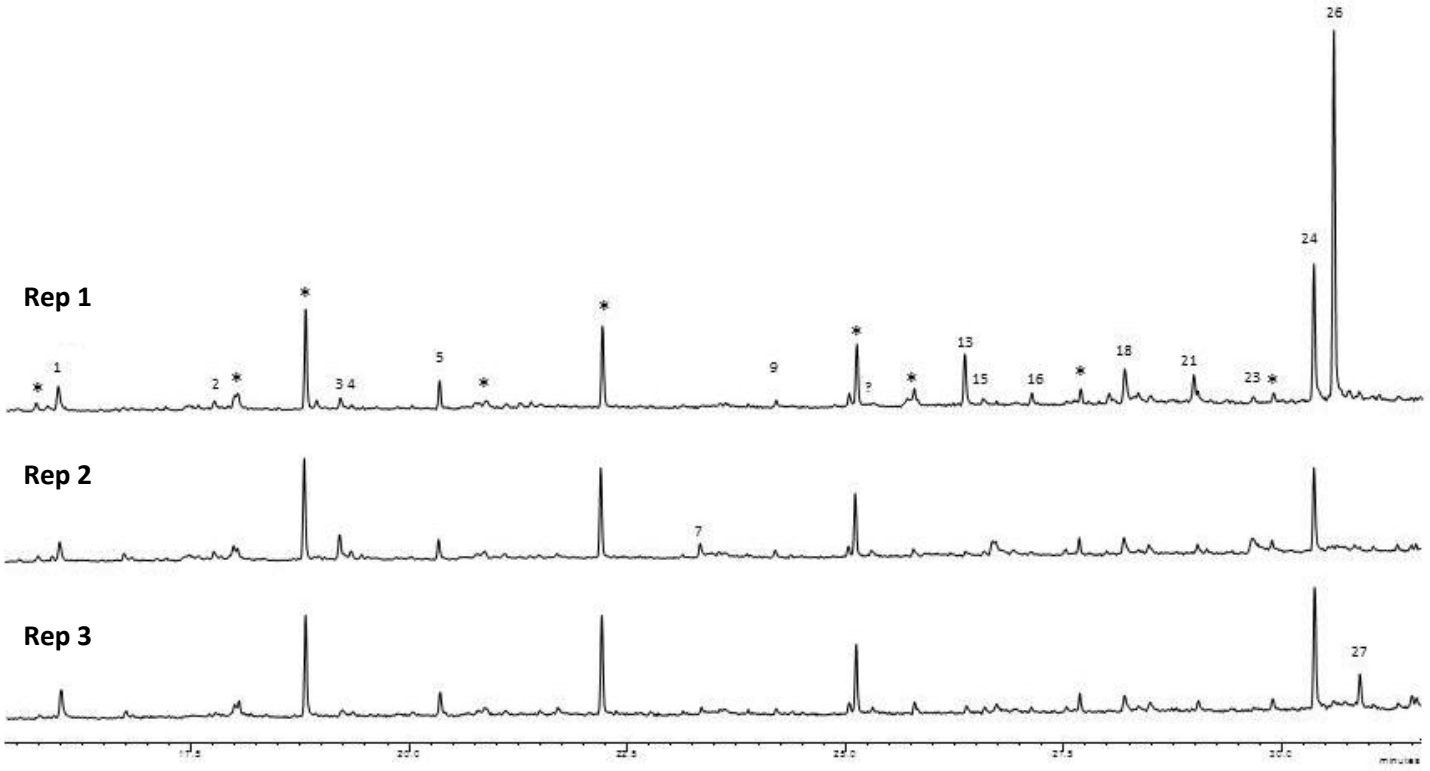

Fig. 16. Replicate GC-MS chromatographs of compounds, collected by the Stir Bar method, emanating from the roots of Innovator potato plants. Numbers above peaks correspond to compounds listed in Table 4. Asterisks indicate contaminants.

Discussion

Lucerne plants were the only bioassay material that were highly significantly attractive to *N. leucoloma* larvae ($P < 0.0001$), but only when tested against pots containing no other plant material (i.e. soil only) using late-instar larvae. When mid-instars were tested or when larvae were presented with a choice between lucerne and other plants, no preference towards lucerne was observed ($P > 0.05$), suggesting either that larvae are unable to discriminate between host-plants or that both choices presented were equally attractive. Furthermore, there was no evidence that the roots of an unfavourable host-plant (sorghum) were repellent to *N. leucoloma* larvae. This result may suggest that non-specific cues emanating from plant roots are important in search behaviour of *N. leucoloma* larvae, such as respiratory emissions of carbon dioxide (CO_2). In the literature, CO_2 is widely reported as the main root attractant to soil-dwelling insects and may act as a 'search trigger' causing insects to search more intensively for more host specific signals (Johnson & Nielsen 2012). However, there is conflicting evidence to suggests that CO_2 gradients generated by respiring roots are 'masked' by other root volatiles, thus avoiding detection (Johnson & Nielsen 2012; Reinecke et al. 2008). Whether the lucerne plants tested exerted stronger emissions of CO_2 than other plants tested was not measured and warrants further investigation to underpin the mechanism of attraction of *N. leucoloma* to lucerne roots.

The only other bioassay to produce a significant difference ($P < 0.05$) in the number of *N. leucoloma* test larvae choosing one arm of the bioassay unit was when a comparison was made between actively feeding *N. leucoloma* larvae placed with sections of carrot and soil only. This suggested that *N. leucoloma* larvae may be attracted to volatiles released either by feeding larvae and/or damaged carrots. Alternatively, it is possible that respiratory CO_2 being released by feeding larvae may have acted as a 'search trigger' causing larvae to search more intensively for more host specific signals. However, this result could not be repeated when a comparison was made between actively feeding *N. leucoloma* larvae placed with sections of carrot and carrot sections only was made.

The majority of bioassays were affected by a high proportion of *N. leucoloma* larvae being found in the neutral zone of the bioassay units. It was unclear whether these larvae had remained stationary within the tubes for the duration of the bioassay or had moved but selected to settle in the neutral zone either because none of the treatments were attractive or they were both equally attractive. If respiratory emissions of CO_2 are important in the orientation of *N. leucoloma* larvae to plant roots, then it is possible that in bioassays where a high proportion of larvae were found in the neutral zone, there were inadequate levels or gradients of CO_2 to trigger searching behaviour.

Analysis of lucerne root exudates sampled by the Stir-bar adsorption method found surprisingly few compounds, despite a detection limit as low as 0.1 ng/L (Baltussen et al. 1999). Only one compound unique to lucerne was found which unfortunately could not be identified based on mass spectra data and Kovats retention indices. Furthermore, the compound was only present at low concentrations and not found in all lucerne samples. Therefore, it is unlikely that this compound was responsible for the observed attraction of *N. leucoloma* larvae to lucerne plants in bioassays. Identification of the compound would require exhaustive chemistry procedures that were beyond the scope of the present study. The compound is unlikely to be available commercially from chemical suppliers and would thus require *de novo* synthesis in the laboratory in order to test its' attraction to *N. leucoloma* larvae. Several studies have shown that the presence of N_2 active fixing root nodules in legumes are important in the attraction of weevil larvae to host-plants (Johnson et

al. 2004, 2005; Quinn & Hower 1986; Wolfson 1987). Although *N. leucoloma* is known to have a preference for feeding on several species of legumes, such as lucerne and peanuts (Matthiessen & Shackleton 2000; Ottens & Todd 1979), it is unknown whether larvae feed preferentially on, or are more attracted to, plants with nodules present. We did not control for the presence of nodules on lucerne plants used in bioassays and their importance in attracting *N. leucoloma* requires further investigation.

In choice experiments, where three *N. leucoloma* larvae were presented with the tubers of five potato varieties, var. Golden Delight tubers were attacked more than other varieties compared although the difference was not significant due to the high variability in the data (Table 3). In no-choice experiments, where *N. leucoloma* larva were confined to the tubers of the same varieties, significantly lower amounts of var. Golden Delight were consumed than var. Nadine or Mozart, even though there was no difference in the number of tubers attacked or feeding holes (Table 4). The difference in the amount of potato consumed was also reflected in the change in larval weights before and after the experiment was conducted, being lowest in larvae fed on var. Golden Delight (Fig. 14). While more replicates are needed, this suggests that although the tubers of var. Golden Delight are attractive to *N. leucoloma* larvae, they are less palatable than other varieties tested. Differences in the attractiveness/palatability of tubers to *N. leucoloma* larvae is likely to be due to differences in the chemical composition between varieties. Chemical analysis of potato root exudates (var. Russet Burbank and Innovator), sampled by the Stir-bar adsorption method, revealed considerable variation in the number and amount of compounds detected both within and between varieties. Relatively few terpenes or terpenoids were isolated from the root systems of potatoes, which are commonly found in potato foliage (Karlsson et al. 2009). However, numerous alcohols, aldehydes and plant secondary metabolites were detected. Compounds of low molecular weight (e.g., alcohols, esters, and aldehydes) tend to underpin the attraction of soil-dwelling organisms to plant roots, whereas secondary plant metabolites are usually regarded as defence compounds and often have repellent properties (Johnson & Nielsen 2012). For example, the sesquiterpenes, solavetivone and phytuberin, which were found in potato root exudates in this study, are known to have fungicidal (Engström et al. 1999) and nematicidal (Lyon et al. 1995) properties, respectively. However, the effect of specific plant compounds on soil-dwelling organisms varies greatly as some root-feeding insects have exploited plant secondary metabolites for host location (Johnson & Nielsen 2012). Some sesquiterpenes and aldehydes are thought to mediate oviposition behaviour in the Guatemalan potato moth (*Tecia solanivora*) (Karlsson et al. 2013). Conversely, the heavier non-volatile steroidal glycoalkaloids exuded by potato roots, are detrimental to *T. solanivora* larval survival (Karlsson et al. 2013). Johnson et al. (2008) also demonstrated a negative correlation between glycoalkaloids production in several potato varieties and their susceptibility to wireworm (*Agriotes* spp.) attack. However, as the correlation was weak, this was unlikely to be the sole mechanism underpinning varietal susceptibility. Cultivar-specific differences in carrot roots exudates can also result in different levels of resistance to the carrot fly *Psila rosae*. Intact roots of resistant carrot cultivars generally emitted reduced concentrations and fewer attractive volatiles (Guerin & Ryan 1984). The alcohol, trans-2-nonenal, which was detected in root exudates collected from both potato varieties in this study (Table 5), also occurs in some cultivars of carrots and is known to have strong repellent properties to the carrot fly, *Psila rosae* (Guerin & Ryan 1984). The possibility that variation in the presence of the compounds detected in potato tubers, affected their

susceptibility/attractiveness to *N. leucoloma* larvae warrants further investigation. If so, it may be possible to select potato varieties that are more tolerant or resistant to *N. leucoloma* attack to limit the economic impact of larvae.

TECHNOLOGY TRANSFER

Communication, training and extension activities over the life of the project are listed in Table 5 and publications are listed in Table 6.

Table 6. PT09027 communication, training and extension activities.

Dates and personnel	Activity	Outcomes
17 September 2009[1] Paul Walker Geoff Allen	Talk presented to growers at Ulverstone, Tasmania	Dissemination of project aims to growers and other industry stakeholders.
5 September 2010[1] Paul Walker	Interview with ABC TV, Tasmania	Interview on project aims aired on ABC State Evening News on 5th September 2010, titled "Potatoes under siege from ravenous grubs" and made available on ABC web-site at http://www.abc.net.au/news/stories/2010/08/05/2974672.htm. Text of the interview appeared as print/web coverage on the ABC News Web-Site (6th September 2010) under the title: "Scientists work on new spud bug lure". Link: http://www.abc.net.au/news/stories/2010/09/06/3003176.htm.
January/February 2011 Paul Walker Geoff Allen Noel Davies	Hosted international scientist Dr Scott Johnson (Scottish Crops Research Institute) for expert advice on project research	Discussions held between Dr Johnson and research team members on procedures for testing the behavioural response of *N. leucoloma* larvae to host plant roots. Field trip to northern Tasmania with Dr Johnson. Discussions held on future collaborative research with Dr Johnson and review paper on *N. leucoloma*. Dr Johnson presented a seminar at the University of Tasmania on his current research.
June 2011 Paul Walker	Meeting with Dr Adrian Nicholas (NSW I & I, Tamworth)	Discussions with Dr Nicholas white-fringed weevil rearing methods, distribution around Tamworth, effect on local lucerne industry and on the establishment of lucerne in mine rehabilitation sites in the Hunter valley.
July 2011 Paul Walker Geoff Allen	Poster presentation at the TIA Vegetable Centre, Vegetable Industry forum held at Longford, Tasmania.	Dissemination of project aims and update on results to growers and other industry stakeholders.
23 November 2012 Paul Walker	Presentation on sampling plans and update on research findings at TIA field day, Forthside Research Farm.	Dissemination of project aims and update on results to growers and other industry stakeholders. Poster displayed and leaflet distributed.
25-28 November 2012 Paul Walker Geoff Allen	Poster presentation at Australian Entomological Society and Australian Arachnological Society Conference, Hobart.	Dissemination of project aims and results to scientific community.
February 26th 2013 Paul Walker	Presentation on sampling plans and update on research findings at Potato R & D workshop held in Ballarat, Victoria (organised by Mr Luke Raggart).	Dissemination of project aims and update on results to industry stakeholders. Leaflet on project distributed.
12th March 2013 Paul Walker	Presentation on sampling plans and update on research findings at Potato R & D workshop held in Sassafras, Tasmania (organised by Mr Luke Raggart).	Dissemination of project aims and update on results to industry stakeholders. Leaflet on project distributed.

[1] Note: these activities were conducted before finalisation of the project contract (February 2011) in anticipation of an earlier start date.

Table 7. PT09027 publications.

Authors	Title	Publication type
Paul Walker and Geoff Allen	Improving the management of white-fringed weevils in potatoes	Article published in Potatoes Australia, June/July 2011 edition, pp. 22-23.
Paul Walker, Geoff Allen, Noel Davis and Scott Johnson.	Improving the management of white-fringed weevils in potatoes	Poster presented at TIA Vegetable Centre, Vegetable Industry forum held at Longford, Tasmania, July 2011.
Paul Walker and Geoff Allen	Improving the management of white-fringed weevils in potatoes	Articles for the Potato Industry Annual Report in 2011/12, 2012/13 and 2013/14
Paul Walker	Spade key weapon in weevil control	Article published in the Primary Producer section of the Advocate newspaper, March 2012
Paul Walker and Geoff Allen	Sampling for white-fringed weevil grubs	Poster presented at TIA field day, Forthside Research Farm, 23 November 2012.
Paul Walker and Geoff Allen	Response of white-fringed weevil (*Naupactus leucoloma*) larvae to host plant roots and tubers	Poster presented at the Australian Entomological Society and Australian Arachnological Society Conference, Hobart, 25-28 November, 2012.
Paul Walker, Geoff Allen and Gretel Sneath	White-fringed weevils beware	Article published in Potatoes Australia, October/November 2012 edition.
Paul Walker and Geoff Allen	Controlling white-fringed weevils	TIA research extension note published in 2012 for distribution at field days.
Paul Walker and Geoff Allen	Improving the management of white-fringed weevils in potatoes	Information leaflet for Potato Industry Extension Program published by Luke Raggart (Ausveg) in January 2013 for distribution at field days.
Paul Walker	New research to learn how weevils find roots	Article published in the Primary Producer section of the Advocate newspaper, January 2013.

RECOMMENDATIONS

1) Continue the extension of *N. leucoloma* sampling plans to potato growers in all regions to ensure sustained uptake and adoption to reduce the incidence of insurance sprays against *N. leucoloma* larvae. This could take the form of further talks organised through the AUSVEG Potato Industry Extension Program and ensuring continuity in the availability of extension material websites such as those operated by AUSVEG, HAL and TIA.

2) Conduct further experiments to examine the attraction of *N. leucoloma* larvae to lucerne roots. The mechanisms underpinning the observed strong attraction of larvae to lucerne roots is still not understood and may involve respiratory carbon dioxide or non-volatile root exudates. The influence of feeding conspecifics and the presence of N_2 fixing root nodules in lucerne and other legumes on the orientation of *N. leucoloma* larvae needs further investigation.

3) Conduct further choice and no-choice experiments to compare the susceptibility of potato varieties to *N. leucoloma* larval attack. A wider selection of varieties need to be tested and the results interpreted with data on the chemical composition of tubers to look for possible compounds that may elicit resistance to larval attack.

ACKNOWLEDGMENTS

We thank all growers who allowed us access to paddocks for sampling white-fringed weevils; Peter Aird, Rebecca Clarkson and Russel Whitmore (ServAg) for assistance in the location of sampling sites; Shasta Jamieson and Vin Patel for assistance in field sampling; Dr Steve Quarrell (TIA) and Associate Professor Noel Davies (Central Science Laboratory, University of Tasmania) for the analysis of root exudates; Paul Horne and Jessica Page (IPM Technologies Pty Ltd, Victoria) for invaluable advice on sampling plans and white-fringed weevils; Dr Scott Johnson (University of Western Sydney) for expert advice on bioassays and root finding behaviour in insects; Mr Stuart Learmonth (Department of Agriculture, Western Australia) for providing white-fringed weevil photos and literature; HAL and the Potato Industry Levy for funding.

APPENDIX

Literature Review on the biology and ecology of the white-fringed weevil, *Naupactus leucoloma* (Coleoptera: Curculionidae), and its pest status in Australia

Introduction

The white-fringed weevil, *Naupactus leucoloma*, is a serious pest of potatoes, lucerne and several other crops in Australia. Native to South America, *N. leucoloma* was first reported attacking lucerne in New South Wales in 1932. Since then it has spread to Victoria, South Australia, Queensland, Western Australia and most recently to Tasmania. The soil-dwelling larva is the damaging stage, attacking the roots and tubers of a wide variety of crop and ornamental plants. While adult *N. leucoloma* also feed on the leaves of a wide variety of crop plants, they do not cause economic damage. Control of *N. leucoloma* larvae is difficult due to their protected location in the soil. Damage is usually worse in crops planted immediately after long rotations of lucerne or pasture containing legumes, such as clover, under which high populations of *N. leucoloma* can build-up unnoticed. Consequently, it is essential that sampling plans for *N. leucoloma* larvae are conducted prior to planting susceptible crops in spring. This literature review summarises the current extent of knowledge on the biology and ecology of *N. leucoloma*.

Taxonomy and nomenclature

Order:	Coleoptera
Superfamily:	Curculionoidea
Family:	Curculionidae
Subfamily:	Polydrosinae
Genus and species:	*Naupactus leucoloma* Boheman 1840
Synonyms:	*Pantomorus leucoloma* Buchanan 1939 *Pantomorus pilosus* Buchanan 1942 *Pantomorus striatus* Buchanan 1942 *Pantomorus dubius* Buchanan 1942 *Graphognathus leucoloma* Buchanan 1947 *Pantoplanes leucoloma* Voss 1954
Common names:	white-fringed weevil (Australia & New Zealand) whitefringed weevil (Australia & New Zealand) whitefringed beetle or white-fringed beetle(USA)[1]

[[1] In the USA, the common name 'white-fringed beetle' or 'whitefringed beetle' has been used to refer to *Naupactus leucoloma* or collectively to a group of three or four closely related *Naupactus* species, including *N. leucoloma*.]

Before 1995, the name *Graphognathus leucoloma* was widely used. Warner (1975) (cited by Lanteri & Marvaldi 1995) synonymized four earlier described subspecies of *G. leucoloma* (*G. l. leucoloma*, *G. l. pilosus*, *G. l. striatus*, *G. l. dubius*) and established that there were four valid species of

Graphognathus in North America (*G. leucoloma*, *G. minor*, *G. peregrinus* and *G. fecundus*). These were transferred to the genus *Naupactus* by Lanteri & Marvaldi (1995).

Geographic distribution and pest status

The centre of origin of *Naupactus* spp. group is South America, with *N. leucoloma* having the broadest distribution, ranging from 12°S to 42°S, west of the Andes and from 15°S to 38°S, east of the Andes (Guzman et al. 2012; EPPO 1999; Lanteri & Marvaldi, 1995).

Australia: *N. leucoloma* was first reported in New South Wales in 1932 attacking lucerne in the Hunter Valley (Hely et al. 1982) and is now found in Victoria, Western Australia, South Australia, Queensland and Tasmania. In NSW, *N. leucoloma* is recognized as one of the most important lucerne pests, and commonly occurs throughout coastal districts, on inland river flats and in irrigation areas (Barnes & De Barro 2009; Nicholas 2009; Hely et al. 1982). It is also a minor pest of potatoes in the Robertson, Guyra and Dorrigo areas of NSW (Learmonth 2005). In Queensland, it has been reported as a pest of peanuts, maize, potatoes, sweet potatoes, lucerne and several other crops at various localities, from the Lockyer and Fassifern Valleys in the south to the Atherton Tableland in the north (Charleston 2013, McDougall 2007; Learmonth 2005; Gough & Brown 1991). In Western Australia, *N. leucoloma* is a pest of pasture and potatoes in cooler and wetter southern areas, primarily in spring-sown crops (Matthiessen 1991). *Naupactus leucoloma* probably entered Tasmania in the mid-1980's but was not officially recognised until 1992 (McQuillan et al. 2007). Infestations were initially found around Scottsdale, Swansea and Ouse but *N. leucoloma* is now present in most agricultural areas of the state where it is a major pest of potatoes and minor pest of several other crops such as poppies and carrots (Wardlaw 2004). The Northern Territory is currently free from *N. leucoloma* where it is a notifiable plant pest under quarantine legislation (Anon. 2013a,b).

North America: Larvae of *N. leucoloma* were first reported in the USA in 1936 devastating cotton and peanut crops in northwest Florida (Okaloosa County) and southern Alabama (Watson 1937). However, it was subsequently discovered that a complex of at least three closely related species of *Naupactus* were present in the USA (*N. leucoloma*, *N. minor* and *N. peregrinus*) and these were often grouped together as species identification of the larval stage was not possible at the time (Zehnder et al. 1998; Harlan 1973). In 1937, *Naupactus* spp. group spread to several localities in Louisiana and Mississippi leading to state quarantine measures being enacted and the establishment of a federal regulatory program (Gross et al. 1972c). Despite these early quarantine efforts, *N. leucoloma* continued to spread throughout south-eastern USA and they have now been collected in Virginia, Arkansas, Georgia, Kentucky, New Mexico, Texas, North Carolina, South Carolina, Tennessee and California (Sites & Thorvilson 1988; Zehnder et al. 1998).

South America: *N. leucoloma* is native to Argentina, Uruguay and southern Brazil, occurring in grasslands and cultivated areas (Guzman et al. 2012). Its range in Brazil includes southern areas (Rio Grande do Sul) while in Argentina it is present in most northern and central provinces (Buenos Aires, Catamarca, Chaco, Córdoba, Corrientes, Entre Rios, Formosa, Jujuy, La Pampa, La Rioja, Mendoza, Rio Negro, San Juan, San Luis, Santa Fe, Santiago del Estero, Salta and Tucumán). It is considered as introduced in Chile and Peru (Lanteri & Marvaldi, 1995; Guzman et al. 2012). In South America, *N. leucoloma* has been listed as a pest of lucerne, beans, potatoes, lettuce, capsicums, strawberries, nursery plants and soybeans (Lanteri and Marvaldi 1995; Lanteri et al. 2013). In Argentina it is

frequent in lucerne fields causing damage under unfavourable weather and soil conditions. In Chile it has been reported in beans and peanuts (Guzman et al. 2012). In Brazil, *N. leucoloma* is one of five species of broad-nosed weevil recently recorded damaging soybean crops in the state of Rio Grande do Sul (Lanteri et al. 2013).

New Zealand: *N. leucoloma* was first discovered in the North Island, near Hawke's Bay, Auckland, in 1945 and in the South Island in 1965 (Todd 1968). It is now well established in lowland pastures throughout the North Island and north-eastern parts of the South Island (Hardwick & Prestidge 1994; Hardwick & Prestidge 1996; East 1977). In New Zealand, *N. leucoloma* larvae have been recorded as pests of lucerne, pastures, peas, potatoes, exotic forest seedlings and horticultural crops (East 1977; East 1982). Additional crops listed by May (1966) as being damaged by *N. leucoloma* larvae in New Zealand were water melon, tomato, wheat and chou moellier (*Brassica oleracea* var. ramose).

Africa: *N. leucoloma* was first recorded in South Africa in 1941 at Rosebank, Cape Town, and was thought to have entered the country in imported fodder (De Jager et al. 1989). It then spread to other areas in the Cape Province: Stellenbosch in 1946; Koekenaap in 1951; Robertson in 1957; Beaufort West in 1962 and Upington (including parts of the Lower Orange River irrigation area) in 1972 where it mainly causes damage to lucerne (De Jager et al. 1989).

Europe: Absent.

Asia: Absent but has been intercepted by quarantine officials in Japan (Masaru et al. 2002).

Biology

Phenology and population development

In the laboratory at a constant temperature of 24 °C, *N. leucoloma* development from egg deposition to adult emergence, takes about 366 days (Masaru et al 2002; Masaki 1998). Similarly, in the field, *N. leucoloma* takes about a year to complete development from egg to adult. However, in cooler areas of southern Australia, such as Tasmania, complete development of a generation may take two years as a range of larval instars can be found in winter (McQuillan 2007; Wardlaw 2004; Horne et al. 2002). Also, in the dry Mediterranean climate region of Western Australia, Matthiessen (1991) observed a steady year round abundance of late-instar larvae, indicating that not all individuals completed their development in one year. In parts of the USA, some *N. leucoloma* larvae also do not pupate until their 2nd year (Bass & Barnes 1969a; Harlan 1973).

In Tasmania, adults are most common in late summer and early autumn. Matthiessen (1991) considered *N. leucoloma* to be poorly adapted to the summer-dry/winter moist Mediterranean-type environment of Western Australia. In this environment in undisturbed pasture, adult *N. leucoloma* are present from summer to early winter (December-June). However, an upsurge in numbers of 1st instars only occurs after the rains begin in April, primarily due to the absence of high quality food (e.g. legumes) essential for oogenesis and due to limited moisture for eggs to hatch (Matthiessen 1991). Under irrigated crops and in environments with wetter summers, population development and survival is less limited by food availability and moisture.

Eggs: Very small (<1 mm diam.), oval, laid in clusters of about 12-60 in the soil on roots, in ground litter beneath plants or on stems and lower leaves of plants. Milky-white when first laid, changing to

dull light-yellow. The larval head capsule and mandibles become visible through the chorion just before eclosion. The eggs are fixed together with a sticky, gelatinous mass which hardens into a protective film (Fig. A1) allowing them to withstand drought for several months. Soil sometimes sticks to an egg mass making detection impossible.

Fig. A1. Egg mass of white-fringed weevil (*Naupactus leucoloma*) laid on tissue paper

Hatching of eggs in the field is limited by rainfall and/or irrigation (Matthiessen 1991). Larvae are able to complete embryonic development under dry conditions but require moisture to emerge (Harlan 1973). In the laboratory, extended storage of eggs can be achieved (63% egg hatch after 8 months) by placing 22-28 day old eggs at 18.3 °C and 95% RH . Bass & Barnes (1969a) noted that in laboratory cultures, *N. leucoloma* eggs placed on damp filter paper in petri dishes could tolerate considerable fungal growth without any apparent effect on hatchability or larval growth. Egg development at 24 °C takes 17.1 days with a developmental threshold of 11.7 °C and thermal constant of 208.7 degree days (Masaki 1998). In WA, eggs hatch in about two weeks after rain or irrigation (Matthiessen 1991). In the USA, eggs laid in summer (July/August) take on average 17 days to hatch but those laid in December take more than 100 days (Harlan 1973).

Larvae: Grey-white body with yellow-brown heads, large black mandibles and no legs. Large larvae tend to turn yellow. The head is retracted into the body so only the mandibles are clearly visible (Fig. A2). Fully grown grubs are about 13 mm long x 6 mm wide and cause the most damage to plant roots/tubers. Found in the soil mostly at depths of 5-15 cm.

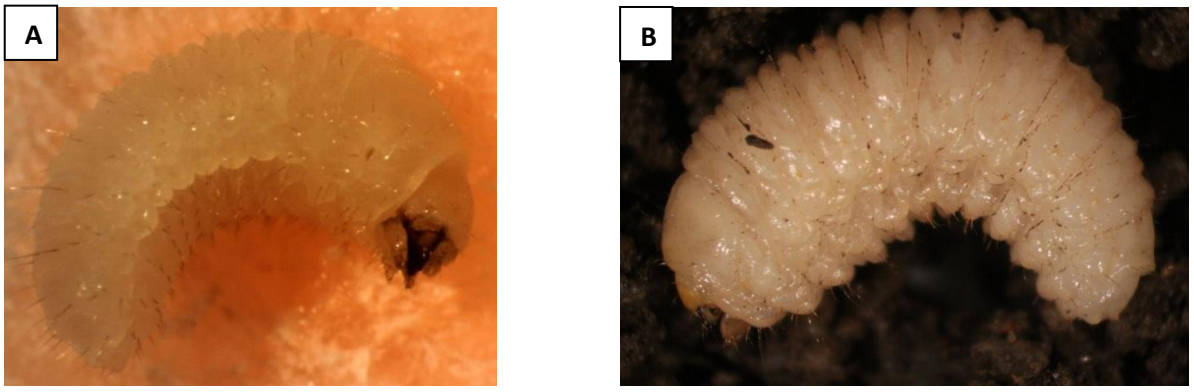

Fig. A2. (A) First instar and (B) eleventh instar white-fringed weevil (*Naupactus leucoloma*) larvae (photo courtesy of Steve Quarrell).

There are eleven larval instars with the first being a non-feeding stage that can survive for several months in the soil before moulting to the second instar to commence feeding on roots (Matthiessen & Shackleton 2000; Hardwick & Prestidge 1996; Gough & Brown 1991). The gut of 1st instars is not fully developed being filled with a yolk-like substance (Gough & Brown 1991). In laboratory experiments at 25 °C, less than 10% of 1st instars moulted to the 2nd instar when placed in soil without food for 10 weeks (Gough & Brown 1991). When some of these 10 week old 1st instars were then transferred to soil with carrot, over 82% moulted into 2nd instars after 10 days compared to 54% incubated without food. Gough & Brown (1991) suggested this moulting resulted through the mechanical handling and removal of larvae from their earthen cells. However, it is possible that other stimuli may promote moulting of 1st instars such as an increase in soil moisture (through placing larvae in freshly moistened soil) or exposure to carbon dioxide while handling.

Matthiessen & Shakleton (2000), incubating 1st instars in plastic vials without food, soil or moisture, found that longevity was longest at 10 °C, with 50% surviving for 35-40 days. At this temperature, the onset of mortality was slow with greater than 90% of larvae surviving for 20 days. As temperature was increased from 15 to 26 °C, survival diminished uniformly with median longevity falling from 15 days at 15 ºC to 7 days at 26 °C.

In an unsuccessful attempt to develop an artificial diet for *N. leucoloma* larvae, Bass & Barnes (1969b) noted that 1st instar larvae were highly susceptible to antimicrobial agents incorporated into the diet to prevent mould growth. All larvae died within 10 days when a range of commonly used antimicrobial agents were tested at concentrations high enough to be of practical use.

When larvae were reared on carrots in the laboratory at 25 °C, each instar from the 1st to the 9th lasted about one week, the tenth about 3-4 weeks while the eleventh instar was extremely prolonged lasting five to six months (Masaru et al. 2002; Gough & Brown 1991). Head capsule width in early instars (1st to 5th) are discrete and readily separated but overlap in older instars (Gough & Brown 1991). Consequently, larval weight is a more convenient way of measuring size and gives a useful approximation of the stage of larval development (Gough & Brown 1991).

Larvae are highly polyphagous and are known to feed on over 240 species of plants but they prefer to feed on plants with tap-roots rather than fibrous roots (McQuillan et al. 2007; Harlan 1973).

Prepupae: The final larval moult (11th) occurs 7-14 days after feeding ceases and there is a considerable interval until pupation occurs (4 months or longer) (Masaru et al. 2002; Gough & Brown 1991; Barnes & Bass 1972). Pre-pupal larvae are difficult to distinguish from actively feeding larvae as the head-capsule widths and weights of 10th and 11th instars are very similar (Gough & Brown 1991). Some older larvae become more yellow (Gough & Brown 1991) but others remain similar in colour throughout the larval stage (PW Walker pers. obs.). Rainfall is thought to stimulate *N. leucoloma* larvae to pupate under some conditions but not in the Mediterranean climate of Western Australia (Matthiessen 1991; Gough & Brown 1991). Gough & Brown (1991) thought that the most likely factor to synchronize pupation is a period of exposure to elevated soil temperatures. Previously, Barnes & Bass (1972) showed that increasing the laboratory rearing temperature (from 22.3 °C to 32.2 °C) stimulated pupation. In pot experiments conducted at Tolga, north Queensland,

Gough & Brown (1991) also found that rapid pupation occurred when ambient temperatures increased to between 25 and 30 °C. This observation was further supported in their laboratory studies as rapid pupation occurred when fully grown larvae were transferred from 25 °C to 30 °C constant.

Whatever the mechanisms needed to stimulate pupation in *N. leucoloma*, the ability of larvae to persist in the soil as non-feeding 11th instars is seen as another adaptation that would assist populations to withstand periods of environmental adversity. It would also help to synchronise the emergence of adult populations when larval development is variable.

Pupae: Creamy white, 10-12 mm long (Fig. A3), found in oval chambers 5-15 cm deep in the soil in spring and early summer. At 24 °C, pupal development takes 15.7 days (Masaru et al. 2002). As in other species of weevils, the prepupa constructs a protective earthen cell by rotating with considerable energy, cementing and smoothing the walls with droplets of a thick fluid secreted from the anus (May 1966). In *N. leucoloma*, the walls of the cell are so thickly coated that they become quite smooth and shiny (May 1966). Two or three days before emergence the pupa turns brown as the adult insect becomes visible through the cuticle. The eyes darken first, then the mandibles, followed. by the other extremities, and finally the cuticle, complete with scales and setae (May 1966).

Fig. A3. Pupa of white-fringed weevil (*Naupactus leucoloma*) in earthen cell (photo courtesy of Steve Quarrell).

Adults: Slate-grey with distinctive white stripe on each side of the wing cover (resembling a sunflower seed on legs),10-13 mm in length (Fig. A4). The elytra are fused together, so they cannot fly but can walk long distances (0.4-1.2 km) (EPPO 1999).

Fig. A4. Adult white-fringed weevil (*Naupactus leucoloma*).

Males have only been recorded from a single location on the banks of the Paraná River, Entre Rìos Province, Argentina (Guzman et al. 2012). Outside this area, all *N. leucoloma* populations consist of parthenogenic females, even in grassland and agricultural areas in other parts of Argentina where it has been introduced (Lanteri & Marvaldi 1995).

Adult *N. leucoloma* are highly polyphagous feeding on the leaves of a very wide range of host plants (over 170 species) including herbs, forbs, shrubs and trees. The combined list of host plants on which *N. leucoloma* species group larvae or adults have been observed to feed on includes 385 species, distributed in 287 genera, 99 families and 41 orders (EPPO 1999; Harlan 1973). Adults feed at the bases of leaf margins, leaving characteristic “notching” but this seldom causes economic damage except when adults are very numerous (McQuillan et al. 2007).

Adults can live for several months depending on food source. Generally, longevity is greater when adults are fed on legumes such as lucerne and peanuts. East (1977) compared the longevity and fecundity of adult *N. leucoloma* fed on different species and cultivars of legumes, brassicas, grasses and cereals. Average longevity was highest at 87 days when adults were fed on the legume sainfoin (*Onobrychis vicifolia*), followed by lucerne at 61 days. Ketchersid & Klingeman (2007) compared adult longevity when fed on a variety of ornamental plants with those fed on peanuts. Average longevity was highest at 101 days when adults were fed on Kobus magnolia (*Magnolia kobus*), followed by peanuts at 67 days.

Several authors have shown that fecundity of *N. leucoloma* is also highly dependent on adult diet and is generally related to longevity. Adults fed on legumes, such as lucerne and peanuts, can lay over 1000 eggs while those reared on fibrous rooted plants, such as sorghum, lay less than 58 eggs (Ketchersid & Klingeman 2007; Ottens & Todd 1979; East 1977). Fecundity is also likely to be effected by the quality of the larval diet although no published studies to show this have been found.

In south-eastern Australia, females emerge from the soil in late November through to April, with peak numbers usually in February (Anon. 2008). Mature females begin laying eggs 5-25 days after emerging and can lay up to 1500 eggs in groups of 20-60 over a two-month period.

Movement and dispersal

Although adult *N. leucoloma* cannot fly they can walk considerable distances (0.4-1.2 km) during their 2-5-month lifetime (EPPO 1999). Also, all life-stages of *N. leucoloma* can be readily spread by humans through trade or agricultural activity by transportation of: adults clinging to hay, other crops, vehicles and agricultural equipment; eggs attached to soil, debri or plant material; and larvae/pupae in the soil of turf, pot plants or in the root balls of tree saplings (McQuillan et al. 2007; EPPO 1999; Dixon 1988). As females are parthenogenetic, the chance of small populations colonizing new regions is increased. It is unknown how N. leucoloma was accidentally introduced into Australia and from which country it originated from.

In Tasmania, the spread of *N. leucoloma* infestations between and within farms is thought to occur along fence lines and/or roads. These areas are often densely vegetated and may act as shelters and provide sources of food for adult beetles emerging in fallow fields.

In South Africa, De Jager et al. (1989) thought that adult *N. leucoloma* were 'dispersed by water' in the Lower Orange River irrigation area as infestations were worse in western areas, the direction of flow of the river and canals. However, it seems more likely that the banks of river and canals (particularly if vegetated) act as natural conduits for the dispersal of walking adult weevils. There is also some anecdotal evidence that dispersing adult weevils seek oviposition sites in more elevated parts of the landscape (P. Horne pers. comm.), perhaps to avoid excessively wet or flood-prone areas.

Damage and economic impact

While adult *N. leucoloma* feed on the leaves of crop plants, it is the larval stage feeding on roots and tubers that causes economic damage. Ottens & Todd (1979) showed that as many as 50 *N. leucoloma* adults per soybean plant were required to significantly reduce pod production.

Watson (1937) considered *N. leucoloma* larvae were not strictly root feeders but feed rather on the portions of the stems underground where they gouge out large cavities which can lead to death of the plants. However, in the laboratory, Hardwick & Prestidge (1996) demonstrated that damage to white clover by early instar larvae consisted of the destruction of root hairs, detachment of fine roots and scarring of larger roots. Late instar larvae (8th-11th) were much more destructive with feeding on clover being characterised by the severing of small roots and main root branches.

In several vegetable crops *N. leucoloma* larvae attack the main roots and underground stem, causing extensive girdling furrowing (Hely et al. 1982). The plants then become stunted and may die. In potatoes and other tuberous plants, such as carrots and parsnips, the larvae gnaw furrows or round pits which may become further damage if invaded by secondary pests or diseases.

Damage to potatoes

Larvae of *N. leucoloma* burrow into potato tubers below the ground, leaving round holes (ca. 5 mm diam.) or a channel-shaped scar. Usually the holes are shallow, just deep enough to hold the larvae, and can look similar to slug damage (Horne et al. 2002). Very low population densities of *N.*

leucoloma can cause economic damage in potatoes. A density of only one larva m-1 row of potatoes (equivalent to about 1 larva 1.5 m^2) resulted in a loss of 9% of average gross return (EPPO 1999). Most *N. leucoloma* larvae are found attacking the underside of tubers towards the bottom of the potato-growing hill (Matthiessen & Learmonth 1993).

As in other vegetable crops, severe damage to potatoes by *N. leucoloma* can occur when crops are planted after pasture, legume crops or peanuts (Horne et al. 2002; Learmonth 2005). In Australia, potato crops sown in August to October usually form tubers in late spring and early summer, coinciding with the presence of rapidly growing, late-instar *N. leucoloma* larvae, thus maximising the potential for damage (Matthiessen 1991). Consequently, pre-crop sampling for *N. leucoloma* larvae is highly recommended to assess the potential risk of damage to potatoes before planting (Learmonth 2005). In northern Queensland, potato crops planted early and subsequently harvested in the cooler months may be at less risk than those planted later, as larvae feed more in the warmer months as they prepare to pupate (Learmonth 2005). Similarly, in Western Australia, crops planted in late spring and harvested in late April may escape damage as *N. leucoloma* larvae would have completed their development before tubers reach a significant size. Crops that remain in the ground for extended periods after senescence are more likely to suffer higher levels of damage if no other food source is available for *N. leucoloma* larvae.

Learmonth (2005) estimated the severity and value of losses by soil insect pests in Australian potatoes (excluding South Australia and Tasmania) as at December 2000. Table A1 summarises the statistics for *N. leucoloma* which was estimated to cause up to 2% damage to potato yields in some regions (Atherton, Qld, and Manjimup, WA). In most regions where *N. leucoloma* was present, 0.2 – 1.0% crop losses were estimated. Overall all regions surveyed, *N. leucoloma* was estimated to cause a total loss of $911,950 in potato yields in one year (as at 2000). Estimates for yield losses by *N. leucoloma* in South Australia and Tasmania were not available.

Damage to other vegetable crops

In NSW, *N. leucoloma* larvae have been recorded as pests of strawberries, beans, carrots, parsnips, cabbage, cauliflower, cucurbits, lettuce and tomatoes (McDougal 2007; Hely et al. 1982). Most damage occurs when young seedlings are planted on land previously under lucerne or clover pasture (Hely et al. 1982). Larval damage usually occurs in autumn or spring but often it is not until November that damage becomes evident as the larvae reach full size. Considerable damage to carrots and parsnips has been recorded on the Northern Tablelands of NSW (Hely et al. 1982). Occasionally, adult weevils attack the foliage of these crops but rarely at levels requiring control (Hely et al. 1982).

In a review of insect pests of 10 selected vegetable crops in mainland SE Australia, McDougall (2007) listed *N. leucoloma* as: a major pest of zucchinis in the Lockyer and Fassifern Valleys, Queensland; a minor pest of zucchinis in the Melbourne area; a minor or infrequent pest of: beans (East and South Gippsland, Sydney Basin, Lockyer Valley); carrots (no regional areas identified); Chinese cabbage (Melbourne, Gippsland, Sydney Basin); cucumber (Sunraysia, Melbourne); pumpkin (Melbourne, Sydney); and sweet potato (Rockhampton, Bundaberg, Cudgen, Mareeba). *Naupactus leucoloma* was not listed as a pest of beetroot, capsicum or celery in this study. In Queensland, *N. leucoloma* is a severe pest of peanuts (Gough & Brown 1991). Charleston (2013) also listed *N. leucoloma* as a pest

of maize during the vegetative stage in Queensland. Infestations are usually patchy with larvae chewing into lateral roots causing death or reduced vigour. In June 2014, *N. leucoloma* was reported damaging pea seedlings in the Bundaberg area, Queensland (Charleston & Brier 2014).

In Tasmania, *N. leucoloma* is a major pest of potatoes and an occasional pest of a wide variety of other crops such as onions, poppies, carrots, beans, brassicas, lettuce and lucerne (McQuillan et al. 2007; Wardlaw 2004). In onions, the larvae chew off roots and bore holes into the bulb. Most damage is caused in spring and summer when larvae are fully grown and are feeding most actively.

In the USA, *N. leucoloma* larvae have also been recorded as pests of young pine trees planted on converted croplands (Steinbeck 1990; Dixon 1988), sweet potatoes (Zehnder et al. 1998), ornamental plants in nurseries (Ketchersid & Klingeman 2007), cotton and corn (Watson 1937).

In small-plot trials, Gross et al. (1972c) showed that damage to soybeans by *Naupactus* spp. larvae was dependant on when infestations were introduced. Seedling numbers of soybeans planted in April in plots infested the previous July and August were significantly less than numbers in plots infested the previous September. Damage to soybean plants by *Naupactus* spp. larvae was typical of that described for other host plants; wilted plants contained large necrotic areas over the entire length of the root with heaviest damage within 4 cm of the soil's surface. Plants frequently broke off at the soil's surface when they were pulled from the soil. The results suggested that reductions in stands of soybeans depended on the soybean planting date, the amount of rainfall and the developmental stage of larvae.

Damage to legumes and pasture crops

Naupactus leucoloma is a major pest of lucerne and pasture crops sown with legumes in Australia, New Zealand, Argentina and South Africa. Infestations in pasture often go unnoticed until paddocks are rotated to annual crops such as potatoes.

Damage to lucerne and other legumes, such as clovers in pasture crops, is caused by *N. leucoloma* larvae feeding on the tap roots of legumes, often resulting in plant mortality and production losses (Barnes & De Barro 2009). Lucerne is particularly susceptible to *N. leucoloma* attack and infestations can reduce the life of crops to a couple of years (McQuillan et al. 2007). In NSW, heavily infested seedling and first year stands can be so badly damaged that resowing is necessary (Munroe 2002). Established plants can support high numbers of larvae with no apparent effect on productivity or longevity provided they are not placed under additional stresses such as inadequate fertiliser, grazing mismanagement or severe drought (Munroe 2002). Similarly, in New Zealand, East (1982) considered established lucerne plants (at least 12 months old) to be highly tolerant to attack by *N. leucoloma* larvae whereas seedlings were highly susceptible, especially when under soil moisture stress (Morton & Roberts 1978). Also, in South Africa most reductions in lucerne yields caused by *N. leucoloma* larvae resulted from damage to stands in their first year before the tap root had thickened (De Jager et al. 1989). Younger plants were often killed by *N. leucoloma* larvae whereas older, well-established plants normally survived, but with a significant loss in yield.

In a small-plot trial conducted in New Zealand over a four year period, East (1982) recognised three classes of legumes in relation to their susceptibility to attack by *N. leucoloma* larvae: 1) susceptible species, which supported high weevil populations (consistently more than 200 m-2) when sown as

pure species and which suffered significant losses in annual herbage production, especially in summer (> 76%) and autumn (> 38%), were white clover (*Trifolium repens*) and *Lotus* spp.; 2) tolerant species, which supported high weevil populations but with no effect on herbage production, were lucerne and red clover (*Trifolium pratense*); 3) species unfavourable, which did not support high populations, were subterranean clover (*Trifolium subterraneum*) and *Desmodium* spp..

East (1982) estimated that root feeding by *N. leucoloma* could reduce dry matter production in pure swards of white clover by up to 28%, and in ryegrass with white clover pasture by up to 10%. Hardwick & Prestidge (1996) suggested that *N. leucoloma* larvae actively select white clover, so that pastures with high larval populations may suffer a decline in white clover content over a period of several years. King & East (1978) showed that in mixed white clover and ryegrass pastures, *N. leucoloma* numbers rose over a period of 1-2 years when white clover content was at its highest and that as the percentage of grass increased, *N. leucoloma* larval populations decreased. In a laboratory study by Hardwick & Prestidge (1996), feeding damage on white clover (*Trifolium repens*) by early instar (2nd-4th) *N. leucoloma* larvae consisted of the destruction of root hairs, detachment of fine roots and scarring of larger roots. Late instar larvae (8th-11th) were much more destructive with feeding on clover being characterised by the severing of small roots and main root branches. Over the 14 day experimental period, several of the plants died and nitrogen fixation rates were significantly reduced.

Not all legumes are suitable hosts plants for *N. leucoloma* larvae. Gough & Brown (1991) reported that while adult weevils feed on the leaves of Glycine (*Neonotonia wightii* (cv. Tinaroo) and lay a moderate number of eggs, survival and development of larvae on the roots of this tap-rooted plant was extremely poor.

Although some control of *N. leucoloma* larvae infesting paddocks where annual crops are to be planted can be achieved using fumigants, they need to be applied to the soil just before sowing and are therefore not practicable to use in already established lucerne or pasture paddocks (Barnes & De Barro 2009).

Foliar feeding by adult *N. leucoloma* in lucerne or pasture crops is not considered to be economically important (East 1981, 1983, McQuillan et al. 2007). However, *N. leucoloma* fed on lucerne can lay 1,600 eggs versus 200 for those fed on grasses and weeds (McQuillan et al. 2007).

Damage to rye grass

Although rye and other grasses are considered to be poor hosts for *N. leucoloma*, Gross et al. (1972c) showed that yield of plots artificially infested with larvae were still significantly reduced. Yields of plots infested with larvae in July were more affected than those infested with larvae in August or September. Rye in the July infested plots had symptoms of severe stress, characteristic of plants with root disorders. Growth was sparse, stalks were thin and relatively short, and foliage was predominately pale green. September infested plots suffered the least amount of damage and yield losses as *N. leucoloma* larvae had not developed sufficiently by April to cause stand reduction.

Damage to irrigation tape in NSW

Nicholas (2009) reported *N. leucoloma* larvae damaging sub-surface irrigation tape in lucerne crops in the Lachlan and Peel Valleys, New South Wales. The 0.34 mm walled polyethylene tape, buried at

a depth of 35-40 cm in the soil, was attacked by overwintering *N. leucoloma* larvae causing leaks in the dripper lines. In severely affected areas, holes numbered in the hundreds and were costly to growers in terms of labour to dig up and repair the irrigation systems. The reasons for larval attack of the tape remains unknown. Nicholas (2009) found no evidence that larvae were attracted to volatile compounds released by the plastic tape. Neither was is it considered likely that larvae were able to detect or were attracted to water inside the tape. Rather, Nicholas (2009) speculated that in winter, when the irrigation system is switched off and the soil moisture declines in the upper soil zone, *N. leucoloma* larvae move down the soil profile in response to drying. As larvae move down, they concentrate into areas where the soil was broken by the ripper used during installation of the irrigation tape. This is when larvae encounter the SDI tape, resulting in a feeding response and damage to the tape. A solution to the problem was found by employing thicker walled plastic tape through which *N. leucoloma* larvae were unable to chew holes (Nicholas pers. comm. 2011).

Sampling for larvae

A sampling plan for insect soil pests, including *N. leucoloma* larvae, in potato crops was devised by Matthiessen & Learmonth (1993) and further developed by Learmonth (2005). A 15 cm spade was used to take a sample of soil to a depth of about 20 cm. Thus each sample unit consisted of a 0.0225 m^2 area of soil. Two sampling methods were tested by Learmonth (2005). For regular sampling, the actual number of units examined per paddock depended on the size and shape of the paddock. For clustered sampling, the method involved sampling at nine evenly placed locations across a paddock, with each location being a cluster of six to nine samples, making a total of up to 81 sampling units. These two sampling methods were compared by Learmonth (2005) on one occasion in a paddock in Western Australia.

The sampling plan relies on the *N. leucoloma* larvae being of a size visible to the unaided eye. Consequently, sampling is best conducted during late winter in south-eastern Australia as this is when most larvae are a large size (P. Horne, pers. comm.). Exceptions may occur if conditions over winter have been too cold and/or too dry for larval development. In pasture paddocks being rotated to potatoes, sampling is also best done before the paddock is worked in preparation for planting as this can make larvae harder to find (Learmonth 2005).

Chemical control

Soil-applied insecticides are most commonly used to control infestations of *Naupactus* spp. However, due to the protection of eggs, larvae and pupae in the soil, control failures are commonly reported (Learmonth 2005; Zehnder et al. 1998; Matthiessen & Learmonth 1995). Gross et al. (1972c) advocated preventive adulticidal treatments against *Naupactus* spp. during critical ovipositional periods to reduce the establishment of larval populations. Zehnder et al. (1998) evaluated several adulticide treatments for the control of *Naupactus* spp. in sweet potatoes in the USA.

The introduction of persistent, organochlorine insecticides during the 1940's gave growers a method of reliably controlling *N. leucoloma* infestations and helped to keep crop damage to low levels (Gross et al. 1972c). The most effective, commonly used insecticides were DDT, aldrin, dieldrin, and chlordane (Boutwell & Watson 1978). All these had relatively long residual toxicity when incorporated into the soil and could provide *Naupactus* spp. control for several years. However, in 1963 personnel of the Plant Protection Division of the USDA in Alabama reported finding *Naupactus*

spp. larvae and adults in a nursery which had been surface treated regularly with dieldrin since 1954 (Harlan et al. 1972). Subsequent surveys found dieldrin resistance in 12 out of 29 *Naupactus* spp. populations sampled in southeastern USA.

With the banning of persistent organochlorine insecticides in the 1970's on environmental grounds, together with the development of resistance to dieldrin, control of *N. leucoloma* larvae became more difficult (Hardwick et al. 1997). This resulted in an increase in reports of *Naupactus* spp. damage to crops such as soybeans and peanuts in the USA (Gross et al. 1972). In Western Australia, the soil fumigant metham sodium became the product-of-choice for the control of *N. leucoloma* after the withdrawal of organochlorine insecticides (Matthiessen & Shakelton 2000; Matthiessen 1991). Metham sodium is a broad-spectrum biocide, widely used prophylactically to control noxious soil-borne organisms in horticulture. However, the high cost of metham sodium (ca $700/ha in 2000) often made it impractical to use in potato production (Matthiessen & Shakelton 2000). Also, the use of a product normally applied for controlling nematodes and fungal pathogens, for insect control raised concerns about its possible long-term impact on soil health. Furthermore, it was found that metham sodium sometimes gave variable rates of control of *N. leucoloma* larvae and may have exacerbated the incidence of the fungal pathogen *Rhizoctenia solani* in potato tubers during trials (Learmonth 2005). Consequently, Matthiessen (1991) led a search for a biofumigant for the control of *N. leucoloma* larvae. Methyl isothiocyanate (the active decomposition product of metam-sodium) was found to be the most active of several fumigants against *N. leucoloma* larvae (Matthiessen et al. 1996; 1997).

Today, the most commonly used insecticides for *N. leucoloma* control in Australian potato crops are fipronil (Regent) and chlorpyrifos (Lorsban), incorporated into the soil prior to planting. Both insecticides give varying levels of control (Learmonth 2005). Unfortunately, there is an increasing trend for fipronil to be used in potato cropping systems prophylactically for the control of *N. leucoloma* without firstly sampling the soil to determine the presence of the pest. This is not only a waste of resources if no *N. leucoloma* populations are present, but it could also lead to the development of insecticide resistance as occurred in the USA with the regular application of dieldrin in nurseries certified as *Naupactus* spp. free (Harlan et al. 1972). Unnecessary application of fipronil may also be detrimental through adverse effects on beneficial soil organisms.

Cultural control

Cultural control, through long fallows or rotations of less preferred host-plants, is currently the best method for reducing established *N. leucoloma* infestations. It is recommended that ground where *N. leucoloma* is thought or known to occur, is thoroughly prepared in the autumn or spring and sown to a legume-free grass forage or cereal crop (such as oats) at a high seeding rate for at least two years, before it is used for susceptible crops like vegetables or lucerne (Hely et al. 1982). It is also recommended that new stands of lucerne should be sown as far away as possible from older stands (Munroe 2002). Matthiesen et al. (1997) suggested that rotation with high-glucosinolate *Brassica* spp. will lead to the release, during their decomposition of their residues, of methyl isothiocyanate, thus providing a means of "biofumigation" against larvae of *N. leucoloma*.

As adult *N. leucoloma* cannot fly, ditches (about 25 cm deep and 25 cm wide) with steep, well-packed sides, have been used to prevent populations from spreading. Holes in the ditches trap adults which can then be destroyed with kerosene (EPPO 1999).

It is thought that abnormally high rainfall kills many *N. leucoloma* larvae. After excessively wet years in New South Wales in the early 1950's, no important *N. leucoloma* damage was reported for several years (Hely et al. 1982). Such declines in larval populations during wet periods is likely to be associated with soil-borne diseases. In the USA, Gross et al. (1972c) noted an annual decline in *N. leucoloma* larval populations during April and May, usually preceded an increase in soil temperatures and soil moisture caused by precipitation of ca 25 mm or more. While this decline was not understood by Gross et al. (1972c), Harlan (1973) stated that after periods of high rainfall, many *Naupactus* spp. larvae which died were infected by fungi e.g. *Metarhizium anisopliae* and *Beauveria bassiana*, unidentified bacteria or nematodes.

Natural enemies and biological control

No insect parasitoids of *N. leucoloma* are known. The first record of surveys for natural control agents of *Naupactus* spp. is that of Berry (1939) (cited by Harlan 1973) who explored throughout countries of South America. He found two species of birds that fed on larvae which probably had little effect in reducing populations as they had access only during cultivation (Harlan 1973).

In the USA, Young et al. (1950) reported carabid beetle larvae, horsefly larvae, wireworms and ants as natural enemies of *N. leucoloma* in the field. They also listed various vertebrates such as toads, mice, snakes and birds as feeding voraciously on adult beetles.

Ahmad (1976) conducted a survey for natural enemies of *N. leucoloma* in the Buenos Aires, Cordoba, La Pampa, Rio Negro and Santa Fe provinces of Argentina, during 1971-73. Larvae were mainly sampled from lucerne crops and were attacked by: nematodes (see below); the bacterium *Corynebacterium* sp.; an entomophagous fungus, *Paecilomyces farinosus*; the mites *Histiostoma feroniarum* (Duf.) and *Caloglyphus* sp.; and the carabids *Anisotarsus cupripennis* (Germ.), *A. laevis* (Curt.), *Barypus clivinoides* (Curt.), *Calosoma argentinense* Csiki, *C. retusum* F., *Cnemalobus gayi* Putz., *Metius circumfusus* (Germ.), *Paramecus cylindricus* Dej., *Pterostichus striatulus* (F.) and *Scarites anthracinus* Dej. Ahmad (1976) thought that some of these, particularly the nematodes and carabids, exerted a considerable influence on the larval populations. A protozoan infection caused by *Nosema* sp. was also found in adult weevils and was thought to be fairly effective (Ahmad 1976).

Nematodes

Research on potential biological control agents for *N. leucoloma* has focussed on entomophagous nematodes. As a soil insect with a 1-2 year life-cycle, *Naupactus* spp. were considered to be ideal for control with parasitic nematodes (Harlan 1973). The use of nematodes as biological control agents against insect pests dates back to the 1930's. However, early attempts often failed due to difficulties in rearing the large numbers of suitable infective stages needed for field application and their high costs in relation to conventional insecticides (Jackson et al. 1981). During the 1970's interest in using nematodes was revived with the development of resistance to dieldrin in *Naupactus* spp. larvae, an increasing awareness of the potential dangers of persistent insecticides and a ban on the use of DDT in many countries (Wouts 1980; Harlan et al. 1972). Also, the development of cheap mass rearing

methods and the rapid increase in the costs of insecticides, further increased interest in the potential of nematodes as biological control agents during the 1980's (Jackson et al. 1981).

Several researchers found nematodes naturally infecting populations of *Naupactus* species, not only in their native range but also in countries where it had been introduced. The first report of nematodes parasitising *Naupactus* spp. (probably *N. leucoloma*) larvae was by Swain (1943). In 1940, nematodes of the genus *Neoaplectana* were found in *Naupactus* spp. larvae collected in Harrison County, Mississippi. Prior to this, no parasitic enemies had been reported from *Naupactus* spp. in the USA or South America, although preliminary experiments with the Japanese beetle (*Popillia japonica*) nematode (*N. glaseri*) by HC Young and HS Hollingsworth in 1939 had shown promising results (Swain 1943). This finding stimulated a co-operative project to search for possible nematode and other parasites as control agents of *Naupactus* spp. and to devise means of utilizing them. By the end of 1942, over 50,000 *Naupactus* spp. had been examined for nematodes. Over half of these specimens came from Harrison County where more than 2% of *Naupactus* spp. larvae were found to contain *Neoaplectana* spp. Species belonging to another nematode genus *Diplogaster*, were also found externally on many *Naupactus* spp. larvae, pupae and adults collected in the south-eastern States (Swain 1945). Repeated attempts to infect healthy *Naupactus* spp. larvae with *Diplogaster* in the laboratory failed. Therefore, it was tentatively assumed to be primarily a saprophagous nematode species and not suitable for consideration as a biological control agent (Swain 1945).

Ahmad (1976) found the nematodes *Neoaplectana* sp., *Rhabditis hambletoni*, and three other species of *Rhabditis*, attacking *N. leucoloma* larvae in Argentina. Stock (1993), also conducting a survey of diseased weevils in Argentina (Rafaela, Province of Santa Fe), came across a field containing several dying dark-purple *Naupactus* spp. larvae. Further observations revealed that larvae were parasitised by an undescribed species of nematode belonging to the genus *Heterorhabditis* and named it as *H. argentinensis*.

In Australia, Sexton & Williams (1981) also found that nematodes of the genus *Heterohabditis* caused a high level of mortality in a population of *N. leucoloma* larvae in lucerne near Geelong, Victoria. Infected larvae turned brick-red in colour. The presence of the nematode in the soil was associated with a 79% reduction of weevil numbers and significantly improved plant condition.

Wouts (1980) isolated the nematode *Neoaplectana bibionis* from infected *N. leucoloma* larvae collected from pastures near Auckland, New Zealand. Infective larvae of this genus of nematodes had previously been isolated from soil samples from a wide range of localities in New Zealand including sparsely vegetated high country, rain forests, agricultural soils and sand dunes.

Field testing of nematodes as a biological control agent against *Naupactus* spp. larvae was conducted in the USA and New Zealand. Harlan et al. (1971) tested the efficacy of the nematode *Neoaplectana dutkyi* against *Naupactus peregrinus* (Buchanan) in a Louisiana grassland field and achieved a 38% reduction in larval numbers when applied at 430,000 nematodes per m^2. Further trials in Mississippi also only achieved a low (50%) reduction in an artificial population of *N. peregrinus* when the nematode was applied at 538,000 per m^2 (Harlan 1973). However, nematodes were recovered from the Louisiana plots 16 months after they were applied. The relatively poor rate of reduction of *Naupactus* larvae was thought to be due to the low reproductive rate of the

nematode in the host. This may have hindered the parasite from being present in a population dense enough for individuals of a sparse beetle population to ingest the nematode (Harlan 1973).

In New Zealand, Jackson et al. (1981) tested the potential of the nematodes *Neoaplectana glaseri*, *Heterorhabditis heliothidis* and *H. bacteriophora* against a range of pasture pests including *N. leucoloma*. All three species of nematodes successfully killed *N. leucoloma* larvae with 90% mortality occurring at the highest nematode release rate (70/ml of soil).

Since the 1980's, research on the use of nematodes as biological control agents against *N. leucoloma* has waned, perhaps due to their low efficacy and high costs when compared to broad-spectrum insecticides. Today, entomophagous nematodes can be purchased commercially through Ecogrow Pty Ltd (Australia), mainly for the control of soil pests attacking high-value turf grass. While none of the nematode species are marketed specifically for *N. leucoloma* control, several are likely to be efficacious against larvae, such as Weevilnem™ which contains a strain of *Heterorhabditis zealandica* for the control of a number of turf and pasture pests.

Fungi

Metarhizium anisopliae and *Beauveria bassiana* have been recorded infecting *Naupactus* spp. larvae in the Gulf coastal states of the USA, usually after rainfall of *ca* 25 mm or more (Harlan 1973). In Western Australia, an entomophagous fungus that can survive the winter in soil was described as having potential for microbial control of *Naupactus* spp. in potatoes (Ralph 1992, cited in Fuxa et al. 1998). However, no further studies on this fungus have been published.

Phytosanitary measures

Several Asian and African countries that are currently free of *N. leucoloma* have imposed strict quarantine regulations on the importation of plant material (such as lilies, gladiolus and dahlia bulbs), requiring phytosanitary certificates from areas of the world where the weevil is present, including Australia (source: http://eng.coa.gov.tw/content_view.php?catid=9035&hot_new=8872). In Europe, no specific measures for *N. leucoloma* are in place but the general phytosanitary measures for soil-borne pests are recommended by the EPPO (EPPO 1999).

Table A1. Estimated economic impact of *Naupactus leucoloma* in potatoes as at December 2000 (data extracted from Learmonth (2005)).

State	Region	Production (tonnes)	Total value of crop	% crop damage attributed to N. leucoloma	Tonnes damaged by *N. leucoloma*	Value of *N. leucoloma* damage
QLD	Lockyer/ Fassifern	34,000	$ 13,600,000	0.5	170	$ 68,000
	Atherton	30,000	$ 12,000,000	2.0	600	$ 240,000
	Bundaberg	18,000	$ 7,200,000	0	0	$ 0
	Darling Downs	23,000	$ 9,200,000	1.0	230	$ 92,000
	Others	11,500	$ 4,600,000	0	0	$ 0
	Total QLD	**116,500**	**$ 46,600,000**		**1,000**	**$ 400,000**
NSW	Sydney	5,800	$ 2,200,000	0	0	$ 0
	Hunter	3,200	$ 1,100,000	0	0	$ 0
	Illwarra	5,500	$ 2,000,000	1.0	55	$ 20,000
	Richmond Tweed	1,800	$ 600,000	0	0	$0
	Mid-north coast	13,500	$ 5,000,000	1.0	135	$ 50,000
	Northern	10,300	$ 3,700,000	1.0	103	$ 37,000
	North Western	2,800	$ 1,000,000	0.5	14	$ 5,000
	Central Coast	5,700	$ 2,000,000	0	0	$ 0
	South Eastern	1,800	$ 600,000	0	0	$ 0
	Murrumbidgee	37,100	$ 13,500,000	0.5	186	$ 0
	Murray	49,000	$ 17,800,000	0.5	245	$ 89,000
	Total NSW	**136,500**	**$ 49,500,000**		**738**	**$ 201,000**

Table A1 continued. Estimated economic impact of *Naupactus leucoloma* in potatoes as at December 2000 (data extracted from Learmonth (2005)).

State	Region	Production (tonnes)	Total value of crop	% crop damage attributed to N. leucoloma	Tonnes damaged by *N. leucoloma*	Value of *N. leucoloma* damage
VIC	Gippsland (s)	16,000	$ 7,200,000	0.2	32	$ 14,400
	Ballarat (s)	8,000	$ 3,600,000	0	0	$ 0
	Otways (s)	5,000	$ 2,250,000	0.2	10	$ 4,500
	King Lake (s)	3,000	$ 1,350,000	0.2	6	$ 2,700
	Portland (s)	2,000	$ 900,000	0.2	4	$ 1,800
	Ballarat (p)	90,000	$ 17,370,000	0	0	$ 0
	Otways (p)	10,000	$ 2,500,000	0.2	20	$ 5,000
	Gippsland (f)	60,000	$ 21,000,000	0.2	120	$ 42,000
	Gembrook fresh	10,000	$ 3,500,000	0.2	20	$ 7,000
	Murray R'vale, SwH (f)	10,000	$ 3,500,000	0	0	$ 0
	Geelong (f)	10,000	$ 3,500,000	0.4	40	$ 14,000
	Kooweerup	30,000	$ 7,500,000	0.2	60	$ 15,000
	Total VIC	254,000	**$ 74,170,000**		**312**	**$ 106,400**
WA	Metro	9,000	$ 3,780,000	0	0	$ 0
	Myalup	5,000	$ 2,100,000	0	0	$ 0
	Busselton	11,600	$ 4,880,000	0	0	$ 0
	Donnybrook	4,400	$ 1,850,000	0.5	22	$ 9,250
	Manjimup	23,000	$ 9,660,000	2.0	460	$ 193,200
	Albany	2,500	$ 1,050,000	0.2	5	$ 2,100
	Total WA	**55,500**	**$ 23,320,000**		**487**	**$ 204,550**
	Total all States	**562,500**	**$ 193,590,000**		**2,537**	**$ 911,950**

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