

**Managing weevils in
pome fruit orchards
with nematodes and
ground covers**

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Department of Agriculture
Western Australia

Project Number: AP00014

AP00014

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Statement about the purpose of the report:

This report details the activities conducted in relation to managing the weevil pests garden weevil, Fuller's rose weevil and apple weevil in pome fruit orchards based on the use of parasitic nematodes and ground covers. Recommendations for future research in relation to management strategies for these weevil pests in pome fruit orchards are included.

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Media Summary

The pests apple weevil, Fuller's rose weevil and garden weevil are important in the production of some fruit crops in Australia. They are most important in Western Australia, but also cause problems in other apple producing states. Adults of all three species are pests because they defoliate crops, disfigure fruit and egg laying blocks mini-sprinklers.

Control of these weevils is based on using broad-spectrum insecticides which are disruptive to integrated pest management in orchards and lack reliable effectiveness.

An earlier project showed that both insect parasitic nematodes and ground cover plants affect the survival of garden weevil larvae. This opened up the possibilities for completely new approaches to control of these species of weevil.

The insect parasitic nematode we worked with was a new strain discovered in NSW and is now commercially available. We undertook a number of field trials to assess their effectiveness against weevil larvae in orchards and vineyards, the latter location was selected because higher populations of garden weevil could be found there more readily. The nematode trials included a range of variables such as method of incorporation and application, different rates and different timing.

After extensive field trials, we conclude that using parasitic nematodes against the soil borne larval stage is not feasible at this time. This is in contrast to situations where the nematode is used commercially in amenity turf. While this is a negative result, we cannot rule out the possibility that as other strains of nematodes become available, or methods of application improve, parasitic nematodes may prove to be of value.

In relation to ground cover plants and suppression of survival of weevil larvae, earlier work showed that survival of garden weevil larvae was very low when fed on clover. This result was considered useful for two reasons – clover had a confirmed effect of reducing survival of weevil larvae and it is already grown as a cover crop in many orchards.

We clarified this effect in a series of pot trials, which showed that clover was not actually killing weevil larvae, but more that it was simply an unsuitable food plant by itself. When clover was grown with apple trees, larvae survived satisfactorily. The same situation was seen for grubs of Fuller's rose weevil. Pot trials also indicated that ryegrass and kikuyu may have a negative impact on larval survival.

Poor survival of weevil larvae on crop hosts such as apple and plum reduced the reliability of pot trial results and tentative conclusions only could be drawn for the best cover crop composition to help reduce weevil abundance.

We were unable to overcome rearing problems to test the effect of different plants on larvae of apple weevil. Laboratory experiments were undertaken with the aim of enhancing our ability to produce apple weevil larvae. We showed that greater egg production could be achieved by rearing adult weevils at 18°C compared to 25°C and that eggs were more likely to hatch when exposed to moisture and temperature of 18°C.

In addition to examining the role of selected cover crop plant species on larval survival, we tested their suitability as food sources for the adult weevils. Grasses such as oats and ryegrass were the least suitable as food plants for all three species of weevil adults and would be a worthwhile for orchardists to consider. They would be especially effective if they could be combined with a canopy exclusion treatment, such as sticky bands.

Further work on the role of ground covers in suppression of these weevil pests is recommended.

Technical Summary

The pests apple weevil, Fuller's rose weevil and garden weevil are important in the production of some fruit crops in Australia. They are most important in Western Australia, but also cause problems in other apple producing states. Adults of all three species feed on foliage and also cause other damage – garden weevil and to a much lesser extent apple weevil feed on fruit causing it to be rejected; apple weevil ringbark fruit stalks reducing fruit size or causing them to fall prematurely; Fuller's rose weevil lays eggs in mini-sprinklers causing erratic watering or complete blockage, or egg masses on fruit cause them to be rejected.

Control of these weevils is based on using broad-spectrum insecticides such as synthetic pyrethroids, organophosphates or carbamates aimed at the adult stage. The application of the pyrethroids is by butt drenching, which needs to be well timed, is time consuming and not always effective. The application of such insecticides can be disruptive to integrated pest management in orchards.

An earlier project showed that both insect parasitic nematodes and ground cover plant species affect the survival of garden weevil larvae. This opened up the possibilities for completely new approaches to control of all three species of weevil.

We undertook a number of field trials in apple orchards and vineyards to assess the effectiveness of nematodes. We moved some of the work to vineyards because garden weevil infestations are more easily found there. The trials included a range of variables: incorporation with and without a rotary hoe; application through mini-sprinklers and drippers; different rates of nematodes; and variations in the timing. Timing of applications was altered in accord with the biology of the weevils – during spring when grubs are at their largest and therefore more easily located by the nematodes, and in autumn when grubs are small, but closer to the soil surface.

After these extensive field trials, we have come to the conclusion that the soft approach using parasitic nematodes against the soil borne grub stage is not feasible in the scenarios we tested. This is in contrast to situations where the nematode works well, that is against larvae of African black beetle in turf. This is because everything is in favour of good infection levels: the grubs are large; nematodes are applied when soil temperatures are well above the nematode activity threshold of 15° C; and there is ample water available to keep the soil profile wet for the nematodes to "swim" to their target. Few of these conditions are met in relation to the orchard weevils. While a negative result, we cannot rule out the possibility of other strains of nematodes becoming available, or other methods of application that may improve their efficacy.

In relation to ground cover plants and suppression of survival of weevil larvae, earlier work showed that when larvae of garden weevil were fed on sub-clover and white clover, survival was very low. This result was considered useful for two reasons – clover had a confirmed effect of reducing survival of weevil larvae and it is already grown as a cover crop in many orchards.

In early studies on cover crops we clarified this effect of clover by testing older grubs. For garden weevil the same general results were found – clovers were undesirable. We then tested clovers grown in combination with apple trees in pots. The results were that the survival of garden weevil larvae was the same whether clover was present or not. We concluded that clover is simply an unfavourable food source and is not actively killing weevil larvae. When the larvae had access to favourable food, in this case apple roots, they survived. These results were also found for Fuller's rose weevil larvae.

Further pot trials selecting a different series of plant species combinations were conducted on garden weevil and Fuller's rose weevil to assess whether any plants actually had a negative impact on survival of larvae. The main effect recorded from these last trials was for

ryegrass, both the readily available annual ryegrass and a perennial ryegrass strain with endophyte fungus present. This endophyte is a symbiont of ryegrass and is toxic to certain species of insects. Survival and rate of development of garden weevil larvae were adversely affected. The effect on survival of garden weevil larvae was almost complete, while for both garden weevil and Fuller's rose weevil, larval development was much slower. By slowing the rate of development, it might be expected that larval mortality in the field would be higher and the resulting weevil adults will be less fit and possibly be more susceptible to insecticide.

Problems with pot trials were evident during this research. For example we cannot explain the low survival rate of garden weevil larvae on apple tree and Fuller's rose weevil larvae on plum trees, noted favoured hosts for these weevils. Another major problem encountered was the inability to overcome rearing problems to test apple weevil larvae in pot trials. Preliminary laboratory experiments were undertaken develop methods to produce larger numbers of eggs and clarify what conditions of moisture and temperature would hasten their hatch rate. We showed that mild temperature of 18⁰C and moisture had some effect, but more work is required if such studies are to be undertaken in the future.

We screened some plant species for their suitability as food plants for adults of the three weevil species. In general, grasses such as ryegrass, kikuyu and oats resulted in the shortest survival times and lowest egg numbers. Such plants could be used by orchardists who may be considering modification of their ground covers. These plant types would be especially beneficial if combined with some form of canopy exclusion treatment for these flightless weevils, such as a sticky band.

We recommend further studies on ground covers, but also that they be undertaken in weevil infested orchards so any positive results will be more quickly able to be implemented by orchardists. Any change to ground cover composition should consider the effects on other pest and beneficial organisms in the orchard ecosystem.

Introduction

The weevils garden weevil (GW), Fuller's rose weevil (FRW) and apple weevil (AW) are amongst the most important pests of pome orchards in Australia because of the damage they can cause and the limited currently available control options (Learmonth, 2000 and Williams, 2000). They are most important in Western Australia but at times cause problems in other apple producing states.

Adults of all three species feed on foliage but cause other damage – GW and to a much lesser extent AW feed on fruit causing it to be rejected; AW ringbark fruit stalks reducing fruit size or causing premature fruit fall; FRW lays eggs in mini-sprinklers causing erratic watering or complete blockage, or lay egg masses on fruit causing them to be rejected.

Among the most unsatisfactory control methods for any pests of pome fruit are those for weevils. Insecticides are relied upon almost exclusively (Sutton *et al*, 2003, Learmonth, 2000, Bailey, 1991 and Allsopp and Hitchcock, 1987). The products used are broad spectrum in their activity which can potentially lead to outbreaks of secondary pests in the orchard complex. Also, where butt drenching of insecticides is applied, orchardists need to time the applications correctly, it is onerous to apply and is not always effective. Sometimes, repeat treatment with insecticide has been necessary.

This study reports on the experiments undertaken to examine two aspects of managing these species of weevils that showed promise in an earlier study (Williams, 2000). These studies were on the role of entomopathogenic nematodes that attack the soil borne larval stage and ground covers that may play a role in the survival and reproductive potential of adult and larvae.

(A) PARASITIC NEMATODES

Introduction

Results of an earlier study (Williams, 2000), showed that insect parasitic nematodes can reduce the abundance of larvae of garden weevil. These findings, while preliminary, suggested the possibility for a completely new approach to managing weevil pests of pome fruit by attacking the soil borne larval stage. Also, the use of nematodes is a biological control approach to weevils and so represents a possible environmentally friendly alternative to the use of broad-spectrum pesticides. Insect parasitic nematodes are relatively selective in the hosts they infect and so also offer the advantage of being selective.

Earlier studies on the use of parasitic nematodes to control FRW in citrus orchards in eastern Australia were reported by Edwards (1996). While activity against FRW was found in pot trials, field work demonstrated small but non-significant reductions in weevil numbers. This work used various strains of the nematode species *H. bacteriophora* and *Steinernema feltiae* and *S. carpocapsae*. Edwards reported that the last species gave some degree of control of FRW in Florida and recommended further work in Australia with other strains of nematode. In contrast, good levels of control of Fuller's rose weevil using *S. carpocapsae* in citrus in USA were reported by Morse and Lindergren (1996).

The discovery and subsequent commercial development of the native insect parasitic nematode *Heterorhabditis zealandica* (Bedding & Nickson, 2001) for the control of some soil dwelling beetle pests paved the way for research to determine whether this natural control agent has activity against larvae of the three species of weevil that are the subject of the present study. The successful pot trials reported by Williams (2000) for controlling GW larvae gave encouragement to undertake the field trials against orchard weevil pests that are reported here.

In order that nematodes are effective in controlling soil borne insects, a number of factors are required. These have been summarised by Bedding (2003). The main factors include the need for the average soil temperature to be at or greater than 15°C, the soil to be moist at the time of application, the nematodes to be applied at high water volume and preferably for the treated site to be watered after application. Also the target stage should be large, both to enhance the success of the nematode to search for and find the host insect and once found, to enhance entry through large body orifices, mainly the mouth and spiracles.

For the main weevil pests in orchards, there are problems in all of these conditions being met. For GW, large larvae are present in early spring (Fisher and Learmonth, 2003) when soil is moist and some rainfall can be relied upon to occur around the time of application. However, soil temperatures are usually cooler than desirable. Less is known on the stage and timing of GW larval development in autumn, but it is reasonable to assume that larvae would be much younger and closer to the soil surface. Small larvae are likely to be more difficult for nematodes to find and enter, but the nematodes would have less soil to search. Soil moisture levels may be a constraint at this time of year also. With the onset of autumn/winter rain in the Western Australian Mediterranean climate, soil temperatures are likely to be falling.

For AW and FRW, constraints to successful control using nematodes exist also. The larvae of these species develop later than GW. So, soil temperature is rising but rainfall and consequently soil moisture levels would be declining. Similar constraints as mentioned for using nematodes to attempt control of GW larvae in autumn would apply also for AW and FRW.

Studies reported here to assess the efficacy of using nematodes to control these three species of weevil have incorporated these environmental and biological constraints as much as possible.

Materials & Methods

Selecting field sites

It was decided that all investigations on the efficacy of nematodes would be undertaken as field trials. The main reasons for this were that pot trials had already been undertaken and showed some positive results (Williams, 2000), and it was necessary to confirm that similar positive results could be obtained under field conditions. Also, by undertaking field trials, realistic variations in application details could be examined and if successful be recommended to orchardists immediately.

A series of field trials was undertaken with the main objectives being to examine the efficacy of the pathogenic nematode *H. zealandica* to larvae of the three species of weevil. In doing this, the effects of different rates, methods of application and incorporation, and time of year were included. Sites ranged from the Perth Hills to Margaret River and south to Manjimup. The sites therefore covered a range of climates and soil types.

Because funding for these studies was provided by pome fruit orchardists, such locations if available were selected first. If suitable field sites were in stonefruit orchards and vineyards, these were used also. Since the arrival of GW in WA in the 1970's, its pest status in apple orchards seems to have changed and AW has become more important in orchards that previously experienced problems with GW. No such situation has occurred in GW infested vineyards. Such vineyards continue to have problems with this pest. For this reason, work with nematodes against GW was often conducted in vineyards. The situation is different in nectarine orchards where GW, once established, has remained the dominant weevil pest. Because the damage threshold for GW in nectarine orchards is very low, such sites were not included in this project where untreated plots were required.

Field sites for these studies were determined by contacting orchardists and vignerons who had a history of problems with weevils. Soil sampling in late winter/early spring was undertaken to confirm the presence of weevils in sufficient numbers and uniformity over proposed trial sites to justify conducting trials there. Soil sampling was undertaken using a spade and examining one square of soil with the side of the square being the width of the spade, 15 cm. Soil samples were taken to a depth of approx. 15cm. Soil was placed in metal trays and examined in the field. Weevil larvae were identified in the field. The depth for soil sampling where AW was the target pest was increased to approx. 25 cm because more mature larvae and pupae can occur to this depth (Andrewartha, 1931, 1933).

Brown-headed larvae were identified as being either GW or AW, depending on the farmer's weevil problems, or spotted vegetable weevil (SVW, *Desiantha diversipes*) (Learmonth, 1988). Other species of weevil with brown head capsules occur in WA, but are rare in orchards and vineyards. SVW is not considered a pest weevil in orchards or vineyards. Larvae of SVW may feed on roots of orchard trees and grapevines, but have never been associated with poor plant vigour, do not reach the high abundance levels of the known pest species of weevils and adults have not been observed feeding on trees or vines. Larvae of SVW were distinguished from GW and AW by size and shape. SVW larvae never get as large as AW/GW larvae and usually taper towards the head (Grimm & Michael, 1989, Learmonth, 1988). Under microscopic examination, SVW larvae have a spine associated with their lateral spiracles. AW/GW lack the spine.

White-headed weevil larvae were identified as being either FRW or whitefringed weevil (WFW, *Naupactus leucoloma*), (Learmonth, 1988). WFW occurs in orchards and vineyards and have been noted as a pest especially in newly established crops. Adults strip the young plants of leaves. This occurrence is rare and as the plants grow, the problems with the weevil diminish. WFW may continue to be found in low numbers but is not usually sufficiently abundant to warrant control measures. WFW & FRW larvae were distinguished from each other mainly on the basis of size, and sometimes on colour. Larvae greater than 10mm long were identified as WFW. Larvae that were light yellow were identified as FRW.

If the site being sampled had a history of problems with FRW, white-headed weevil larvae were identified as this species, subject to the size and colour criteria mentioned already. There are other species of weevil whose larvae have white head capsules Learmonth, 1988). Their abundance in orchards and vineyards is usually very low.

Nematode applications

Batches of the insect parasitic nematode, *H. zealandica* for the trials, were obtained from Ecogrow (Craig Wilson, pers. comm.) and sent to WA via air freight. The nematodes were requested just before they were needed so they would arrive fresh and require minimal storage before application. Nematodes were applied with a stimulant at 0.2% to aid their searching action in soil once they were applied.

The following aspects of application of nematodes varied across trial and demonstration study sites:

(a) method of application.

Three methods were used in order to apply the nematode solution to the trial sites. The first of these methods was using a tractor mounted boom spray to apply the nematodes directly onto the orchard floor. The boom was equipped with HARDI 18 INJET flooding flat fan nozzles 1 metre apart, with the first nozzle 0.5 metre from the tree or vine row. The boom was set up with three or two nozzles for orchard and vineyard spraying respectively. Each side of the vine or tree row was sprayed to obtain a spraying zone from half way across the interrow on either side. The boom spray was been calibrated to deliver 540 litres spray solution per hectare.

The second method involved the use of mini-sprinklers in orchards, but not vineyards. The reason for this was to follow commercial irrigation practices in the respective crops to apply the nematodes. The mini sprinkler type used was a Philmac Orbitor Anti-Ant OAA 13 White, which delivered 65 litres per hour at 2 bar pressure. These sprinklers were placed adjacent to each tree in each treatment plot. The sprinklers were run for 5 minutes for an output of around 27L of nematode solution per treatment plot. Many orchardists place the sprinklers at alternate trees, but for the purpose of maximising the likelihood of even distribution of the nematodes, they were placed at each tree in this series of experiments.

In most vineyards and many orchards, irrigation is applied through a dripline. This system was also used to apply nematodes. Kramer and Grunder (1998) reported on the requirements for even application of nematodes using trickle irrigation and Curran and Patel (1988) provide an example of the application of nematodes through trickle irrigation to control weevil larvae. In vineyards, the dripline used was Netafim Dripmaster 17 (R17D36-060) rated at 3.5 L/hr. The dripline had outlets at 60 cm intervals. Two lines were laid out, one on each side of the treated vine row in a staggered pattern so nematodes were applied at 30cm spacings. As vineyards varied in their row and vine spacings, the concentration of the nematode solution was varied to deliver the desired rates.

(b) rate of application

Sufficient development work had been undertaken by the company Ecogrow on beetle pests in amenity turf to indicate possible rates to compare for this study on weevil larvae. The actual rates used were recommended by Bedding (pers. comm.). The rates used ranged from 0.1 to 1 million active stages per square meter.

(c) method of incorporation

The volumes of water used for the three application methods varied, with the water rate for the boom spray application the lowest at 540L/ha. The application of nematodes utilising the irrigation systems involved volumes of between 6 and 10 times this amount. The efficacy of the nematode applications by boom spray was compared with and without incorporation after

application using a side mounted rotary hoe. The depth to which the area treated with nematodes was hoed was around 5 to 10 cm, a depth considered sufficient to protect the nematodes from desiccation and to aid their entry into soil to begin the search for weevil larvae.

(d) time of year

Because the survival and ability of nematodes to seek host is affected by soil temperature and moisture level, and stage of the host, it was considered important to examine the efficacy of nematode applications at different times of the year. This would correspond to variations in these parameters.

Measuring the efficacy of nematode applications

Two methods were used to quantify the effects of applying the nematodes to control weevils – soil sampling both to estimate larval abundance and collect insects to determine whether they were infected with nematodes, and monitoring the subsequent abundance of adults weevils. For comparison with a commercial treatment, most sites included the application of a butt drench with the synthetic pyrethroid insecticide alphacypermethrin. This insecticide is registered for use to control both AW and GW in deciduous fruit tree orchards, and an Australian Pesticides and Veterinary Medicines Authority (APVMA) minor use permit was available for its use in vineyards. In some trial areas, another insecticide product containing a synthetic pyrethroid insecticide was tested for its efficacy as a butt or foliar spray. In apple orchards, the butt drench was applied at 1L spray solution per tree; in the apricot orchard and grapevines, 0.5L spray solution per tree or vine and vine post was applied.

Soil sampling for larvae was undertaken by spade sampling at trial/demonstration sites. Only untreated and the highest rate of the nematode treatments were sampled because of the time constraints in undertaking soil sampling. Each sampling unit was a square spade of soil (15 cm long spade blade) to a depth of 15 cm. Soil was examined in the field and larvae categorized as described above for species identity. All pest weevil larvae were collected into perspex tubers and reared singly with carrot as a food source in the laboratory to assess the proportion infested with nematodes.

The abundance of adults of AW and GW were monitored using single faced cardboard bands placed on the trunk of fruit trees or the butt of grapevines just below the crotch (Fisher and Learmonth, 2003). Bands were examined weekly to fortnightly during the main period of weevil activity. Monitoring was continued to about January.

The abundance of Fuller's rose weevil adults was assessed by foliage tapping over a tray, 30 cm by 20 cm. Usually four areas of foliage were tapped per treatment plot.

Results

2000/01 season

Four replicated field trials were conducted during the 2000/01 season. Three were conducted in apple orchards and one in an apricot orchard. The apple orchards were located at Jarrahdale, approx. 50km south-east of Perth, Donnybrook and Manjimup and the apricot orchard was in Manjimup. All sites were infested with apple weevil, and the apricot orchard also infested with Fuller's rose weevil. At all sites, nematodes were applied by boom only, with and without incorporation using a rotary hoe. At two sites, one treatment involved use of the rotary hoe without the application of nematodes.

To confirm whether the nematode application had resulted in any soil insect stages becoming infected, weevil larvae and pupae were collected in untreated plots and those treated with the highest rate of nematode at each of the four trial sites. Of the 39 brown head weevil larvae, 14 white head weevil larvae and 10 pupae collected from untreated plots, none were infected with nematodes. Similar numbers were collected from the nematode treated

area and no infection was found. In the latter area, two of the brown head weevil larvae emerged as AW. The incidence of unexplained mortality was very high in these field collected insects for both treatments.

The abundance of apple weevil adults as an average of the treatments for the four orchards is given in Figs. 1 to 4.

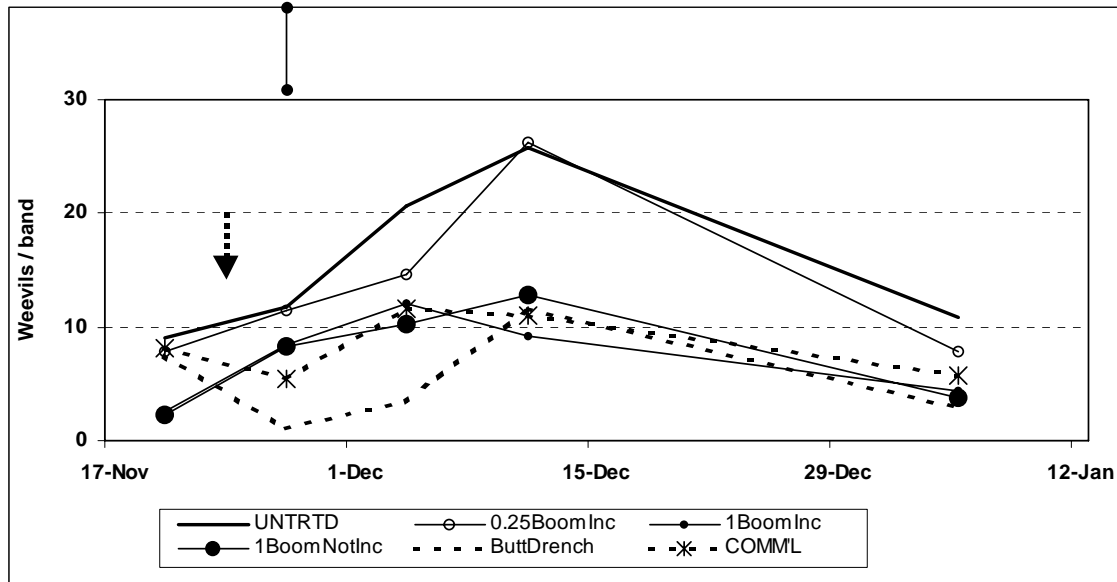


Fig.1. The abundance of apple weevil adults in trunk monitoring bands in apple trees at Jarrahdale, WA where parasitic nematodes had been applied on 19 October 2000 at 0.25 m/m² or 1 m/m², with or without incorporation using a side mounted rotary hoe. Comparison with application of a synthetic pyrethroid butt drench applied at the time indicated by the arrow and weevils in a commercially managed part of the orchard were included. The bar above the graph shows the LSD_{0.05} value where a statistically significant difference in mean abundance levels was found from ANOVA.

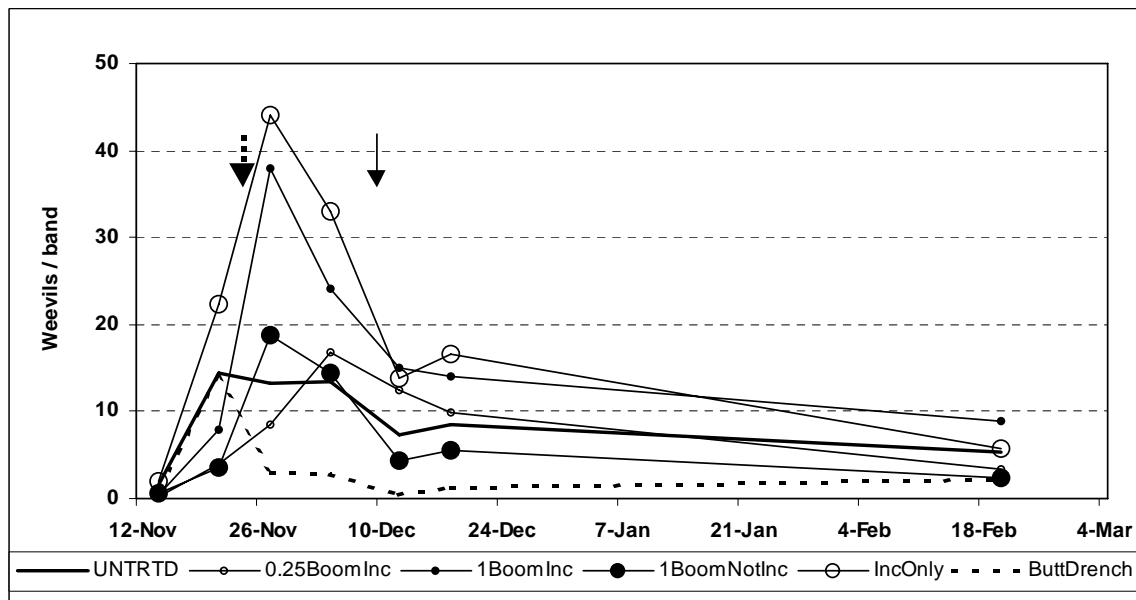


Fig.2. The abundance of apple weevil adults in trunk monitoring bands in apple trees at Donnybrook, WA where parasitic nematodes had been applied on 24 October 2000 at 0.25 m/m² or 1 m/m², with or without incorporation using a side mounted rotary hoe. Rotary hoeing without the addition of nematodes was included as a treatment at this site. Comparison with application of a synthetic pyrethroid butt drench applied at the time indicated by the arrow on the left was included. The trial area was subjected to a foliar spray of insecticide at the time indicated by the arrow on the right.

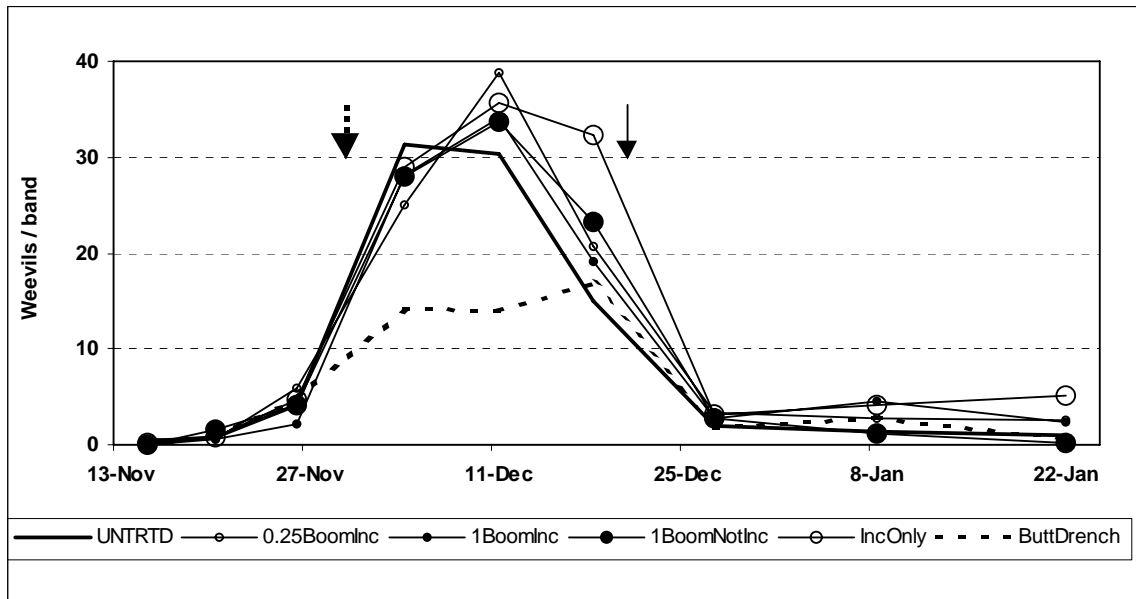


Fig.3. The abundance of apple weevil adults in trunk monitoring bands in apple trees at Manjimup, WA where parasitic nematodes had been applied on 23 October 2000 at 0.25 m² or 1 m², with or without incorporation using a side mounted rotary hoe. Rotary hoeing without the addition of nematodes was included as a treatment at this site. Comparison with application of a synthetic pyrethroid butt drench applied at the time indicated by the arrow on the left was included. The trial area was subjected to a foliar spray of insecticide at the time indicated by the arrow on the right.

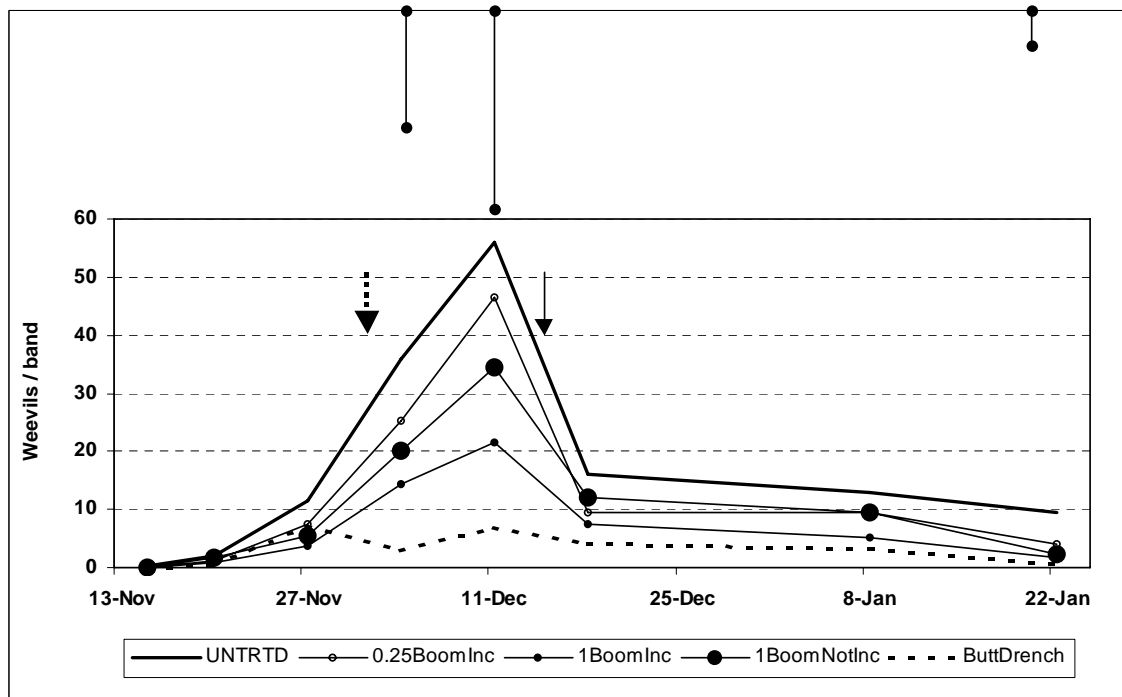


Fig.4. The abundance of apple weevil adults in trunk monitoring bands in apple trees at Manjimup, WA where parasitic nematodes had been applied on 20 October 2000 at 0.25 m² or 1 m², with or without incorporation using a side mounted rotary hoe. Comparison with application of a synthetic pyrethroid butt drench applied at the time indicated by the arrow on the left was included. The trial area was subjected to a foliar spray of insecticide at the time indicated by the arrow on the right. The bars above the graph shows the LSD_{0.05} value where statistically significant differences in mean abundance levels were found from ANOVA.

The main significant difference among treatments was the effect of the insecticide butt drench which had the greatest effect in reducing the numbers of weevil adults. Even though some effects of the nematode to reduce weevil abundance appears to have occurred, when the graphs are examined, the variability in the abundance across the replications was so great that significant differences were not found.

This was also the case for the abundance of Fuller’s rose weevil also present in the orchard depicted in Fig. 4. Despite treatment means of 10.8, 2.8, 4.4, 4.2 and 6.8 for the five treatments, the associated standard deviation of the means was 13.7, 2.4, 5.0, 2.6 and 6.1 respectively.

Therefore no effect of either rate of nematode application or whether they were incorporated could be considered to have occurred.

2001/02 season

Three replicated field trials were conducted during the 2001/02 season. One was conducted in a vineyard and two in apple orchards. The vineyard was located at Karridale, approx 300 km south of Perth. The apple orchards were at Mullalyup, approx. 200km south-east of Perth, and Manjimup. The target weevil pest in the vineyard was GW, while in the apple orchards it was AW. At these sites, nematodes were applied through mini-sprinklers, dripper line or a tractor mounted boom. None of the treatments involved incorporation using a rotary hoe.

The abundance of weevil adults as an average of the treatments for the three sites is given in Figs. 5 to 7.

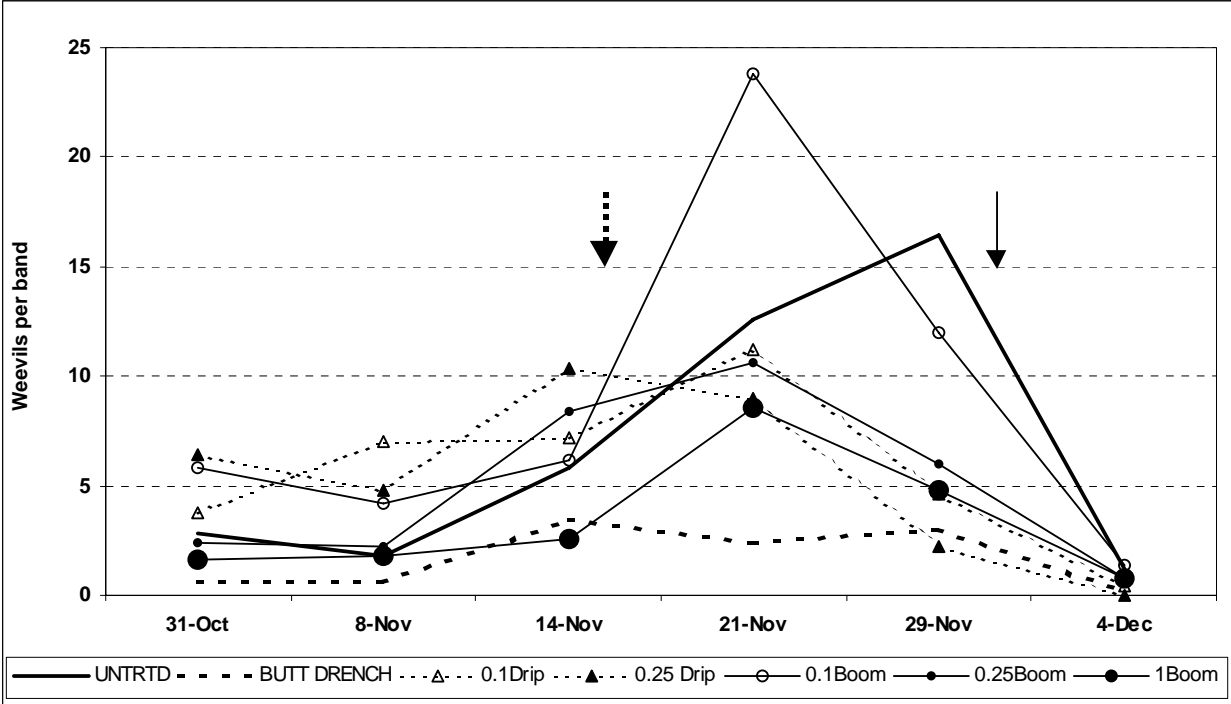


Fig.5. The abundance of garden weevil adults in trunk monitoring bands in grapevines at Karridale, WA where parasitic nematodes had been applied on 21 September 2001 at 0.1 m/m² and 0.25 m/m² through trickle irrigation line and at 0.1/m², 0.25/m² and 1 m/m² through a boom spray. Comparison with application of a synthetic pyrethroid butt drench applied at the time indicated by the arrow on the left was included. The trial area was subjected to a foliar spray of insecticide at the time indicated by the arrow on the right.

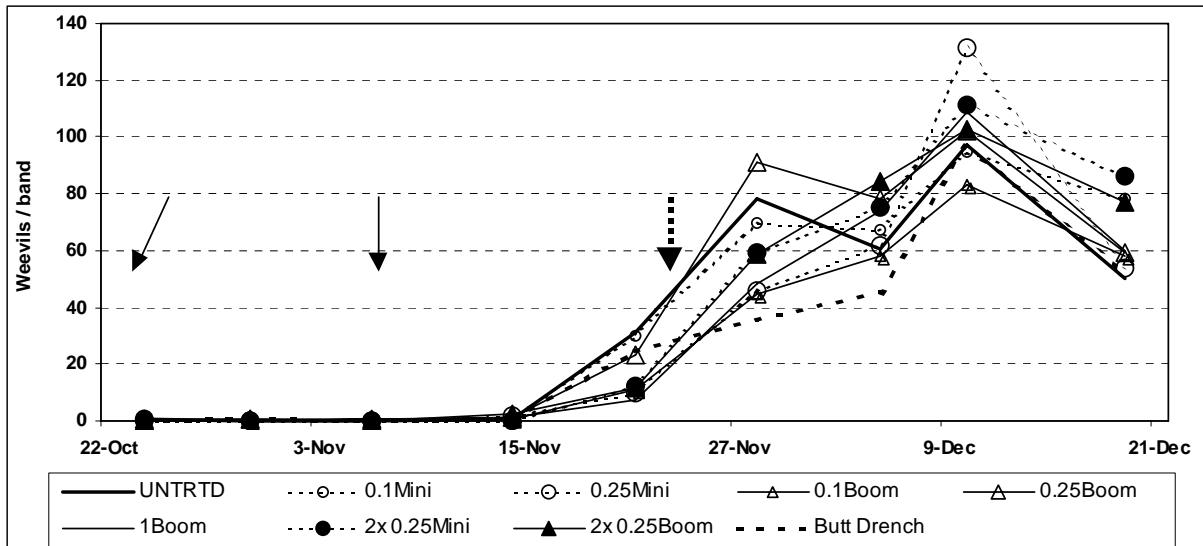


Fig.6. The abundance of apple weevil adults in trunk monitoring bands in apple trees at Mullalyup, WA where parasitic nematodes had been applied on 27 September 2001 at 0.1 m/m² and 0.25 m/m² through mini-sprinklers and at 0.1 m/m², 0.25 m/m² and 1 m/m² through a boom spray. On , a second application of nematodes by both mini-sprinkler and boom was made at 0.25 m/m² to a second series of plots treated earlier at this rate. The timing of the nematode application is indicated by the two arrows on the left. Comparison with application of a synthetic pyrethroid butt drench applied at the time indicated by the arrow on the right was included.

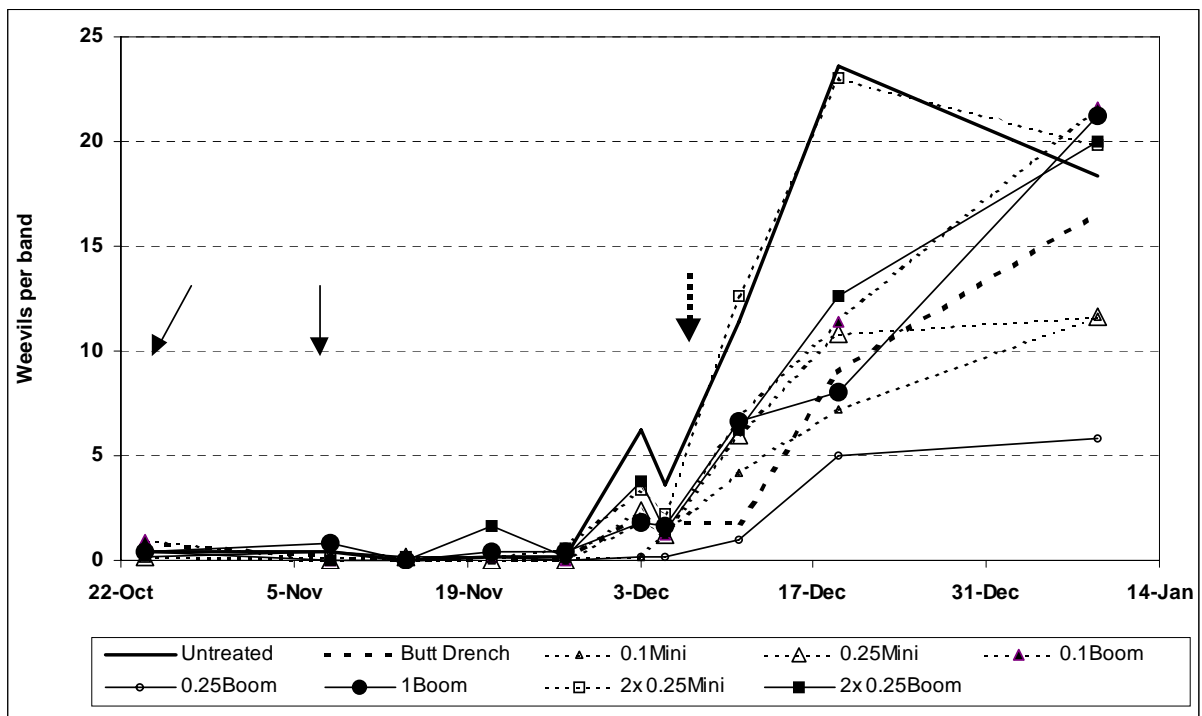


Fig.7. The abundance of apple weevil adults in trunk monitoring bands in apple trees at Manjimup, WA where parasitic nematodes had been applied on 24, 25 September 2001 at 0.1 m/m² and 0.25 m/m² through mini-sprinklers and at 0.1 m/m², 0.25 m/m² and 1 m/m² through a boom spray. On 7 November, a second application of nematodes by both mini-sprinkler and boom was made at 0.25 m/m² to a second series of plots treated earlier at this rate. The timing of the nematode application is indicated by the two arrows on the left. Comparison with application of a synthetic pyrethroid butt drench applied at the time indicated by the arrow on the right was included.

As was the case for the series of trials in the previous season, the variability of abundance in weevils within replications resulted in no significant differences being recorded in any of the trials using ANOVA. In any case, there was no consistent effect of the nematode treatments in reducing weevil abundance. Also, in both AW trials sites, the effectiveness of the insecticide butt drench was quite poor. This type of effect has been observed in the past with this weevil control treatment in commercial orchards.

2002/03 season

During this season, one replicated trial and two demonstration studies were conducted. The replicated trial was undertaken in a plum orchard in Manjimup infested with FRW. The demonstration studies involved the application of parasitic nematodes to large plots without replication. One of the demonstration study sites was in a vineyard in Pemberton where the target pest was GW. The other demonstration study site was in an apple orchard in the Perth hills where AW was the target pest. At all sites, nematodes were applied through a tractor mounted boom. None of the treatments involved incorporation using a rotary hoe.

The abundance of weevil adults as an average of the treatments for the three sites is given in Figs. 8 to 10.

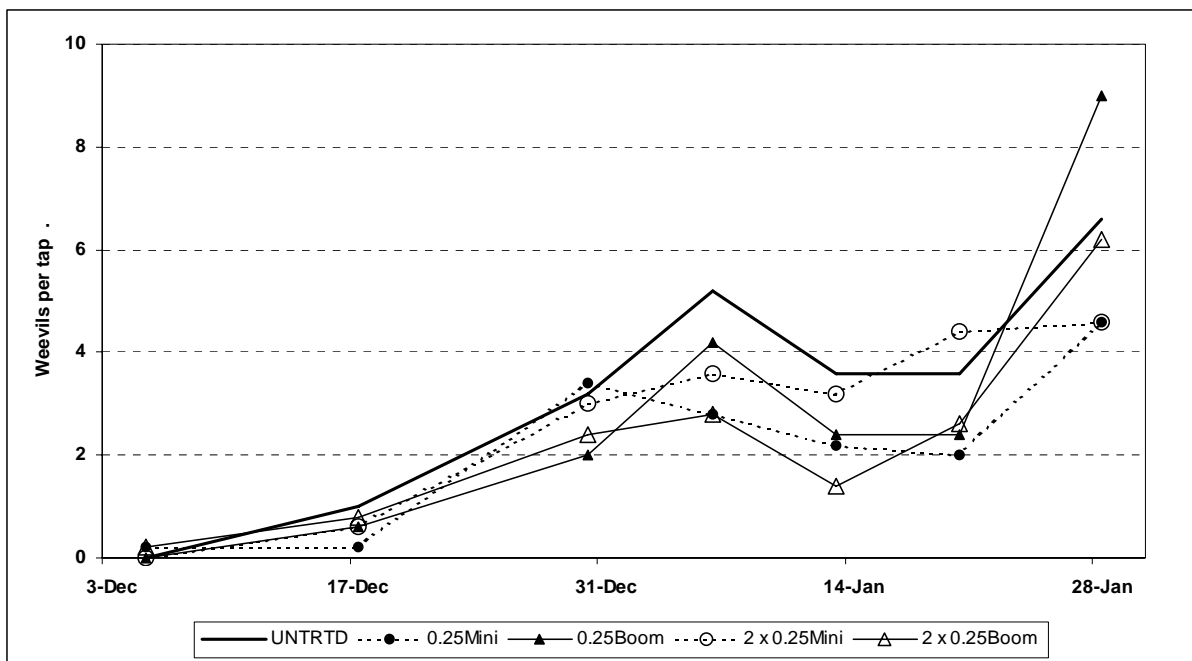


Fig.8. The abundance of Fuller's rose weevil adults from foliage tapping in plum trees at Manjimup, WA where parasitic nematodes had been applied on 24, 25 September 2001 at 0.1 m/m² and 0.25 m/m² through mini-sprinklers and at 0.1 m/m², 0.25 m/m² and 1 m/m² through a boom spray. On 7 November, a second application of nematodes by both mini-sprinkler and boom was made at 0.25 m/m² to a second series of plots treated earlier at this rate. The timing of the nematode application is indicated by the two arrows on the left. Comparison with application of a synthetic pyrethroid butt drench applied at the time indicated by the arrow on the right was included.

No significant treatment effects were found for weevil density in the trial involving FRW. For the demonstration study involving GW, a greater number of weevils was recorded in the treated area. This result can only have been produced through the variability of the weevil abundance in the two plot areas. With respect to the demonstration studies, there appears to have been a treatment effect as far as AW is concerned (Fig. 10). When compared with other studies undertaken and considering that there was no replication involved, this result may have occurred also because of the variability in abundance of the weevils present.

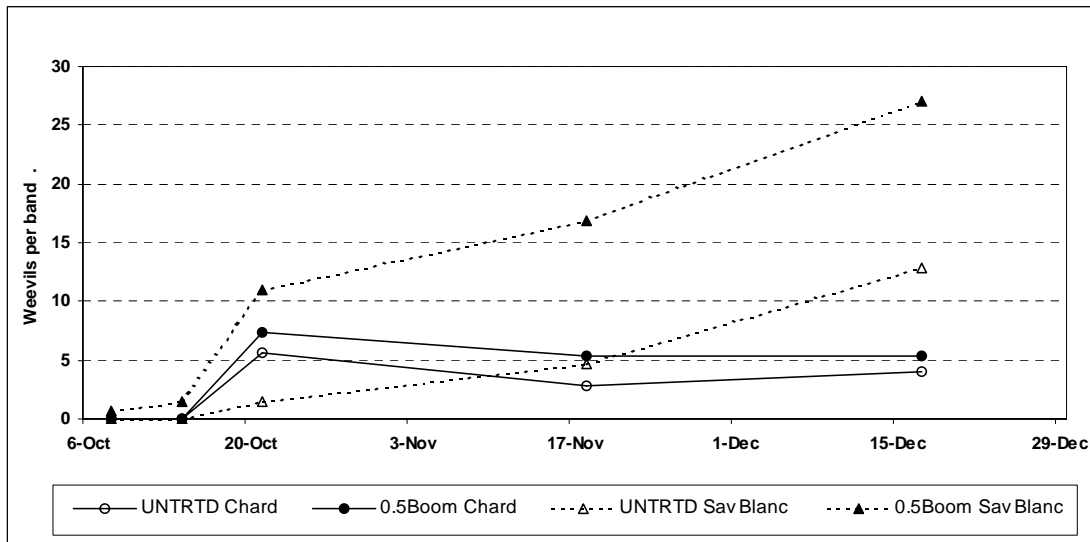


Fig.9. The abundance of garden weevil adults in trunk monitoring bands in two varieties of grapevines at Pemberton, WA where parasitic nematodes had been applied on 26 June 2002 at 0.5 m/m² through a boom spray.

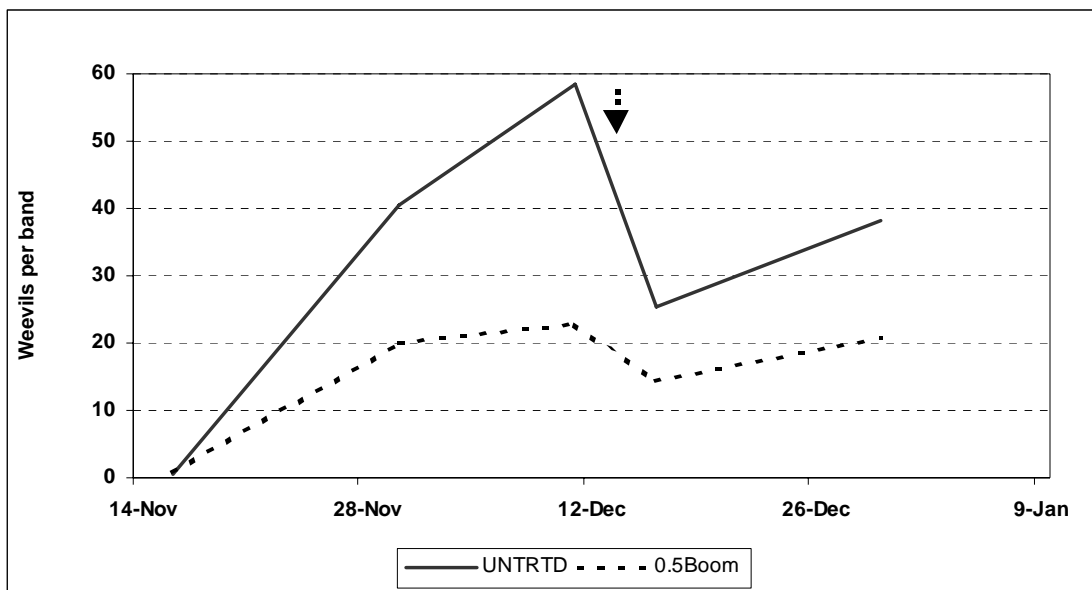


Fig.10. The abundance of apple weevil adults in trunk monitoring bands in apple trees at Karragullen, WA where parasitic nematodes had been applied on 20 June 2002 at 0.5 m/m² through a boom spray. A butt drench of synthetic pyrethroid was applied to the monitored area at the time indicated by the arrow.

Discussion and Conclusions

This section of the project sought to determine whether the efficacy of an insect parasitic nematode demonstrated in laboratory trials was applicable to field populations of three species of weevil pests in pome fruit orchards. This relatively newly discovered species of nematode *Heterorhabditis zealandica* was available commercially and is used against beetle larvae pests of turf. The commercial availability of the nematode as well as its demonstrated activity against garden weevil larvae (Williams, 2000) were the main reasons for assessing its potential role in orchards.

Replicated field trials were undertaken in apple, apricot and plum orchards as well as vineyards. A range of rates, methods of application, with and without incorporation and time of year were examined. Results from these studies showed that the nematode does not have a role to play in managing the weevil pests. Despite soil sampling to confirm the presence of reasonable numbers of weevil larvae in the various trial sites selected, there was a great deal of variation in the infestation level across trial sites. While considerations such as increasing the number of replications would have helped overcome this issue, if the nematodes had a reasonable level of efficacy, numbers in treated areas would be expected to be low enough to still result in significant treatment effects.

Results of preliminary laboratory experiments conducted concurrently by Bedding (2003) cast doubt over the effectiveness of *H. zealandica* against AW and FRW. From these results in a controlled situation, the results of the field work here were not unexpected. A report by Morse and Lindergren (1996) on nematodes for control of Fuller's rose weevil in citrus indicated that some control of this weevil with nematodes is possible.

Use of nematodes for managing insect pests has a history of success, but the situations where this has been achieved have been special cases (Bedding, 2003). The application of parasitic nematodes in orchards and vineyards represents a challenge for this biological agent to be successful. The weevil larvae are soil borne and scattered over a wide area, even though they may be more concentrated along the dripline (in Williams, 2000). Weevil larvae are most susceptible when they are large, but for garden weevil this occurs at a time of year when soil temperatures are low. For apple weevil and Fuller's rose weevil in WA, this occurs at a time of year when soil moisture levels are declining.

Nematodes are obligate parasites requiring the continual presence of a host and therefore would need to be applied each season in which weevil numbers were considered to be a threat to orchards (Bedding, pers. comm.), although a different conclusion was reached by Morse and Lindergren (1996) in their study.

Parasitic nematodes are relatively expensive compared to chemical insecticides. For them to be adopted, their efficacy would need to be reliable as well as being more competitively priced. In the case of the weevils in this study, the availability of more active strains would provide some optimism for their utilisation.

(B) GROUND COVERS

Introduction

In addition to the positive results with parasitic nematodes, the results reported by Williams (2000) of poor survival of weevil larvae when reared on sub-clover and white clover also provided encouragement to undertake further work on this aspect. The objective of studies to identify food plants that are not favourable to the survival of weevil larvae has implications for plant selection for the ground cover flora of orchards. The role of certain weeds as favourable food plants that enhance the survival of weevil larvae of some of these species of weevil has been well known (Williams, 2000 and Fisher & Learmonth, 2003). The role of cover crops in management of arthropod pests in vineyards has been reviewed by Ingels *et al* (1998). They refer to particular examples in relation to leafhoppers and make general comments on pest suppression and the role of cover crops as part of an Integrated Pest Management approach rather than a stand alone tool.

The objective of the present study was to progress from the earlier preliminary studies to identify plants that are detrimental to the survival of weevil larvae, but which are compatible with being grown as ground cover in orchards. Like nematodes, assessing the use of different ground cover swards represents a shift away from reliance on broad-spectrum insecticides in controlling weevils.

A series of pot trials was run to clarify the suitability of a range of food plants for their effect on survival of weevil larvae. Plants were selected on the basis of suitability as orchard cover crops. Pot trials were undertaken in the first instance as it was thought to be a first stage screening process without the problems that may occur in conducting field trials. A smaller list of appropriate candidate plant types could then be used in the field in weevil infested orchards.

In addition to assessing the role of a range of plants in the survival of larvae, their suitability as food plants for the adult stage of the weevils was also examined in laboratory trials.

Materials and methods

Pot trials with weevil larvae

Candidate plant species were grown in pots and infested with newly hatched weevil larvae obtained from laboratory cultures of weevil adults. The weevil adults for the laboratory colonies were collected from field infestations in vineyards and orchards and held in 850ml plastic take-away containers with air holes punched in the lids to allow air exchange but to limit moisture loss so the food source for adults did not dry out. Adults of GW were fed on grapevine leaves, while adults of FRW and AW were fed on apple leaves. Food was changed twice weekly and eggs were collected. As GW prefers injecting their eggs into spaces, moistened folded paper towelling 5cm wide and stapled in the centre to form a fan was provided as an egg laying substrate. Eggs of FRW were laid on leaves, the surface of the rearing container and occasionally through the puncture holes and onto the lid of the rearing container. AW laid most eggs on the leaves, but also on the floor and sides of the container.

Eggs were collected using a fine hair brush and placed on moistened filter paper inside plastic petri dishes with the lid sealed with plastic film. Petri dishes were placed in self seal plastic bags. Unless a sufficient number of eggs was obtained ready for hatching and infesting pots, the eggs were accumulated and stored in a refrigerator running at about 4°C.

When required eggs were removed from the fridge and placed under different conditions depending on the weevil species. For GW and AW, petri dishes were unsealed and placed in re-sealable plastic bags at 25°C. For FRW, petri dishes were also unsealed and eggs were cleaned to remove and help prevent subsequent infection with what appeared to be fungal growth. Egg masses were cleaned by placing them in a fine stainless steel mesh

strainer and washing them in a solution of 1% sodium hypochlorite. After this, eggs were returned to moistened filter paper and placed in plastic re-sealable bags. They were subjected to 30°C for 72 hours and then held at 25°C to hatch.

Upon hatching larvae were divided evenly into the number of pots allocated for each trial. For example if a trial involved 9 treatments, 5 replications and 3 times of assessment, a total of 135 pots and 540 weevil larvae were available from the laboratory colony, then each pot would receive 4 larvae. This procedure was repeated daily until the total number of larvae required for each pot was reached.

The appropriate number of newly hatched larvae was moved from the petri dishes to 30 g portion control cups using a fine haired brush. These containers were then inverted on the soil surface to allow the larvae to burrow into soil.

As for previous pot trial studies, similar methods were used for growing plants. Black plastic plant pots approximately 200mm deep and 200mm in diameter were used for single plant species. Where test plants were grown in combination with a small fruit tree to test whether low survival of larvae on single species was a result of starvation or some degree of toxicity, larger pots 370 mm deep and 300 mm diameter were used. Folded shade cloth was placed in the bottom of pots to prevent escape of larvae and coarse gravel for drainage placed on top of that. Pots were then filled to about 30 mm below the top with a potting mix with a pH of approximately 6 and consisting of the following per cubic metre:

Pine bark, medium composted	0.5m ³
Coco peat	0.25 ³
River sand	0.25 ³
Superphosphate	1kg
Ammonium nitrate	1kg
Potassium sulphate	0.3kg
Macromin	0.2kg
Ferrous sulphate	0.5kg
Limestone	2kg

Plants were grown in a glasshouse where the air temperature was maintained at between 24 and 28°C and watered twice daily for 5 minutes, with overhead misting sprinklers. Watering was altered throughout the season to allow for plant water usage. Plants were prepared about two months prior to infestation of weevil larvae to allow adequate root growth.

Osmocote® slow release fertiliser was applied to correct apparent nutrient deficiencies in plants exhibiting symptoms of leaf discolouration such as yellowing. Occasionally aphids were a problem and the aphid specific insecticide Pirimor® was applied.

Assessment of trials for larval survival was carried out destructively. Potting mix and root material from each pot was placed on a metal tray then hand sorted, relying on visual detection to collect surviving larvae, pupae or adults. Throughout the process any clumps or mats of roots were teased apart to thoroughly assess each treatment. This material was checked twice to try to overcome deficiencies related to relying on hand sorting, rather than a mechanical process such as floatation.

More than one assessment time was included to gauge the correct time to assess treatments where the rate of development of weevil larvae was not well known. Pots assessed too early could result in missing larvae if they were very small; pots assessed after a very long time

such that some larvae had developed to adults, could also compromise accuracy because of the mobility of adults.

After collecting the weevils from pots, the stage and number of insects was recorded. Larvae and pupae were weighed and the head capsule width of larvae was measured using a dissecting microscope with a calibrated eyepiece scale. Data on head capsule widths is not included in this report and weight of larvae was used as a measure of larval development.

The details on plants used for each species of weevil and commencement and observation times are listed below in the Results section.

Laboratory trials on food plants for adult weevils

As well as comparing a range of potential cover crop plants for their suitability for survival of the larval stage of the three species of weevil, the suitability of some of them and others as food plants for the survival and reproduction of the adult stage was compared.

For this, adults were reared in the laboratory using the same type of plastic container described above. Weevil adults were supplied with leaves of the plants selected for comparison. The leaves were replaced with fresh ones twice a week, and at the same time dead weevil adults and eggs were counted and removed.

Garden weevil lays eggs in masses, often injecting them in the paper fan provided. Because these eggs are laid without being cemented together, counting was reasonably accurate. Fuller's rose weevil lays egg masses, with eggs cemented and sometimes overlapping each other. To improve the accuracy of counting eggs of both Fuller's rose weevil and garden weevil, both a dissecting microscope and a magnifying lens equipped with a fluorescent lamp were used. Apple weevil lays eggs singly so counting these eggs was more accurate.

At the time weevil containers were checked, the amount of food consumed was rated, using the following scores: 0 = no or minor feeding; 1 = moderate feeding; 2 = obvious feeding.

The details on plants used for each species of weevil and commencement and observation times are listed below in the Results section.

Laboratory trials on apple weevil egg production and hatch

Limited pot trials could be undertaken on assessing survival of apple weevil larvae on different plant species because of poor egg production and a low percentage of egg hatch in the laboratory colony. To attempt to overcome these problems, two experiments were undertaken.

The first experiment involved rearing apple weevil adults under two temperature regimes to determine whether temperature would affect egg production. For this, 10 apple weevil adults were held in individual containers as described above and fed on apple leaves either in an insectary at 25 °C, or in an incubator at 18 °C. The containers were examined twice a week. The number of eggs and dead adults were recorded and removed, and the amount of feeding was scored using the same system described above. There were 6 replications at each temperature and the experiment was run over 66 days.

The second experiment examined the effect of temperature and moisture on the timing of hatching of apple weevil eggs. This involved exposing two lots of 20 newly laid apple weevil eggs to a series of temperature and moisture regimes and durations listed in Table 1.

Moisture regimes were either wet or dry, depending on whether the filter paper on which eggs were laced in petri dishes was wet up with distilled water or not. The experiment was commenced on 25 May 2004 and concluded after 66 days on 30 July 2004. Eggs were

checked twice weekly and the number that had hatched was recorded and the larvae removed.

Table 1. List of temperature and moisture regimes to which eggs of apple weevil were exposed to in the laboratory in an attempt to achieve quick and uniform hatching. The final incubation temperature for all treatments, unless otherwise indicated, was 25°C.

Tr t No.	Moisture	Temp. (°C)	Chill Time	Shock Trt.
1	Dry	4	1 week	-
2	Dry	4	2 weeks	-
3	Dry	4	4 weeks	-
4	Dry	4	8 weeks	-
5	Dry	4	1 week	1 day @ 30 °C
6	Wet	4	1 week	-
7	Wet	4	2 weeks	-
8	Wet	4	4 weeks	-
9	Wet	4	8 weeks	-
10	Wet	4	1 week	1 day @ 30 °C
11	Dry	18	-	-
12	Wet	18	-	-
13	Dry	25	-	-
14	Wet	25	-	-
15	Dry	30	-	1 day @ 30 °C
16	Wet	30	-	1 day @ 30 °C
17	Wet after 7 days	18	-	-
18	Wet after 14 days	18	-	-

GARDEN WEEVIL

Results

Pot trials with weevil larvae

A series of three experiments was conducted on the survival of garden weevil larvae on different plant species during the project.

Pot trial 1

The first experiment had two aims – to compare different plants as single species for development of garden weevil larvae, and secondly to determine if more advanced larvae reared on sorrel initially would survive when transferred to clover. The second aim followed on from results in earlier experiments reported by Williams (2000) where clover was found to be a poor food plant when infested with first instar larvae. The second aim of the experiment was examined by infesting two types of sub-clover with more advanced larvae obtained after rearing on the favourable food plant sorrel for around 40 days.

The plant species and transfer treatments included for assessment are listed in Table 2. Each plant type or series of plants was replicated 5 times for each of three assessment times. All pots were infested with 25 first instar larvae each, over the period 11 to 26 June 2002. The insect repellent clover was obtained from Murdoch University (O'Hara and Flores

Vargas, pers. Comm.), as part of that institution's program on developing insect resistant plants such as Prima gland clover (Nutt and Loi, 2002).

Table 2. Treatments used to compare the survival of garden weevil larvae in a pot trial involving different plants as single species per pot and including treatments where garden weevil larvae were transferred to some plants having initially having been reared on a known favourable food plant, sorrel.

Plants tested	Measurements (days after infestation)
<i>Single species for the duration of the trial:</i>	Weevil development assessed at three dates
Carrot cv. Western Red	(times) after initial infestation for all treatments:
sub-clover cv. Dalkeith	Early - 15 Aug. (approx. 58 days)
insect repellent clover	Mid - 2 Sept (approx. 76 days)
kikuyu	Late - 30Sep (approx. 94 days)
Ryegrass cv. Betta Tetilla	
sorrel	
<i>Larvae reared on sorrel then transferred to:</i>	Transfer dates (times) from initial sorrel infestation:
sorrel	Early - 30 Jul (approx. 42 days) assessed 15 Aug.
sub-clover	Mid - 5 Aug (approx. 48 days) assessed 2 Sept.
insect repellent clover	Late - 14 Aug (approx. 57 days) assessed 30 Sept.

For the treatments involving rearing larvae on the known favourable food plant sorrel initially before transferring them to sorrel, sub-clover or insect repellent clover, the average number of larvae transferred per pot is shown in Fig. 11. Because larvae were to be transferred to other pots for further rearing, larvae recovered from the initial sorrel pots were categorised to size by naked eye based on body size. The number of larvae for each treatment was quite variable, with the numbers for the sorrel to sorrel treatments the least.

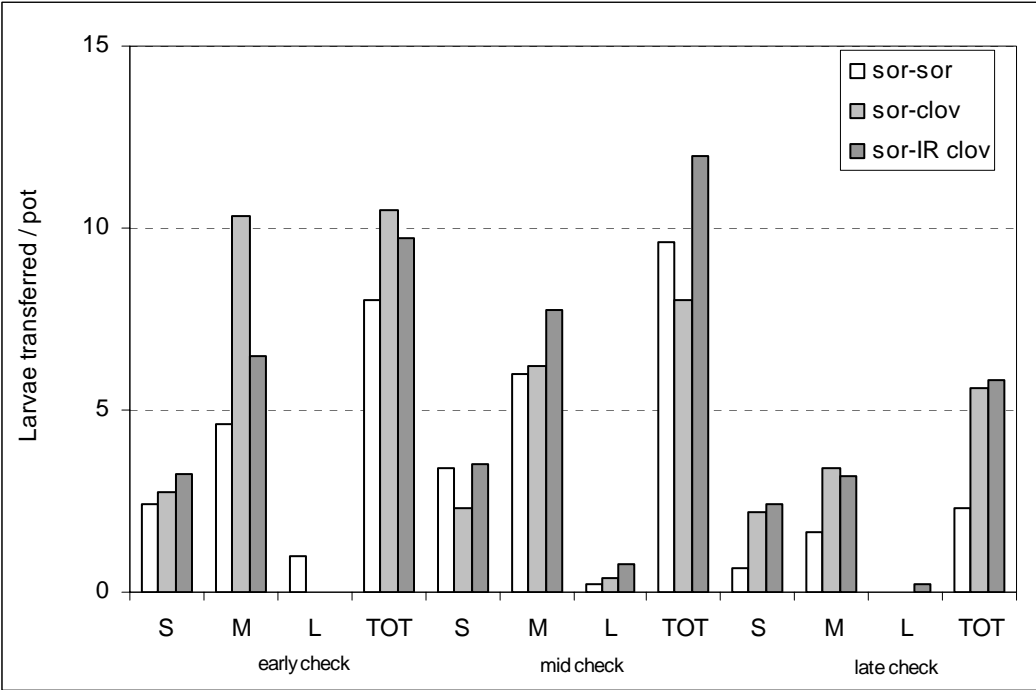


Fig. 11. The average number of S (small), M (medium), L (large) and TOT (total) garden weevil larvae per pot transferred from sorrel to sorrel, sub-clover or insect repellent clover in an experiment to assess whether more advanced larvae could survive on sub-clover or insect repellent clover.

The three times of assessment were included in this experiment to ensure larval development was such that reliable comparisons among treatments were possible. The proportion of weevils as larvae, pupae and adults found at the last time of assessment is given in Fig. 12. It is possible larvae may have been too small in some treatments at the first time of assessment (see below) and some adults were found at the last assessment.

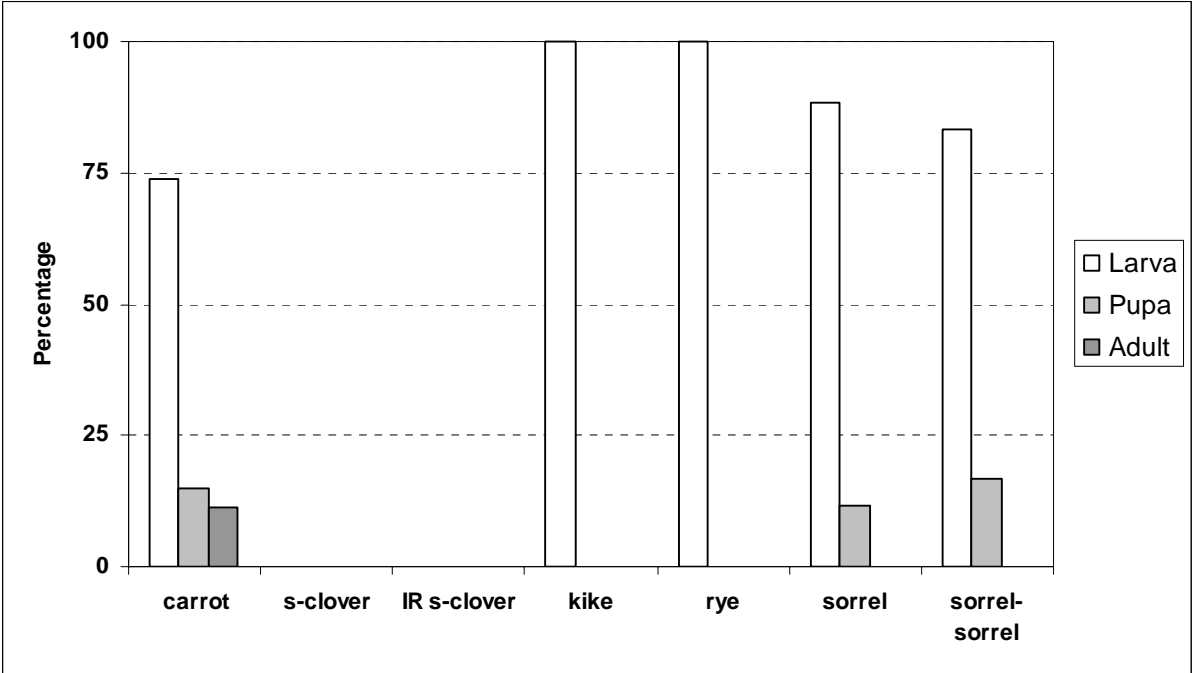


Fig. 12. The proportion of recovered garden weevil as larvae, pupae and adults at the third time of assessment in a pot trial to compare their survival when infesting with first instar larvae for single plant species, or more advanced larvae after initially infesting the favourable food plant sorrel.

The average percentage survival of first instar larvae surviving for the three times of assessment for each treatment is given in Fig. 13. These data were also subjected to an analysis using a generalized linear mixed model (see Table 3).

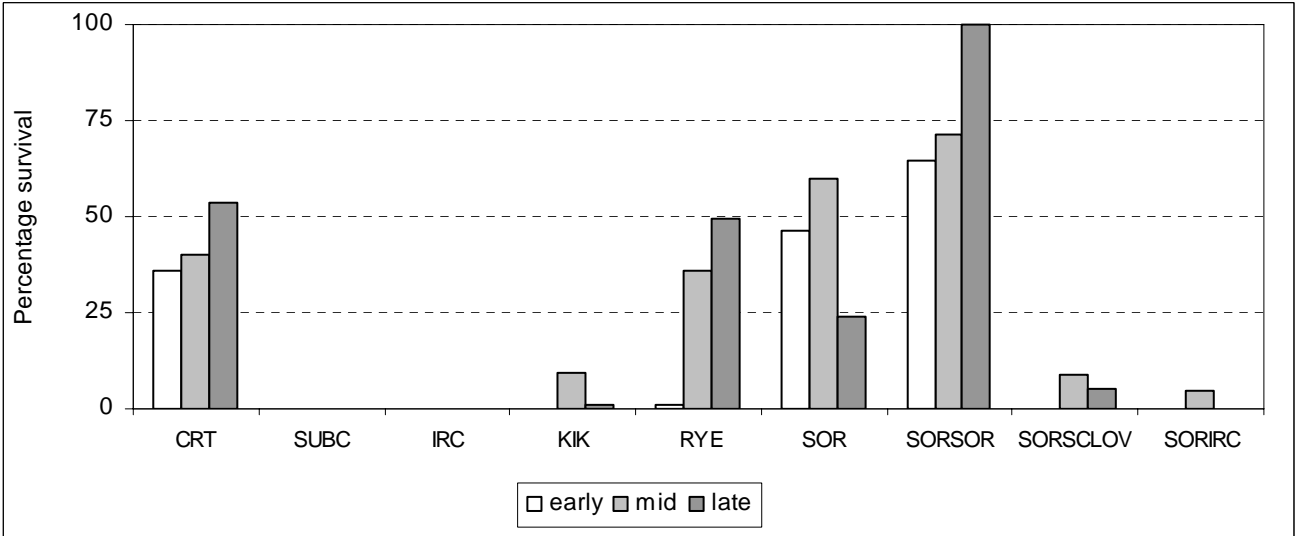


Fig. 13. The average percentage survival of garden weevil larvae in pots with single species of plants, either for the duration of the experiment or transferred from the favourable food plant sorrel to sorrel, sub clover and insect repellent clover at three times of assessment after the initial infestation. See Table 2 and text for details.

In terms of comparing the different plant treatments, the second time of assessment has advantages over the earlier and later assessments. For the early assessment, some of the larvae in the pots of less favourable food plants may have not been big enough to be reliably

found when sorting the soil. At the last time of assessment, some adults were found in the pots containing carrot suggesting the number of weevils surviving may have been compromised. Nevertheless, the comparisons for all three times of assessment are included.

Table 3. Average survival levels for garden weevil larvae in pots with different plants or transfer of larvae part way through their development at three times of assessment after initial infestation or transfer. Also included are results of an analysis of survival levels using a generalised linear mixed model; means followed by the same letter were not significantly different ($P < 0.05$).

Treatment	Assessment and % survival		
	Early	Mid	Late
<i>Single species for the duration of the trial:</i>			
carrot	34 b	40 a	54 a
sub-clover	0 c	0 c	0 b
insect repellent clover	0 c	0 c	0 b
kikuyu	0 c	10 c	1 b
ryegrass	1 c	36 b	50 a
sorrel	54 b	60 a	24 a
<i>Larvae reared on sorrel then transferred to:</i>			
sorrel	72 a	75 a	100 a
sub-clover	0 c	8 c	3 b
Insect repellent clover	0 c	4 c	0 b

At the first time of assessment, the highest survival was for those larvae were placed in pots with sorrel and then transferred to sorrel again. Where larvae were in pots containing carrot and sorrel, survival was also high. Almost no larvae were found in pots containing the other plant types – the grasses ryegrass and kikuyu, and the clovers, either from the time of initial infestation, or after transfer of more advanced larvae that had initially been placed in pots containing sorrel.

A similar result occurred at the second time of assessment, except that larvae in pots containing ryegrass showed around one-third had survived, suggesting that larvae may have been too small to find at the first time of assessment. Also, around ten percent of larvae were recovered in the pots containing kikuyu.

At the last time of assessment, the survival rate for larvae placed on pots containing ryegrass was equivalent to that for carrot and sorrel. Further evidence of the favourability of carrot and sorrel as food plants was the proportion of recovered weevils in the pupal and adult stages at the third time of assessment with only pots containing carrot having weevils that progressed to the adult stage (see Fig. 12). For the grasses ryegrass and kikuyu, all weevils recovered were in the larval stage. For the two earlier times of assessment, all weevils recovered were in the larval stage.

The average weights of larvae recorded for each of the three times of assessment for each treatment are given in Fig. 14. These data were analysed using a linear regression model (ANOVA). Where significant treatment effects were found, means were compared in pairs, a requirement given the range in variance within treatments. For this, an $LSD_{0.05}$ was calculated for each pair of treatment means to decide whether they were significantly

different. These calculations were made for all treatments where some larvae survived. Naturally comparisons of average weights could not be made for those treatments where no larvae were recovered. The results of these comparisons between pairs of means are listed in Appendix 1.

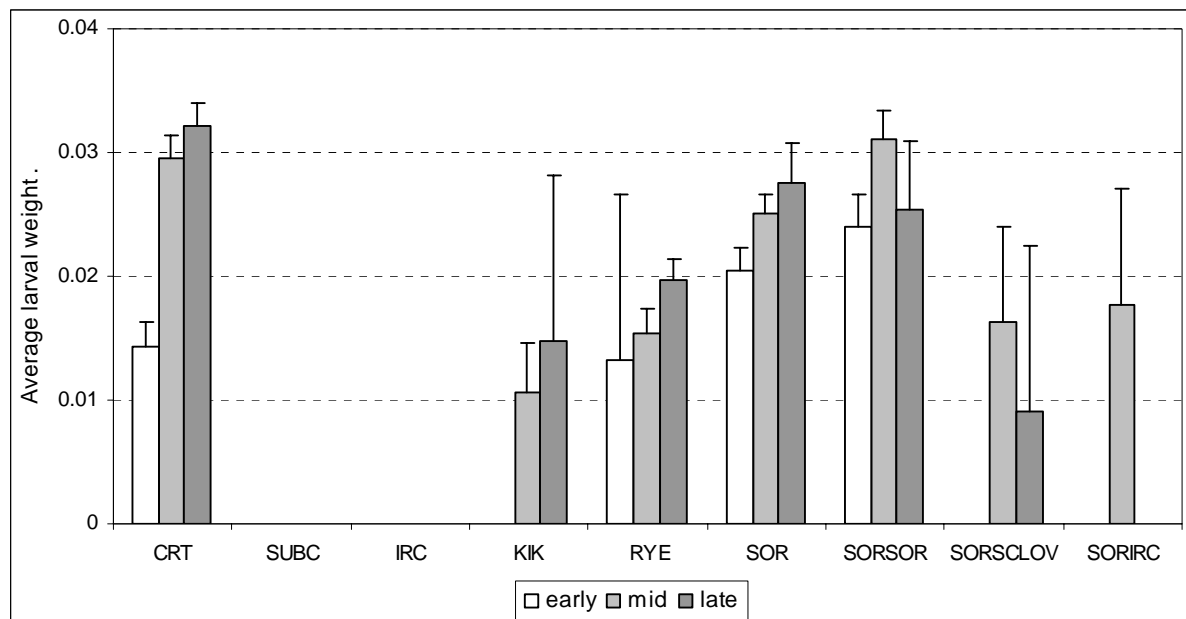


Fig. 14. The average weight with standard error, of garden weevil larvae in pots with single species of plants, either for the duration of the experiment (CRT = carrot, SUBC = sub-clover, IRC = insect repellent clover, RG, KIK = kikuyu, RYE = ryegrass, SOR = sorrel) or transferred from the favourable food plant sorrel to sorrel (SORSOR), sub-clover (SORSCLOV) and insect repellent clover (SORIRC) at three times of assessment after the initial infestation.

At the early assessment, larvae in pots of sorrel and those transferred from sorrel and placed on sorrel again were significantly heavier than larvae from the carrot and ryegrass pots.

At the second time of assessment, larvae from pots with carrot were significantly heavier than those from kikuyu and ryegrass but not from any of the pots involving sorrel, including the few larvae that survived on clover and insect repellent clover after transfer from sorrel. Larvae from pots with kikuyu and ryegrass were significantly lighter than those from sorrel alone, or sorrel after transfer from sorrel earlier, but were statistically similar in weight to the larvae on clover or insect repellent clover after transfer from sorrel. Larvae from the sorrel to sorrel transfer treatment were significantly heavier than those from the sorrel alone treatment.

At the late assessment, the only significant difference in average larval weight was that larvae from the pots containing ryegrass were significantly lighter than those from carrot or sorrel.

As another indicator of the relative suitability of the different plants as food for garden weevil larvae, the average total weight of larvae per pot for each treatment at each time of assessment is included (see Fig. 15). Details of the results of statistical analysis are given in Appendix 1. The numbers of larvae recovered in some treatments was quite variable. This variability made it difficult to detect significant differences between some treatments even though the limited data indicated that there may have been a treatment effect.

Because some larvae may have been too small at the first (early) time of assessment and some larvae had developed to pupae at the late (third) assessment time (see Fig. 12), the most relevant time of assessment upon which to base comparisons of the different plants/treatments is the mid (second) assessment. At the second assessment, the greatest average total weight was for pots planted to sorrel. The weight of larvae in pots with carrot

and the sorrel/sorrel transfer treatment were not significantly different (see Appendix 1). Weight of larvae from pots planted to ryegrass was not significantly different to that for pots with carrot and the sorrel/sorrel transfer treatment. Treatments involving other plants resulted in significantly lower weights of larvae.

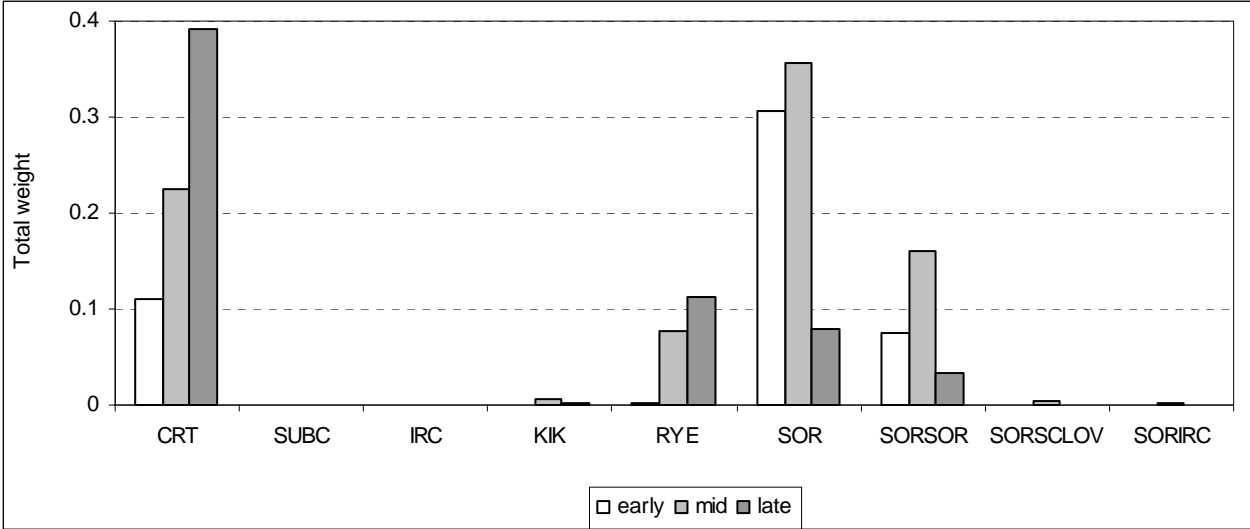


Fig. 15. The average total weight of garden weevil larvae in pots with single species of plants, either for the duration of the experiment (CRT = carrot, SUBC = sub-clover, IRC = insect repellent clover, RG, KIK = kikuyu, RYE = ryegrass, SOR = sorrel) or transferred from the favourable food plant sorrel to sorrel (SORSOR), sub-clover (SORSCLOV) and insect repellent clover (SORIRC) at three times of assessment after the initial infestation.

The average total weight of garden weevil pupae from pots at the last time of assessment for all treatments is given in Table 4. For the purpose of statistical analysis, the data required a log transformation. The means in the table are the backtransformed data. Pots sown to carrot resulted in the greatest number (see Fig. 13) and therefore weight of pupae. Larvae feeding on sorrel for the duration of the experiment produced the next highest weight. Other treatments produced few or no pupae.

Table 4. The average total weight of garden weevil pupae at the last time of assessment from pots infested with first instar larvae and either feeding on single species of plants or transferred from sorrel part way through the experiment. The data were analysed using regression analysis ANOVA; means followed by the same letter are not significantly different at the 5% level.

Treatments	Average total pupal weight
carrot	0.052 a
s-clover	0 bc
IR s-clover	0 bc
kike	0 bc
rye	0 bc
sorrel	0.006 b
sorrel-sorrel	0.001 bc
sorrel-s-clover	0 bc
sorrel-IR s-clover	0 bc

Pot trial 2

The second experiment to assess and compare plants for their suitability as food plants for garden weevil larvae involved mixtures of plants grown in the same pot (see Table 5 for details). Each plant type or series of plants was replicated 5 times for two assessment times. All pots were infested with 20 first instar larvae each, over the period 5 May to 18 June 2003.

Two times of assessment were planned so that for at least one of them weevils would be sufficiently developed to enable efficient retrieval from the sorting of soil and roots by eye. At the first time of assessment, pupae and adults were found (see Fig. 16). Because of this, the second time of assessment was brought forward and conducted one week after the first. All data from both assessments were combined, so that there were effectively 10 replications.

Table 5. Treatments used to compare the survival of garden weevil larvae in a pot trial involving mixtures of plants.

Plants tested	Measurements (days after infestation)
apple	Weevil development assessed at two dates (times) after initial infestation for all treatments: 11 Aug 2003 (approx. 85 days) 18 Aug 2003 (approx. 92 days)
apple + sub-clover cv. Dalkeith	
apple + insect repellent clover	
apple + ryegrass cv. Betta Tetilla	
sorrel	
sorrel + sub-clover	
sorrel + insect repellent clover	

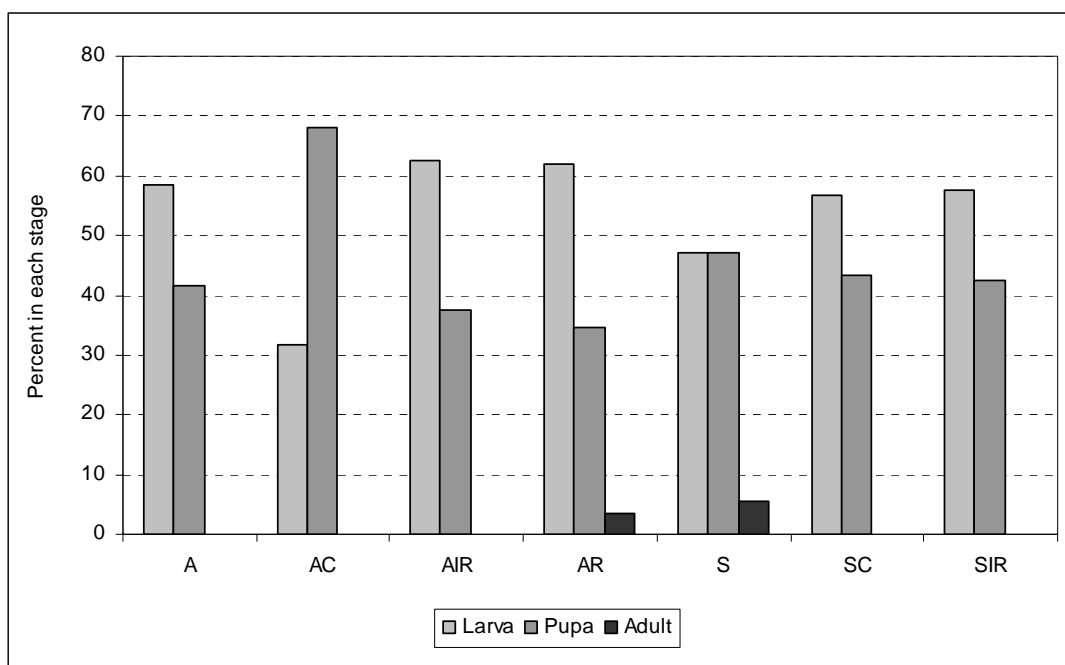


Fig. 16. Proportion of garden weevil recovered as larvae, pupae or adults after infesting pots containing single or mixed plants with first instar larvae. Plants: A = apple, AC = apple + sub-clover, AIR = apple + insect repellent clover, AR = apple + ryegrass, S = sorrel, SC = sorrel + sub-clover, SIR = sorrel + insect repellent clover.

The proportion of the initial first instar larvae placed in the pots that were recovered as any stage of garden weevil when soil and roots were examined is shown in Fig. 17. The data is also presented in Table 6, where the results of an analysis using a generalised linear mixed model for significance are given. The levels of significance are reported on the

backtransformed means. The highest level of survival was recorded for sorrel and the lowest for apple. This result for larvae infesting roots of apple trees, a major host for garden weevil, was unexpected and places some doubt over the validity of results from this experiment. This is discussed more fully below.

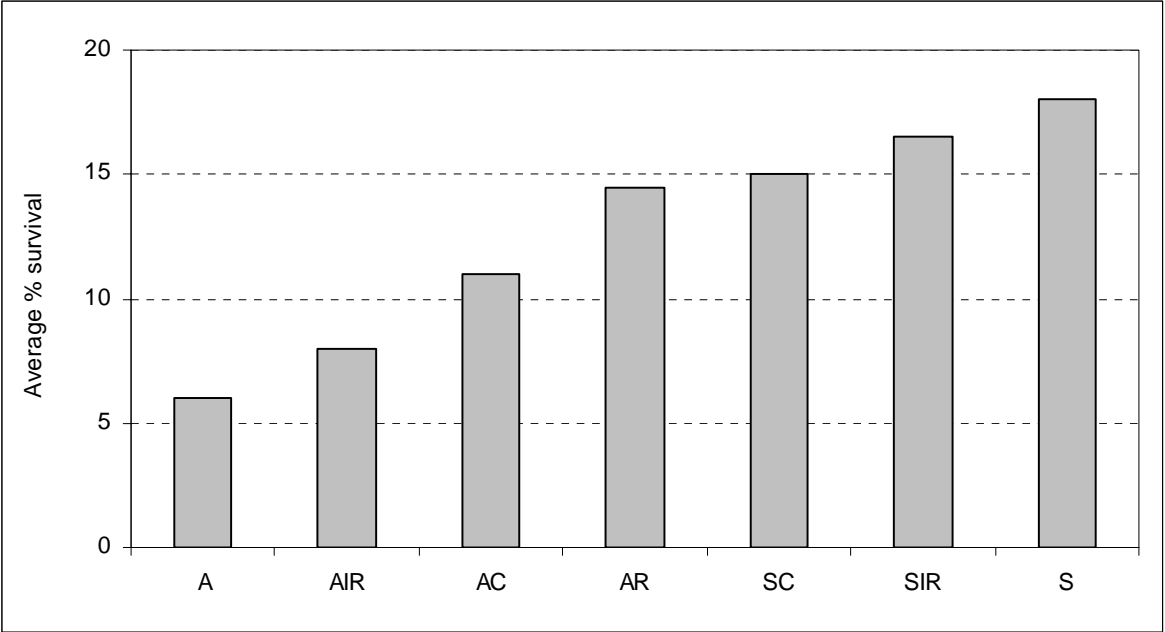


Fig. 17. The average percentage survival of garden weevil larvae in pots containing single or mixed plants. Plants: A = apple, AIR = apple + insect repellent clover, AC = apple + sub-clover, AR = apple + ryegrass, SC = sorrel + sub-clover, SIR = sorrel + insect repellent clover and S = sorrel.

Table 6. Average survival levels for garden weevil larvae in pots with single species or two different species. Also included are results of an analysis of survival levels using a generalised linear mixed model; means followed by the same letter were not significantly different ($P < 0.05$).

Treatment	% survival
apple	6 c
apple + insect repellent clover	8 bc
apple + sub-clover	11 abc
apple + ryegrass	14 ab
sorrel + sub-clover	15 ab
sorrel + insect repellent clover	16 ab
sorrel	18 a

The average weight of larvae and pupae recovered from pots for each treatment is shown in Fig. 18. These data were subject to statistical analysis using regression ANOVA which showed there were no significant differences among the means.

The average total weight of weevils recovered from all treatments is shown in Fig. 19. These data were analysed using regression ANOVA which showed that there were significant differences only for total weight of pupae per pot (see Table 7). The greatest number (weight) of pupae were recovered in pots sown to the sorrel treatments – either plants grown alone or as mixtures with one other plant type. The lowest weight was recorded for pots with apple trees alone.

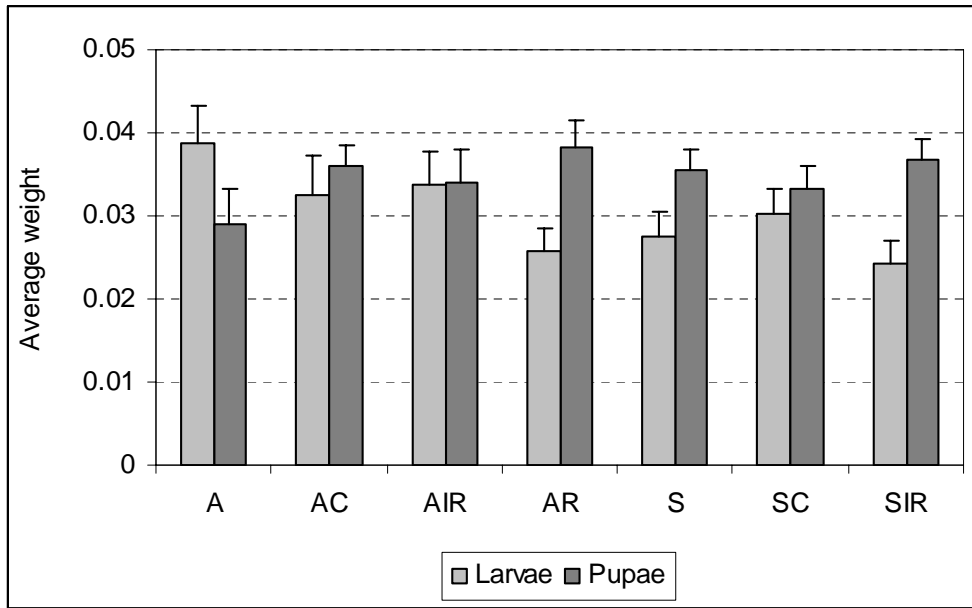


Fig. 18. The average weight with standard error, of garden weevil larvae and pupae in pots containing single or mixed plants. Plants: A = apple, AC = apple + sub-clover, AIR = apple + insect repellent clover, AR = apple + ryegrass, S = sorrel, SC = sorrel + sub-clover, SIR = sorrel + insect repellent clover.

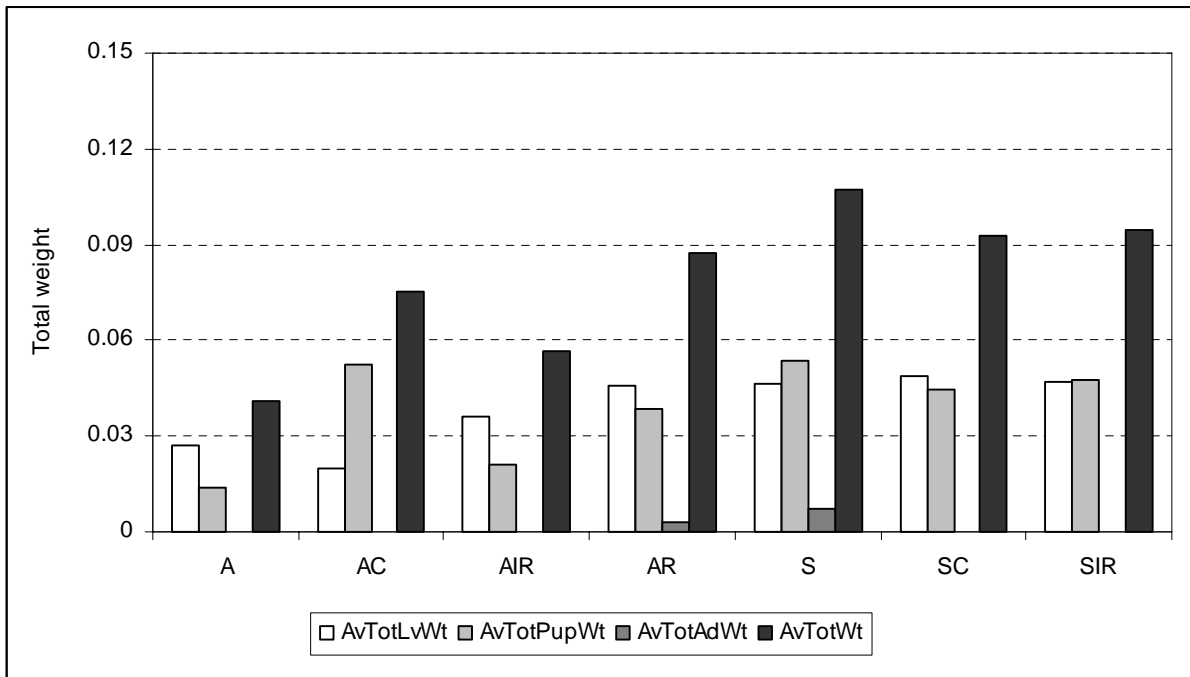


Fig. 19. The average total weight of garden weevil larvae, pupae, adults and all stages in pots containing single or mixed plants. Plants: A = apple, AC = apple + sub-clover, AIR = apple + insect repellent clover, AR = apple + ryegrass, S = sorrel, SC = sorrel + sub-clover, SIR = sorrel + insect repellent clover.

Table 7. The average for total weights of garden weevil pupae recovered from pots with different plants infested with first instar larvae. Data was subjected to a regression ANOVA and means followed by the same letter were not significantly different at $P < 0.05$. Means shown in the table are the backtransformed values.

Treatment	Weight of pupae
apple	0.004 c
apple + insect repellent clover	0.009 bc
apple + sub-clover	0.015 ab
apple + ryegrass	0.014 abc
sorrel + sub-clover	0.057 a
sorrel + insect repellent clover	0.038 a
sorrel	0.032 ab

Pot trial 3

The third experiment was to assess and compare a range of plants for their suitability as food plants for garden weevil larvae involved mixtures of plants grown in the same pot (see Table 8 for details). Each plant type or series of plants was replicated 5 times, with only one time of assessment. All pots were infested with 25 first instar larvae each, over the period 3 to 17 May 2004.

Table 8. Treatments used to compare the survival of garden weevil larvae in a pot trial involving mixtures of plants.

Plants tested	Measurements (days after infestation)
apple	Weevil development assessed at one date (time) after initial infestation for all treatments: 2 Aug 2004 (approx. 84 days)
apple + mustard cv. Fumus	
apple + lupins cv. Belara	
apple + oats cv. Wandering	
apple + ryegrass cv. Betta Tetilla	
apple + insect repellent clover	
apple + chicory cv. Puna	
apple + chickpea cv. Sona	
apple + field pea cv. Dunpeas	
apple + vetch cv. Barloo	
apple + endophytic ryegrass cv. Canon	

The proportion of larvae that were recovered during the assessment for each treatment is shown in Fig. 20. Despite the wide range in proportion recovered from around 1% for apple plus endophytic ryegrass (Kemp *et al*, 2003) to 16% for apple plus lupins, there was no significant difference among treatments.

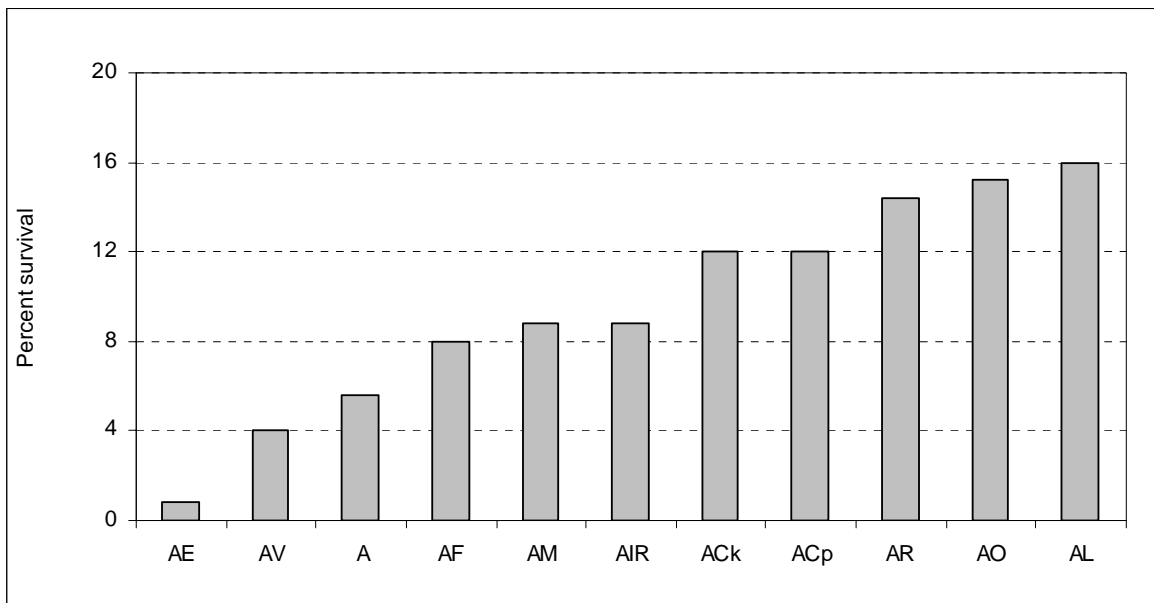


Fig. 20. The average percentage survival of garden weevil larvae in pots containing single or mixed plants infested with first instar larvae. Plants: AE = apple + endophytic ryegrass, AV = apple + vetch, A = apple, AF = apple + field pea, AM = apple + mustard, AIR = apple + insect repellent clover, ACk = apple + chicory, ACp = apple + chickpea, AR = apple + ryegrass, AO = apple + oats, and AL = apple + lupin.

Of the weevils recovered the proportion that was in the larval, pupal and adult stages are given in Fig. 21. A statistical analysis of the number weevils alive in the larval stage showed no significant difference among treatments for proportion in that stage.

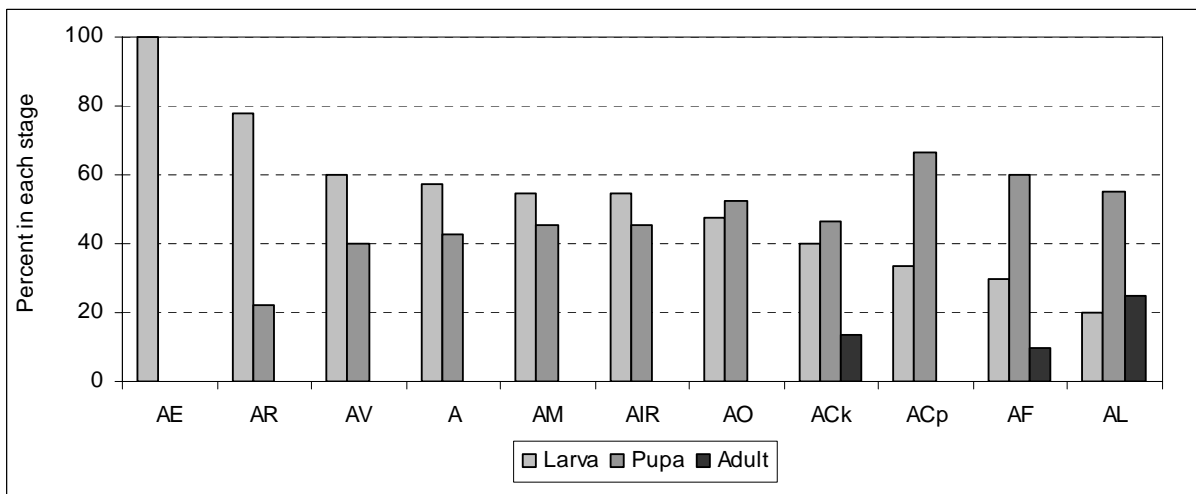


Fig. 21. . Proportion of garden weevil recovered as larvae, pupae or adults after infesting pots containing single or mixed plants with first instar larvae. Plants: AE = apple + endophytic ryegrass, AR = apple + ryegrass, AV = apple + vetch, A = apple, AM = apple + mustard, AIR = apple + insect repellent clover, AO = apple + oats, ACk = apple + chicory, ACp = apple + chickpea, AF = apple + field pea and AL = apple + lupin.

The average weight of larvae and pupae in each of the treatments is given in Fig. 22. Using a regression ANOVA analysis, no significant differences among means were found.

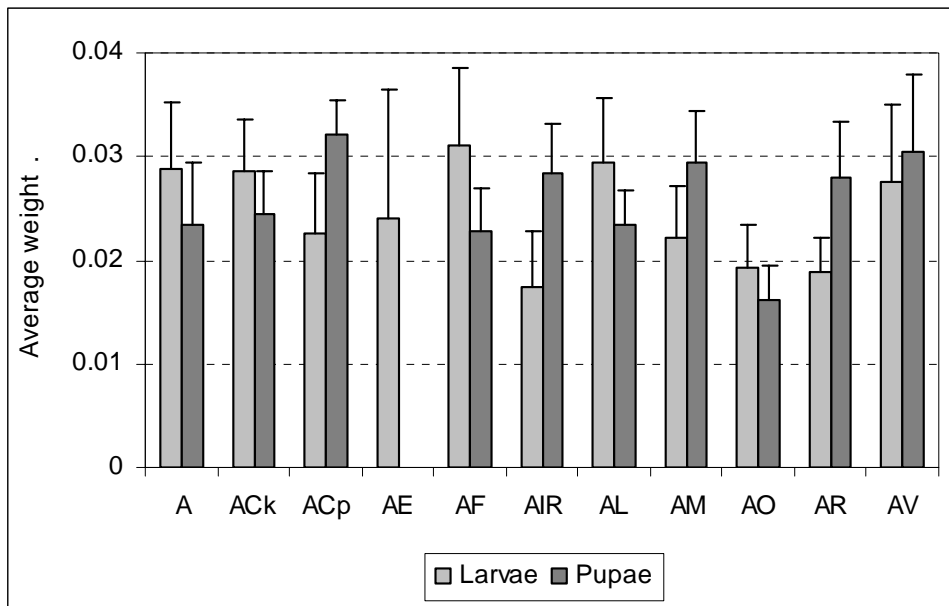


Fig. 22. The average weight with standard error, of garden weevil larvae and pupae in pots containing single or mixed plants. Plants: A = apple, ACK = apple + chicory, ACp = apple + chickpea, AE = apple + endophytic ryegrass, AF = apple + field pea, AIR = apple + insect repellent clover AL = apple + lupin, AM = apple + mustard, AO = apple + oats, AR = apple + ryegrass and AV = apple + vetch.

The average total weight of larvae and pupae recovered are given in Fig. 23. Using a regression ANOVA analysis, no significant differences among means were found.

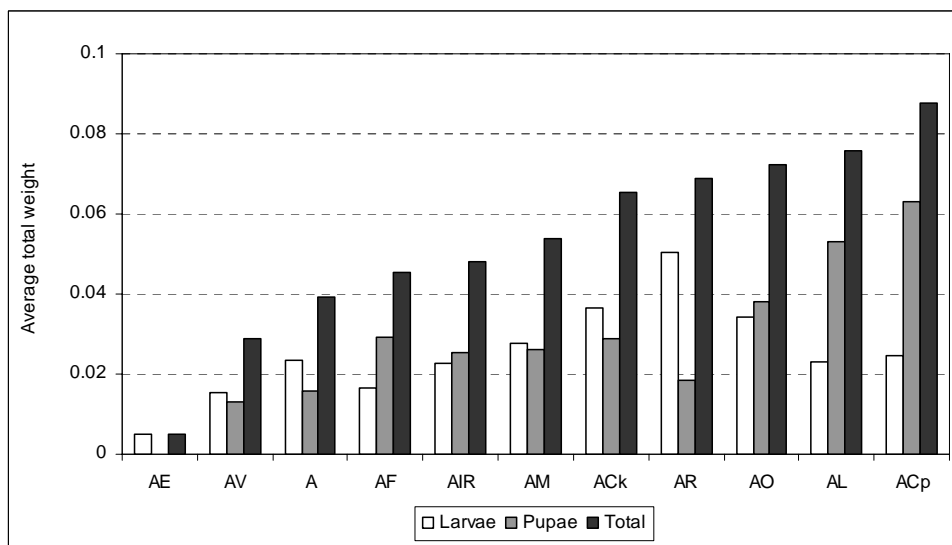


Fig. 23. The average total weight of garden weevil larvae, pupae and total for these stages in pots containing single or mixed plants. Plants: A = apple, AC = apple + sub-clover, AIR = apple + insect repellent clover, AR = apple + ryegrass, S = sorrel, SC = sorrel + sub-clover, SIR = sorrel + insect repellent clover.

Laboratory trials on food plants for adults

This experiment was commenced on 5 Jan 2004. The range of plants that garden weevil were reared on to compare their favourability for survival and fecundity, including just the paper fans provided as oviposition substrates either wet or dry, are listed in Table 8. Also included in the table are the average duration of weevil survival expressed in WeevilDays, the total number of eggs laid and the total cumulative feeding score based on the rating system described above.

The number of WeevilDays was obtained by adding the product of the number of weevils found dead on any time of checking and the duration since the set up of the experiment, for example, if 5 weevils were found to have died 12 days after the treatment commenced, the contribution to weevil days would be 60. Weevils still alive at the conclusion of the experiment were for the purpose of calculating WeevilDays, assumed to have died on the last day. For each treatment, 10 weevils were placed in the rearing containers - 5 males and 5 females. There were 5 replications.

Table 8. The list of plants garden weevil adults were reared on to compare them for weevil survival (WeevilDays, see text for explanation), fecundity (total number of eggs) and feeding score (see text for scoring system used). The data were analysed using ANOVA and LSD's are included, where significant differences among means were found ($P < 0.05$).

Treatment	WeevilDays	*Total no. eggs	Feeding score
Dry paper fan	97.6	0 (0)	0
Wet paper fan	243.2	3.8 (14)	0
Ryegrass	248.2	0.97 (1)	16.4
Oats	262.2	4.73 (22)	18.0
Vetch	295.8	1.34 (2)	13.0
Chicory	306.4	6.39 (41)	26.8
Lupin	341.8	5.37 (29)	26.8
Sorrel	341.8	14.29 (204)	29.0
Mustard	379.8	3.48 (12)	29.8
Grapevine	556.4	14.22 (202)	36.6
Apple	573.6	11.13 (124)	37.6
<i>LSD</i> _{0.05}	83.6	5.14	3.9

*Total number of eggs transformed to square root for the analysis; backtransformed means in brackets.

Surprisingly and in contrast to feeding the weevils nothing but including the paper towelling fan for oviposition, the moistened paper fan allowed weevils to survival for an average of 24 days, compared to round 10 days for weevils that had access to dry paper only. Having access to the moistened paper was equivalent to feeding on ryegrass, oats, vetch and chicory. The most suitable sources of food for weevil survival were apple and grapevine. Lupin, sorrel and mustard were intermediate in their suitability as food plants in terms of weevil survival.

The greatest number of eggs was laid when weevils fed on sorrel, grapevine and apple. Paper towelling either wet or dry did not allow weevils to lay many eggs. A similarly low number of eggs were laid when weevils fed on ryegrass, vetch, mustard and oats. Weevils feeding on lupin and chicory laid an intermediate number of eggs. In general terms, the feeding score for a particular food source was similar in relative magnitude to the number of WeevilDays.

Discussion - garden weevil

This series of glasshouse experiments sought to clarify the role of selected currently used or potential cover crop plants for their role in the survival of the larval stage of garden weevil in a pome fruit orchard. If certain plants were found to be at least non-food plants for garden weevil, or had some antibiotic properties, then these could be encouraged.

The objectives of first experiment were to determine whether the apparent toxic effect of clover on first instar garden weevil larvae seen in previous work (Bowden, 1997, Endersby *et*

al, 1998 and Williams, 2000) was also the case for more advanced larvae. Also, the experiment examined the suitability of two common grasses grown as ground covers in orchards, ryegrass and kikuyu, as food for garden weevil larvae.

When first instar larvae of garden weevil were initially reared on the favourable food plant sorrel, then transferred to either sorrel or two different types of clover, the survival of those placed on clover was very low. This result confirmed the unsuitability of clover as a food source for garden weevil larvae.

The study of the survival and development of garden weevil larvae in the first pot trial experiment also demonstrated the suitability of carrot, used as a check plant for comparative purposes, and the weed sorrel, as favourable food plants for garden weevil (see Figs. 13, 14 and 15). These plants resulted in the highest survival levels and average and total weights of larvae. Also, in the case of carrot, the greatest proportion of larvae reached the pupal stage and was the only plant that allowed larvae to develop to adults during the experiment.

Ryegrass allowed reasonable development of larvae but the results suggested that larval growth may have been slow initially as few larvae were recovered at the first time of assessment (Fig. 15). If larvae were small they may not have been found during soil examination. Larvae from pots sown to ryegrass were also lighter than those from carrot or sorrel pots.

Kikuyu was an unfavourable food plant in terms of level of survival, but those larvae that did survive grew to a size similar to those on more favourable food plants. Because so few larvae survived and their weight was quite variable, a statistical comparison of their average weight was not significantly different to that of larvae reared on carrot or sorrel.

Both sub-clover and an insect repellent clover were confirmed as unfavourable food plants when larvae had access to these plants only. There was no evidence that the insect repellent clover was any more detrimental to the survival of garden weevil larvae than a common type of sub-clover.

The objectives of the second pot experiment were to determine whether garden weevil larvae would survive in the presence of clover roots when grown in conjunction with favourable food plants such as apple or sorrel and also to study the effect on survival of larvae in pots with an apple tree grown in combination with ryegrass.

This experiment was compromised to some extent by the significantly lower survival rate of garden weevil larvae compared to results for the first pot trial. Survival rates were around $\frac{1}{3}$ to $\frac{1}{4}$ of those in the first experiment. More importantly, the survival on apple alone was significantly lower compared to sorrel. This result was unexpected because garden weevil is a major pest of apple and sampling infested orchards in weed free driplines has yielded high populations of larvae (Learmonth, 2000 and Williams, 2000). The difference in the density of the root systems of apple, sorrel and in particular ryegrass was apparent in the pots. Roots of the apple rootstock trees were not as extensive as those of the other plants. This may have led to some of the small first instar garden weevil larvae not being able to find roots to commence feeding, resulting in the lower level of survival. Also, by not placing larvae necessarily near the stem of the apple tree compared to where an adult may have placed eggs in a field situation, could have added to the task of larvae seeking roots after burrowing into the soil of the pot. Despite this deficiency, because some larvae survived with many developing to the pupal stage in all treatments, some conclusions are considered.

The results of the experiment demonstrated that rather than being toxic, clover appeared to be a non-favoured food plant. The proportion of larvae surviving in pots of both apple and sorrel growing in combination with two types of clover were not significantly different to pots containing either apple or sorrel alone. The most likely reason for poor survival of larvae which have access to clover only is that they simply starve to death.

The addition of ryegrass to pots with either apple or sorrel was not detrimental to the survival of larvae. In fact, pots with apple plus ryegrass as well as those with sorrel alone were the only treatments where larvae had completed development to reach the adult stage.

The third experiment had the objective of comparing a range of plants combined with an apple tree for their suitability as food sources for garden weevil larvae. A similar deficiency occurred in this experiment as for the second experiment – unexpectedly low survival across all treatments including the check treatment of apple alone. Survival rates ranged from 16% for apple plus lupin to 1% for apple plus endophytic ryegrass. Despite this range, statistical analysis failed to show significant difference because of the large variability of survival within treatments. The reason for the variable and low survival rates across treatments is not known. As discussed above, the availability of roots for the small larvae may have been a problem. The variability in survival suggests either some refinement in technique or the need to use greater numbers of larvae when infesting pots. If more larvae are used, the retrieval of a greater number of larvae could lead to more definitive conclusions about plants being compared.

Despite this it was apparent that some of the plants tested in combination with apple were not antibiotic to the survival of garden weevil. The most obvious plant that appeared to have some effect was the endophytic ryegrass. Where this was grown in combination with apple, the least number of larvae survived and it was the only treatment where no pupae were recorded.

The laboratory experiment on garden weevil adults had the objective of comparing a range of plants for their suitability as food plants for adult survival and egg laying capacity. The results showed the importance of moisture for the survival of garden weevil adults and indicated which plants would be best to include as ground covers. Suitable plants would be the grasses oats and ryegrass and the broadleaf plants vetch and mustard. The results confirm the recommendation to remove the weed sorrel from orchards, because it allowed a high number of eggs to be produced and a reasonable duration of adult survival. Of the crop types represented, grapevines and apples were suitable food sources. Of potential cover crops, chicory and lupins would appear to allow moderate egg production, while oats, mustard, vetch and ryegrass would allow for low to very low levels of egg production.

Results

Pot trials with weevil larvae

Three pot experiments were undertaken to assess and compare a range of plants for their suitability as food plants for Fuller's rose weevil larvae.

Pot trial 1

The first experiment involved examining single plant species per pot. The plants that were to be compared are listed in Table 9. Pots were infested on 24 June 2002 with 20 larvae per pot. For each treatment, there were two times of assessment and five replications.

Table 9. The list of plants assessed as single species for their suitability as food plants for larvae of Fuller's rose weevil larvae in a glasshouse.

Plants tested	Measurements (days after infestation)
Carrot cv. Western Red	Weevil development assessed at two dates (times) after initial infestation for all treatments: 26 Aug. (63 days) 23Sept (91 days)
sub-clover cv. Dalkeith	
insect repellent clover	
kikuyu	
Ryegrass cv. Betta Tetilla	
sorrel	
fathen	
capeweed	

Weevil survival was quite low in this experiment. At the first time of assessment, only 2 pots of sorrel were infested with one larva in each. At the second time of assessment 10 larvae were present in one of the sub-clover pots, and two of the sorrel pots had one weevil in each – a larva and a pupa. Possible explanations for this low survival level are discussed below.

Pot trial 2

In the second pot experiment of FRW larvae, 5 plant treatments were compared. These consisted either of single plant species or mixtures of two plant species (see Table 10 for details). Pots were infested over the period 9 to 16 May 2003 with 50 larvae per pot. For each treatment, there were two times of assessment and five replications.

Table 10. The list of plants assessed as single or mixed species for their suitability as food plants for larvae of Fuller's rose weevil larvae in a glasshouse pot trial.

Plants tested	Measurements (days after infestation)
apple	Weevil development assessed at two dates (times) after initial infestation for all treatments: 20 Aug. (approx. 100 days) 8 Sept (approx. 119 days)
apple + sub-clover cv. Dalkeith	
apple + insect repellent clover	
apple + ryegrass cv. Betta Tetilla	
plum	

The proportion of larvae retrieved at each time of assessment is shown in Fig 24 and Table 11. The proportion of weevils recovered was greater at the second assessment, probably because larvae were larger (see below). The only significant difference between the treatments was that all treatments involving apple had greater survival than larvae placed on pots with plum trees.

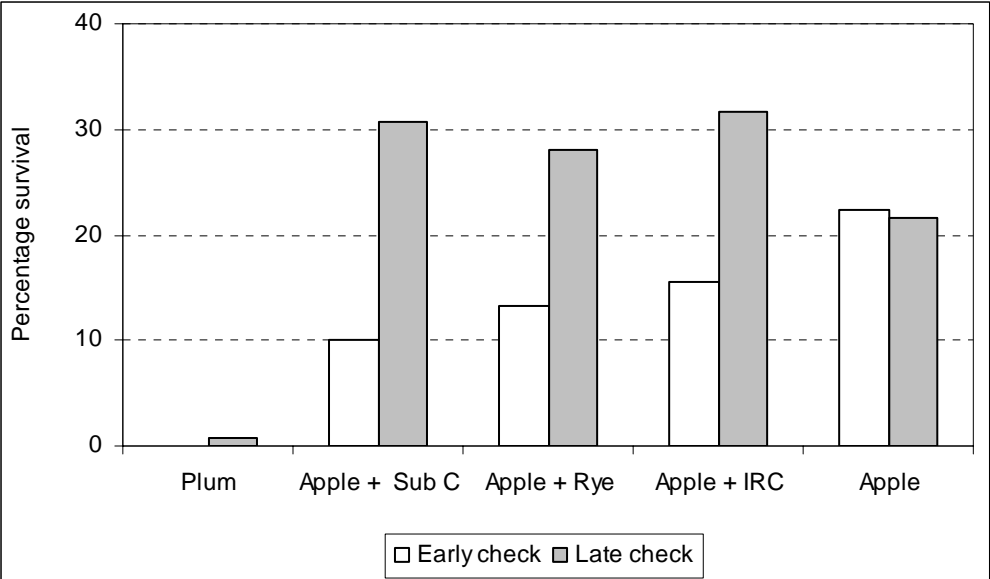


Fig. 24. The percentage survival of Fuller's rose weevil larvae in pots containing single or mixed plants in a glasshouse pot trial at two times of assessment after initial infestation with first instar larvae.

Table 11. Average survival levels for Fuller's rose weevil larvae in pots with different plants at two times of assessment after initial infestation. Also included are results of an analysis of survival levels using a generalised linear mixed model; means followed by the same letter were not significantly different ($P < 0.05$).

Treatment	Assessment and % survival	
	Early	Late
plum	0	0.8 b
apple + sub-clover	10.0	30.8 a
apple + ryegrass	13.2	28.0 a
apple + insect repellent clover	15.6	31.6 a
apple	22.4	21.6 a

The average weights of larvae recovered from the two times of assessment are shown in Fig. 25. No larvae were recovered from the plum tree pots at the first time of assessment, so this treatment could not be included in the analysis. For the other treatments, larvae recovered at the second time of assessment were significantly heavier than those recovered at the first time. Within each time of assessment, there was no significant difference in average weight of larvae per treatment. The number of larvae recovered from the plum pots was small and the weights very variable as indicated by the large standard error (Fig. 25).

The total weights of larvae recovered from all pots at the two times of assessment are shown in Fig. 26. These results reflect the combination of a greater number of larvae recovered and their average weight being higher at the second time of assessment. As was the case for

average larval weight, the only significant difference was for pots with plums being lower than all treatments involving the apple tree.

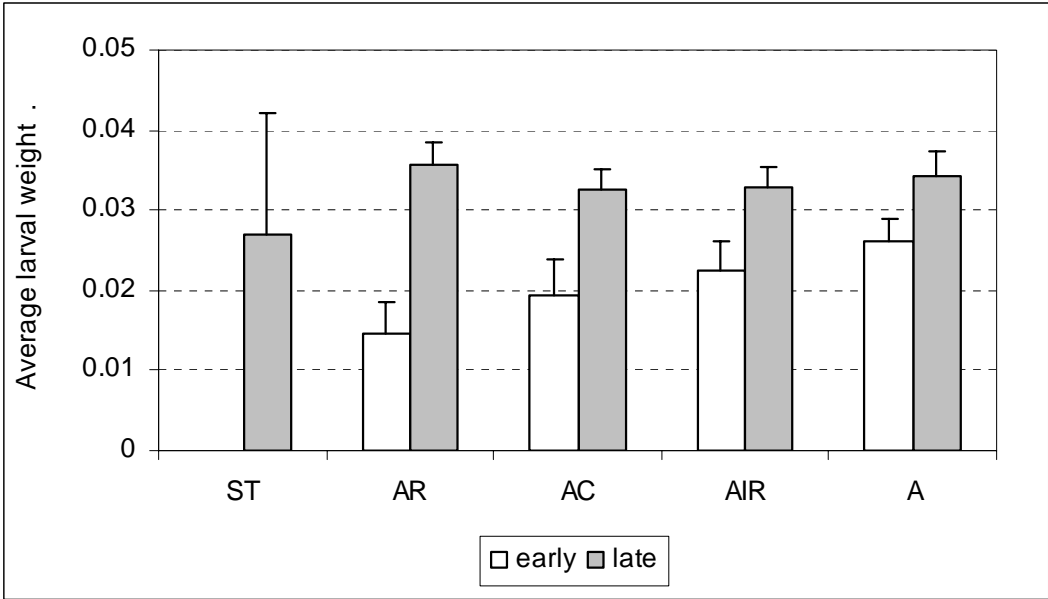


Fig. 25. The average weight with standard error, of Fuller's rose weevil larvae in pots containing single or mixed plants at two times of assessment. Plants: ST = plum, AR = apple + ryegrass, AC = apple + sub-clover, AIR = apple + insect repellent clover and A = apple.

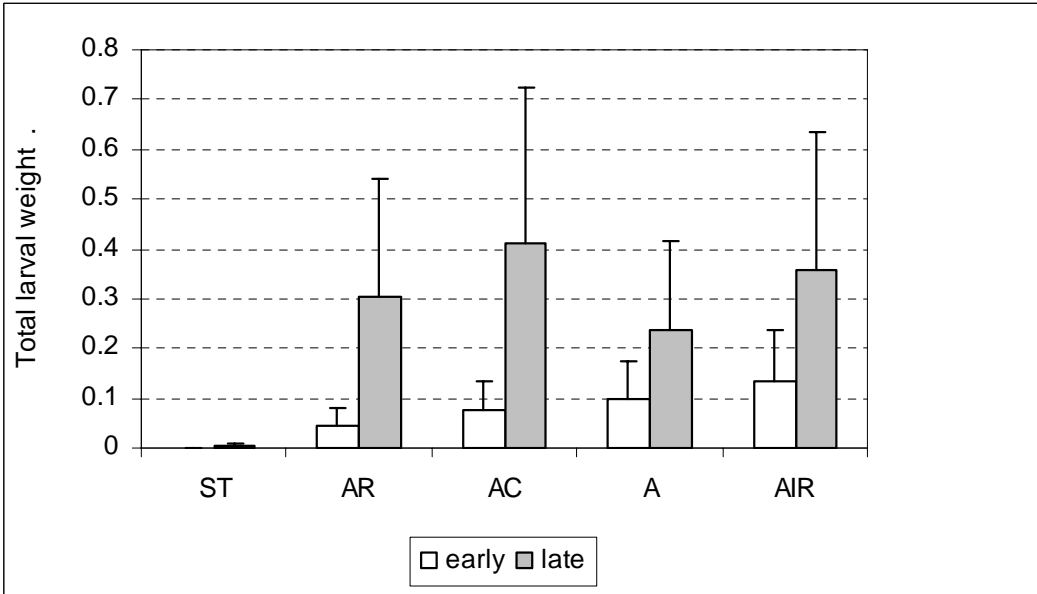


Fig. 26. The average total weight with standard error, of Fuller's rose weevil larvae per pot containing single or mixed plants. Plants: ST = plum, AR = apple + ryegrass, AC = apple + sub-clover, A = apple and AIR = apple + insect repellent clover.

Pot trial 3

In the third pot experiment on FRW larvae, 11 plant treatments were compared. These consisted either of apple alone or a mixture of apple and one other plant species (see Table 12 for details). Pots were infested over the period 20 to 31 May 2004 with 25 larvae per pot. For each treatment, there was one time of assessment and five replications.

The proportion of weevil larvae recovered is shown in Fig. 27 and, together with the results of analysis using a generalised linear mixed model, in Table 13. Treatments involving apple in

combination with endophytic ryegrass, oats and ryegrass resulted in a significantly higher proportion of larvae surviving. There was no significant difference for the other treatments despite the wide range in the proportion of weevils surviving. To some extent this would have been a result of the generally low level of survival across all treatments. Also, as was the case in some of the other pot trials, the large variability in the numbers of larvae surviving would have made it difficult to register a significant difference.

Table 12. The list of plants assessed as single or mixed species for their suitability as food plants for larvae of Fuller’s rose weevil in a glasshouse pot trial.

Treatments	Measurements (days after infestation)
Apple	Weevil development assessed at one date (time in days) after initial infestation for all treatments: 23 Aug. (90 days)
Apple plus Mustard cv. Fumus	
Apple plus Lupins cv. Belara	
Apple plus Oats cv. Wandering	
Apple plus Ryegrass cv. Betta Tetilla	
Apple plus <i>T. strictum</i> 053094 (insect repellent clover)	
Apple plus Chicory cv. Puna	
Apple plus Chickpea cv. Sona	
Apple plus Field peas cv. Dunpeas	
Apple plus Vetch cv. Barloo	
Apple plus endophytic ryegrass cv. Canon	

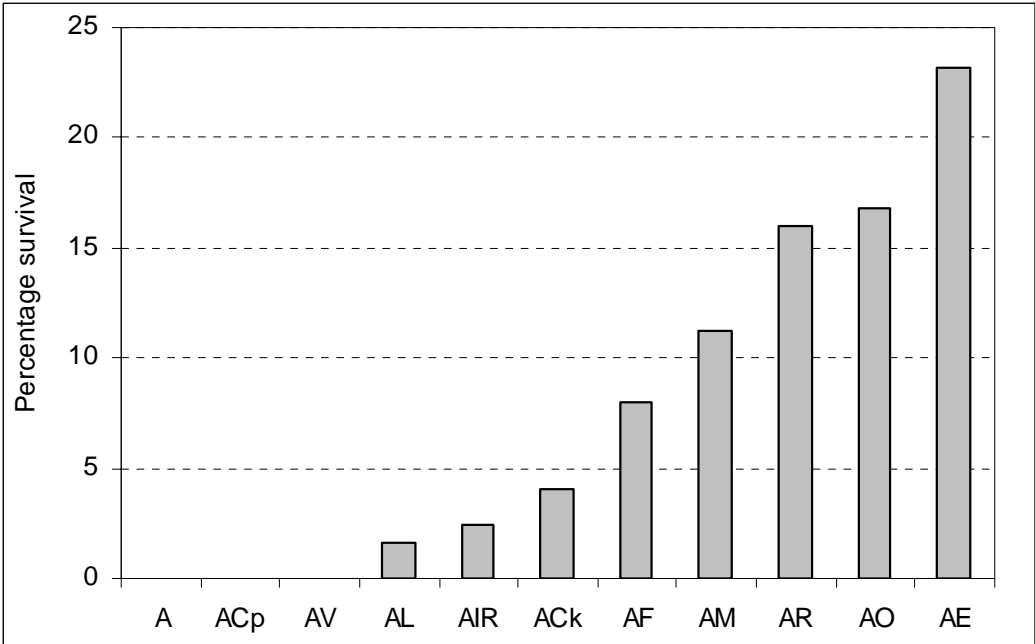


Fig. 27. The percentage survival of Fuller’s rose weevil larvae in pots containing apple only or apple in combination with other plants in a glasshouse pot trial after initial infestation with first instar larvae. Plants: A = apple, ACp = apple + chickpea, AV = apple + vetch, AL = apple + lupin, AIR = apple + insect repellent clover, ACK = apple + chicory, AF = apple + field pea, AM = apple + mustard, AR = apple + ryegrass, AO = apple + oats and AE = apple + endophytic ryegrass.

Table 13. Average survival levels for Fuller’s rose weevil larvae in pots with different plants. Also included are results of an analysis of survival levels using a generalised linear mixed model; means followed by the same letter were not significantly different ($P < 0.05$).

Treatment	% survival
Apple	0 b
Apple plus Chickpea	0 b
Apple plus Vetch	0 b
Apple plus Lupins	1.6 b
Apple plus Insect repellent clover	2.4 b
Apple plus Chicory	4.0 b
Apple plus Field peas	8.0 b
Apple plus Mustard	11.2 b
Apple plus Ryegrass	16.0 a
Apple plus Oats	16.8 a
Apple plus endophytic ryegrass	23.2 a

The average weight of FRW larvae for each treatment where some larvae were recovered is given in Fig. 28. There was no significant difference among treatment means when the data was analysed using a generalised linear mixed model.

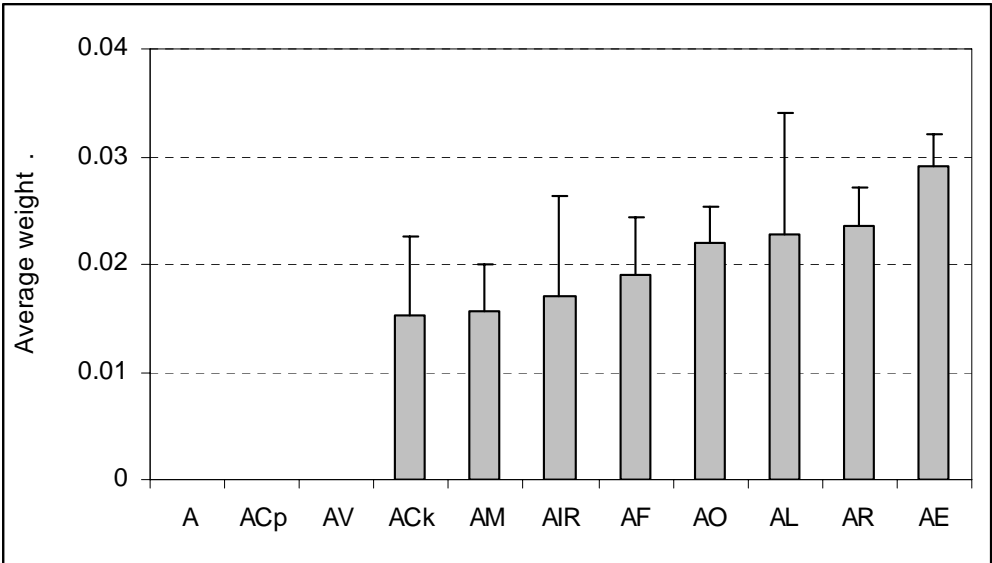


Fig. 28. The average weight with standard error, of Fuller’s rose weevil larvae in pots containing single or mixed plants. Plants: A = apple, ACp = apple + chickpea, AV = apple + vetch, ACk = apple + chicory, AM = apple + mustard, AIR = apple + insect repellent clover, AF = apple + field pea, AO = apple + oats, AL = apple + lupin, AR = apple + ryegrass and AE = apple + endophytic ryegrass.

The average total weight of larvae recovered from each treatment is given in Fig. 29 and, including the results of statistical analysis using linear regression ANOVA, in Table 14. For pots containing apple in combination with endophytic ryegrass, ryegrass, oats and field peas, the average total weight of larvae was not significantly different. The weights for larvae from pots with apple plus chicory or mustard were not significantly different from pots with apple plus oats or field peas. As already mentioned, no larvae were recovered from pots with apple alone or apple plus cowpeas or vetch.

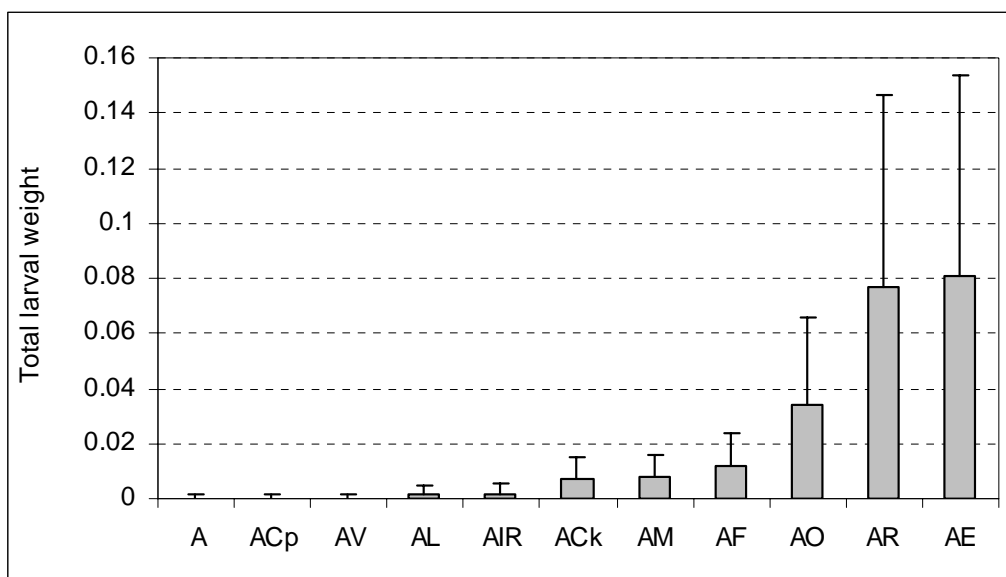


Fig. 29. The average total weight with standard error, of Fuller's rose weevil larvae per pot containing apple or apple in combination with another plant. Plants: A = apple, ACp = apple + chickpea, AV = apple + vetch, AL = apple + lupin, AIR = apple + insect repellent clover, ACk = apple + chicory, AM = apple + mustard, AF = apple + field pea, AO = apple + oats, AR = apple + ryegrass and AE = apple + endophytic ryegrass.

Table 14. The average total weight of Fuller's rose weevil larvae recovered from pots sown to a range of plants. The data shown are backtransformed means from statistical analysis using regression ANOVA; means followed by the same letter are not significantly different ($P < 0.05$).

Treatment	Total weight
Apple	0 d
Apple plus Chickpea	0 d
Apple plus Vetch	0 d
Apple plus Lupins	0.002 cd
Apple plus Insect repellent clover	0.002 cd
Apple plus Chicory	0.007 bcd
Apple plus Mustard	0.008 bcd
Apple plus Field peas	0.012 abc
Apple plus Oats	0.034 ab
Apple plus Ryegrass	0.077 a
Apple plus endophytic ryegrass	0.081 a

Laboratory trials on food plants for adults

This experiment was commenced on 5 Jan 2004. The range of plants that adults of Fuller's rose weevil was reared on to compare their favourability for survival and fecundity are listed in Table 15. Also included in the table are the average duration of weevil survival expressed in WeevilDays, the total number of eggs laid and the total cumulative feeding score based on the rating system described above. The number of WeevilDays was obtained as described above for garden weevil. For each treatment, 5 weevils were placed in the rearing containers – FRW adults are all females, being parthenogenetic. There were 5 replications.

Table 15. The list of plants Fuller's rose weevil adults were reared on to compare them for weevil survival (WeevilDays, see text for explanation of calculating this data), fecundity (total number of eggs) and feeding score (see text for scoring system used). The data was analysed using ANOVA and LSD's are included, where significant differences among means were found ($P < 0.05$).

Treatment	WeevilDays	*Total no. eggs	Feeding score
Nothing	44.6	5.8 (34)	0
Oats	175.2	3.3 (11)	23.0
Ryegrass	175.2	10.8 (117)	30.8
Vetch	223.4	11.8 (139)	32.0
Sorrel	229.8	11.9 (142)	36.6
Mustard	232.0	17.1 (292)	32.6
Lupin	297.0	22.2 (493)	39.0
Chicory	308.0	20.4 (416)	38.4
Apple	317.6	28.0 (784)	36.4
Apricot	361.2	36.0 (1296)	37.2
<i>LSD_{0.05}</i>	67.8	3.7	5.6

*Total number of eggs transformed to square root for the analysis; backtransformed means in brackets.

Although some eggs were laid where weevils had no access to food, this was more likely the result of collecting the weevils from an orchard where they already had access to food.

The longest lived weevils were those provided with apricot, apple and chicory leaves. Lupin enabled weevils to live a significantly similar duration to apples and chicory. Weevils fed on mustard, oats and vetch lived a similar duration. Those fed on the grasses ryegrass and oats lived the shortest duration. Weevils deprived of food died sooner than any treatment where food was provided, and lived an average of 9 days (5 weevils used per treatment).

As was the case for weevil survival, most eggs were laid by weevils that had access to apricot leaves. Weevils fed apple leaves laid the second highest number of eggs followed by weevils fed on lupins and chicory. Mustard was intermediate in the fecundity of weevils, with sorrel, vetch and ryegrass being equal but poorer food sources. Of the food sources provided, weevils fed on oats laid fewest eggs.

With respect to feeding activity, there was less difference among the various plant types. Weevil feeding was least on oats. Feeding activity of ryegrass, vetch and mustard was similar. The highest levels of feeding activity were on lupin, chicory, apricot, sorrel and apple.

Discussion – Fuller's rose weevil

This series of glasshouse experiments had the same objectives as for garden weevil with the exception that there had been no information on the role of clover as a potential non-food plant. The experiments sought to clarify the role of selected currently used or potential cover crop plants for their role in the survival of the larval stage of Fuller's rose weevil in a pome fruit orchard. If certain plants were found to be at least non-food plants as seems to be the case with clover for garden weevil, or had some antibiotic properties, then these could be encouraged as a way of helping to manage FRW in orchards.

The first pot experiment sought to assess the role of the weeds sorrel, capeweed and fathen, the clovers – sub-clover and an insect repellent clover – and the two grasses ryegrass and kikuyu, on their suitability as food plants for Fuller's rose weevil larvae. Recovery of larvae was very low at the two times of assessment and larvae were recovered only from pots planted to sorrel and sub-clover. The possible reasons for this apparent low survival rate were unclear.

In the second pot experiment, survival was much higher at up to around 1/3 of larvae being recovered in the second time of assessment. Survival on plums was surprisingly low, considering this is a major orchard type where FRW is a pest. It is inconceivable that larvae of FRW would not feed and survive well on roots of this orchard crop where they are considered a pest. However, the few larvae that were recovered at the second time of assessment had an average weight not significantly different to that for other treatments. As was the possible reason suggested for erratic results for garden weevil, the low survival of FRW on plums may have been associated with the relatively limited root system of the potted plants and the position where larvae were placed as pots were infested.

For the other treatments, the apparent increase in survival of larvae was seen between the two times of assessment. This was no doubt related to the larvae increasing in size and therefore the recovery rate improving at the second time of assessment. For all treatment attributes measured in relation to FRW larvae, there were no significant differences among the treatments involving apple tree. This suggests that the plants combined with apple were not antibiotic to FRW larvae. The plants may act as food plants for the larvae, but this conclusion could not be reliably made without infesting these plants alone.

The third pot trial that examined the survival of FRW larvae, compared apple alone with a range of potential cover crop plants. Survival reached a maximum of about 1/4, but no larvae survived in pots planted to an apple only. Therefore, results in this experiment are again questionable. This pot trial was run for about 30 days less than the second trial where an increase in the number of larvae recovered was recorded. By running the third trial for a longer time a higher level of recovered larvae may have been achieved.

Average larval weights were reasonably uniform among treatments where some larval survival was recorded, suggesting that if larvae could find some roots to feed on initially, they could grow at a similar rate. This suggested that none of these plants were antibiotic to FRW. Where no survival of larvae was recorded in pots with apple in combination with chickpea and vetch, the fact that no larvae survived on apple alone precludes an assessment that these plants are antibiotic.

The average total weights were quite variable, a reflection that the methodology of the pot experiments needs refinement. In relative terms, it seems that FRW larvae are not adversely affected by endophytic ryegrass or the grasses ryegrass and oats.

The experiment on food plants suitable for adults showed that survival and egg laying were greatest on the crop plants apricot and apple. Potential cover crop plants chicory, lupin and mustard would allow moderate egg laying, while the weed sorrel and potential cover crop plants vetch and ryegrass would allow low levels of egg production. Oats was a poor food plant for adults. A recent study by Maher and Logan (2004), showed that FRW adults fed more and laid more eggs when supplied the weed dock and the ground cover white clover, compared to the crop plant kiwifruit and buttercup.

Results

Pot trials with weevil larvae

One pot experiment was attempted to assess and compare a range of plants for their suitability as food plants for apple weevil larvae.

This experiment involved examining single plant species per pot. The plants that were to be compared are listed in Table 16. Pots were infested over the period 16 April 2003 to 22 May 2003 with only 5 larvae per pot. This was all the larvae that could be obtained from the laboratory culture. Despite this number being much lower than planned, the plants had been prepared for infestation and the experiment was undertaken anyway. For each treatment, there were two times of assessment and five replications.

Table 16. The list of plants assessed as single species for their suitability as food plants for larvae of apple weevil larvae in a greenhouse.

Plants tested	Measurements (days after infestation)
apple	Weevil development assessed at two dates (times) after initial infestation for all treatments: 21 Aug 2003 (approx 109 days) 8 Sep 2003 (approx 127days)
capeweed	
Carrot cv. Western Red	
insect repellent clover	
Ryegrass cv. Betta Tetilla	
sub-clover cv. Dalkeith	

Weevil survival was quite low in this experiment. At the first time of assessment, 5 pots were infested – two pots planted to capeweed with 1 and 2 larvae, and three pots planted to apple with one larva in each. Average weights of larvae were 0.011g and 0.021g for capeweed and apple respectively. At the second time of assessment only 2 larvae were recovered - 1 larva in each of pots planted to capeweed and apple. Possible explanations for this low survival level may be similar to suggestions made above for garden weevil and Fuller's rose weevil.

Because of problems with getting apple weevil eggs to hatch, no further pots trials could be undertaken. This prompted the experiments discussed below to try to improve egg production and egg hatch for apple weevil.

Laboratory trials on apple weevil egg production and hatch

The first experiment involved rearing apple weevil adults under two temperature regimes to determine whether temperature would affect egg production (see above for details on methods, page 19). The results for the two regimes are given in Fig. 30 and Table 17, where the results of an ANOVA are included.

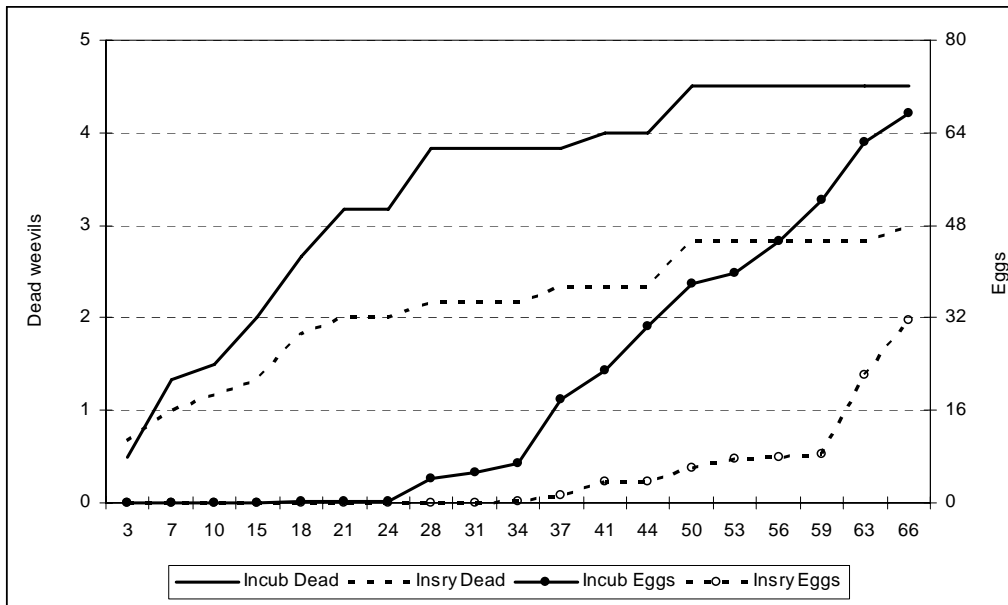


Fig. 30. Cumulative mortality of apple weevil adults and the cumulative number of eggs laid when reared at 18°C in an incubator or 25°C in an insectary.

Table 17. The average cumulative number of WeevilDays, total eggs laid and total feeding score for apple weevil adults when reared at 18°C in an incubator or 25°C in an insectary. The data were subject to AVOVA and where significant differences were found, the LSD_{0.05} (P<0.05) is included.

Regime	WeevilDays	Eggs	Feeding
Incubator 18 C	188	67.3	31.67
Insectary 25 C	266	31.5	30.83
<i>LSD_{0.05}</i>	<i>ns</i>	<i>ns</i>	<i>0.83</i>

The only significant difference was that weevils reared at 25°C had a higher feeding score than those at 18°C. There was no significant difference in the duration of survival or number of eggs laid, but the variation in the experiment was quite large. An examination of the average effects indicates there may be a difference with weevils dying sooner but laying more eggs at the lower temperature (Fig. 30). The probability values for significance for eggs laid and WeevilDays were 6.4 and 8.2 respectively, making these attributes nearly significant at the 5% level. The inclusion of more replications may have resulted in a significant difference.

The second experiment examined the effect of temperature and moisture on the timing of hatching of apple weevil eggs. This involved exposing two lots of 20 newly laid apple weevil eggs to a series of temperature and moisture regimes and durations, listed in Table 1 (page 20). The results of this experiment are given in Fig. 31.

Over the range of treatments, the highest level of egg hatch was less than 20%. The data was subjected to a statistical analysis using a generalised linear mixed model. The main significant effect was that treatments involving moisture resulted in a significantly greater or faster hatch of eggs. There was no significant effect of the low or very high temperature shock treatments on the rate of egg hatch.

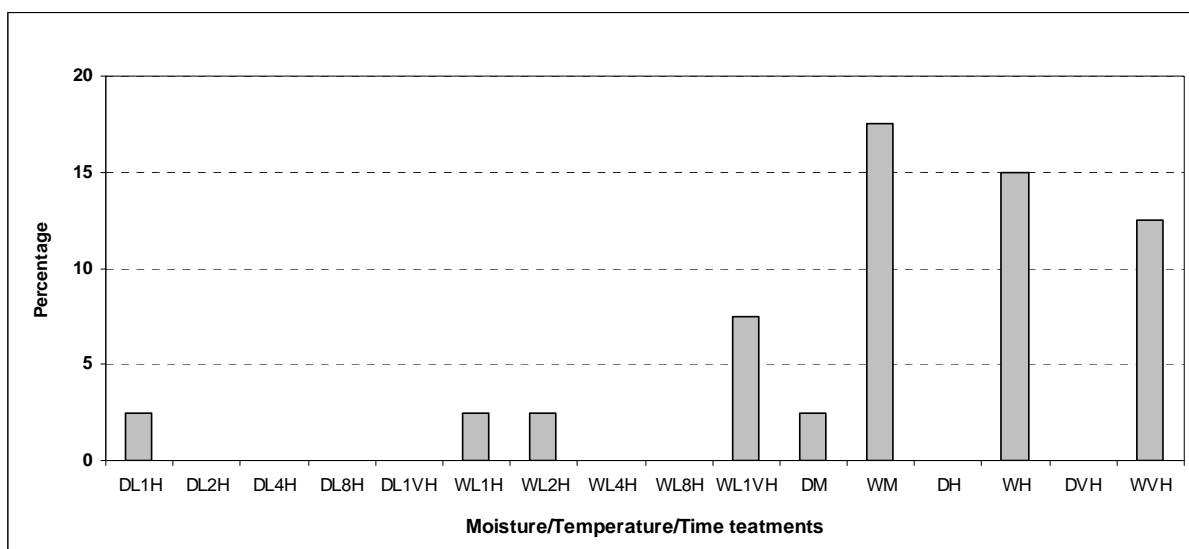


Fig. 31. The percentage of apple weevil eggs hatching after being subjected to a range of temperature and moisture regimes. For details see Table 1 page 20. Abbreviations: D = dry, W = wet, L = 4°C, M = 18°C, H = 25°C, VH = 30°C, the numbers refer to time in weeks.

Laboratory trials on food plants for adult weevils

The range of plants that apple weevil adults were reared on to compare their favourability for survival and fecundity is listed in Table 18. This experiment was commenced on 22 March 2004 using field collected adults. For each treatment, 5 weevils were placed in the rearing containers – AW adults are all females, being parthenogenetic. There were 5 replications. Also included in Table 18 are the average duration of weevil survival expressed in WeevilDays (see above for description on how this was calculated), the total number of eggs laid and the total cumulative feeding score based on the rating system described above.

Table 18. A list of the plant species apple weevil adults were reared on to compare them for survival (WeevilDays, see text for explanation), fecundity (total number of eggs) and feeding score (see text for scoring system used). The data were analysed using ANOVA and LSD's are included where significant differences among means were found (P<0.05).

Treatment	WeevilDays	*Total no. eggs	Feeding score
Nothing	82	0 (0)	0
Apricot	92	4.5 (20.3)	2.6
Olive	114	1.5 (2.3)	4.5
Field pea	165	2.5 (6.3)	5.7
Ryegrass	166	1.6 (2.6)	1.5
Chicory	166	4.8 (23.0)	10.0
Lupin	202	9.4 (88.4)	19.7
Mustard	264	4.8 (23.0)	7.5
Apple	270	3.6 (13.0)	18.4
Sorrel	311	3.8 (14.4)	18.7
<i>LSD</i> _{0.05}	110	3.1	4.1

*Total number of eggs transformed to square root for the analysis; backtransformed means in brackets.

The longest lived weevils were those provided with sorrel, apple, mustard and lupin leaves. Chicory enabled weevils to live a significantly similar duration to ryegrass, field pea, olive and apricot, as well as weevils deprived of food, which survived an average of 16 days.

Fecundity of apple weevil was not entirely related to duration of survival. Most eggs were laid by weevils fed on lupin. Weevils fed on mustard, chicory, apricot, sorrel and apple laid a similar but smaller number of eggs. Weevils with access to ryegrass and olive leaves laid the least number of eggs.

Weevils fed on lupin leaves demonstrated the greatest feeding activity, and this was at a similar level to sorrel and apple leaves. A similar but lower feeding activity score was recorded for chicory and mustard. Feeding activity was lower again on field pea, olive and apricot leaves, with the lowest activity score for weevils fed on ryegrass leaves.

Discussion – apple weevil

The pot trial experiments that were planned on larval food plants for apple weevil were compromised by the unavailability of sufficient larvae from a laboratory culture. An attempt was made to rectify this problem by undertaking two laboratory experiments. These experiments must be regarded as preliminary, but suggest that apple weevil adults would lay more eggs if held at mild temperatures of around 18°C, and that a greater proportion of eggs will hatch more promptly if held under moist conditions. Despite these results, more work is required to define rearing regimes that will allow for a greater production of apple weevil larvae.

The laboratory experiment on suitability of food plants in relation to survival and fecundity of apple weevil adults produced some surprises. Sorrel, apple, mustard and lupins were the plants that resulted in the longest survival of apple weevil adults. Lupin, mustard, chicory, apricot, sorrel and apple were the most suitable plants in terms of eggs laid. Apple weevil is a major pest of olives in WA and it was surprising that this food source resulted in both low survival and the lowest number of eggs laid of all plants tested. Whether the selection of olive leaves was biased to older less palatable ones, or some other reason, this result places some doubt over the outcomes of this experiment. Of potential cover crops for orchards that are least favourable to apple weevil adults, ryegrass would have to be considered the least.

Conclusions – ground covers

The experiments on ground covers in this project sought to identify suitable plants to recommend to orchardists to help reduce the abundance of garden, Fuller's rose and apple weevils in deciduous fruit tree orchards.

The experiments were planned as pot trials with candidate plants, assessed for their suitability as larval food plants, and laboratory experiments to assess their suitability as food plants for adults.

For garden weevil larvae, the experiments confirmed that clover is an unsuitable food plant for both newly emerged and advanced stages of larvae, and that it is not antibiotic. The experiments with other plants species for both garden weevil and Fuller's rose weevil were inconclusive in identifying other plant species that have the same attributes as clover, or any that could be confirmed as antibiotic.

The pot experiments on both garden weevil and Fuller's rose weevil larvae demonstrated that some plant species are either neutral like clover or may be favourable food sources. Because of the unexpectedly low survival rates of larvae on what are considered favourable food plants such as apple and plum, firm conclusions as to whether some plant species were antibiotic to weevil larvae are not possible.

However, where reasonable survival levels were obtained for plants grown in combination with apple, it is expected that such plants are unlikely to have antibiotic properties. For

garden weevil larvae, plant species unlikely to possess antibiotic properties would include ryegrass, sub-clover, insect repellent clover, oats, chickpea and chicory. For Fuller's rose weevil larvae, such plant species would include endophytic ryegrass, ryegrass and oats.

Pot experiments undertaken presented some unexpected results. Further work in this area may well benefit from a review of the methods used or consider extending such studies by undertaking work directly in the field. The justification for undertaking the pot trials was to expose different potential cover crop plants to artificial, uniform infestations of weevil larvae in the controlled environment of a glasshouse. It was assumed that such a situation would have led to clear comparisons of the plants tested. Because this was not the case, the procedure for testing different plant species requires careful consideration. Undertaking such studies directly in the field has the advantages that any results obtained would have direct application and more readily justify implementation in commercial situations. Others have commenced studies for general considerations of manipulation orchard ground cover crops (for example Harrington *et al*, 2000).

The applicability of studies of cover crop plants for adult weevils can be seen in situations where some form of exclusion band may be employed in orchards to prevent these flightless weevils from gaining access to the tree canopy (for example, Barnes *et al*, 1996 and Learmonth, 2000). It is apparent in the studies here that the crop plants themselves almost consistently provide the best food source for survival and reproduction of the weevils. The advantages of keeping weevils from accessing the tree canopy could be further enhanced by controlling weevils on the orchard floor with the use of predatory birds (Witt *et al* 1995, Learmonth 1999).

With just a few exceptions noted above, the results of the laboratory studies on plants suitable for adults produced some consistent results. In terms of cover crops least suitable for adult weevil survival and ability to lay eggs, the grasses and in particular ryegrass would appear to be suitable candidates. Oats and vetch appear to be reasonable plants to use where garden weevil and Fuller's rose weevils may be pests, but these plants were not examined for apple weevil.

One plant not included in these studies because it was not available at the time of testing, was clover. This species is very common in orchards and should be a priority to be tested if further studies on this topic are considered. Preliminary studies conducted on insect repellent clover at Murdoch University suggested these types of clover are less favoured than normal subterranean clover (Flores Vargas, pers. Comm.). Also, any eggs produced in such studies need to be held to confirm their viability.

Technology Transfer

Written material produced in relation to this project is listed in Appendix 2.

The aims and results of much of the project work in WA have been made known to orchardists in WA at orchard improvement group meetings, at training workshops on deciduous fruit tree pest management in WA and the results of experiments conducted in orchards and vineyards have been communicated directly to property owners and managers.

Recommendations - scientific and industry

At this time the use of parasitic nematodes for control of weevil pests in orchards cannot be recommended. It should be noted that the formulation of the parasitic nematode used in this study was very suitable for application through boom spray, and trickle and mini-sprinkler irrigation systems.

Future work with parasitic nematodes should only be undertaken if new more virulent strains become available. Workers in this field are encouraged to keep these orchard weevil pests in mind when screening new strains for efficacy.

Parasitic nematodes are unlikely to be used by industry unless they are cheaper and can be shown to be effective given the vagaries of the field situation.

Studies on orchard floor plants to clarify their contribution to garden weevil survival in terms of both adults and larvae confirmed the recommendation that the weed sorrel be controlled.

Suitable ground cover plants for weevil management include grass plant species such as ryegrass and kikuyu. Such plants are poor food sources for adults and seemed to at least retard larval development.

Further studies on the role of ground cover management are recommended. The effect on weevil survival could be dramatic if combined with weevil exclusion from the tree canopy. The effect of changing ground cover composition on other organisms, pest and beneficial, in the orchard ecosystem should be taken into account in such studies.

Future studies on ground cover management should be conducted in orchards rather than as pot trials. Field studies would overcome unexplained problems experienced in pot trials. Results from field experiments would be more robust and more rapidly lead to recommendations for new practices to be adopted by orchardists.

Future studies on ground cover management should include clover as this plant is very common in orchards and in this study, was confirmed to be neutral in relation to survival of larvae. Its suitability as a food plant for adults was not tested in this study.

If future studies on ground covers involve controlled glasshouse or laboratory experiments with artificial infestation of apple weevil, more work will be necessary to clarify egg laying and egg hatching requirements for this species.

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Appendix 1. Details of statistical analyses in garden weevil pot trial 1.

Treatment details are listed in Table 2, page 21.

1. Average weight of larvae in Pot Trial 1.

In the following tables, the average weight of larvae are given for each treatment and the LSD_{0.05} values calculated for pairs of means are given in the two way table to the right of the table of averages. Where a **significant difference** was found between two means the relevant **LSD value is in bold type and underlined**.

Early assessment

Treatment	Average weight (g)
CRT	0.014274
SUBC	*
IRC	*
KIK	*
RYE	0.013293
SOR	0.020487
SORSOR	0.023997
SORSCLOV	*
SORIRC	*

LSD comparisons			
	RYE	SOR	SORSOR
CRT	0.027354	<u>0.00544</u>	<u>0.0066</u>
RYE		0.027289	0.027536
SOR			0.006288

* = no larvae were recovered in these treatments.

Mid assessment

Treatment	Average weight (g)
CRT	0.029463
SUBC	*
IRC	*
KIK	0.010674
RYE	0.015385
SOR	0.025085
SORSOR	0.031085
SORSCLOV	0.016264
SORIRC	0.017617

LSD comparisons						
	KIK	RYE	SOR	SORSOR	SORSCLOV	SORIRC
CRT	<u>0.008885</u>	<u>0.005713</u>	0.005009	0.00596	0.01606725	0.019666
KIK		0.009016	<u>0.008587</u>	<u>0.009174</u>	0.01751564	0.020866
RYE			<u>0.005237</u>	<u>0.006152</u>	0.01613962	0.019725
SOR				<u>0.005505</u>	0.01590408	0.019533
SORSOR					0.01622851	0.019798
SORSCLOV						0.024791

* = no larvae were recovered in these treatments.

Late assessment

Treatment	Average weight (g)
CRT	0.032167
SUBC	*
IRC	*
KIK	0.014717
RYE	0.019637
SOR	0.027606
SORSOR	0.025368
SORSCLOV	0.009132
SORIRC	*

LSD comparisons					
	KIK	RYE	SOR	SORSOR	SORSCLOV
CRT	0.02737	0.005198	0.007335	0.011789	0.027313
KIK		0.027329	0.027815	0.029306	0.038286
RYE			0.007182	0.011695	0.027272
SOR				0.012789	0.027759
SORSOR					0.029253

* = no larvae were recovered in these treatments.

2. Total weight of larvae in Pot Trial 1.

Treatment	Time of assessment and total larval weight					
	EARLY		MID		LATE	
	*Trans mean	Backtr. mean	*Trans mean	Backtr. mean	*Trans mean	Backtr. mean
CRT	-2.17876 ab	0.111182	-1.47853 ab	0.225973	-0.93437 a	0.390835
SUBC	-6.14124 c	0.000152	-6.14124 d	0.000152	-6.14124 c	0.000152
IRC	-6.14124 c	0.000152	-6.14124 d	0.000152	-6.14124 c	0.000152
KIK	-6.14124 c	0.000152	-4.75532 c	0.006606	-5.70743 c	0.001321
RYE	-5.58967 c	0.001736	-2.53468 b	0.077287	-2.17271 b	0.111869
SOR	-1.17963 a	0.305393	-1.02716 a	0.356022	-2.50475 b	0.079696
SORSOR	-2.55495 b	0.075696	-1.8164 ab	0.16061	-3.37204 b	0.03232
SORSCLOV	-6.34054 c	0	-5.15904 cd	0.003747	-6.14633 c	0.000141
SORIRC	-6.35459 c	0	-5.60847 cd	0.001667	-6.50093 c	0
<i>Av. LSD_{0.05}</i>	<i>1.210821</i>		<i>1.164263</i>		<i>1.224329</i>	

*Means followed by the same letter are not significantly different at 5% level.

Appendix 2. A list of publications written during the project.

Learmonth, S. (2000). Taking the war against fruit weevils downunder. Primary Focus. WA Primary Industry magazine.

Learmonth, S. (2003). Weevils a hard nut to crack. Pome Fruit Australia. Progress report to APAL, Dec 2003.

Learmonth, S. and Trainer, S. (2004). Two steps forward & one step back with weevil research. Tree Fruit. Oct issue: 18-19.

Trainer, S. (2003). Parasitic nematodes for control of weevils in orchards and vineyards. WA Dept Agriculture. Bunbury AGMEMO APRIL 2003.

Trainer, S. and Learmonth, S. (2004). The suitability of different plants as food sources for larvae of garden weevil (*Phlyctinus callosus* Boh.), and Fuller's rose weevil (*Asynonychus cervinus* Boh.). 22nd International Congress of Entomology, Brisbane, Australia 15-21 August 2004. Poster.