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# WOOLLY APPLE APHID: INTERACTIONS WITHIN AN ORCHARD SYSTEM

**BY**

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TO HAROLD F. MADSEN
1921 - 1987

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# SUMMARY

Woolly apple aphid, *Eriosoma lanigerum*, is a sporadic, secondary pest found in the orchards of the 'Adelaide Hills' region of the Southern Mount Lofty Ranges of South Australia. Control is effected through the use of resistant rootstocks, chemical intervention and to a lesser extent, with a parasitic wasp, *Aphelinus mali.*

This study was initiated in response to an increased concern voiced by the growers to the local Department of Agriculture over the apparent prevalence of woolly apple aphid and the increased difficulty in control.

Apple growers of this region were surveyed to (1) determine a profile of orchard management practises, including spray protocol for woolly apple aphid, (WAA), codling moth and phytophagous mites and (2) to ascertain their perception of woolly apple aphid as a pest. Results of the survey indicated that WAA was viewed as a problem by more than half the growers surveyed, but was not considered to be on the increase. Control was maintained mainly through the use of insecticides; the parasite was not mentioned by the growers as an alternative method.

In laboratory studies conducted to determine the efficacy of insecticides commonly used in the orchard, the organo-phosphates, vamidothion and chlorpyriphos, were highly effective against WAA while fenoxycarb (insegar) did not differ significantly from a water control. The same insecticides tested against *A. mali* at three different life stages (larva, early mummy and adult) indicated that the organo-phosphates and organo-chlorines had a high kill rate at larval and adult stages. The mummy stage was less affected by these chemicals suggesting that timing of sprays is critical for maximum protection of the parasite.

High levels of aphid infestation had a significant, negative effect on tree height, total leaf number and total leaf area. This was confirmed in both a laboratory and field experiment on non-bearing Granny Smith trees. Trees were not affected in the first year of the field study, however, highly infested trees tended to break dormancy later and/or died in the second year.

A two year study of the spatial distribution and seasonal increase of aphid colonies showed that colonies were not equally distributed throughout the tree. A marked increase in the number of colonies was found in the upper stratum of the tree, which was evident from the onset of infestation and continued throughout the season. A greater than expected number of colonies were found in the southern aspect of the tree. Populations reached a seasonal peak in the early autumn (March).

Studies using sticky barrier traps throughout the tree showed that there were no seasonal migrations of aphids between the roots and canopy. Trap catches showed similar trends to the colony numbers throughout the season, with a seasonal peak in late summer/early autumn (February/March).

# DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying provided that an acknowledgment is made in the instance of any reference to this work.

Frances FitzGibbon

(August 1996)

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# CHAPTER ONE

# INTRODUCTION

Apple orchards are one of the most intensively managed systems in horticulture with a complex of over 500 insect species that feed on the trees and fruit (Croft and Hoyt 1983). The high value of apples and consumer demand for high quality fruit, have led to intensive study of apple pests and concentrated efforts to improve pest management practices.

With the advent of the synthetic organic insecticides in the 1940s and 1950s research into the control of fruit pests was directed towards chemical control (Hoyt and Burts 1974; Metcalf and Luckmann 1982). This research lead to spray programs being developed and used as regular, preventative measures regardless of whether the pest was present in damaging numbers or not. At first the results were dramatic - increased yields of high quality produce were obtained at very little cost. However, this era of prophylactic insecticide application was beginning to show signs of breaking down with increased incidences of resistance.

In the 1960s and 1970s however, the emphasis swung towards the study of the biology, population dynamics and biological control of the insects within orchards. This shift was due to several factors. By this time, pest insects were developing resistance more and more quickly to insecticides resulting in greater costs to the producer attempting to control the pests, which were ultimately passed on to the consumer. Natural enemies were unable to cope with the barrage of pesticides and insects not previously considered as major pests began to assume that status with a consequent further increase in insecticide usage. The cost of developing and testing insecticides was also escalating, concurrently with a decrease in the life expectancy of the pesticide once it reached the marketplace.

A final, and possibly the greatest incentive to the search for novel methods of pest control, was a new pressure exerted by consumers. By the mid-century, a fruit of extremely high quality and low price was expected and little thought was given to how that quality was achieved. In 1962, however, Rachel Carsen's book *Silent Spring* initiated a groundswell of resistance to the broad and often indiscriminate use of pesticides; in particular, it alerted consumers to the hazards of pesticide residues. By the 1970s, an increase in programs that involved the reduction of chemicals coupled with a greater reliance on natural enemies (biological control) was already evident. This integrated approach, which required comprehensive studies on the complex biological interactions within cropping systems, was later called Integrated Pest Management (IPM) and was to be the way of pest control in the future.

IPM is an approach to pest management which draws on the techniques and methods of both traditional and modern disciplines. As its name implies, IPM attempts to manage pest populations at acceptable levels by integrating a number of techniques instead of relying on a single method (Rabb and Guthrie1970). Crops resistant to insect damage may be used, whether selected from the range of existing cultivars developed using traditional plant breeding approaches or genetically engineered. Land management practices may include crop rotation, or novel tillage regimes. In particular, attempts are made to enhance the effectiveness of natural biological control agents. Land management practices may be modified to encourage populations of natural enemies or natural enemies may be released en masse at particular times of the year. Insecticides and spraying regimes are chosen to minimise the destruction of natural enemies. Whatever tactics are employed to manage the pest, the goal remains the same: to reduce pest populations below the economic threshold while minimising inputs and maintaining high quality produce. IPM systems should be economically sound, environmentally safe and socially acceptable.

The first instance of IPM in apple production was in Nova Scotia (Whalon and Croft 1984). Export restrictions during the Second World War forced a reduction of production costs in order to save the industry. 'Harmonised' control, which relied on natural regulation of pest populations and the selective use of pesticides was introduced. Yet, despite initial successes of this program and others world-wide, IPM did not immediately become routine practice (Prokopy *et al.* 1990; Gruys 1982; Whalon and Croft 1984) and growers eventually returned to intensive, high pesticide use. Only in the 1970s, with pressures brought to bear by increasing pesticide resistance, high costs of chemical control and a change in public opinion, was attention once again focused on IPM.

In the 1970s and 1980s, the emphasis in IPM was placed on monitoring the first appearance and subsequent abundance of pests and beneficials in order to determine the timing of selective pesticide applications against those insects not controlled by their natural enemies. While other techniques such as pheromone traps and sterile male release programs have been investigated (Madsen and Vakenti 1973; Proverbs *et al.* 1982), chemical control has nevertheless remained the main thrust of most IPM programs.

Within the last 10 years, IPM in apple management has gone one step further integrating behavioural and biological with pesticidal methods (Roitberg and Angerilli 1986; Prokopy *et al.* 1990). This was stimulated by the realisation that 'first-stage' IPM (monitoring and spraying) was proving to be of limited effectiveness in reducing the number of insecticide applications. To make IPM even more effective, its practitioners had to look at the orchard as an interacting complex of insects, plants and the environment rather than as a number of separate interactions, each involving a single pest. An advanced IPM program requires a sound knowledge not only of the biology of each specific pest, but the web of interactions that involve the whole pest complex within the orchard. In particular, the interactions between all pest species and their natural enemies must be considered. IPM programs must also be tailored to specific geographic regions. An optimum IPM program requires a systems approach tailored to the crop, its geographic region and even local market expectations and economics. In effect, IPM must replace the simplistic reliance on the automatic spraying schedules of the past with a fully interactive relationship between the unfolding of biological events and the knowledge and experience of the IPM operator. Regardless of the complexities involved in implementing IPM programs, economics and public concern about environmental and health issues will inevitably ensure this approach to pest control in the future.

Apple IPM has been most successfully implemented in North America and Europe ( Croft and Hoyt 1983; Dickler and Schafermeyer 1991). Other countries, such as Australia, have lagged behind in the reduction of pesticide usage, despite a growing interest in IPM over the last 15 years (Readshaw 1975; Miles *et al.* 1979; Wicks and Granger 1989; Bower 1987).

Woolly apple aphid (*Eriosoma lanigerum*) (WAA) is considered an indirect, secondary pest of apples (Croft 1975) and is found in all apple producing areas of the world (DeBach 1964). It attacks the roots, pruning scars and woody tissues of new growth and, in heavy infestations, the fruit. Principle damage to the fruit comes from the production of sooty molds developing on aphid honeydew which makes the fruit unsaleable and from lowered tree vigour.

Control of WAA has been maintained by the use of resistant rootstocks, a parasitoid, *Aphelinus mali,* and the use of insecticides. In South Australian orchards, this combination gave satisfactory control of WAA until the mid 1980s but orchardists have now begun to increase chemical controls specifically targeting this pest and have expressed concern over its apparent resurgence in damaging infestations. That concern, expressed to the South Australian Department of Agriculture, instigated the present study.

As a step towards the development of an overall IPM program for apple orchards, the goal of this project was to gain an understanding of the biology of woolly apple aphid and its parasitoid; of the impact of the pest on the crop; and of the effects of pesticides used routinely in South Australian orchards on both woolly apple aphid and its parasitoid. The immediate task addressed was to identify current management practices of apple growers in South Australia and to determine grower perception of woolly apple aphid as a problem. Aspects of the biology of WAA and *A. mali* were examined as a complement to previous studies in South Australia (Sen Gupta 1969). Work was also done on the toxicity against WAA and *A. mali* of insecticides used in the control of codling moth and phytophagous mites. The effects of woolly apple aphid infestation on the growth of young trees were then investigated to determine whether there was a real potential for loss of productivity over the life time of the tree. The seasonal activity of WAA on mature trees was determined over a two season period to gain information for the development of a more efficient sampling method. Finally, the implications of the research regarding the control of this pest were considered.

# CHAPTER TWO

# A SURVEY OF APPLE GROWERS OF THE ADELAIDE HILLS REGION OF SOUTH AUSTRALIA

## 2.1. ABSTRACT

In 1987 the apple growers of the Adelaide 'Hills' region (Lower Mount Lofty Ranges) of South Australia were surveyed to ascertain their perception of woolly apple aphid (WAA) as an orchard pest. The survey was also used to develop a profile of orchard management practices. Forty-six out of 63 (73%) growers responded to a postal survey consisting of 31 questions regarding the orchard layout, management and spray protocol for codling moth, spider mite and woolly apple aphid. Seventy-five per cent of the growers sprayed specifically for WAA and 60% thought that woolly aphid was a problem, but only 29% thought that it was an increasing problem. A variety of pesticides were used for controlling WAA, codling moth and two spotted spider mite. Vamidothion was the most commonly used control agent against WAA. Northern Spy was the most popular rootstock used in orchards (93%) followed by MM106. Only two growers mentioned the presence or use of the parasitoid, *Aphelinus mali*.

Grower response highlighted strong similarities in the physical characteristics of the orchards and in the tree varieties planted. The four predominant varieties under production were Red Delicious, Golden Delicious, Granny Smith and Jonathan but there were indications that newer varieties are being planted.

## 2.2. INTRODUCTION

Orchard management varies widely from region to region throughout the world. Soil type, climate (rainfall, temperature, prevailing winds) and tree cultivar all influence orchard characteristics and management. The level of education, experience and attitude of the orchard manager may also determine the types and amounts of insecticides, fungicides or herbicides applied, the tree varieties grown, their subsequent training and the use of new or innovative techniques.

With such differences in management practices, it can be difficult for workers new to the field to obtain an effective overview. The experience of workers already in the area is invaluable but use of surveys can provide an efficient means not only to target a particular group of people but also to obtain a quick overview of specific characteristics or practices. In this case, the area of specific concern was the woolly apple aphid (WAA).

In the autumn of 1986 personnel at the local Department of Agriculture (Lenswood Research Centre) felt that they were receiving a greater number of requests from orchardists for advice concerning WAA and its control than in previous years. Casual observation at grower meetings indicated that some growers believed that WAA was an increasing problem both in prevalence and in control (A. Granger, S.A. Department of Agriculture, personal communication). To find out if this was an accurate reflection of the growers as a whole, a decision was made to survey the orchardists. A survey would indicate how many growers had this opinion and perhaps provide an indication of where control may be breaking down.

The purpose of the survey was three-fold - firstly, to obtain the basic background information concerning current orchard management in the area. This would include the physical lay-out of the orchard as well as tree variety, and type of irrigation. Secondly, the survey would determine if WAA was a problem and, if so, whether it was on the increase. Finally, the data compiled from the survey would be used in determining future experimental designs both in the laboratory and in the field. This information would be used to locate suitable sites for further field experiments and orchardists that would be willing to allow their orchards to be used in experiments or as monitoring sites.

While the main aim of the survey was to determine the growers' attitude towards woolly apple aphid as a pest, it also provided an opportunity to get an overview of the way orchards in South Australia are managed and a description of their physical properties.

## 2.3. MATERIALS AND METHODS

A survey would gather the greatest amount of general information concerning the orchards and their management in the most efficient way possible. A telephone survey or visits to orchards to gather this information were considered too costly in time and funds. It was also felt that growers would be more willing to fill out a short questionnaire at their leisure than to engage in a telephone conversation or break their daily schedule with an orchard visit.

A list of the apple growers of South Australia was obtained from the Apple and Pear Growers Association. Growers were chosen from the 'Adelaide Hills' region because of the high density of orchards in the area and its proximity to the Waite Institute. All of the growers surveyed were considered to be commercial growers by the Apple and Pear Board and did not include anyone considered to be a 'backyard' gardener.

The survey was divided into three sections (Appendix One). The first covered a general physical description of the orchard and its exact location. This section asked questions about the size of the orchard, its slope (predominant and secondary), frost prevalence and soil type. The second section was concerned with tree varieties planted, their age, the rootstocks used, training system, tree spacing (within and between rows), ground cover and irrigation. The final section covered spray schedules for codling moth, woolly apple aphid, and phytophagous mites, as well as the presence of WAA and its pest status in the orchard; the type of spraying employed (dilute or concentrate spraying) was also included.

The questions were set out in such a manner that there would be minimum ambiguity in the answers. The appropriate answer in the multiple choice questions was to be marked with an (X) or a (√) and the 'yes/no' questions were to be answered by circling the appropriate response. The questionnaire was prefaced by a short description of the reasons for its implementation and instructions in completing it. Also included in the preface was a contact number in case the growers had any difficulty in completing the questions or concerns about the survey in general. It was stressed that all information was confidential and would not be used for any other purpose or given to any other group. This proviso was felt to be very important in achieving the growers' cooperation in filling out the survey.

Prior to sending out the surveys, an announcement was placed in the local Apple and Pear Growers Newsletter to alert growers to its arrival. Included in the questionnaire package was a self-addressed, stamped envelope for returning it to the Waite Institute. The survey was sent out to the growers in the spring of 1987 (October).

One month was allowed to elapse before follow-up calls were initiated. More than 50% of the surveys had been returned by that time and it was felt that a single phone call was all that was warranted to determine whether the growers had received the questionnaire and whether they intended filling it out. Some confusion was created by a certain number of growers having the same name and an address that was no more than a road name. Growers who had not received the questionnaire but were interested in responding were sent another package or, if they were willing, answered the questions in a telephone interview.

Once all the questionnaires had been returned, the data were tabulated and the frequencies determined for each multiple choice and yes/no answer (SAS 1985). A copy of the survey and the answers to the questions are found in Appendix One.

## 2.4. RESULTS

A total of 63 questionnaires was posted and a return rate of 73% (46 surveys returned) was achieved. All but one of the forms had been filled out in an unambiguous way and all but one gave a name and/or address. Generally, growers had taken care in filling out the survey and some added comments about their orchard or pest control without prompting. Later information offered by the Department of Agriculture indicated that, in general, growers had reacted favourably to the survey.

Just over half of the growers who had responded had orchards that were over 10 hectares (25 acres ) (Figure A1.1A). A small number were made up of several separate blocks of land. There was no indication about how many orchards were larger than 10 hectares except one respondent who indicated that his orchard was over 36 hectares (90 acres).

It was expected that few of the orchards would not be on a slope as the topography of the Adelaide Hills is one of steep hills and deep valleys. All but two orchards were on sloping land, the two most predominant slopes being towards the north and east (Figure A1.1B). The north facing slope would be the most favourable slope for agriculture as it gets the late morning and early afternoon sun. It was clear from the questionnaires that most orchards were on land that generally had more than three predominant slopes. Some growers indicated that their orchards had predominant slopes in all cardinal and intermediate directions, often with secondary slopes as well.

Forty-four per cent of the orchards were not frost-prone (Figure A1.1C). Some areas of the Hills experience much cooler temperatures than others and the entire area is generally cooler than the Adelaide Plains (S.A. Department of Meteorology). These temperature characteristics make the Adelaide Hills suitable for apple production.

Nearly all the orchards had soil that was clay, loam or a combination of the two (35% were loam over clay). Only four growers indicated that their orchards had a sandy type of soil (Figure A1.1D), three of them being within two kilometres of each other.

The most commonly used inter-row (alley) ground cover was a grass or grass-clover mixture, 47% and 36%, respectively (Figure A1.1E). The area between trees within the rows was generally controlled by herbicides (76%) (Figure A1.1F).

Nearly all the growers (82%) pruned the trees in the winter or dormant season and a small percentage (18%) pruned in both summer and winter (Figure A1.2A). No orchardist pruned only in the summer. Almost half of the growers (48%) indicated that they trained their trees to a vase shape (an inverted 'V') which allows light into the centre of the tree canopy but still protects the fruit from the sun. Twenty-four and 22% of the growers, respectively, trained the trees to a central leader or open shape (Figure A1.2B). A tree that has been trained to a central leader has one main trunk with laterals along its height. The open shape is similar to the vase shape but is less defined in its outline. These two shapes are variations on the same training style and are done for the same reason; both allow more light into the centre of the tree canopy and still allow enough shade to protect the fruit from the sun.

All orchards were irrigated. The main type of irrigation was under-tree sprinklers (Figure A1.2C), which could be considered the 'traditional' method of irrigation. Some growers used more than one type of irrigation, presumably in different areas of the orchard. It was not determined whether the growers who indicated 'yes' for both undertree and drip irrigation actually used only drip irrigation and were trying to clarify the position of the drippers.

Twenty-one different apples varieties were grown in the surveyed orchards (Figure 2.1A). The four most commonly grown varieties were Granny Smith, Jonathan, Golden Delicious and Red Delicious. Eighty per cent of the orchards had Red Delicious trees that were under five years old (Figure 2.1B). Only 29% of the growers had Jonathans of this age. However, 58% had Jonathans that were over 40 years old. Sixty-nine per cent of the growers had Granny Smith trees that were between 11 and 20 years old. Only one grower indicated that more than 75% of the orchard was planted to one variety of apple. Most growers had less than 25% of the orchard planted to a single variety and 92% had a quarter of the orchard planted to a variety other than one of the 'traditional' cultivars. There was no indication what that variety might be. Only two of the varieties were spur type; Red and Golden Delicious. Of these two, 82.5% of the growers had spur Red Delicious (Figure A1.3A).

All but three of the growers who stated that they grew the four major varieties of apples used Northern Spy as a rootstock (Figure A1.3B). Northern Spy is resistant to root infestations of WAA. Of the three who did not, two used MM106 which has some resistance to WAA and the third grower gave no indication of the rootstock used ('no' was given as the response for every alternative including 'other').

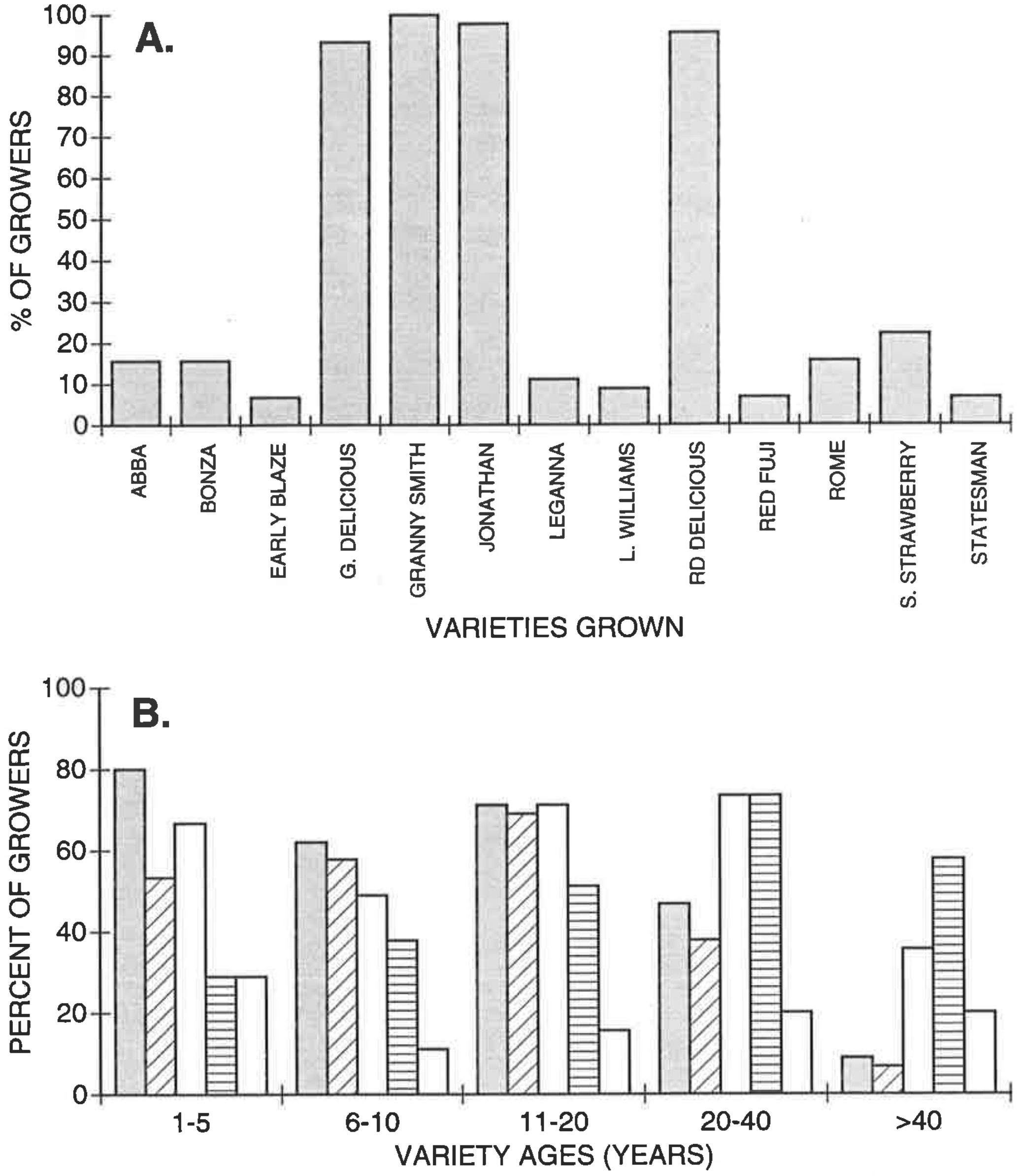


**Figure 2.1.** Per cent of growers' affirmative response to survey questions. (A) apple varieties grown, 'G. Delicious' = Golden Delicious, 'L.Williams' = Lady Williams, 'Rd Delicious' = Red Delicious, 'S. Strawberry' = Summer Strawberry. 'Other' includes Akane (4.4%), Cleo (2.2%), Crofton (2.2%), Red Granny Smith (2.2%), Rokewood (4.4%), Spartan (2.2%), Winesap (2.2%) and Maiden's Blush (2.2%).
(B) age frequencies of the major varieties grown in the orchard.

▩ Red Delicious

▨ Golden Delicious

☐ Granny Smith

▤ Jonathan

☐ Other

Sixty per cent of the growers indicated that they used MM106 as well as Northern Spy (Figure A1.3C). This rootstock was used on the younger trees, usually on the spur Red Delicious. Seedling rootstock was also mentioned as a rootstock and one grower indicated that interstock was used on some of the young Red Delicious.

All growers surveyed sprayed for codling moth, with the majority (73%) of them spraying between three and five times a season (Figure 2.2A). A total of six insecticides was used in controlling codling moth (Figure 2.2B). The most common was azinphos-methyl (APM or Gusathion), with carbaryl (Sevin®) second in usage. Mite sprays were used no more than two times per season by the majority (78%) of the growers (Figure 2.2C). Nine different insecticides were used against phytophagous mites (generally *Tetranychus urticae*). The most commonly used were propargite (Omite®; 47%) and clofentezine (Apollo®; 46%) (Figure 2.2D). Thirteen per cent of the growers indicated that they used three different miticides against mites. Only two growers mentioned the use of predatory mites. Some growers indicated the use of chemicals that were no longer registered for use, specifically, cyhexatin (Plictran®) and azocyclotin (Peropal®). Eighty per cent of the growers used a dormant oil spray for pests other than woolly apple aphid (Figure 2.2E).

Seventy-six per cent of the growers sprayed specifically for woolly apple aphid (Figure 2.3A); slightly less (60%) thought that it was a problem in their orchard (Figure 2.3B). Only 29% thought that it was an increasing problem. Seventy-eight per cent of the respondents recognised that certain areas of the orchard and varieties were more likely to have WAA than others. The most common response was that the Granny Smith trees or vigorously growing trees were the 'hotspot' areas. Vamidothion (Kilval®) was the most commonly used spray (42%) with oil (25%) as second choice (Figure 2.3C). Two growers mentioned the presence of the parasitoid, *Aphelinus mali*, in the orchards but only one of these considered it an effective method of control.

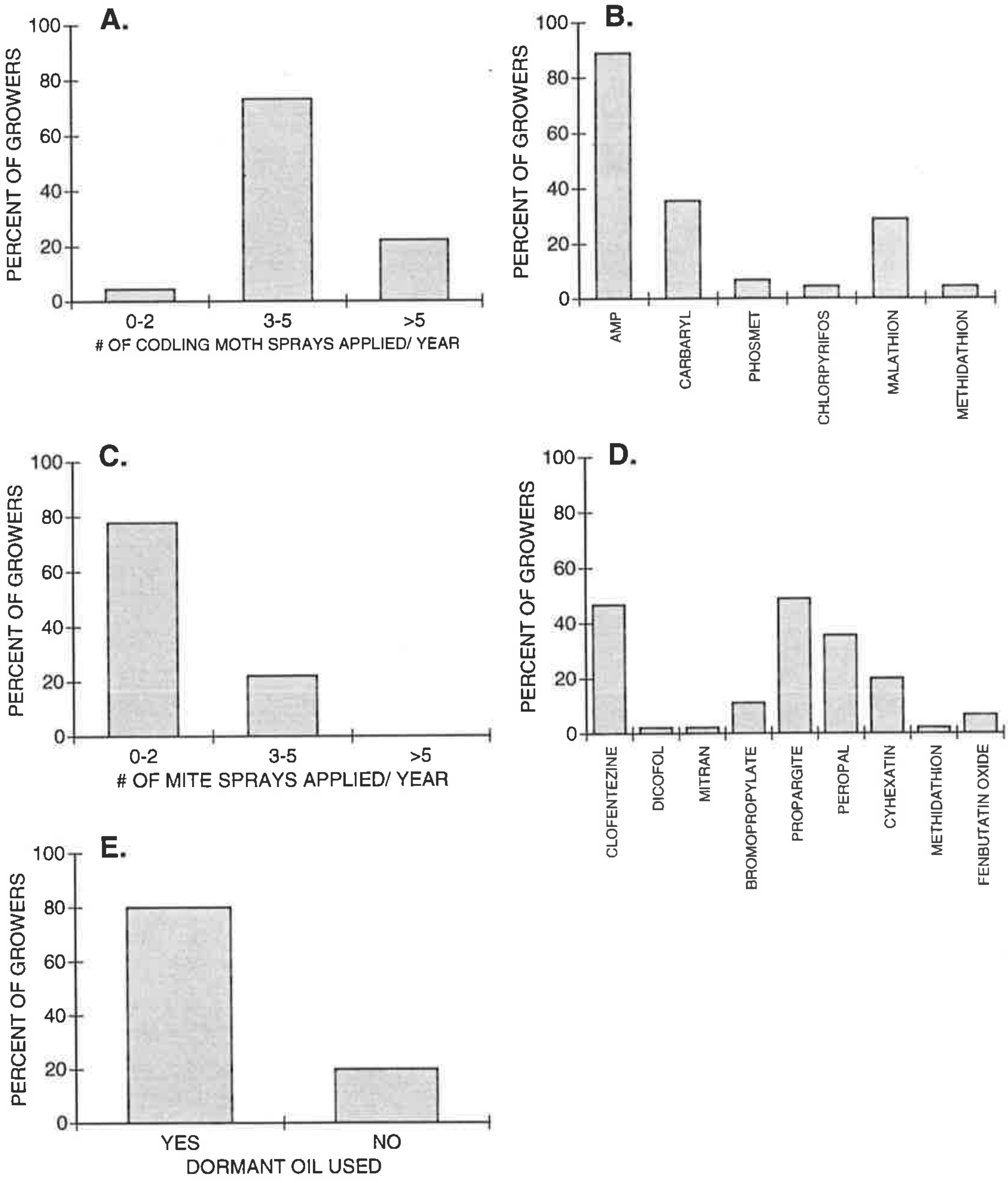


**Figure 2.2.** Per cent of growers' affirmative responses. (A) number od sprays applied per year to control codling moth, (B) sprays applied specifically to control codling moth, (C) number of sprays applied per year to control phytophagous mites, (D) sprays applied specifically to control mites, (E) whether dormant oil is used for pests other than woolly apple aphid.

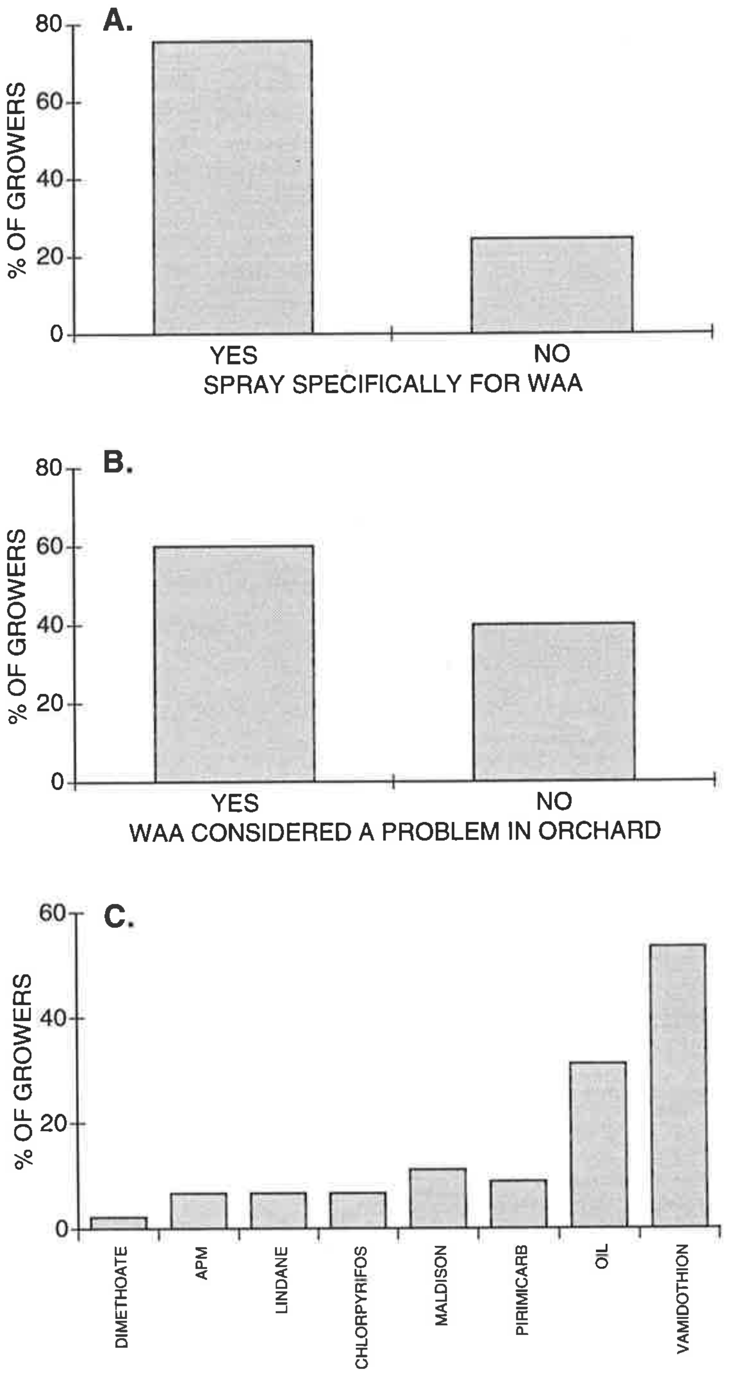


**Figure 2.3.** Per cent of growers' affirmative response to survey questions. (A) whether the grower sprays specifically against WAA, (B) whether the grower considers WAA a problem in the orchard, (C) sprays applied specifically against WAA.

## 2.5 DISCUSSION

There was a great deal of similarity among the orchards of this region of South Australia. An orchard that could be considered as typical of this area would be a block of land that was over 10 hectares with a predominant slope to the north or east. The soil would be either clay, loam or a combination of the two (loam over clay). The orchard would be subject to some frosts but would not be considered as frost-prone, probably due to the sloping nature of the block. Ground cover would consist of grass or a grass/clover combination within the alleys, while the vegetation within the rows would be controlled with herbicides. Irrigation would be provided by undertree sprinklers or drippers.

The preferred slopes of north and east take advantage of the morning and early afternoon sun. A west slope would be too hot and dry for apple production and would lead to the fruit being burnt. An east slope would provide extra moisture and was an important factor before irrigation became a common practise. Irrigation is a relatively recent innovation in orchards being introduced approximately 10 to 15 years ago. Until that time the orchards relied on rainfall for irrigation.

The orchards of the Adelaide Hills region are well established with a predominance of old trees planted in a 'standard' (large inter-tree and inter-row spacing) format. The trees in this typical orchard would be pruned in the winter and would be trained in one of three styles; vase, open centre or central leader. Four apple varieties are dominant in the orchards: Red Delicious, Golden Delicious, Granny Smith and Jonathan. There is evidence of a shift away from older varieties such as Jonathan, especially in younger plantings. Apple trees have a long life as a crop hence age frequency analysis can point out when certain cultivars became popular. For example, a greater percentage of growers have Jonathans that are 40 years old or more compared with those who have non-bearing (less than five years old) Jonathans. Red Delicious became a popular cultivar approximately 20 years ago and has remained popular in the last five years. This may reflect the shift to spur type and 'specialty' Red Delicious within the past decade. Granny Smith appears to have remained a popular variety over the past forty years. The survey was not able to determine what new varieties are being planted because growers were not asked to specify what 'other' cultivars were grown. Some growers did, however, indicate cultivars in the 'other' alternative in the question concerning varieties, and the most commonly mentioned ones were Red Granny Smith and Red Fuji. Nevertheless, Red and Golden Delicious and Granny Smith cultivars are still dominant. None of the growers surveyed had the entire orchard planted to one cultivar. It was most common for there to be at least four cultivars under production at the same time reflecting a replacement of old and no longer popular varieties with new ones. Ninety-two per cent of the growers indicated that up to 25% of the orchard was planted to 'other' varieties, again showing the trend towards new varieties.

Codling moth still appears to be most commonly controlled by chemical means, and methods such as pheromone disruption or trapping are still in an experimental stage. Growers were not asked if they used pheromone traps to time sprays but it is known that a number of them do (A. Granger, personal communication). Codling moth sprays are applied according to the calendar (ie: every two to three weeks depending on the pesticide used) starting about two weeks after petal fall (early October). The number of applications is reduced after December and the timing of the remaining sprays is dependent on the experience of the grower. The Lenswood Research Centre offers a codling moth monitoring service for the Lenswood area and growers outside the vicinity also use it to time their sprays.

At the time of the survey there was no Integrated Mite Program (IMP) being utilised in the area and phytophagous mites were considered a problem. Earlier attempts to introduce such a program failed, but with pressure to reduce the number of sprays used in orchards and a number of orchardists (albeit small) using predacious mites successfully, there is now more interest in IMP. Up to the time of the survey, growers used a wide variety of chemicals against phytophagous mites, including some that were no longer registered for use. Twenty per cent of the growers indicated that they used cyhexatin, even though it was no longer officially available. It had been withdrawn from the market in the year that the survey was sent out and their response presumably indicated the presence of stockpiles of the chemical. Some of the growers were aware of resistance problems that could be encountered with mites and gave this as the reason for using more than one product. It was surprising to see that methidathion (Supracide®), was used as a miticide by even one grower, considering that its harmful effects against predacious mites is widely recognised.

The number of growers who felt that WAA was a problem of increasing importance was less than expected. It is unlikely that the survey underestimated growers with this view. Rather, it seems more likely that growers who were experiencing problems with WAA and its control may have been particularly vocal in their concerns and that Department of Agriculture personnel had, therefore, become more aware of these concerns. It was not clear, in retrospect, whether the growers sprayed for WAA because it was a problem or whether they believed that it was a problem in their orchard despite spray application. What did become clear was that growers who used vamidothion to control WAA appeared to be spraying at a time of year other than that recommended by the manufacturer. The recommended application time is late spring/early summer to maximise the systemic effects of the aphicide and give good control for that season and for most of the following season as well. When growers were questioned informally they indicated that they used vamidothion in the late summer and that they were spraying on a yearly basis. This shift may account for the reduced efficacy of the chemical as perceived by the growers, as late season application would not utilise its systemic properties to maximum effect. Applied as the manufacturer suggests, in the spring or early summer when the tree is actively growing, multi-season control might still be achieved.

In general, the survey and follow-up telephone interviews indicated that growers placed a heavy reliance on chemical control for pests such as codling moth, phytophagous mites and woolly aphid, and that as long as control was achieved they were satisfied. Due to the direct damage to the fruit caused by codling moth, thereby reducing market value, and the obvious damage to the tree caused by WAA there is a certain reluctance to consider alternative methods of control. Integrated Pest Management has not yet been embraced as a viable alternative to chemical control and with the continued use of pesticides secondary pests such as phytophagous mites and WAA will continue to be considered as major pests in the orchard.

With this continued reliance on pesticides to control WAA, the survey results pointed out the need for an easy and effective method of sampling in order to maximise the effects of the pesticide and at the same time reduce the amount of pesticide usage - either in amount used per application or number of applications per season. The implications of the use of such an array of pesticides would have to be investigated to determine the effects they may have on the aphid, in terms of resistance, and the effects on beneficial insects such as *Aphelinus mali*. The introduction of new techniques such as predacious mites and the use of pheromones was slow and grower resistance to these techniques was encountered. However, with increased pressure by consumers for pesticide-free fruit, increased insect resistance to pesticides and stricter government controls on pesticide usage and residues, grower attitude is changing and this change was reflected in the survey. The apple growers, as a group, plan to reduce chemical application in the orchard by 50% within the next five years. The shift to decreased pesticide usage in a specified time limit will encourage growers to apply the methods of IPM to their orchard management if for no other reason than to remain competitive in the market place.

The survey did not specifically ask which apple variety was affected by WAA, however, of the 35 growers that mentioned 'hotspots' in the orchard, 16 stated that Granny Smith was the affected variety compared to 7 and 5 for Delicious and Jonathans respectively. The remaining responses referred to the area of the orchard that was affected rather than variety. With this in mind it was decided to use Granny Smith trees in further experiments or field trials. This decision was strengthened by the fact that this variety still comprised a large proportion of the orchards.

While there were other questions that could have been addressed in the survey, it proved a useful tool for rapidly gaining large amounts of information quickly and relatively easily. Some answers also pointed out areas that should have been more clearly defined such as use of new varieties, grower awareness of alternative control techniques, and personal information (e.g. such as level of education). Desire to gain even more information must be balanced, nevertheless, against the willingness of growers to spend more time on answering questions they may feel are unimportant to the running of their orchard, or that divulge personal information. Experience has shown that there is a fine line between being considered interested and, on the other hand, being considered intrusive. It can be all too easy to overstep these bounds in the eagerness to gain information.

# CHAPTER THREE

# BIOLOGY OF WOOLLY APPLE APHID, *Eriosoma lanigerum* AND ITS PARASITE, *Aphelinus mali*

## 3.1. ABSTRACT

The biology of *Eriosoma lanigerum*, woolly apple aphid (WAA) and its parasitoid, *Aphelinus mali*, were investigated in a series of laboratory experiments. Developmental times for each instar of WAA were calculated at three temperatures (15, 20, and 25°C). A greater proportion of time was spent as a first instar than any other life stage, excluding the adult. The lower temperature threshold for overall development was estimated as 5.7°C.

Developmental times were determined for two stages of *Aphelinus mali*; egg lay to mummification and mummification to emergence. The lower temperature threshold was estimated to be 9.3°C for total development. Longevity and sex ratio were also determined. Females lived significantly longer than males. Sex ratio of field populations was approximately 1M:1.8F. The proportion of males increased with increasing temperature.

The total development time was similar for the two insects at temperatures around 25°C but a divergence in developmental times was seen as temperatures decreased. The aphid developed more rapidly than the parasitoid at lower temperatures.

## 3.2. INTRODUCTION

### 3.2.1. Woolly apple aphid

The woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae), has its origins in the eastern United States (Patch 1913; Greenslade 1936) and is now found in all apple growing regions of the world. It was introduced to Australia in the latter part of the 19th century (Nicholls 1932).

Its life cycle and biology have been well described (Baker 1915; Monzen 1926; Bodenheimer 1947; Evenhuis 1958; Bonnemaison 1965; Asante *et al.* 1991). In eastern North America woolly apple aphid has a holocyclic life cycle, overwintering and producing sexual morphs on American elm, (*Ulmus americana*), its primary host; the asexual morphs spend the summer on apple (Patch 1912, 1913; Baker 1915; Becker 1918). In other areas of the United States and the world, the aphid, in the absence of American elm no longer migrates to an alternate host (Bodenheimer 1947, Bonnemaison 1965). The life cycle is reduced to a varying number of generations of viviparous, parthenogenetic females. Alate and sexual morphs are still produced but the eggs laid by the fertilised females either do not hatch or the resulting fundatrix invariably dies (de Fluiter 1931; Bodenheimer and Swirski 1957; Evenhuis 1958; Staübli and Chapuis 1987). This part of the life cycle is not considered important in the spread of the aphid.

In the absence of its primary host, the aphids overwinter as young nymphs (usually first instars) on apple trees. In the moderate climates of South Australia, all stages have been seen throughout the winter months (personal observation). The exact overwintering location is controversial (Chapter Six). It has been reported that the overwintering site is the root collar and/or root system in North America and Europe (Theobald 1921; Jenser 1983; Staübli and Chapuis 1987). Other workers have stated that the young nymphs spend the winter in cracks and pruning scars on the trunk and branches of the tree (Dumbleton and Jeffreys 1938; Evenhuis 1958).

In the summer the aphids reproduce parthenogenetically, forming colonies on pruning scars, wounds and new growth. Alate forms are produced in the spring (September-November) and autumn (March-May). Production of alates appears to be associated with daylength and temperature (approximately 12L:12D and 18°C), however, they are not commonly found in the field in South Australia (Sen Gupta 1969; Chapter Six). Hughes *et al.* (1965) recorded only four alates caught in aphid traps in Victoria in a 13 month period. Under laboratory conditions, alates can be produced in large numbers over a range of temperatures and at daylengths exceeding 12 hours (personal observation; Sen Gupta 1969). They will give rise to sexuparae, the female of which will produce a single egg. Eggs have not been recorded in the field in Australia or New Zealand and, under laboratory conditions, do not result in a viable fundatrix (Sen Gupta 1969).

The durations of the various instars (in days) have been estimated by workers in the United States (Marcovitch 1934), Germany (von Ehrenhardt 1940), Israel (Bodenheimer 1947) and the Netherlands (Evenhuis 1958). Estimation of developmental thresholds for the various instars have been given by Bodenheimer (1947), Evenhuis (1958) and Asante *et al.* (1991) with comparable results.

The aphids form dense colonies which are easily recognised by the white flocculant covering that is secreted by wax glands along their dorsum. The aphids feed on the woody tissues, new growth and the roots of the tree forming unstructured galls or intumescences. Some varieties of apple are more susceptible to the aphid (for example Granny Smith) and feeding can cause large scale distortion of the new growth, resulting in serious debilitation (Miles 1989a). High populations can lead to early leaf drop at the feeding sites, excessive honey dew deposition on leaves and fruit, and infestation of the fruit itself (Madsen and Bailey 1958). Root infestation has been considered more destructive than aerial infestations, especially to young trees, with high infestations leading to the death of the tree (Patch 1913; Marcovitch 1934; Stanley 1951)

### 3.2.2. *Aphelinus mali*

*Aphelinus mali* (Haldeman) is the primary parasitoid of *Eriosoma lanigerum* and, like its host, the parasitoid is thought to have originated in the Eastern United States (Lundie 1924; Greenslade 1936). Its life cycle has been well documented in the United States (Lundie 1924) and in areas where it was introduced to control WAA (Bodenheimer 1947; Evenhuis 1958).

*A. mali* has been introduced to most areas of the world where WAA has become established. It was first introduced to Australia from New Zealand in 1923 and 1924 to all apple growing states (Newman 1924; French 1924; Jarvis 1925). The introduction of *A. mali* was hailed as the answer to a troublesome problem, but its success has been uneven and chemical control is still widely used against WAA (Bengston 1965; Bower 1987; Holdsworth 1970). It is considered to be a successful example of biological control in Western Australia (Sproul 1981), but in South Australia it is not considered by orchardists as an alternative to chemical control (Chapter Two).

The parasitoid is a solitary endoparasitoid. While several eggs may be laid in a single host, only one adult parasitoid emerges (Bodenheimer 1947; Evenhuis 1958). All life stages can be parasitised including the alates, but third instars are the preferred stage (personal observation; Bodenheimer 1947; Mueller *et al.* 1992). As the parasitoid larva matures the parasitised aphid becomes restless, often moving away from the colony and wax production ceases. The aphid then turns black, dies and the resulting mummy is attached to the substrate along the ventral surface. The adult emerges through a hole cut in the dorso-ventral portion of the mummy. The parasitoid overwinters in the mummy as a fully mature larva or pupa in a diapause induced by short-day photoperiod (Hagen and van den Bosch 1968).

*A. mali* is haplo-diploid, with unfertilised females producing only males and fertilised females giving rise to males and females at a ratio of approximately 1.8F:1M (Evenhuis 1958). The sex ratio varies depending on whether the parasitoids are emerging from diapause (Trimble *et al.* 1990) or from a summer population (Evenhuis 1958). Other factors such as host size, host stage, colony shape and density can also affect the sex ratio (Mueller *et al.* 1992; Asante and Danthanarayana 1993).

The duration of the various life stages of *E. lanigerum* and *A. mali* was determined under laboratory conditions. The longevity and sex ratio of *A. mali* was also estimated to provide background information necessary for further experimentation using this insect. A recent extensive study of aphid and parasitoid development was undertaken in New South Wales (Asante et al. 1991; Asante and Danthanarayana 1992; Asante and Danthanarayana 1993). This study resulted in similar developmental thresholds and developmental times for both insects as those found in other parts of the world (Marcovitch 1934; Bodenheimer 1947; Evenhuis 1958). With this in mind, a set of confirmatory experiments was conducted. If there was any significant deviation from the results elsewhere more detailed experiments would be conducted.

## 3.3. MATERIALS AND METHODS

All apple seedlings, aphids and parasitoids used in the developmental experiments were taken from a laboratory culture (Appendices Two and Three).

### 3.3.1. Woolly apple aphid

Two adult woolly apple aphid females were placed on young Granny Smith (cv) seedling apple trees. A total of 20 trees and 40 aphids were used for each replicate. The aphids were left for one hour to settle on the tree before being transferred to a constant temperature room set at 20±1°C, 12L:12D photoperiod. The aphids were left undisturbed until they produced one or more nymphs. The adults were then removed from the trees and the trees were randomly divided into groups of five. Each group of trees was randomly assigned to one of three temperature treatments: 15±1, 20±1, 25±1°C with a 12L:12D photoperiod. At 12 hour intervals the aphids were observed with a stereomicroscope for evidence of ecdysis (in the form of exuviae) until all nymphs had become adults. The experiment was replicated three times.

### 3.3.2. *Aphelinus mali*

### 3.3.2.1. Developmental times

Twenty, third instar aphids were placed on each of four apple seedlings (Granny Smith cv.) and allowed to settle for two hours. After the aphids had settled, the trees were moved to a 20±1°C constant temperature room. Each tree was caged within a lantern globe (Maxbilt Trading Co., Lantern Glass 285, approximately 1 litre volume; Figure A3.1). Two mated adult female *Aphelinus mali* were introduced to each cage and left for 24 hours. The parasitoids were observed until they started to parasitise the aphids after which they were checked periodically. If the parasitoids did not start to parasitise the aphids after half an hour, they were removed and replaced with a second pair. After 24 hours had elapsed the parasitoids were removed and the trees randomly assigned to four temperature treatments; 15±1, 20±1, 25±1 and 30±1°C. The aphids were observed at 12 hour intervals using a stereomicroscope for signs of parasitism and the time of mummification and emergence was recorded. The experiment was replicated three times. Developmental rates, lower threshold temperatures and thermal constants were determined in the same manner as that used for the aphids.

#### 3.3.2.2. Sex Ratio

At three times during the season (early spring, summer and autumn) 120 mummies were randomly selected from Granny Smith trees in the orchard of the Waite Agricultural Research Institute. The mummies were divided into groups of fifteen and placed in small (7.5 cm) petri dishes and sexed as they emerged. Data were analysed to determine if sex ratios varied with respect to when the mummies were taken from the orchard.

#### 3.3.2.3. Adult life span

In order to determine the life span of the adult parasitoid a large number (> 400) of mummies were removed from the laboratory culture and each one was placed in a small glass vial that had a drop of dilute honey as a food source. The mummies were kept at 20±1°C and checked at 24 hour intervals for emergence. As they emerged, the parasitoids were sexed and placed in one of three constant temperature chambers (15±1, 20±1 and 25±1°C) and either a varying humidity (mean ca. 50%; range 49 - 70%) or a constant RH of 75%. If there were not enough parasitoids emerging to make up groups of a minimum of four females and/or males per treatment per day, they were discarded. Constant humidity was maintained with a saturated solution of NaCl (Winston and Bates 1960). The collection of emerging adults was continued for seven days. The number of days to death were recorded for each sex. The influence of humidity, sex and temperature on parasitoid longevity was compared by mixed model regression analysis (SAS 1985).

### 3.3.3. Statistical analysis

The developmental rates were determined by taking the inverse of the total developmental time. The influence of temperature on developmental rate was analysed by regression analysis (SAS 1985). Lower temperature thresholds for development were calculated by the x-intercept method used by Campbell *et al.* (1974). Thermal constants (degree-day accumulation required to complete development) were estimated at each temperature using the equation: DD=Y(T-t), where Y is the developmental time in days, T is the experimental temperature (a constant temperature), and t is the lower developmental threshold.

Developmental thresholds were compared to previous studies in the following manner. The developmental threshold is defined as the temperature at which the developmental rate is zero (Dent 1991). This is determined by solving the linear equation (Developmental rate=a+bT), describing the relationship between the developmental rate and temperature. The lower developmental threshold therefore is, t=a/b. To compare the estimated threshold with previous studies (Asante *et al.* 1991; Asante and Danthanarayana 1992) the 95% confidence limits were calculated for the estimated slope and intercept. The true upper and lower confidence limits for the threshold were defined as:

$$\text{Upper limit of } t = (a + 95\%\ \text{CI})/(\text{slope} - 95\%\text{CI})$$
$$\text{Lower limit of } t = (a - 95\%\text{CI})/(\text{slope} + 95\%\text{CI})$$

where CI is the confidence interval. These limits were also used to assess the confidence of the DD estimates.

## 3.4. RESULTS

### 3.4.1 Woolly apple aphid

WAA moulted four times before the adult stage. Infrequently, (less than 1%) an aphid would moult five times; these aphids were not included in the analysis. Time spent as a first instar was greater than the duration of the other instars and consistently comprised over a third of the total developmental time (Table 3.1). The other instars ranged from 20 to 23% of the total developmental time. Mean developmental times (either for individual instars or total time) increased with decreasing temperatures.

**Table 3.1.** The durations (mean ± standard error) of the life stages of WAA reared on small apple seedlings at three constant temperatures. Range is presented in round brackets. The values for Asante *et al.* (1991) are presented in square brackets. N is the number of nymphs in that stage.

| | | Life stage/Duration (days) | | | | |
|---|---|---|---|---|---|---|
| Temperature °C | | 1 | 2 | 3 | 4 | total |
| 15 | | 9.4±0.6 | 5.9±0.3 | 5.3±0.2 | 5.5±0.1 | 25.9±0.7 |
| | N | 15 | 14 | 14 | 13 | 13 |
| | | [12.09] | [4.81] | [4.57] | [5.17] | [26.48] |
| 20 | | 5.7±0.2 | 3.8±0.1 | 3.8±0.2 | 4.1±0.1 | 17.1±0.4 |
| | N | 20 | 20 | 17 | 13 | 13 |
| | | [7.65] | [3.18] | [2.95] | [3.15] | [16.85] |
| 25 | | 4.4±0.1 | 3.1±0.1 | 3.0±0.1 | 3.3±0.1 | 13.9±0.1 |
| | N | 18 | 16 | 16 | 16 | 16 |
| | | [6.24] | [2.74] | [2.56] | [2.84] | [13.91] |

There was a significant positive linear relationship (P<0.01) between temperature and developmental rate (Figure 3.1). The lower temperature threshold for total development was estimated as 5.7°C. A total of 250 degree-days are needed above this threshold for the development from birth to adult. Asante et al. (1991) reported a lower developmental threshold of 5.2°C with a total of 268 degree-days needed for development.

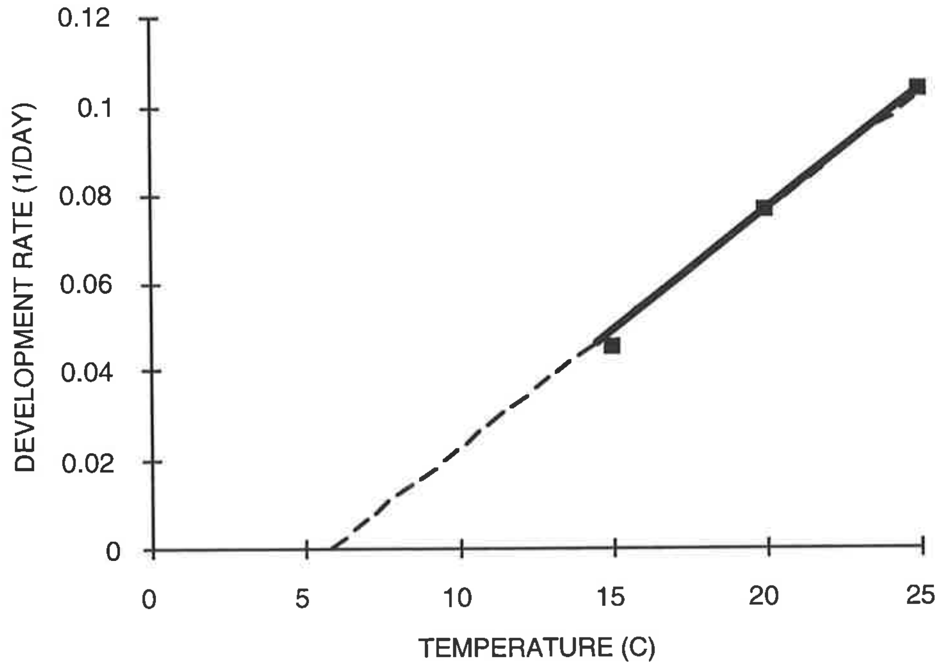


**Figure 3.1.** Relationship between temperature and development rate (1/days to complete development) of WAA. Developmental rate = -0.030716 + 0.0053495 ∗ temperature, $R^2 = 0.86$. Dashed line is the extrapolation to the lower developmental threshold of 5.7°C.

### 3.4.2. *Aphelinus mali*

The duration of the life stages of *A. mali* decreased with an increase in temperature (Table 3.2). Over 50% (range 52 - 59%) of the total development was spent in the pre-mummy stage. There was no significant difference in total development times between males and females and the data were pooled to determine the lower developmental threshold of 9.9°C (Figure 3.2). Asante and Danthanarayana (1992) reported a developmental threshold of 8.3°C for males and females combined. A total of 237 degree-days above the lower threshold is required to complete development compared to 255 degree-days estimated by Asante and Danthanarayana (1992). Their study represented a larger range of temperatures that included 30°C. In the present study the data for 30°C were not included in the determination of the developmental threshold or thermal requirements. The high mortality prior to emergence at this temperature coupled with the deviation from a linear relationship in the regression analysis was justification for its exclusion. There were clear indications in all replicates of an increase in the proportion of males associated with increasing temperature.

The sex ratio of adult parasitoids emerging from mummies collected from the field ranged from 1M:1.8F to 1M:0.7F and did not differ among the three sampling times (P>0.05; spring, summer, autumn). The overall sex ratio was 1M:1.8F.

**Table 3.2.** The durations (mean ± standard error) of development from egg lay to mummification and from mummification to emergence of *A. mali*. Also included are the emergence rates and sex ratios at each temperature.

| | | | Life stage/ Duration (days) | | | |
|---|---|---|---|---|---|---|
| Temperature °C | N | Emergence rate (%) | to mummy | to emergence | total | Sex ratio (M:F) |
| 15 | 53 | 94.3 | 23.0±0.7 | 19.2±0.2 | 42.1±0.7 | 1:9.6 |
| 20 | 141 | 99.3 | 14.9±0.3 | 10.0±0.1 | 24.9±0.3 | 1:7.6 |
| 25 | 41 | 100 | 8.2±0.1 | 7.5±0.1 | 15.6±0.2 | 1:4.1 |
| 30 | 127 | 63 | 7.7±0.1 | 6.2±0.1 | 13.9±0.1 | 1:3 |

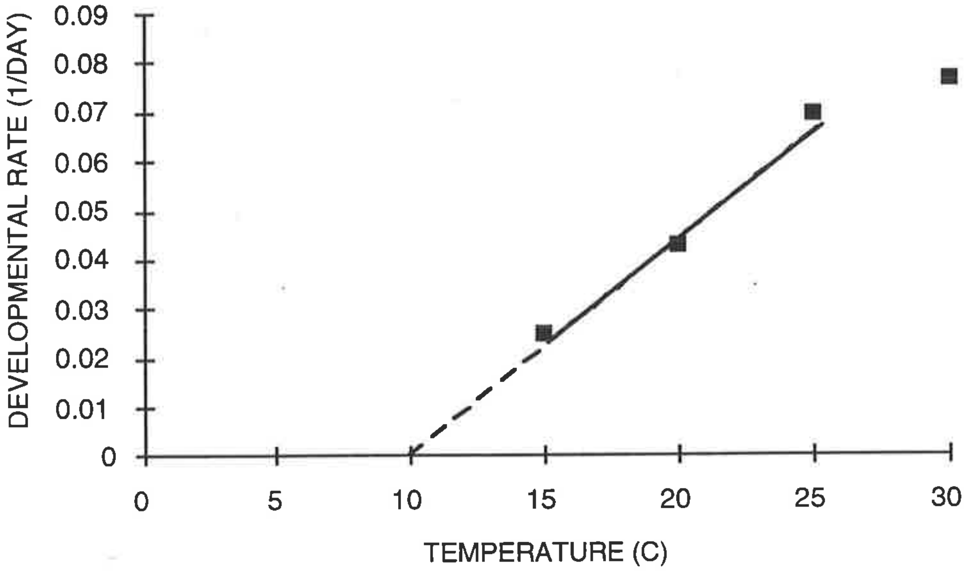


**Figure 3.2.** Relationship between temperature and development rate (1/days to complete development) of *A. mali*. Developmental rate = -0.043356 + 0.0043814 $*$ temperature, $R^2 = 0.86$. The data for 30°C was not used in estimating the lower developmental threshold. Dashed line is the extrapolation to the lower developmental threshold of 9.9°C.

Adult female *A. mali* lived longer ($P<0.05$) than males at all temperatures (Table 3.3). Life span was longest at 15°C and shortest at 30°C for both sexes but there was a great deal of variation in the life span at all temperatures. Changes in humidity had no effect on the mean life span of either sex.

**TABLE 3.3.** Mean (± standard error) life span (days) of male and female *A. mali* at three constant temperatures.

| | | Temperature °C | | | |
|---|---|---|---|---|---|
| | | 15 | 20 | 25 | 30 |
| Life span | | | | | |
| Male | | 13.4±1.5 | 4.8±0.3 | 4.1±0.3 | 3.4±0.3 |
| | N | 43 | 44 | 44 | 55 |
| Female | | 19.1±1.8 | 6.7 ± 0.6 | 5.2 ± 0.3 | 3.5±0.3 |
| | N | 61 | 59 | 60 | 51 |

## 3.5. DISCUSSION

The estimated lower temperature threshold was slightly higher but not significantly different than that determined for WAA populations in New South Wales (Asante *et al.* 1991; Asante and Danthanarayana 1992). However, the total developmental times (in days) were in close agreement (Table 3.1). There was less agreement between the estimated thermal constants with a difference of 17 degree-days between Asante *et al.* (1991; 268 DD) and the present study (250 DD). This is probably due to the larger sample sizes and greater range of temperatures used by Asante *et al.* (1991). There are other possibilities for the differences such as geographical locality, rearing conditions of the experimental populations or even light intensity (Campbell *et al.* 1974; Taylor 1981; Dixon *et al.* 1982). Factors such as density, source of insects (ie; laboratory culture or field), apple variety and humidity can all exert an influence on insect development which would lead to differences in estimates of thresholds and developmental times. The similarity between the values in other studies world-wide (Bodenheimer 1947; Evenhuis 1958; Bonnemaison 1965; Asante *et al.* 1991) most probably reflects the fact that the aphid is using the same host (apple) and the host plant tends to be grown in areas of similar climatic conditions.

The time spent as a first instar larva was the largest proportion of the life cycle, with the exception of the adult stage, and this confirmed earlier studies (Marcovitch 1934; Bodenheimer 1947; Evenhuis 1958; Asante *et al.* 1991). This was not the case for woolly apple aphid populations in India where all instars were of equal duration (Gautam and Verma 1983).

Total developmental times for *A. mali* were greater than those of its host and correspondingly, the lower temperature threshold was higher (Table 3.2). The lower developmental threshold was about 1.5°C higher than the estimate by Asante and Danthanarayana (1992). However, this difference is not significant. As temperature decreased, the difference between the developmental times for the two insects increased (42 and 26 days for *A. mali* and WAA respectively at 15°C). Thus, WAA has a competitive edge as temperatures drop below 25°C. The theoretical temperature threshold for post diapause *A. mali* was estimated at 9.4°C (Trimble *et al.* 1990). The insects used in the present study were not from pre or post diapausing mummies and the total development times were similar to earlier studies (Bodenheimer 1947; Evenhuis 1958; Bonnemaison 1965).

Aphelinid females are synovigenic; unfertilised females produce only males while fertilised females produce both sexes (Viggiani 1984). The sex ratio observed was similar to that for *A. mali* populations in the Netherlands (1M:.8F in South Australia compared to 1M:1.5F). There is a certain degree of variability within populations depending on host size, stage and colony size (Mueller *et al.* 1992; Asante and Danthanarayana 1993) as well as the availability of males. Sex ratio can also be determined by environmental factors such as temperature (Godfray 1994). This may explain the increased proportion of males with increased temperature. This is an area that has had little research and should be further investigated.

Female *A. mali* lived longer than males at all temperatures which is characteristic for some Aphelinids (Viggiani 1984) and has been reported elsewhere (Bonnemaison 1965). Life span was shortest at 30°C indicating an upper threshold in agreement with other studies (Bodenheimer 1947). At this temperature emergence rates were also low which was not specifically stated but implied by Asante and Danthanarayana (1992).

The developmental rates and subsequent temperature thresholds of woolly apple aphid and *A. mali* give an indication of the complex interactions between them. At warmer temperatures (ca. 25°C) their developmental durations are similar but as temperatures drop the difference in developmental time between the two increases, resulting in a competitive edge for the aphid. With higher temperatures around 30°C - a not uncommon phenomenon in South Australian summers - the parasitoid suffers from low emergence rates and short adult life spans. Once again the aphid is able to increase unchecked, albeit at reduced rate. With *A. mali*'s relatively high temperature threshold of 9.4°C for post-diapause development (Trimble *et al.* 1990), WAA has time at the beginning of the season to increase to levels that cannot be successfully controlled. In moderate climates *A. mali* is considered a successful method of control (Mueller *et al.* 1992), but this control is erratic for reasons that remain unclear. Recent studies have indicated that this parasitoid has greater success in parasitising small, less crowded aphid colonies and that the waxy covering provides a measure of protection to colonies (Mueller *et al.* 1992). However, these types of colonies would be largely present in the spring when the parasitoid is still dormant. In South Australia parasitoid populations do not increase rapidly enough in the spring allowing the aphid to increase to levels that are difficult to control. In spite of the fact that *A. mali* is able to match the generation time of WAA by the summer it is still unable to control the aphid. Other factors that may be causing this lack of control are investigated in later chapters.

# CHAPTER FOUR

# INSECTICIDE TOXICITY TO WOOLLY APPLE APHID AND ITS PARASITOID, *Aphelinus mali*, UNDER LABORATORY CONDITIONS

## 4.1. ABSTRACT

Nine insecticides were tested at recommended rates of application against woolly apple aphid colonies on small trees under laboratory conditions. Chlorpyrifos was most toxic and killed more than 95% of the colonies and winter oil was least toxic. The waxy exudate of the aphids appeared to give some degree of protection against insecticidal sprays, especially to those individuals near the centre of colonies.

The toxicity of 10 orchard insecticides was tested on three life stages of the hymenopteran parasitoid, *A. mali*. Life stages tested were pre-mummy (early larval), post-mummy (late larval) and adult. The longevity of adults surviving treatment when early or late larvae was compared to a control. Adult mean time to death after being treated with the pesticides as adults was compared to a water control. The insecticides represented the organochlorines, organophosphates, carbamates, pyrethroids and a dormant oil. Parasitised aphids in the post-mummy stages were treated on small apple seedlings and in a Potter's Tower.

There was little difference in adult longevity of parasitoids that emerged from aphids treated at the pre-mummy stage. However, treatments had an effect on the parasitised aphids, resulting in a reduced number of parasitoids. Parasitoids treated at the post-mummy stage on trees gave mixed results suggesting

other factors may have come into play. Adult longevity was significantly reduced in parasitoids that were treated in the post-mummy stage in a Potter's Tower with azinphos-methyl, malathion and carbaryl.

Adult parasitoids were susceptible to all of the organophosphates and the organochlorine within the first 24 hours of treatment. Oil, fenoxycarb, pirimicarb and fluvalinate had no effect on adult survival.

## 4.2. INTRODUCTION

Control of woolly apple aphid (WAA) has been achieved with varying degrees of success by three different methods: resistant rootstock, introduction of a parasitoid and use of chemical sprays.

Certain rootstocks (Northern Spy and the MM series) can inhibit root infestation but some aphid biotypes have been able to overcome this resistance (Giliomee *et al.* 1968; Sen Gupta 1969; Rock and Zeiger 1974; Cummins *et al.* 1981; Young *et al.* 1982). In spite of this, resistant rootstocks are still the most effective and widely used method of controlling root infestations in South Australian orchards (Chapter Two).

Biological control is being used against above-ground infestations of WAA. *Aphelinus mali*, the only parasitoid attacking WAA, has been introduced to most apple growing regions in the world (Howard 1929; Yothers 1953). Its success has been variable, however, (Evenhuis 1962) and in South Australia adequate control of WAA has not been achieved, nor did growers recognise it as a control method (Chapter Two). Predators, primarily Coccinellidae, Hemerobiidae and Chrysopidae reduce aphid numbers most frequently in low pesticide or 'organic' orchards (Ravensberg 1981). High populations of the European earwig, (*Forficula* *auricularia* (L)), reduce WAA colonies in the Netherlands (Noppert *et al.* 1987; Stap *et al.* 1987; Mueller *et al.* 1988), but this earwig is considered a pest in its own right in some orchard areas of America (Carroll and Hoyt 1984).

Above-ground colonies are most commonly controlled by pesticide application. The organophosphorous compound, vamidothion, is the standard and most effective aphicide for WAA but a range of other insecticides are also registered against it (Worthing 1991). Orchardists in the Adelaide Hills region use vamidothion and seven other insecticides against WAA (Chapter Two; Baker unpublished data). In Australia vamidothion has a withholding period (last application prior to harvest) of 42 days which prevents multiple applications in a season. Applications of vamidothion in the Adelaide Hills have increased from one application every two to three years to one per year.

In most apple growing areas, WAA is considered a secondary rather than a major pest (Croft and Hoyt 1983) with the Netherlands being the exception (Noppert *et al.* 1987). Major pests such as codling moth (*Laspeyresia pomonella* (L)) normally dictate the main features of an orchard spray schedule and control of less economically important pests must be compatible with these programs. Fortuitously, the application of azinphos-methyl (APM) against codling moth also reduces WAA numbers throughout the season (Penman and Chapman 1979, 1980). However, control by APM ceases prior to harvest when application has to be stopped. With its rapid rate of increase (Evenhuis 1958; Asante *et al.* 1991) WAA can then still reach high levels by the end of the growing season. The organochlorines can be effective in controlling WAA, but both organophosphates and organochlorines have adverse effects on other insects in the orchard complex, notably parasitoids and generalist predators (Brown 1977; Theiling and Croft 1988).

One of the disadvantages of chemical intervention in any horticultural system is the pressures that are placed upon the beneficial insects. While a large number of pest species can rapidly and very effectively develop resistance to pesticides, many predators and parasitoids remain susceptible (Brown 1977). There are exceptions to this, such as the phytoseiid mites, *Typhlodromus occidentalis* Nesbitt and *T. pyri*, which are resistant to organophosphates (Hoy 1985). Unfortunately situations such as this are rare (Havron *et al.* 1987) and until suitable alternatives to pesticides can be found to control key pests like codling moth and leafrollers there will always be enormous pressure on beneficials within orchards and biological control will remain difficult.

Woolly apple aphid is only one of a variety of pests in South Australian orchards that is controlled by pesticide applications. The number and amount of pesticides used in South Australian apple orchards to control insects is considerable with some applied as frequently as every seven to 10 days (Chapter Two; Wicks and Granger 1989). Thus, all life stages of the woolly apple aphid's parasitoid, *Aphelinus mali,* come into contact with one or more pesticides. Life stages within the mummified bodies of aphid hosts are considered least susceptible to pesticides, suggesting opportunities for selective timing of pesticide application (Stary´ 1970). However, it was shown that insecticides can have a negative effect on parasitoids inside mummies (Purcell and Granett 1985; Hsieh and Allen 1986; Krespi *et al.* 1991). Malathion and parathion differentially affected emergence of adult *A. mali* from treated mummies, with the late pupal stage most susceptible (Evenhuis 1958; El-Haidari and Georgis 1978). Full grown larvae were not affected at all.

With such a variety of insecticides used in the orchards of South Australia it was of interest to determine what effect they have on WAA and its parasitoid. Survival of aphid populations was measured following treatment by the pesticides. A laboratory-based experiment using small trees was designed to minimise effects such as weather (rain, sunlight), other chemicals or destruction of colonies through predation or parasitism. The effects of commonly used pesticides on three life-stages of *A. mali* were investigated. Post treatment emergence rates and subsequent adult longevity of the early and late larval stages were recorded. Adult parasitoids were treated with the same pesticides and their survival rate and longevity were compared to a water control. The insecticides included carbamates, organophosphates (OP), an organochlorine (OC), a pyrethroid and winter oil.

## 4.3. MATERIALS AND METHODS

All rearing and experimentation was done at 25±2°C, under a 16L:8D.

### 4.3.1. Aphid colonies

Colonies of WAA were established on three-month old Granny Smith apple seedlings similar in height and vigour. Infestation was established by placing 10 fourth instar aphids on the top leaves of the trees. A layer of Tac-Gel™ around the lower stem kept aphids from clustering around the soil-stem interface where they would be protected from any sprays applied to the tree. The trees and aphids were placed in a controlled environment chamber for 11 days so that colonies could become established and all life stages would be present.

Trees were randomly placed in groups of five and assigned to one of nine sprays; eight pesticides, water plus a wetting agent and a water control (Table 4.1). The order of sprays was randomly assigned. Immediately before application each spray was mixed with distilled water to field rate concentrations as described on the label. To each spray, a commercial surfactant (Cittowet®) was added at the field rate (0.1% v/v). Prior to spraying, colonies on each tree were counted. Each tree was placed on an electric rotating wheel and sprayed for 10 seconds for complete and even coverage. Earlier trials showed that this was the point of 'run-off' for the size of tree and speed of wheel rotation. Sprays were applied with a pressurised hand sprayer (Hills™; one litre capacity) pumped to maximum pressure. Sprayer output was 200 ml/minute and was consistent over several applications. Once sprayed, the trees were left in a fume-hood to air dry before returning to the controlled environment chamber for 24 hours.

**Table 4.1.** Trade names, common name, class and field rates of 10 pesticides tested against WAA colonies and *Aphelinus mali*. Fenoxycarb was used only on *A. mali*. AI = active ingredient.

| Trade Name | Common name | Class | AI Field Rate (gm/100 litres) |
|---|---|---|---|
| Pirimicarb | pirimicarb | dimethylcarbamate | 25.0 gm |
| Insegar | fenoxycarb | carbamate | 10.0 gm |
| Sevin | carbaryl | methylcarbamate | 100.0 gm |
| Kilval | vamidothion | organophosphate | 50.0 gm |
| Gusathion | azinphos-methyl (APM) | organophosphate | 5.25 gm |
| Lorsban | chlorpyrifos | organophosphate | 25.0 gm |
| Malathion | malathion | organophosphate | 100.0 gm |
| Thiodan | endosulfan | organochlorine | 66.5 gm |
| Klartan | fluvalinate | pyrethroid | 4.8 gm |
| Oil | - | petroleum | 15 l |

Treatments were kept as far away from each other as possible to minimise possible effects of chemical volatilisation. Chlorpyrifos was tested separately with a water control due to its high volatility. An initial spray trial compared two possible controls; water and water with 0.1% Cittowet® to investigate any aphicidal effects that the surfactant may have had.

After 24 hours the number of living and dead colonies were recorded. Within each colony the number of live and dead aphids were counted; mortality was confirmed by the absence of movement when touched with the tip of a fine brush. The experiment was repeated three times.

### 4.3.2. *Aphelinus mali*

#### 4.3.2.1. Pre-mummy (larval) stage

Colonies of WAA were initiated in the same manner described in Section 4.3.1. After eight days infested trees were placed singly in lantern-globe cages (Appendix Three) with four mated female *Aphelinus mali*. The parasitoids were placed in glass vials to mate and observed until the first copulation after which they were left for a minimum of eight hours before being separated and the females introduced to the lantern globes. After 24 hours the parasitoids were removed and the trees left in the chamber for another four days. The trees were then randomly assigned to groups of five and allocated a spray treatment. The trees and colonies were sprayed to run-off with a hand-held sprayer (Section 4.3.1). The trees were left to dry in a fume hood and replaced in the controlled environment chamber for 48 hours. Newly formed mummies were removed from the trees and individually placed in small glass vials which had a drop of diluted honey as a food source for the emerging parasitoid. As the parasitoids emerged, sex and time to death (days) were recorded. Emergence or survival rates were also determined. The experiment was replicated three times.

#### 4.3.2.2. Mummies

Aphids were parasitised in the same manner described in the previous section. Trees with parasitised colonies were left for eight days so that the maximum number of mummies had developed. Spray application and post spray treatment was done in the same manner as for the early larval stage. Mummies were not treated with chlorpyrifos because of its volatility and effect on the insects on other trees within the room. Emergence, sex and longevity were recorded for each parasitoid. The experiment was replicated three times.

In a second experiment, 495 mummies were divided into groups of 15 and each group assigned to one of 11 treatments; the pesticides listed in Table 4.1. The experiment was repeated three times. Mummies were placed on pieces of filter paper (Watman #1) in 7 cm petri dishes and sprayed in a Potter's Tower (Potter 1952) with 2.5 ml/treatment at a pressure of 103 kPa. All pesticides were applied at field rates. After drying, the mummies were placed in individual vials with honey and held until emergence. Emergence rates, sex and longevity were recorded.

#### 4.3.2.3. Adult parasitoids

Newly emerged (less than 24 hours) male and female parasitoids were placed into three groups of equal numbers for each sex. There were 21 to 40 females and 11 to 20 males in each group depending on emergence levels for that day. Each group was assigned to one of three treatments: a water control and two pesticides.

Cages similar in design to those described by Kühner *et al.* (1985) were made by sandwiching an aluminium frame (100 mm length, 100 mm width, 20 mm height) between two sheets of 3 mm glass and held together with rubber bands (Figure 4.1B). Two sides of the frame had three holes (7 mm diameter). All three holes on one side and two of the holes on the second side were covered with fine gauze to provide ventilation. The centre hole of the second side was left uncovered so that parasitoids could be introduced to the cage and the air source attached. Six cages were attached to a double-ended vacuum pump by 5 mm plastic tubing; three cages on each side (Figure 4.1A). Air flow was at a rate of 340 cm$^3$ per minute and was passed through a water trap before entering the pump to prevent cross-contamination of the cages.

Pesticides were applied at field rates to the glass plates with a hand-held pressurised sprayer. Enough spray was applied to the glass in a two second burst to provide a complete, even film of droplets. The glass was allowed to air dry before the cage was assembled. Parasitoids were always introduced to the cage on the day that the plates had been sprayed (approximately 5 hours later). Drops of honey and water were placed on the aluminium sides of the cages to provide a food source. After assembly, the parasitoids were introduced to the cages and left for 24

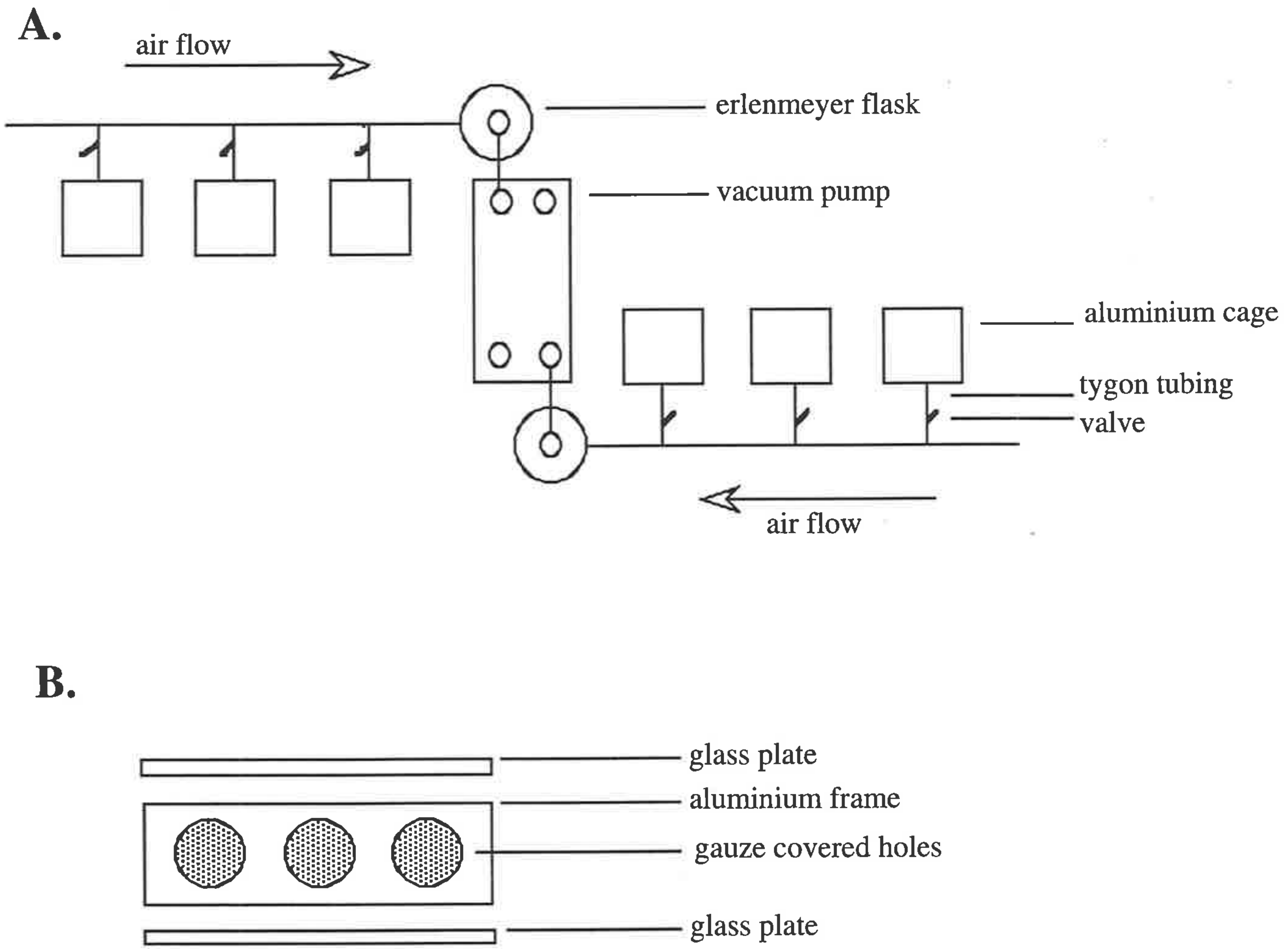

A.
air flow
erlenmeyer flask
vacuum pump
aluminium cage
tygon tubing
valve
air flow
B.
glass plate
aluminium frame
gauze covered holes
glass plate

**Figure 4.1.** Aluminium spray cages for adult *A. mali*. (A) overall plan. Each cage held either male or female wasps. Plates were sprayed with a water control or a pesticide. Air from the cages was filtered through 200 ml water in the erlenmeyer flask to prevent contamination. (B) exploded plan of cage. Gauze covered holes were on the front and back of the cage; sides were left as solid aluminium. The sandwich was held in place by thick rubber bands.

hours before being removed. Each living parasitoid was placed in a vial with a food source. Deaths were recorded daily.

Due to limitations of equipment, space and parasitoids, only two pesticides and the control could be tested at any one time. Treatments were replicated three times unless all parasitoids in the pesticide-treated cages were dead after 24 hours. In these cases treatments were replicated twice.

### 4.3.3. Statistical analysis

Per cent reduction of unparasitised WAA colonies was estimated by the equation:

$$R = 100-(A/B) * 100$$

where A is the number of live colonies post-treatment and B is the number of live colonies pre-treatment (Abbott 1925). Per cent reduction in live colonies was transformed (arcsin $\sqrt{x}$). Comparisons among treatments were made by main factor ANOVA and SNK multiple comparison test (SAS 1985).

Parasitoid life spans after treatment at the pre- and post-mummy stages were compared among treatments and replicates by a factorial model (with interaction) ANOVA. As males and females have different life spans (Chapter Three) the sexes were analysed separately. Dunnett's test (SAS 1993) was used to compared the longevity of adults emerging from treated mummies to that of the adults emerging from mummies treated with water (control).

Differences in adult mortality after treatment (cage experiment) were compared by analysis of deviance (ANODE; Genstat 1987) because the data had a poisson distribution. Differences among sprays were sufficiently marked that further testing by multiple comparison was not warranted. Due to the variability in life spans within control treatments, comparisons were not made among 'triplets' (control and two insecticides) but only between replicates of each triplet.

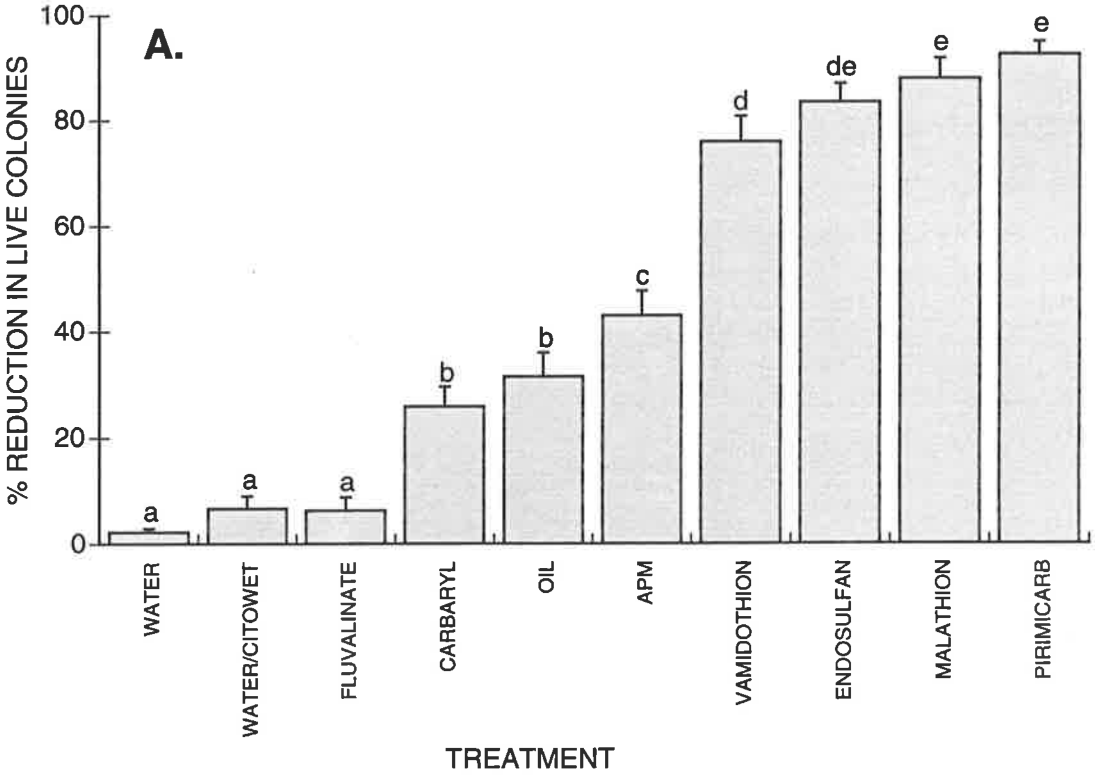
## 4.4. RESULTS

### 4.4.1. Aphid colonies

Aphid numbers in colonies varied greatly prior to treatment in spite of the care taken during initial infestation. Pre-treatment numbers could not be estimated accurately without disturbing or destroying colonies resulting in poor estimates of the reduction in aphid numbers. Therefore, no further attempt was made to estimate aphid numbers and only the deaths of whole colonies were analysed.

There were no differences in colony number ($P>0.05$) after being sprayed with water or water mixed with Cittowet®. Therefore, only water was used as a control in the remaining experiments.

Reduced colony numbers were most marked when treated with vamidothion, endosulfan, malathion, pirimicarb and chlorpyrifos (Figure 4.2A, B). Within eight hours the 'wool' covering of the colonies had deteriorated and was falling off. The aphids in these colonies were exhibiting signs of stress, wandering down stems, dropping to the ground and dying. After 24 hours, the remaining colonies had few aphids, usually first instars, and the majority of these were obviously dying. The most protected colonies, such as those in the leaf axils, remained intact except for minor damage to the wool and aphids around the edges. Colonies that had been sprayed with vamidothion appeared to have a greater number of moribund individuals remaining in the colonies. The APM treatment had significantly less of a reduction of colony numbers ($P<0.05$) than the other organophosphates (Figure 4.2A). Colonies with one or two aphids remained after being sprayed.



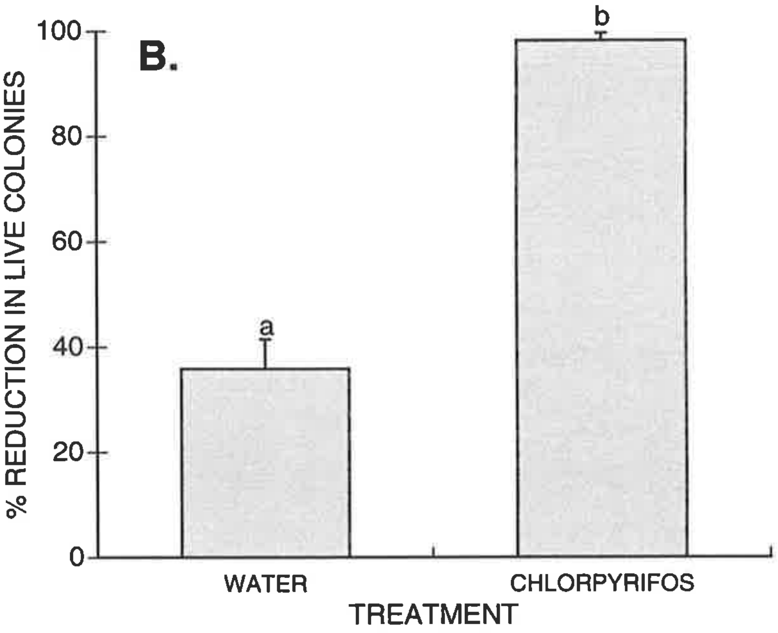


**Figure 4.2.** Mean per cent reduction (± standard error) of live WAA colonies 24 hours post spray.
(A) Treatments were done concurrently.
(B) Chlorpyirfos was done separately with a water control due to its high volatility. Values were adjusted using Abbott's formula.
Treatments with the same letter are not significantly different ($P>0.05$).
APM = Azinphos-methyl.

There was no difference in colony reduction between the treatments with winter oil and carbaryl, however, they both differed significantly from the control ($P<0.05$; Figure 4.2A). The carbaryl appeared to reduce the numbers of aphids in each colony (evidenced by dead individuals) but did not destroy many of the colonies entirely. The oil spray disintegrated the wool covering but left considerable numbers of young aphids apparently unharmed. Colonies that appeared most heavily affected were those that had a greater number of adult aphids.

Chlorpyrifos had a dramatic effect on the colony numbers of both treated and control trees. There were considerably more colonies killed on the control trees than in the experiments with the other insecticides. Reduction in colony numbers was significantly greater ($P<0.05$) in the chlorpyrifos treatment than in the water treatment (Figure 4.2B).

### 4.4.2. *Aphelinus mali*

#### 4.4.2.1. Pre-mummy (larval) stage

All parasitised aphids treated with pirimicarb, vamidothion and endosulfan were killed, resulting in the complete absence of parasitoid mummies. Only four parasitised aphids developed to the mummy stage following treatment with malathion. With the difficulty in accurately estimating the total number of parasitised aphids prior to spraying it is not possible to determine the survival rate after treatment. Therefore, N refers to the total number of mummies removed from the tree and not the total number of parasitised aphids. With the exception of fluvalinate and malathion the number of mummies formed were similar to the water controls (Table 4.2). There were no differences in the development time (time to emergence) among treatments. With the exception of the larvae treated with fluvalinate emergence rates were above 90 per cent and none of these levels differed significantly from the control. Of the unemerged mummies whose stage could be identified the largest proportion (56%) were at the early larval stage which indicates that the spray may have had an immediate effect on the insect. There were no differences in male life span among the treatments (P>0.05; Figure 4.3). However, females that were treated with carbaryl and fenoxycarb had significantly greater life spans than their counterparts in the water treatment. Sex ratio was variable ranging from near normal (1M:1.4F) in the controls to heavily female biased in the APM treatment (1M:3.4F). When compared using a Chi Square test (SAS 1989) the sex ratio of the APM and fenoxycarb treated parasitoids were significantly different from the overall sex ratio (1M:1.8F) ($X^2_{(6)}=29.74$, $P<0.0001$).

**Table 4.2.** Per cent emergence, mean time to emergence, sex ratio and mean life span of *A. mali* after being treated with pesticides at the pre-mummy (larval) stage.

| Pesticide | N | % emergence | $\overline{X}$ time to emergence (days±SE)† | sex ratio (M:F) | | $\overline{X}$ adult life span (days±SE) |
|---|---|---|---|---|---|---|
| water | 206 | 90.8 | 18.8±0.12 | 1:1.4 | male | 10.5±0.89 |
| | | | | | female | 12.4±0.87 |
| carbaryl | 135 | 97.0 | 17.1±0.09 | 1:2.6 | male | 13.8±1.29 |
| | | | | | female | 17.1±0.95 |
| APM | 173 | 96.5 | 17.1±0.09 | 1:3.4 | male | 11.3±1.31 |
| | | | | | female | 13.1±0.79 |
| fenoxycarb | 235 | 94.0 | 17.5±0.07 | 1:1.2 | male | 11.7±0.69 |
| | | | | | female | 16.5±1.70 |
| fluvalinate | 85 | 85.9 | 16.8±0.13 | 1:2.1 | male | 8.6±1.27 |
| | | | | | female | 13.4±0.94 |
| malathion | 4 | 100.0 | 16.5±0.87 | 1:3 | male | 21.0 |
| | | | | | female | 21.3±1.20 |
| oil | 201 | 94.5 | 17.0±0.09 | 1:1.6 | male | 11.5±1.00 |
| | | | | | female | 15.2±1.93 |

† There was no difference between sexes for time to emergence, therefore the data were pooled.

#### 4.4.2.2. Mummies

The emergence rates of parasitoids treated in the mummy stage while on the trees were consistently high and there were no differences in emergence rates among treatments. (Table 4.3).

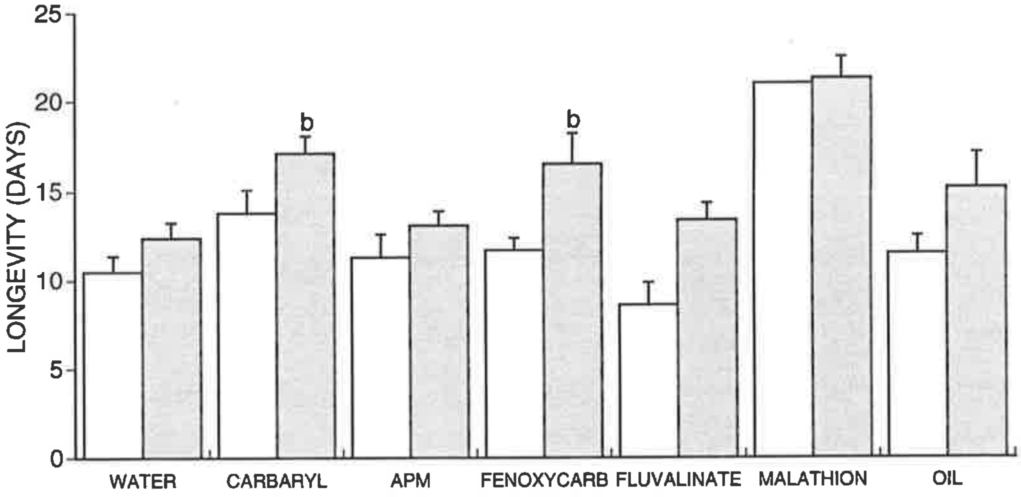


**Figure 4.3.** Life span (mean ± standard error) (days) of *A. mali* adults emerging after treatment at the pre-mummy stage (four days after oviposition). There were no significant differences in life span among treatments for males (P>0.05).
Bars capped with a letter are significantly different from the water control (P<0.05) for females.
APM = azinphos-methyl.

Males ☐ Females ▨

**Table 4.3**. Per cent emergence, mean time to emergence, sex ratio and mean life span of *A. mali* after being treated with pesticides at the post-mummy stage. Mummies were sprayed while still on the tree.

| Pesticide | N | % emergence | $\overline{X}$ time to emergence (days±SE)† | sex ratio (M:F) | $\overline{X}$ adult life span (days±SE) | |
|---|---|---|---|---|---|---|
| water | 98 | 96.9 | 16.4±0.16 | 1:4.3 | male | 9.4±1.36 |
| | | | | | female | 10.1±0.90 |
| pirimicarb | 173 | 97.7 | 16.5±0.12 | 1:3.1 | male | 6.9±0.90 |
| | | | | | female | 6.1±0.70 |
| carbaryl | 228 | 96.1 | 17.2±0.14 | 1:1.9 | male | 6.8±0.66 |
| | | | | | female | 7.3±0.66 |
| APM | 81 | 93.8 | 16.2±0.17 | 1:0.9 | male | 10.3±0.90 |
| | | | | | female | 4.8±1.33 |
| fenoxycarb | 123 | 96.7 | 16.7±0.16 | 1:1.6 | male | 9.5±0.85 |
| | | | | | female | 8.0±0.92 |
| vamidothion | 134 | 97.8 | 15.8±0.12 | 1:3 | male | 7.1±1.00 |
| | | | | | female | 9.3±0.80 |
| fluvalinate | 124 | 95.2 | 16.8±0.15 | 1:3.0 | male | 11.6±1.07 |
| | | | | | female | 17.0±0.84 |
| endosulfan | 143 | 98.6 | 15.9±0.12 | 1:2.4 | male | 11.9±0.89 |
| | | | | | female | 13.3±0.80 |
| malathion | 105 | 96.2 | 16.9±0.15 | 1:1 | male | 17.1±0.81 |
| | | | | | female | 16.8±1.10 |
| oil | 142 | 96.5 | 15.9±0.13 | 1:3 | male | 8.1±0.99 |
| | | | | | female | 10.9±0.78 |

† There was no difference between sexes for time to emergence, therefore the data were pooled.

Of the unemerged mummies, the greatest proportion (47%) were in the pupal stage compared to 37% and 18% for the adult and larval stages respectively. There were no differences between treatments ($P>0.05$) in the time to emergence. Females in the pirimicarb and APM treatments had significantly shorter life spans than the females in the water treatment ($P<0.05$). In contrast, the females in the fluvalinate, endosulfan and malathion treatments lived significantly longer than the females emerging from the water treated mummies ($P<0.05$; Figure 4.4A). Males in all but one of the treatments had similar life spans to those in the control treatment. As with the females, the males emerging from mummies that had been treated with malathion had longer life spans than the males emerging from the water treated mummies. This increase in life span for individuals from malathion treated mummies was consistent in all replicates for both sexes. Again sex ratio was variable with a highly female bias (1M:4.3F) in the water treatment to a slightly male bias in the APM treatment (Table 4.3). Chi Square analysis showed that the water, APM and malathion treatments were significantly different from the overall sex ratio (1M:2.2F) ($X^2_{(9)}=54.32$, $P<0.0001$).

Mummies that were removed from the trees and treated in a Potter's Tower showed the greatest variation in emergence rates (Table 4.4) with malathion and chlorpyrifos treated mummies having the lowest rate of emergence (68.9 and 15.6% respectively). Emergence rates from the carbaryl, APM, malathion and chlorpyrifos were significantly different from the water treatment ($P<0.05$). The other treatments showed similar emergence rates to the mummies treated on the trees. There were no differences among treatments in the average time taken to emerge. Nearly all of the unemerged mummies were fully formed adults - 86% - compared to 11% and 3% for pupal and late larval stages respectively. There was more variation in adult longevity among the treatments in the Potter's Tower experiment than in the adults emerging from mummies that were treated on the trees. Females in five of the treatments had significantly different ($P<0.05$) life spans than the females in the control treatment (Figure 4.4B). The average life span of these females was less than those in the water treatment. All males emerging from treated mummies had significantly shorter life spans than the males from the control treatment with the exception of the chlorpyrifos treatment ($P<0.05$; Figure 4.4B). There was less variation in the sex ratios among treatments with no significant departure from the overall ratio of 1M:1.8F ($X^2_{(10)}=0.113$, $P>0.05$).

**Table 4.4**. Per cent emergence, mean time to emergence, sex ratio and mean life span of *A. mali* after being treated with pesticides at the post-mummy stage. Mummies were removed from the tree and sprayed in a Potter's Tower.

| Pesticide | N | % emergence | $\overline{X}$ time to emergence (days±SE)† | sex ratio (M:F) | $\overline{X}$ adult life span (days±SE) | |
|---|---|---|---|---|---|---|
| water | 45 | 97.8 | 13.4±0.26 | 1:3 | male | 23.5±2.08 |
| | | | | | female | 21.8±1.61 |
| pirimicarb | 45 | 91.1 | 13.9±0.27 | 1:2.4 | male | 15.3±2.00 |
| | | | | | female | 18.6±1.71 |
| carbaryl | 45 | 84.4 | 13.4±0.27 | 1:2.2 | male | 4.2±2.00 |
| | | | | | female | 10.5±1.81 |
| APM | 45 | 82.2 | 13.6±0.24 | 1:2.7 | male | 6.8±2.19 |
| | | | | | female | 4.4±1.78 |
| fenoxycarb | 45 | 91.1 | 13.2±0.30 | 1:1.6 | male | 9.6±1.73 |
| | | | | | female | 13.0±1.88 |
| vamidothion | 45 | 97.8 | 12.8±0.24 | 1:1.3 | male | 14.2±1.58 |
| | | | | | female | 15.0±1.84 |
| fluvalinate | 45 | 100 | 14.0±0.24 | 1:2.5 | male | 14.0±1.92 |
| | | | | | female | 15.9±1.66 |
| endosulfan | 45 | 97.8 | 13.2±0.26 | 1:0.8 | male | 13.8±1.41 |
| | | | | | female | 19.8±2.06 |
| malathion | 45 | 68.9 | 13.7±0.26 | 1:2.1 | male | 9.0±2.19 |
| | | | | | female | 7.4±2.06 |
| oil | 45 | 91.1 | 13.3±0.23 | 1:1.2 | male | 15.2±1.59 |
| | | | | | female | 22.8±2.01 |
| chlorpyrifos | 45 | 15.6 | 14.3±0.84 | 1:2.5 | male | 14.0±4.89 |
| | | | | | female | 16.0±4.12 |

† There was no difference between sexes for time to emergence, therefore the data were pooled.

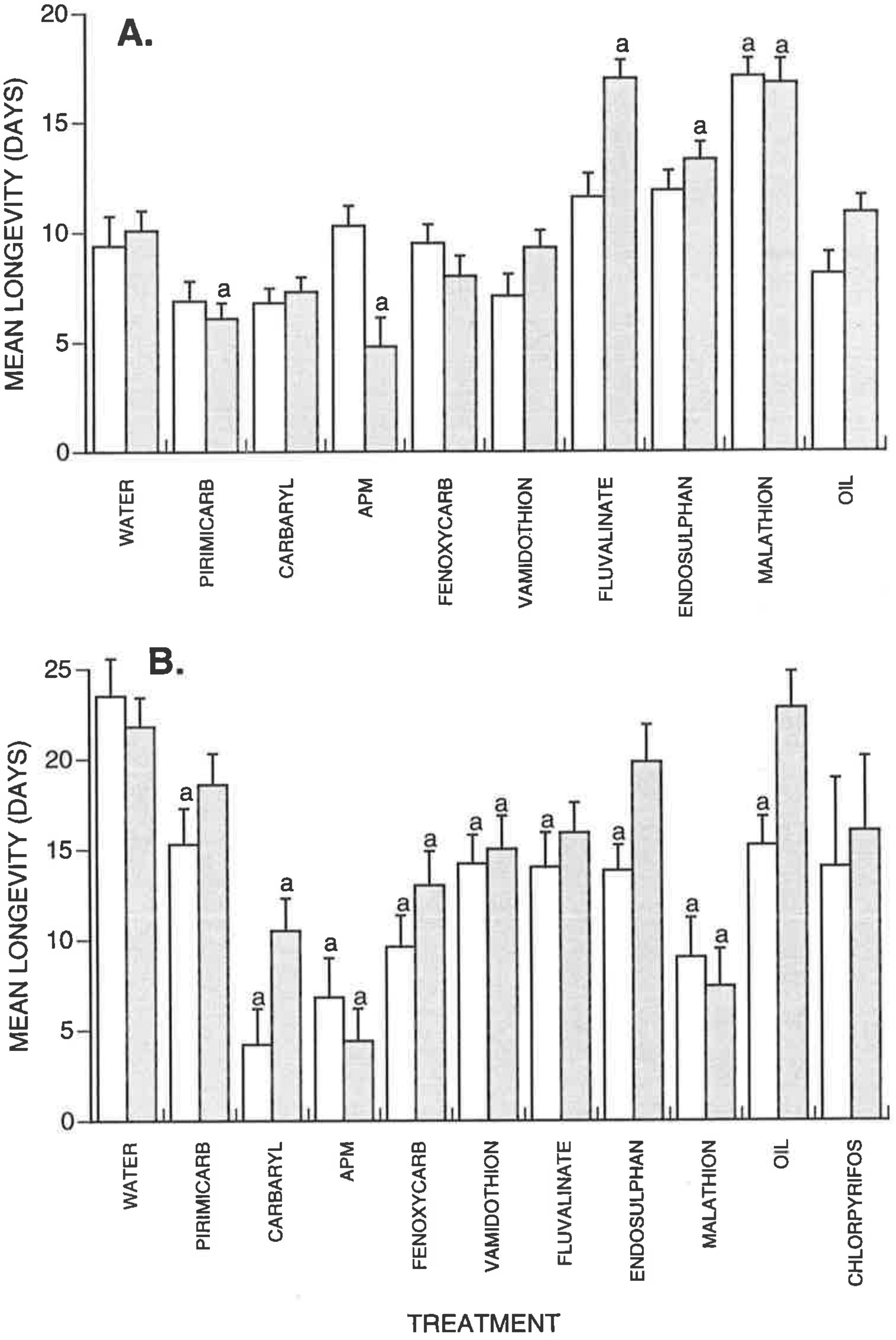


**Figure 4.4.** Life span (mean ± standard error) of emerged adult *A. mali* after treatment with pesticides at the post-mummy stage.

(A) Mummies were sprayed while on the trees and then removed.

(B) Mummies were removed from the tree and treated with the pesticide using a Potter's Tower. Bars capped with a letter are significantly different from the water control, within each sex ($P<0.05$). APM = azinphos-methyl.

Males ☐ Females ▨

#### 4.4.2.3. Adult parasitoids

After being put in the treatment cages the parasitoids were observed periodically during the first five hours. No avoidance of the treated surfaces was seen in any treatment except oil. Adult survival was nil with all of the organophosphates, and one of the carbamate treatments and death occurred within four hours. All males in the endosulfan treatment died within 24 hours (Figure 4.5). The effects of the chlorpyrifos were almost immediate with the parasitoids dying within 30 minutes exposure to the pesticide. If the parasitoids survived the first 24 hours of exposure to the pesticide there were no differences (P>0.05) in the time to death between the adults treated with water or those treated with a pesticide (Figure 4.5). The only exceptions to this were the females treated with fenoxycarb and endosulfan.

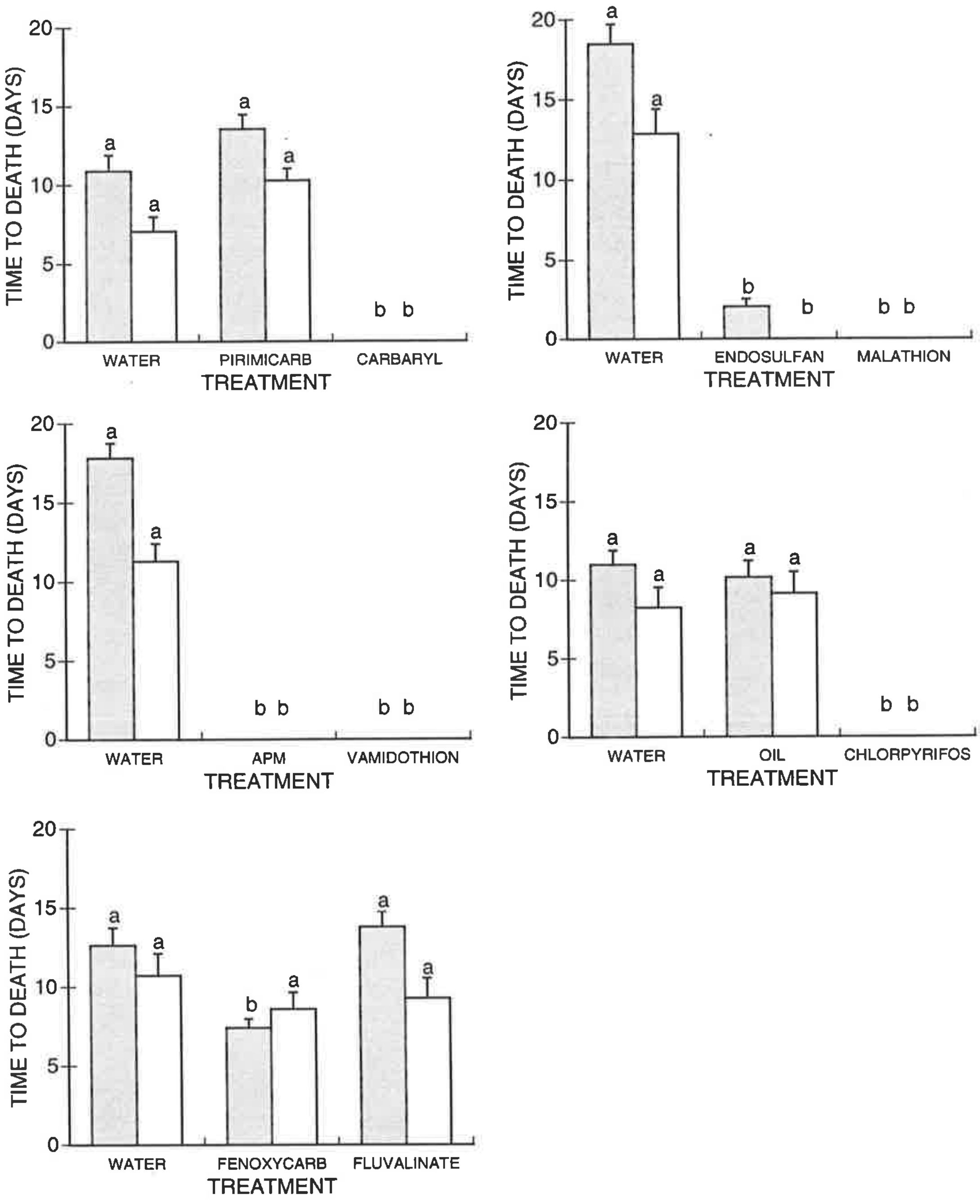


**Figure 4.5.** Time to death (mean ± standard error) (days) of adult *A. mali* after 24 hours exposure to pesticides in 'sandwich' cages. In all treatments, there was a significant difference in time to death between sexes, therefore letters above the bars refer to treatment differences only. Treatments with the same letter are not significantly different ($P>0.05$).
APM = azinphos-methyl.

Males ☐ Females ▨

## 4.5. DISCUSSION

Under laboratory conditions, vamidothion, malathion, chlorpyrifos (OPs), endosulfan (OC) and pirimicarb (carbamate) clearly had a negative effect on WAA colonies compared to the water control and fluvalinate. An exception to the general effectiveness of the OPs was azinphos-methyl (APM) which is the most commonly used insecticide in orchards. APM reduced WAA colonies by ca. 40% compared to more than 80% for the other OP compounds (Figure 4.2). This result is at variance with a previously reported laboratory test in which APM reduced colonies on watersprouts by ca. 66% (Penman and Chapman 1979). Pirimicarb, which is an aphicide was as effective as the organophosphates tested here. All of these chemicals act as cholinesterase inhibitors and either systemics and/or contact/stomach insecticides.

Vamidothion was effective in reducing colony numbers when applied as a foliar spray and prevented reinfestation for some weeks after treatment (personal observation). It was manufactured as a persistent systemic aphicide with WAA as a specific target. It has been used since the late 1960's and there have been no published reports suggesting decreasing efficacy until recently (Swart et al. 1991; Pringle *et al.* 1994). Even so, apple growers of the Adelaide Hills region indicated in a survey in 1985 that WAA was more difficult to control with a single application of vamidothion during the season (Baker, unpublished data) and this was reiterated in a survey done in 1987 (Chapter Two). Such loss of control may be due to changing orchard management, ie: a time shift in the application from early to late season allowing the chemical to act as a contact rather than a systemic spray. A combination pre- and post harvest application of vamidothion was found to give reasonable control of WAA on extremely heavily infested Starking trees in South Africa (Swart and Flight 1990), although, a pre-harvest spray of vamidothion combined with a post-harvest spray of chlorpyrifos was found to give even better control. It was noted by the authors that post-harvest sprays of vamidothion (a systemic) and chlorpyrifos (a contact insecticide) could not be directly compared due to the reduced feeding activity of aphids and declining sap movement in the trees.

Chlorpyrifos is highly volatile and leaves insecticidal deposits on nearby surfaces (Worthing 1991). It was most effective in controlling WAA when used as a dormant or spring application on Granny Smith trees in New South Wales although a second application was required before the end of the season (Bower 1987). In the present study its effect on the aphids were dramatic, causing rapid mortality.

Winter oil gave poor control under laboratory conditions even though it is recommended as a control measure against WAA (James *et al*. 1989). Due to its phytotoxic properties it is recommended as a dormant spray and, at this time, WAA colonies have less wool presumably leaving individual aphids more vulnerable to its effects. In the present experiments, colonies, while young, were well established and had a covering of wool which protected the aphids from the oil. In contrast, the other insecticides (OP, OC and pirimicarb) appeared to dissolve the wool on contact. Oil used as a dormant spray was ineffective against WAA colonies on apple trees in NSW, although, its efficacy was increased by the addition of insecticides (chlorpyrifos, diazinon or methidathion) (Bower 1987).

Woolly apple aphids are protected by their waxy exudate, thus reducing the efficacy of pesticides unless it can be dissolved. Under field conditions turbulence caused by sprayer discharge will remove a portion of the wool but only on those colonies that are directly exposed to the turbulence. In addition, colonies may be protected from the sprays by their position on the tree. Those developing in leaf axils and within the centre of trees may largely escape control and initiate a resurgence of populations (Chapter Six).

While some of the insecticides tested here appeared to give excellent control, the results must be considered with caution. The laboratory trials were carefully controlled, using trees of similar shape, size and age, colonies of equal age and optimum coverage of trees with the insecticides was obtained. Conditions in the field are far removed from the laboratory and results can be quite different. For example, pirimicarb, in spite of its high control in the laboratory has given disappointing results in the field (A. Granger, personal communication; Staübli and Chapuis 1987) while APM, which would appear to give only moderate results in the laboratory, gave good control in the field (Penman and Chapman 1980). Factors such as tree canopy density (or tree shape), travel speed of the tractor, as well as the application volume (high or low volume spraying) affect the deposition of insecticides (Travis *et al.* 1987a,b). Changes in deposition and distribution of a pesticide on trees will ultimately affect its efficacy. Weather will also play a part in the efficacy of a pesticide under field conditions. For example rain and wind will decrease the volatile effects of chlorpyrifos.

The ability of a parasitoid to regulate its host population will depend on its demographic characteristics, such as developmental duration, stage specific survival and adult reproductive potential. These characteristics will in turn be affected by exposure to the pesticides used in the orchard. The most important effect will be the degree of mortality experienced by the different life stages of the parasitoid, ie: larval, pupal and adult. High aphid mortality will lead to high parasitoid mortality if the parasitoid is in the larval stage. The results presented here demonstrate that aphids and hence the early larval stage of the parasitoid is very susceptible to pesticides. If, as in the case with chlorpyrifos, nearly all of the aphids are killed then it stands to reason that few parasitoids will emerge regardless of the level of initial parasitism. While not quantified, the mortality of parasitised aphids was similar to that found for unparasitised aphids in the pirimicarb, vamidothion, malathion and endosulfan treatments. Similar results were observed by Krespi *et al.* (1991) who found that no mummies of *Aphidius uzbekistanicus* (Luz) were formed after the application of an organophosphate in spite of the fact that more than 50% of the aphids were parasitised.

There was great difficulty in producing predetermined numbers of parasitised aphids. Allowing two or more females to freely parasitise a set number of colonies of the same size and age does not guarantee that all aphids will be parasitised. Sometimes parasitism rates were quite low for no apparent reason. Unlike other aphids, WAA cannot be removed from the host, parasitised and put back on to the tree without high mortality. First instars are the most easily transferred but least preferred stage for parasitism while third instars, which are the most preferred host stage do not settle readily after being disturbed (Chapter Five; Mueller *et al.* 1992). It is because of these difficulties that aphid/parasitoid mortality following exposure to pesticides was not directly quantified.

Mummified aphids, ie: those where the parasitoid is in the late larval or pupal stage, are most protected. However the protection is not absolute. This was evident when comparing the experiments where the mummies were still on the tree and the Potter's Tower experiment where the mummies were removed from the trees prior to treatment. Mummies that were treated in a Potter's Tower showed greater variability in emergence rates with carbaryl, APM, malathion and chlorpyrifos producing the greatest mortality (Table 4.4). In this experiment 86% of the unemerged mummies were fully formed adults indicating that some development had occurred after treatment. In an earlier study, Evenhuis (1959) noted that malathion was highly toxic to late pupae treated on the tree and that the emergence rate was nil. Contrary to the Evenhuis results, these experiments found little mortality following treatment with malathion (Table 4.3). El-Haidari and Georgis (1978) also found that malathion was toxic to *A. mali* pupae, however, they used a dip technique to apply the chemical rather than spray application. Their study is in closer agreement with the present findings of the Potter's Tower experiment where the emergence rate was significantly less than the control treatment. Chlorpyrifos again had a marked effect with only seven individuals emerging from the 45 mummies. The remaining individuals died as fully formed adults while still in the mummy.

No effect on the duration of development post treatment could be detected among the pesticides. However, this is an area that would benefit from further investigation. If the time taken to emerge was negatively affected (ie: lengthened) by pesticide treatment this would mean that generation times would increase, thus reducing the intrinsic rate of increase. This in turn would result in the parasitoid population lagging further behind that of the aphid and control would be seriously hampered.

Significant differences in the sex ratio of emerged adults were detected among treatments when the parasitoids were treated both in the early and late larval stages. Ratios varied from highly female biased to highly male biased. How much significance should be placed on these observations is uncertain. While the pesticides apparently affected the survival of males and females differently other factors could have produced these variable sex ratios. While the parasitoids were observed until a copulation occurred it is possible that the female was not successfully fertilised. Unfertilised eggs give rise to males only (Evenhuis 1958) and it would require only one such female present in the lantern cage to skew the resulting sex ratios. Host size also plays an important role in sex determination with small hosts producing males and large hosts producing a strongly female biased population (Mueller *et al.* 1992). Colonies were allowed to develop long enough to produce aphids ranging in size and age to offset this effect, however, a small shift in mean aphid size could result in a notable shift in sex ratio.

If pesticides do alter the parasitoids' normal sex ratio this could have repercussions with respect to its ability to control aphid populations. A population that is heavily female biased could result, at the worst, in male only progeny or at least very heavily male biased populations. Conversely, if the early generations are heavily male biased this will lead to fewer females resulting in lower populations and therefore less effective control of the aphid.

The life spans of parasitoids that survived exposure to pesticides in the early larval stages were unaffected by the pesticide treatment. The life span of adults emerging from treated mummies, however, were affected by some of the pesticides but the effects differed depending on whether the mummies were treated while still on the tree or treated in the Potter's Tower. When the mummies were treated on the trees there was no significant difference in the life span of treated males compared to the controls with the exception of the malathion treatment. Treatment with malathion resulted in males (emerging from mummies treated on the tree) that lived significantly longer as adults than males not exposed to a pesticide. This result was observed in all replicates. Although not statistically significant, longer adult male life spans were also observed after mummies were treated with fluvalinate and endosulfan (Figure 4.4A). This trend was mirrored in the results for treated females where longer life spans were evident in the fluvalinate, endosulfan and malathion treatments. However, long female life spans were not the norm and adult females had shorter life spans than the controls in almost half of the treatments, though the differences were not significant in all cases. Interestingly, the mummies treated with malathion in the Potter's Tower produced both males and females that had significantly shorter life spans than the controls and this was reflected in all replicates (Figure 4.4B).

The results of these two experiments indicate that the insecticides may have differing effects on the emergence and survival of the parasitoid depending on the degree to which the mummy comes into contact with the insecticide. When the mummies are treated with the insecticide *in situ* some will not come in to direct contact with the spray because they are protected either by parts of the tree or aphids within the colony. While parasitoid mortality was apparently reduced under these circumstances some pesticides appeared to have a sublethal effect. The consequences of the sublethal effects were variable resulting in males having significantly increased or decreased life spans compared to the controls. The reasons for these effects and the apparent differences between the sexes are unknown.

Consideration of the effects of the pesticides on the lifespan of surviving parasitoids is important as female survival and life span have a direct impact on fecundity and therefore, the number of individuals in the next generation. It is likely that *A. mali* is synovigenic - the eggs develop and mature continuously throughout the life of the insect (Viggiani 1984; Jervis and Kidd 1986). While it has been suggested that the majority of eggs are laid in the first few days of the females' life this is not known for certain. Data on the actual fecundity of aphelinids is scarce (Viggiani 1984) and this area was not investigated in the present study due to the technical difficulties involved. However, if one assumes that lifetime fecundity depends on the number of eggs/female per day, the reproductive value (R) will be reduced drastically if the parasitoid life span is shortened. Once again the control potential of the parasitoid is reduced. Even in those cases where exposure to a pesticide during development did not affect female life span it is possible that they may be less effective in ovipositing, searching for hosts or feeding under field conditions due to a reduction in vigour. This may not have been noticeable under experimental conditions where they had free access to food and water, however being 'unhealthy' after treatment may be exacerbated in the field where greater stresses come into play.

Testing the adults gave more consistent results than the earlier stages as experimental conditions were easier to control. While the treated glass can simulate the surface that the parasitoid would be walking on in a natural situation, it does not take into account interactions that may occur between leaf and chemical (Kühner *et al.* 1985). The present tests show that in general, the organophosphates are highly toxic to adult *A. mali*. This has been reported elsewhere for other endoparasitoids (Purcell and Granett 1985; Krespi *et al.* 1991; Shean and Cranshaw

1991) and for *A. mali* in particular (Staübli and Chapuis 1987). The three types of carbamate had differing effects on the mortality rate of the adults. Carbaryl (a methyl-carbamate) killed all parasitoids within 24 hours; the survival of parasitoids treated with pirimicarb (dimethyl-carbamate) was not significantly different than that of the control, while parasitoids treated with fenoxycarb, an ethyl-carbamate, had a significantly lower rate of survivorship than the controls.

Every life stage of *A. mali* is vulnerable to destruction by one or more of the commonly used insecticides. The most protected stage, the mature pupa, is between stages that are vulnerable leaving little chance for populations to increase during the season. Azinphos-methyl is not as effective in reducing aphid numbers and, therefore, larval parasitoids as the other organophosphates but this has the advantage of leaving a residual population of parasitised aphids. Unfortunately, APM is very effective in killing adult *A. mali*. Compounds like fenoxycarb are being tested as replacements for APM. Fenoxycarb is thought to be safe to beneficials but this needs careful scrutiny both in the laboratory and in the field. While there was no effect on the adult life span of mummies treated on the both males and females had significantly shorter lives when the mummies were directly treated with fenoxycarb in the Potter's Tower. Adult females also had significantly shorter lives than their untreated counterparts when exposed to fenoxycarb on the glass plates (Figure 4.4A, B).

Clearly, the pressures on *A. mali* are intense throughout the season. If a spray profile of a typical orchard is determined from the results of the survey (Chapter Two) it can be seen that there really is no time during the season that some stage of the parasitoid is not killed. Oil is applied as a dormant or spring spray in conjunction with chlorpyrifos both of which will kill emerging adult parasitoids. Applications of APM every three weeks, starting in late spring and continuing through the summer will have a detrimental effect on the larval, pupal and adult stages. In addition, there are applications of miticides (which may or may not have a detrimental effect on the parasitoid), aphicides or malathion both of which will reduce parasitoid numbers. Finally, applications of chlorpyrifos or vamidothion specifically for WAA at either end of the season will kill the parasitoids. Therefore it is unlikely that *A. mali* will be effective against WAA until the number and variety of pesticides used in the orchard are decreased.

# CHAPTER FIVE

# EFFECTS OF WOOLLY APPLE APHID INFESTATION ON THE GROWTH OF YOUNG APPLE TREES UNDER LABORATORY CONDITIONS AND IN THE FIELD

## 5.1. ABSTRACT

The effects of above-ground infestation of woolly apple aphid (WAA) on the growth of young apple trees under laboratory and field conditions were investigated. Trees were treated with three levels of infestation and tree height, leaf number and leaf area, measured at regular intervals, were used as indicators of tree growth.

In the laboratory, plots of seedling apple trees (Granny Smith cv.) were infested and kept at 23±1°C and 16L:8D photoperiod. In all of the three experiments at least one of the growth indicators was significantly reduced after four to eight weeks of aphid infestation. High infestations led to tree death after six weeks of infestation in two of the three experiments.

In the field three blocks of Granny Smith cv., grafted on to either Northern Spy or seedling Granny Smith rootstock, were infested and measured for a period of twenty months. Aphid infestation had no effect on the chosen growth indicators in the first year of study in two of the three blocks, but differences in all

three variables were detected in the following year. There was no difference in tree growth between the two rootstocks in either year. High aphid infestations had a negative effect on tree height, leaf number and leaf area in two of the three blocks but effects could not be detected in the third block. High infestations delayed the break of dormancy and killed more than 25% of the trees.

## 5.2. INTRODUCTION

In general, aphids are thought to cause relatively little damage to their hosts, not only in comparison to plant chewing insects but also when compared to other sucking insects. The nature and extent of the damage to the host plant by aphid feeding depends on many factors such as density, feeding site, host reaction and the insects' potential role as virus vectors. As the majority of aphid species feed on the leaves of their hosts (Blackman and Eastop 1984) rather than the woody portions, research has tended to concentrate on the interactions between these particular species and their hosts. While the damage is often positively related to aphid numbers per host this is not always the case. For example, small numbers of spotted alfalfa aphids, *Therioaphis trifolii* f. *maculata* (Buckton) can do considerable damage to some varieties of its host, *Medicago sativa*, by causing chlorosis at the growing tips and reducing plant growth (Miles 1989a). In contrast, the green peach aphid, *Myzus persicae* (Sulzer) causes minor damage to some hosts even when present in very large numbers, provided virus transmission is not a significant factor (Van Emden *et al.* 1969).

When a negative impact of mass feeding by aphids has been detected the effects tend to be manifested as a general debilitation of the host resulting in an overall reduction in growth rate (Dixon 1971a,b). Dixon (1971a) noted a reduction in root growth and weight of young lime trees when fed upon by the lime aphid, *Eucallipterus tiliae* (L). The trees also produced smaller leaves during and after infestation. In sycamore trees a reduction in leaf size was also found to be a consequence of infestation by the sycamore aphid, *Drepanosiphum platanoides* (Schr.). In addition, infested sycamores tended to lose their leaves in the autumn while they were still rich in nitrogen (Dixon 1971b). Hamilton *et al.* (1986) reported that *Aphis pomi* De Geer density significantly reduced the yield and sugar content of fruit on Golden Delicious apple trees as well as reducing shoot growth within a single season.

Information concerning the effects of aphids which feed on the woody tissues as opposed to the leaves of their host is limited. The woolly balsam aphid, *Adelges picea* (Ratz.), feeds on new shoots and causes the distortion of twigs and smaller branches of its host, the balsam fir (Balch 1952). Moderate infestations have been found to reduce the life expectancy of trees from in excess of 100 years to less than 20, while heavy stem infestations can kill trees in under three years (Balch 1952).

Woolly apple aphid (WAA), like the woolly balsam aphid, attacks the woody tissues of the tree rather than the leaves and in addition infests the roots. Swellings or intumescences are formed at the feeding sites causing leaf drop and splits in the tissue (Madsen and Bailey 1958). WAA has been shown to have a significant negative effect on the growth of young apple trees under laboratory and field conditions (Gambrell and Young 1950; Sproul 1981; Weber and Brown 1988). All studies investigating the effects of WAA on apple trees have examined trees that experienced both root and above-ground infestation. In general, these studies have shown that WAA infestation can reduce the growth rate and fruit yield of affected trees (Stanley 1951; Brown and Schmitt 1990; Brown *et al.* (1995). The growth rate of infested potted M7 seedlings was significantly less than uninfested trees after 16 weeks of WAA infestation (Weber and Brown 1988). Brown and Schmitt (1990) reported that non-bearing Red Delicious/M7A trees were significantly affected by WAA infestations after three growing seasons.

In both these studies, the aphids infested both the above-ground and root portions of the trees and the effects of the two kinds of infestation were not clearly separated; nor was the severity of effects on tree growth related to different densities of WAA. Yet the aerial and root regions present very different food sources for the insects (Sen Gupta and Miles 1975) and the site of infestation could well influence the interaction between aphid feeding and tree growth. Most studies have concentrated on the root infestation as it has generally been assumed that these infestations are of greater significance than above-ground infestations although there has been no appropriately designed experiments to test this assumption. Questions related to the relative importance of root versus above-ground infestations have become of less concern with the development and selection of rootstocks resistant to WAA. The development of these rootstocks has been a successful method in controlling root infestation by WAA (Greenslade 1936; Bengston 1965; Sen Gupta and Miles 1975; Ferree and Carlson 1987). The use of resistant rootstocks such as Northern Spy and its derivatives, the Malling-Merton series, is world-wide and used extensively in South Australia. While resistant rootstocks limit root infestation, above-ground infestations still occur and the impact of such infestations has not been investigated.

As there have been no studies undertaken to assess the impact of above-ground infestations on apple tree growth a series of experiments were undertaken in the laboratory and the field. By conducting the experiment under controlled laboratory conditions the effects of other factors such as weather, soil and other pests were eliminated, thereby highlighting the relationship between tree growth and WAA. The field experiment mirror the conditions found in a young orchard. The laboratory experiments will be presented first with the field experiment second followed by a unified discussion at the end of the chapter.

## 5.3. MATERIALS AND METHODS - LABORATORY EXPERIMENT

### 5.3.1. Experimental design

A random complete block design consisting of two blocks was used in each experiment. There were three levels of infestation - no aphids, low and high - within each block and five trees per block treatment combination. The five trees were enclosed within a cage covered with fine mesh gauze. There was no evidence to suggest that there was any aphid movement between trees within a cage which enabled each tree to be considered as an experimental unit.

Tree height, total leaf area and total leaf number were measured 24 hours prior to infestation, to give an indication of tree growth or vigour. The rim of the pot was used as the base line from which tree height was measured. Total leaf area was calculated for all leaves on the tree and determined by the method set out in Appendix Five. Total leaf number was the sum of the leaves on the main stem and side shoots. The data obtained were ranked (SAS 1985) and six groups of five trees were selected such that each group represented a range of tree sizes. The groups were randomly assigned to one of the block treatment combinations. The experiment was repeated three times.

### 5.3.2. Plant conditioning and initiation of the experiment

Prior to the experiment the trees were maintained in semi-controlled conditions (22-28°C; 16L:8D; 40-60%RH). Thirty, three-month-old trees approximately 10 cm in height with similar numbers of leaves were selected and placed in a controlled environment chamber (23±1°C), 16L:8D and approximately 65%RH (range 60 - 70%). The trees were transplanted to 150 mm pots to reduce any effects of root-binding during the course of the experiment. Sterilised recycled soil was used in all experiments. The potted trees were placed in large galvabond™ trays (length

700 mm, width 600 mm and depth 40 mm), sub-irrigated every three days and allowed to acclimatise to the conditions of the growth room.

The low infestation treatment was initiated by transferring 15 first instar aphids (crawlers) onto the uppermost leaves of each tree with a fine sable hair brush. Thirty crawlers were placed on each tree in the high infestation treatment. Placing the aphids on the upper leaves allowed them to wander over the tree and find a suitable place to settle. First instars were used because they were easily identified, ensuring that all aphids were approximately the same age. The crawlers were also less disturbed by transfer to new plants than the more sedentary older instars and, therefore, less likely to die.

After the aphids were transferred, the five trees in each treatment were enclosed in a cage (height 1000 mm, length 600 mm and width 500 mm) covered with fine mesh gauze. After 18 hours the trees were inspected and the number of aphids recorded. The number of aphids per tree was then reduced to five in the low and 15 in the high treatments. Control or 'no infestation' treatment trees were also checked for aphids and if present, they were removed. It was found that it was easier to remove excess aphids than to add additional individuals. Trees were watered every three days and fertilised with 23N:4P:18K (Aquasol™; 1.6 gm/l) every fortnight for the duration of the experiment. Measurements of tree height, total leaf number, total leaf area, colony area and colony number were taken fortnightly and the cages were re-randomised within the block.

During experiments 2 and 3 all trees were sprayed once for powdery mildew (Bayleton®, 0.5 gm/l). No other fungicides or pesticides were used. At the termination of the experiment, the roots of each tree were examined for the presence of aphid colonies.

### 5.3.3. Statistical analysis

At the end of the experiment tree height, total leaf number and area for the last sample time was analysed using ANOVA (SAS 1985) as a randomised complete block design. No significant block effect ($P > 0.05$) was found in any of the three experiments and the blocks were pooled in subsequent analyses within each experiment. However, using the same response variables, significant differences were found between experiments and as a result each of the experiments were analysed separately.

Regression analysis was used to investigate the effect of WAA density on tree growth. The change in all three response variables with respect to time was described using a quadratic equation of the form

$$y= a+b_0x-b_1x^2$$

where y is the variable in question, a is the intercept, $b_0$ and $b_1$ are the coefficients corresponding to time and time squared respectively. This equation provided a good empirical description of the change of the response variables through time (see $R^2$ values in Appendix Six). To determine whether the pattern of tree growth differed between treatments the regression lines were compared using an overall test for coincidental regressions (Zar 1984). Since trees were standardised between treatments with respect to height, leaf number and area at the beginning of the experiment no differences in the intercepts between treatments within each experiment were expected. This was indeed the case, thus the comparisons of the regression lines are, in fact, comparing $b_0$ and $b_1$. If there was a significant difference in the overall comparison the analysis was further broken down to compare the controls with the combined treatments (low and high infestation) and to compare the two levels of infestation. Using the observed data and the quadratic formula a set of predicted values were estimated for each treatment level and each variable. These are presented graphically in Figures 5.1-5.3. Tabulation of tree height, leaf number, leaf area and aphid number for both experiments are presented in Appendices Six and Seven.

Total aphid number was assumed to follow an exponential growth curve with the form

$$N_t = N_0 \exp (rt)$$

where $N_0$ is initial aphid density, r is the intrinsic rate of increase, t is time and $N_t$ is aphid number at any given time. The data (Figure 5.4 A-C) were log transformed and fitted using linear regression to obtain values for the intrinsic rate of increase. The total aphid number was also analysed by a factorial with interaction ANOVA using the MS of the 'block*level' interaction as the error term (due to the experimental design - random complete block) (SAS 1985). This was to determine if there were any differences in aphid number between treatments throughout the experiments.

## 5.4. RESULTS - LABORATORY EXPERIMENT

At the end of each experiment tree height, total leaf number and area for the last sample time was analysed using ANOVA (SAS 1985) as a random complete block design. No significant block effect (P=0.05) was found in any of the three experiments and these data were pooled in subsequent analyses within each experiment. However, using the same response variables, significant differences were found between experiments and as a result each of the experiments was analysed separately.

In the three experiments, the control trees and the roots of all treatment trees remained free of aphids. After four weeks, the aphid numbers in the low infestation treatment had increased markedly so that by the end of the experiment there was no qualitative difference between the low and high infestations. However, trees in the high infestation treatment had sustained a greater load of aphids for a longer time resulting in the death of the trees by week 8 in Experiments 2 and 3. On the final sampling date in both these experiments, leaf number represented both dead and living leaves, however for the estimation of leaf area only living leaves were included.

In all three experiments a consistent trend was seen in the values for tree height, leaf number and area. Averages for the three variables in the low infestation treatment were always intermediate between the other two treatments by the end of the experiment.

### 5.4.1. Experiment 1.

Experiment 1 was terminated after six weeks due to an infestation of thrips and two spotted spider mites. At this time the trees in the uninfested treatment were taller than the infested trees (Figure 5.1A). Tree height in the low infestation treatment was intermediate between the other treatments and the trees were, on average, about 24% taller than those in the high infestation treatment. The rate at which the trees were increasing was similar in all three treatments for the first two weeks of the experiment. After this time the trees in the high infestation treatment were obviously beginning to grow more slowly than the trees in the other two treatments. When comparing the three regression lines there was a significant difference between the uninfested and combined infested trees, but no differences between the two levels of infestation (Table 5.1).

**Table 5.1.** P values of the F statistic comparing the regression lines for tree height, total leaf number and total leaf area of trees infested with different densities of woolly apple aphid in Experiment 1.

| | P value | | |
|---|---|---|---|
| Variable | All | Control *versus* combined treatments | Low *versus* high |
| Tree height | P<0.001 | P<0.001 | P>0.1 |
| Total leaf number | P<0.001 | P<0.001 | P<0.01 |
| Total leaf area | P<0.001 | P<0.001 | P>0.5 |

values

Mean leaf number on the uninfested trees was greater than those on the low and high treatment trees after six weeks of infestation (Figure 5.1B).

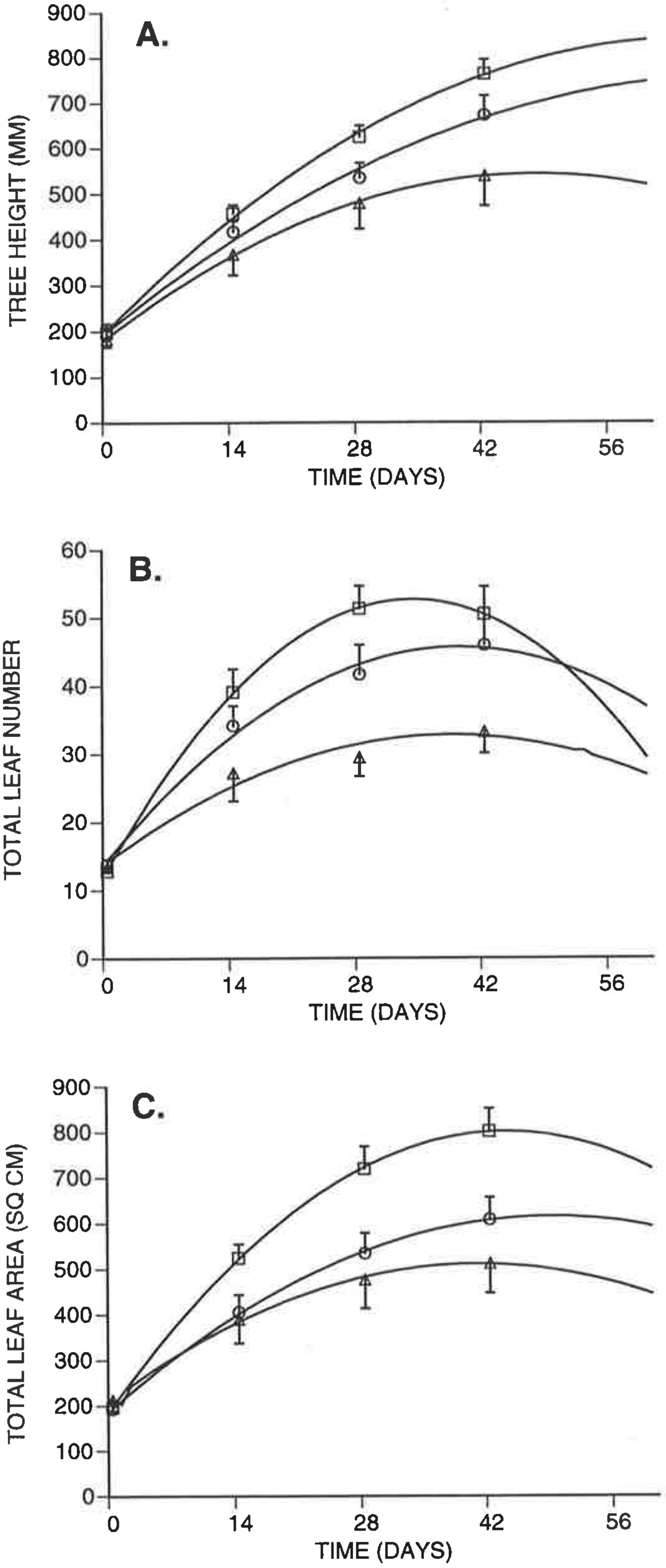


**Figure 5.1.** Relationship between tree parameters (mean ± standard error) and woolly apple aphid densities over time in Experiment 1. (A) tree height, (B) total leaf number, (C) total leaf area. Infestation commenced 24 hours after time 0. Solid lines represent predicted values. Symbols represent actual values.

No aphids □ Low infestation ○ High infestation △

However the leaf number in this treatment appeared to have levelled off while the number of leaves continued to increase, albeit slowly, in the other two treatments. By the end of the experiment the high infestation treatment trees had 62% fewer leaves than the uninfested trees. Leaf number in the uninfested and low infestation treatments appeared to have increased at similar rates while the highly infested trees showed a slower rate of increase after the first two weeks of infestation. Differences in leaf number are summarised in Table 5.1.

no [illegible]

After only two weeks, the rate at which leaf area was increasing in the infested treatments was starting to decline compared to the uninfested trees with the high infestation treatment trees showing the greatest decline (Figure 5.1C). There was a significant difference between the regression line of the uninfested trees compared to the combined infestation treatments but no differences were detected between the two levels of infestation (Table 5.1).

The total number of WAA, estimated by colony area (Appendix Four), increased dramatically between four and six weeks of infestation (Figure 5.4A). After two weeks of infestation there were already differences ($P<0.05$) in the total WAA number between treatment levels. After six weeks of infestation, however, there was no longer a difference in aphid numbers between the two initial infestation levels. The intrinsic rate of increase was estimated to be 0.158 and 0.104 for low and high treatments respectively (Table A6.7). Estimated aphid days (determined by calculating the area beneath the curves in Figure 5.4A) showed little difference between the two levels of infestation (63879 and 67333 for low and high levels respectively).

### 5.4.2. EXPERIMENT 2

Aphid numbers increased rapidly in both the low and high infestation treatments such that by the sixth week of infestation 20% of the high infestation trees showed signs of stress (leaf flagging and reduced water uptake). By the eighth week all trees in this treatment and 80% of the trees in the low infestation treatment had died. The aphids had left the trees either as alates or by simply dropping to the ground as the tree died. The trees in the control treatments remained healthy, growing and free of aphids and at this point the experiment was terminated.

For the first four weeks after infestation trees in all treatments grew at approximately the same rate. After the fourth week the trees in the high infestation treatment started to grow more slowly while the other two treatments continued to grow rapidly. However, by the sixth week post infestation the trees in the low infestation treatment were also showing signs of decreased growth rate. The uninfested trees continued to grow at an essentially constant rate (Figure 5.2A). A comparison of the regression coefficients showed a significant difference between the uninfested trees and those of the combined infested treatments as well as between the two infestation levels (Table 5.2).

Leaf number showed a similar trend to that of tree height with the uninfested trees showing a consistent rate of leaf production during the first six weeks after infestation (Figure 5.2B). Leaf number in the two levels of infestation started to decline after the fourth week. There was a significant difference between the regression lines when comparing the control trees to the combined infested treatments with no differences when comparing the low and high infestation treatments (Table 5.2).

As with leaf number, there was no difference between the slopes of the infested treatments, though there was a difference ($P<0.001$) when comparing the uninfested treatment to the combined infested treatments. Leaf area on the dead trees was entered as a missing data point. After four weeks of infestation leaf area on the highly infested trees was decreasing while leaf area production in the low infestation treatment was similar to that of the uninfested trees (Figure 5.2C).

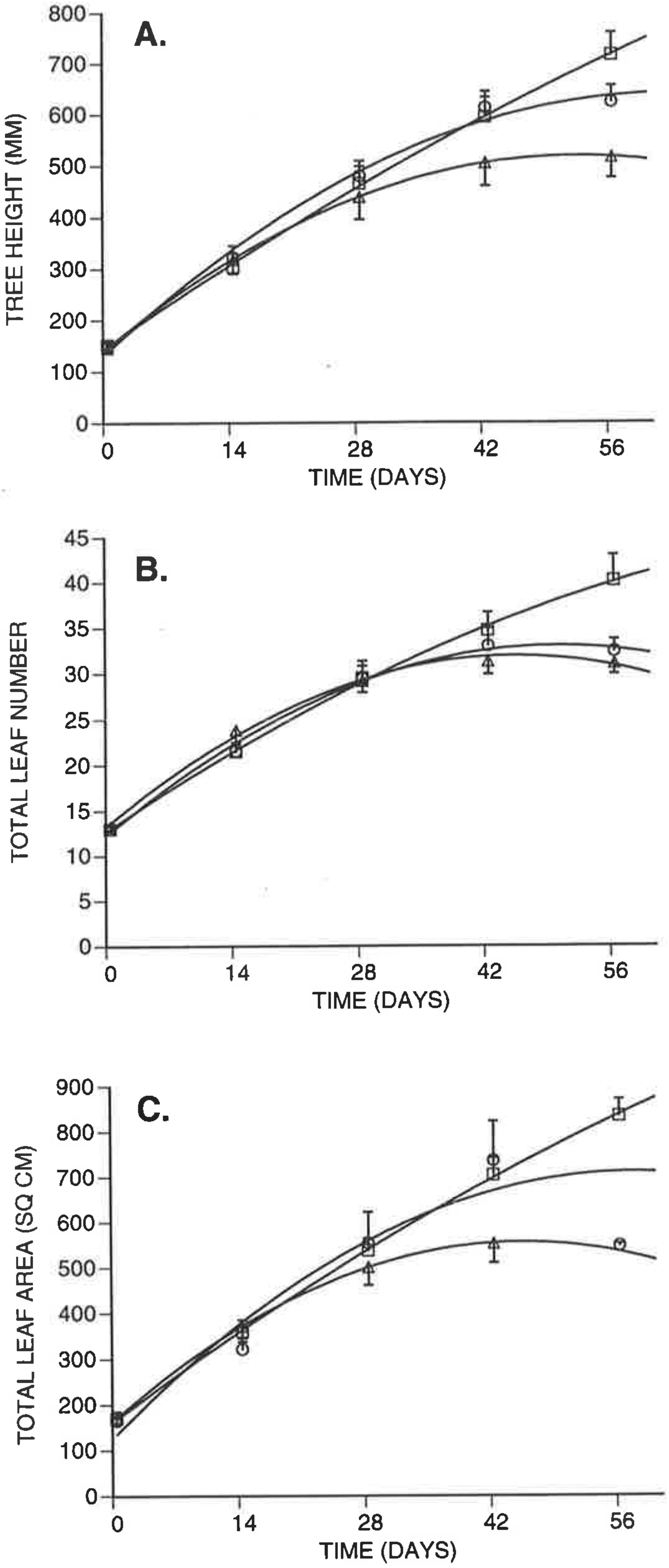


**Figure 5.2.** Relationship between tree parameters (mean ± standard error) and woolly apple aphid densities over time in Experiment 2. (A) tree height, (B) total leaf number, (C) total leaf area. Infestation commenced 24 hours after time 0. Solid lines represent predicted values. Symbols represent actual values.

No aphids □ Low infestation ○ High infestation △

At the end of the experiment there was a marked decrease in leaf area of the trees in the low infestation treatment due to the reduced number of leaves on the trees.

**Table 5.2.** P values of the F statistic comparing the regression lines for tree height, total leaf number and total leaf area of trees infested with different densities of woolly apple aphid in Experiment 2.

| | P value | | |
|---|---|---|---|
| Variable | All | Control *versus* combined treatments | Low *versus* high |
| Tree height | P<0.001 | P<0.01 | P<0.02 |
| Total leaf number | P<0.001 | P<0.001 | P>0.5 |
| Total leaf area | P<0.2 | P<0.001 | P>0.2 |

The numbers of aphids in the infested treatments increased at a similar rate to those in the first experiment until the last week, when there was a sudden decline due to the death of the trees (Figure 5.4B). Mean aphid number differed significantly (P<0.001) between treatments after two weeks of infestation. This difference was reduced just prior to the death of the trees. The intrinsic rate of increased was estimated to be 0.150 for the low infestation treatment and 0.096 for the high (Table A6.7). Estimated aphid-days were similar for both treatment levels (67877 and 68736 for low and high levels respectively).

### 5.4.3. Experiment 3

Similar to the previous experiment, after eight weeks all trees in the high infestation treatment had died as well as 30% of trees in the low infestation. The uninfested trees remained free of aphids and continued to grow.

A steady decline in the rate of tree growth in the high infestation plots was observed after two weeks of infestation (Figure 5.3A). The trees in the low infestation treatment were slightly taller than the uninfested trees until six weeks post infestation when their growth rate also started to level off (Figure 5.3A). The uninfested trees appeared to grow at a constant rate throughout the experiment.

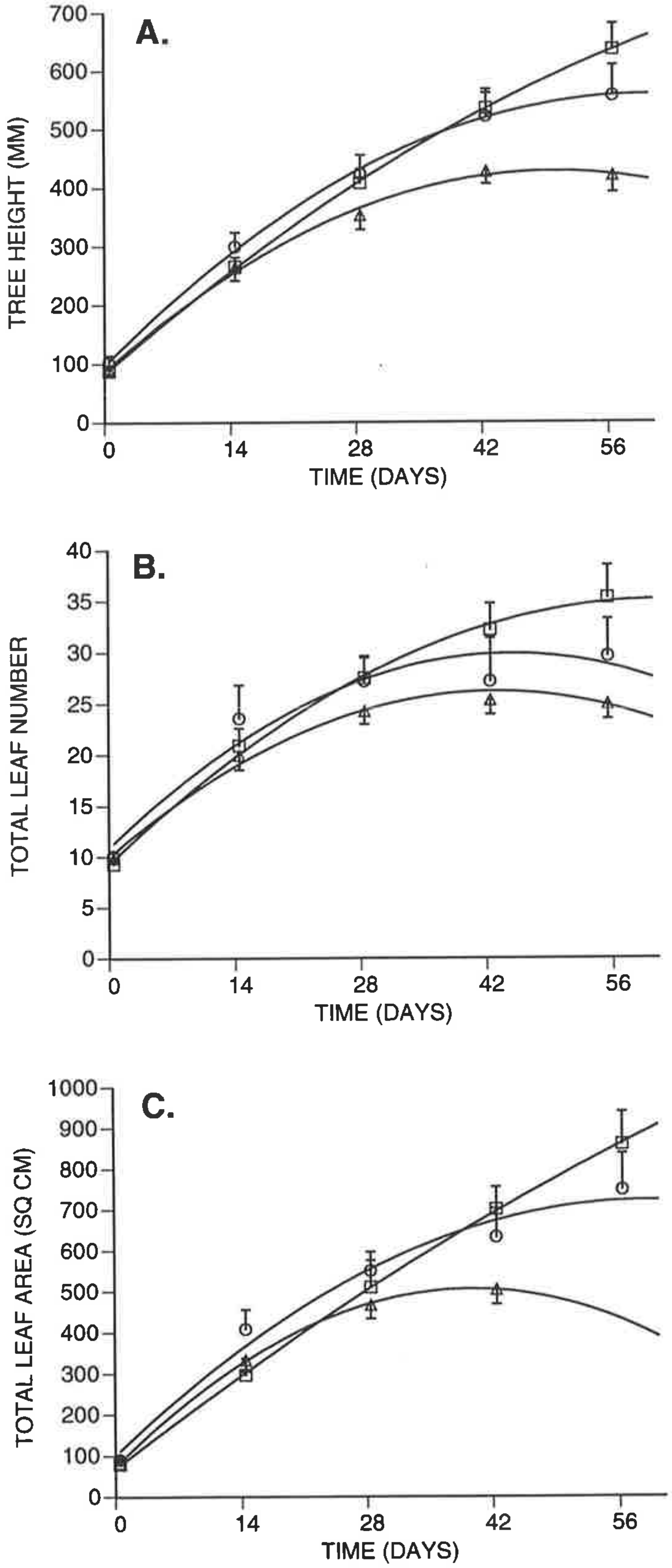


**Figure 5.3.** Relationship between tree parameters (mean ± standard error) and woolly apple aphid densities over time in Experiment 3. (A) tree height, (B) total leaf number, (C) total leaf area. Infestation commenced 24 hours after time 0. Solid lines represent predicted values. Symbols represent actual values.

No aphids □ Low infestation ○ High infestation △

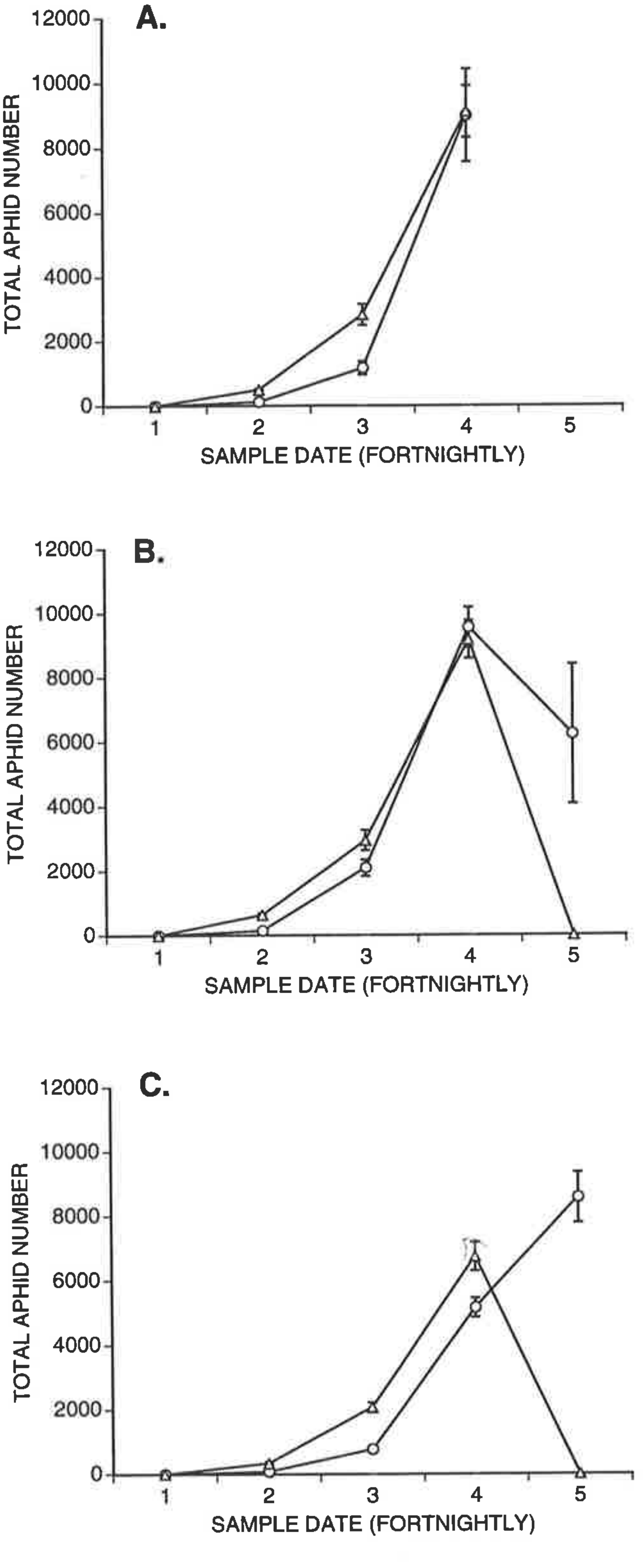


**Figure 5.4.** Number (mean ± standard error) of aphids on trees infested with different densities of woolly apple aphid in three experiments: (A) Experiment 1; (B) Experiment 2; (C) Experiment 3.

Low infestation ○ High infestation △

When comparing the regression lines there was a significant difference (P<0.001) between the uninfested and combined infested treatments and between the two infested treatments (Table 5.3).

In all treatments, leaf number increased similarly for the first two weeks. After this time, mean leaf number levelled off in the infested treatments while the number of leaves in the uninfested trees continued to increase though at a slightly slower rate (Figure 5.3B). Upon comparison, the regression line for the uninfested treatment was significantly different from the combined infested treatments (Table 5.3). There were no differences between the low and high infestation treatments.

Leaf area followed the same general pattern as that of leaf number with a decline in the leaf area of the infested trees four weeks post infestation (Figure 5.3C). In the final week of the experiment leaf area of the uninfested trees was approximately 68% greater than that in the low infestation treatment. There were significant differences between the regression lines of the uninfested and combined infested trees (Table 5.3). There was also a significant difference between the slopes for the two infestation treatments.

**Table 5.3.** P values of the F statistic comparing the regression lines for tree height, total leaf number and total leaf area of trees infested with different densities of woolly apple aphid in Experiment 3.

| | P value | | |
|---|---|---|---|
| Variable | All | Control *versus* combined treatments | Low *versus* high |
| Tree height | P<0.001 | P<0.001 | P<0.001 |
| Total leaf number | P<0.01 | P<0.001 | P>0.2 |
| Total leaf area | P<0.01 | P<0.001 | P<0.001 |

Total aphid number showed an increasing difference (P<0.05) between the high and low treatments in the first four weeks after infestation (Figure 5.4C) but by the end of the experiment there was no difference between the two. Similar to the other two experiments the intrinsic rate of increase for the low and high treatments were 0.156 and 0.11 respectively (Table A6.7). Estimated aphid-days in the low infestation trees was about 26% less than in the high infestation trees (36604 and 49540 respectively).

## 5.5. MATERIALS AND METHODS - FIELD EXPERIMENT

How old the trees

### 5.5.1. Experimental design

The experiment was laid out as a completely randomised block design comprised of three orchards (16.5 m length by 2 m width) subdivided into six plots (1.5 m length by 2 m). Plots within the orchard were separated by a gap of 1.5 m. Each plot had three rows of four trees spaced 0.5 m within rows and 1.0 m between rows. Prior to planting, the plots were randomly assigned to one of two rootstocks, Northern Spy or Granny Smith and to one of three levels of infestation; no aphids, low and high.

Each of the three orchards or experimental sites will be referred to in the text as 'blocks' or orchards. This term is not used in a statistical connotation but in a descriptive sense unless otherwise stated.

### 5.5.2. Year One

Northern Spy and Granny Smith were the chosen cultivars. 108 year-old trees of each cultivar were purchased in late March 1989. The semi-dormant trees were transplanted to 200 cm pots with sterilised recycled soil and placed outdoors to become completely dormant over the course of the winter.

In the last week of August, when the trees were still dormant, they were transplanted into the prepared sites (Blocks A, B, and C) at the Waite Institute. Lack of available space lead to one of the blocks being separated from the other two but within a half kilometre. Rows in all three experimental orchard blocks had a north-south orientation.

Blocks A and B were situated in Claremont Orchard which was a fallow pasture on a slight east-west slope and were approximately 10 meters apart. The soil consists of a mix of red-brown (B1 type) and brown earth (lime enriched C1) which has a coarse cloddy characteristic that will crack when dried out. It is considered to be suitable for apple cultivation given adequate irrigation. Predominant winds are from the east. The orchard block was bounded on the east by a lucerne block, on the south by pasture, on the west by a vineyard and on the north by a plot of cultivated banksia plants. The closest established apple planting was approximately 300 m to the west and down wind from the experimental blocks.

Block C was situated in the Alverstoke Orchard approximately 300 m north-west from the other site. The site was bordered on the east by a planting of mature apricot trees, on the south by seedling pistachio trees, on the west by a large plastic tube shade-house and on the north by a orchard of mature almond trees. The closest apple planting consisted of three rows of mature trees approximately 150 m to the south-east. The soil in this area is also a red-brown earth, slightly lighter than that of the other site as a result of the addition of top soil in the past. The east-west slope of the Alverstoke orchard site was slightly less than that of the Claremont blocks. This orchard was also slightly more protected from the strong 'gully' (katabatic) winds that occur in this region of Adelaide in the summer months.

Before planting the trees, the sites were rotovated twice to break up the soil. Holes, 50 cm deep and 40 cm in diameter, were dug by hand to avoid soil compaction around the roots. Immediately after planting, the trees were well watered and fertilised with 20N·20P·20K (1.5 gm/l water). The pH did not need to be adjusted at either site.

In the third week of September, as the sap was beginning to rise, all trees were T-budded with Granny Smith budwood. The budwood was obtained from a block of mature Granny Smith trees at the Lenswood Research Station (South Australian Department of Agriculture) in the 'Adelaide Hills' region of the Mount Lofty Ranges. The wood was collected in August while the trees were still dormant, wrapped in dampened paper and plastic and stored at 5±2°C until ready for use. After budding, the trees were left to break dormancy and the main stems were then cut back to the level of the bud union to force the bud to break.

A microjet drip irrigation system was set up and the trees watered on a weekly basis throughout the remainder of the season. This system was used because the bore water, which was being used for irrigation can 'burn' apple leaves with its high salt content. The orchards were treated in the same manner as a newly planted orchard regarding fertilisers, weed control and irrigation. Weeds around the base of the trees were removed by hand and the remainder were cut with a WhipperSnipper™ mower.

Once the new buds were growing, the trees were assessed and the four trees most similar in size and vigour in each plot were chosen as the 'sample' trees. This approach was taken due to a percentage of trees dying, buds not taking, or poor bud growth which prevented the use of all trees as had been intended. Every attempt was made to choose similar trees between as well as within orchards.

Aphids were not introduced to the treatment trees until the new shoots were at least 15 cm. The first introduction was in the second week in December 1989 when the trees were rapidly growing. The low infestation was initiated by placing ten first instar crawlers in the bottom of a gelatine capsule and attaching the capsule to the tree stem. High infestation treatments were initiated with 50 first instar crawlers. The aphids were released in the evening to give them a greater chance of survival and finding refuges on the tree before the heat of the day. A second batch of aphids was released one month later after the first infestation failed to establish. The stem of the tree at the release site was encased in a 'cage' of fine gauze mesh to both protect the aphids from predators and prevent them from leaving the area. The 'cage' was tied at each end with a Twist-Tie™ around the stem. Two final releases were made seven and 10 days after the second release on trees that had no sign of infestation. The gauze mesh was removed once the aphids had established colonies. No attempt was made to restrict the movements of the aphids after this and colonies appeared along the length of the stem. As the season progressed there was an influx of ants attracted to small colonies of *Aphis citricola*. These colonies were manually removed and did not reappear. The ants did not appear to interfere with the woolly apple aphids in any way but they did become a nuisance while measuring the trees. To prevent ants moving up the trees, a narrow band of Tac-Gel™ (polybutene) was applied to the base of the tree below the graft union. This treatment was done to all trees regardless of the presence of *A. citricola*. It also retarded the WAA from colonising the roots.

Tree height, total leaf number, total leaf area, and side shoot number, leaf number and area were measured at approximately monthly intervals throughout the season, starting in December until leaf drop in late June. Total colony number and area (which is used as a measure of total aphid number; Appendix Four), were also measured after infestation was established. When the total aphid number in the low infestation treatment rose over an estimate of 1500 per tree, excess colonies were manually removed. To determine the coefficient required in estimating the true leaf area, a selection of leaves were picked from non-experimental trees within the orchards and areas measured as set out in Appendix Five.

### 5.5.3. Year Two

The trees were allowed to become fully dormant over the winter before pruning. Pruning and fertilisation were to be as similar as possible to that employed in a typical orchard. Trees in young orchards are now most commonly trained to a central leader system, therefore this system was chosen. In August 1990, the trees were pruned to a height of approximately 460 mm. Where the tree did not reach this height the previous year, it was left unpruned or very lightly pruned to removed the dead wood at the tip. All lateral shoots were removed as well. If a lateral was at the point of cutting, the closest inter-lateral space was used. The cut stems and shoot ends were painted with pruning paint to prevent 'bleeding' and possible introduction of infection. All trees in the orchard were treated in the same way regardless of whether they were to be measured or not. Immediately after pruning, the sites were fertilised with complete fertiliser (20N.20P.20K). When the buds had broken, the second and third leaf rosettes were removed to allow the topmost bud to become dominant and form the trunk.

At each sampling date, trees that still appeared to be dormant were closely examined to determine if they had died. Dead trees could be easily differentiated from dormant trees by the texture and appearance of the stem bark. If a tree was alive, the bark was firm and had the normal greyish colour of a dormant tree; the buds had a healthy unshrivelled appearance. Dead trees had dark reddish bark that was wrinkled and dry and if a small portion of the bark was peeled back the underlying tissue was brown and brittle, whereas the dormant trees had a layer of green tissue under the bark.

Two attempts were made to reinfest treatment trees to replace the colonies of WAA which had died out during the winter. The same system used in the first year was employed but without success. With the failure of these attempts it was decided to leave the trees free of aphids for the entire season if possible. Measurements were taken in the same way as the previous year, starting in October. Due to the length of time needed to measure the trees (up to ten days for the three blocks) as the season progressed, time between measurements was expanded to two months. A sudden influx of WAA into all treatments occurred in mid-March however, and the experiment was terminated earlier than expected: the last measurement was taken in April. The trees were removed after the final measurement and the roots examined for the presence of aphids or old galls.

### 5.5.4. Statistical analysis

The data from the field experiment was analysed in the same manner as the laboratory experiment (Section 5.3.3) using regression analysis. Data for the total side shoot number, side shoot leaf number and area were analysed by main factor ANOVA and differences in means further compared by Tukey's (HSD) test (SAS 1985). Total colony and aphid number in the first year of the experiment were analysed by factorial model (with interaction) ANOVA and, if necessary, Tukey's (HSD) test (SAS 1985). With measurements made at monthly intervals it was not possible to accurately estimate the intrinsic rate of increase of the aphid under field conditions.

## 5.6. RESULTS - FIELD EXPERIMENT

As there were significant differences ($P<0.05$) among the three orchard blocks for all growth parameters the blocks were analysed separately. This requirement unfortunately complicates presentation of the results in the text and figures that follow. Nevertheless, certain overall trends will be considered in the discussion at the end of the chapter. No differences ($P>0.05$) were found between the two types of rootstock so rootstock was not considered in subsequent analyses. One tree in the high infestation treatment in Block C died after the first month of sampling, resulting in a reduced sample size for the remainder of the experiment.

### 5.6.1. Year One

#### 5.6.1.1. Biological overview

The trees in all three orchards remained dormant until November when there was a sudden increase in shoot growth. Once growing, the trees in Block C (in

Alverstoke Orchard) appeared to grow more rapidly than the trees in the other two blocks. In the first month of growth, mean tree height in this orchard was ca. 135 mm greater than the trees in the other orchards. The trees in Block A (Claremont Orchard) appeared to be affected by the inadvertent overspray of bore water from the adjacent lucerne paddock which was started in January. The trees stopped growing and the leaves became thickened and burnt; these are classic signs of salt damage. While the duration of this extra watering was not long (eight hours per week for less than three weeks) it was enough to affect this particular group of trees for the remainder of the experiment.

As the WAA colonies became larger and more numerous there was an influx of adult *Scymnodes lividigaster* (Mul.) (Coleoptera: Coccinellidae), larval and adult *Harmonia conformis* (Coleoptera: Coccinellidae) which were present for less than a month. They did not appear to affect the density of aphid colonies and no attempt was made to control them. Stem deformation, in the form of localised swellings, was observed on all infested trees regardless of the degree of infestation. While it was not possible to observe and measure galls accurately during the experiment without destroying colonies, a number of these swellings appeared to be splitting the bark of trees.

Late in the season (mid-April in Block C and early May in Blocks A and B), aphid colonies in all three orchards were parasitised by *Aphelinus mali*.. In spite of this aphid numbers continued to increase in the high infestation treatments in all orchards and all but one of the low infestation treatments (Block C). As control or elimination of the parasitoids would have been impossible without major destruction of aphid colonies, no effort was made to reduce their numbers.

Aphid numbers increased in the high density treatments towards the end of the season (May) and was accompanied by increased movement of first and second instar aphids along the length of the stem. While there seemed to be two way movement up and down the stem, the predominant direction of travel was towards the apical bud. On very heavily infested trees, crawlers also congregated along the midrib on the ventral side of the uppermost leaves. No alate forms were observed at any time.

#### 5.6.1.2. Tree growth

By the end of June, the trees stopped growing but still retained green leaves. There was no production of new leaves and most trees had lost a small number of the older, senescing leaves. Leaf drop did not occur until August, a phenomenon typical of this area.

Mean tree height in both blocks (A and B) in Claremont Orchard did not differ among treatments at any time (Table 5.4). In both orchards there was a sharp increase in height the first month, after which growth was minimal (Figure 5.5A, 5.6A). In Block A, the growth rate remained similar in all treatments. Trees in the high infestation treatment of Block B were taller than the other trees (Figure 5.6A) and remained so for the entire season. The uninfested trees were intermediate between the infested trees. In Block C, tree height increased quickly for the first three months before reaching a plateau (Figure 5.7A). Trees in the high infestation treatment remained shorter than the other trees after the initial sampling date and grew at a reduced rate. There was little difference between the uninfested trees and those in the low infestation treatment. There were no significant differences in the regression lines of the three treatments in either Block A or B (Table 5.4). In Block C there was a significant difference ($P<0.001$) between the low and high infestation treatments but no differences between the uninfested and the combined infested treatments (Table 5.4).

**Table 5.4.** P values of the F statistic comparing the regression lines for height of trees infested with different densities of woolly apple aphid in three orchards, Year 1.

| Block | P value<br>All | Control *versus* combined treatments | Low *versus* high |
|---|---|---|---|
| A | $P>0.05$ | $P>0.05$ | $P>0.05$ |
| B | $P>0.05$ | $P>0.05$ | $P>0.05$ |
| C | $P<0.001$ | $P>0.05$ | $P<0.001$ |

### 5.6.1.3. Leaf growth

In all three orchards there was a sharp increase in leaf production followed by a gradual decline in numbers (B in Figures 5.5, 5.6, 5.7). Mean leaf numbers peaked in Block A in early March (Julian Date 60) in the uninfested and high infestation treatment and at the end of January in the low infestation treatment (JD 30). There were no differences in the comparison of the three regression lines in this orchard (Table 5.5). Peak leaf numbers were reached in Block B in March (JD 60) in the uninfested and low infestation treatment and at the end of April (JD 115) in the high infestation treatment (Figure 5.6B). Leaf number was similar in all treatments for the first sample date, however for the remainder of the season the number of leaves on the highly infested trees was actually greater than on the uninfested trees; mean leaf number in the low infestation treatment was intermediate. When comparing the regression lines there was a significant difference ($P<0.02$) between the uninfested and the combined infested trees, however there were no differences between the two levels of infestation (Table 5.5). In Block C the leaf numbers peaked at the end of April (JD 115) for all treatments. There were also more leaves on the trees in this orchard than in the other two orchards. This was most marked in the uninfested trees where there were almost three times as many leaves in Block C on these trees as there were on the corresponding trees in Blocks A and B. There were significant differences in the regression lines between the combined infestation treatments and the uninfested treatment (Table 5.5).

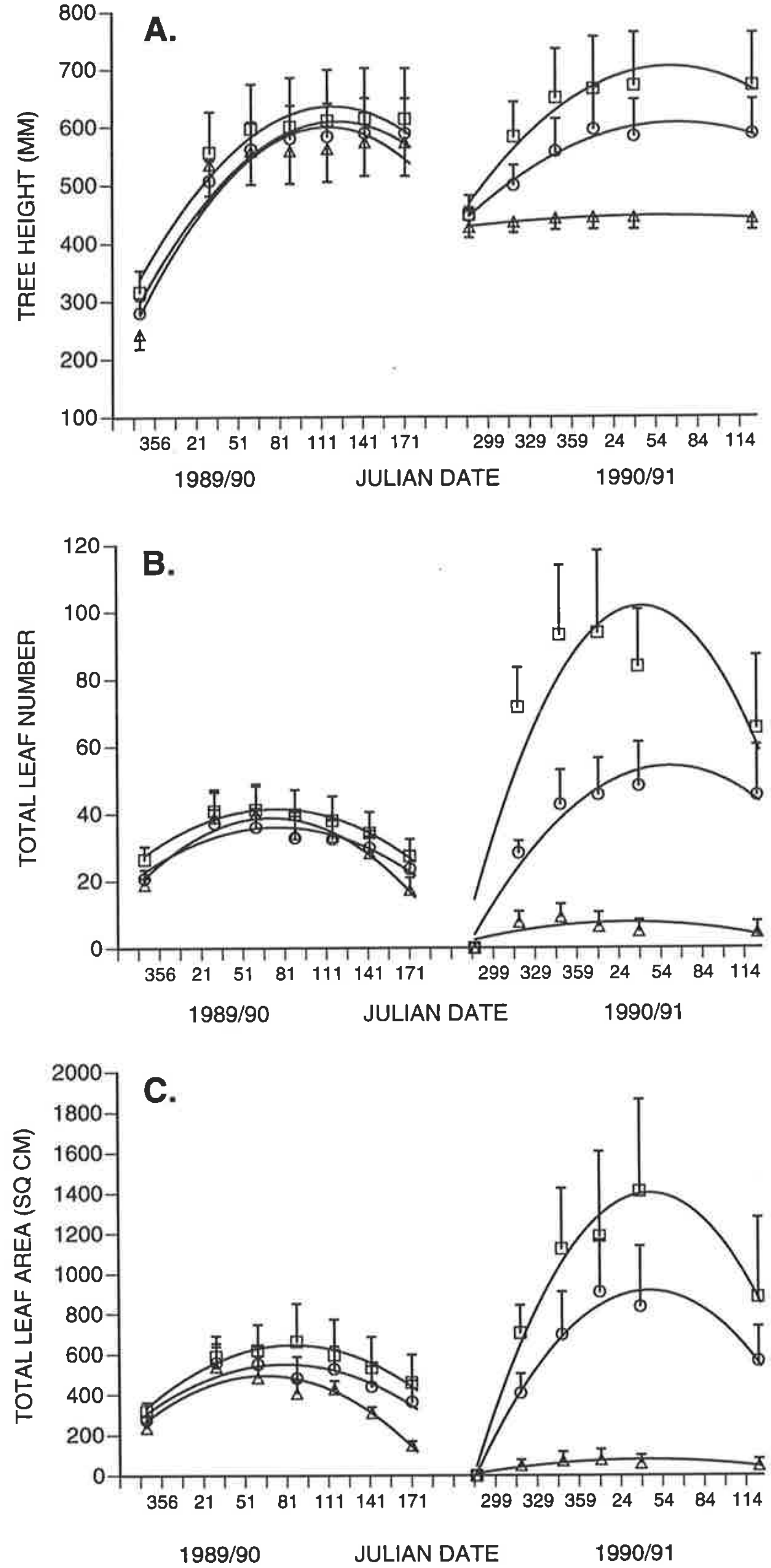


**Figure 5.5.** Relationship between tree parameters (mean ± standard error) and woolly apple aphid densities during Year 1 (1989-1990) and Year 2 (1990-1991) in Block A. (A) tree height, (B) total leaf number, (C) total leaf area. Solid lines represent predicted values. Symbols represent actual values.

No aphids □ Low infestation O High infestation Δ

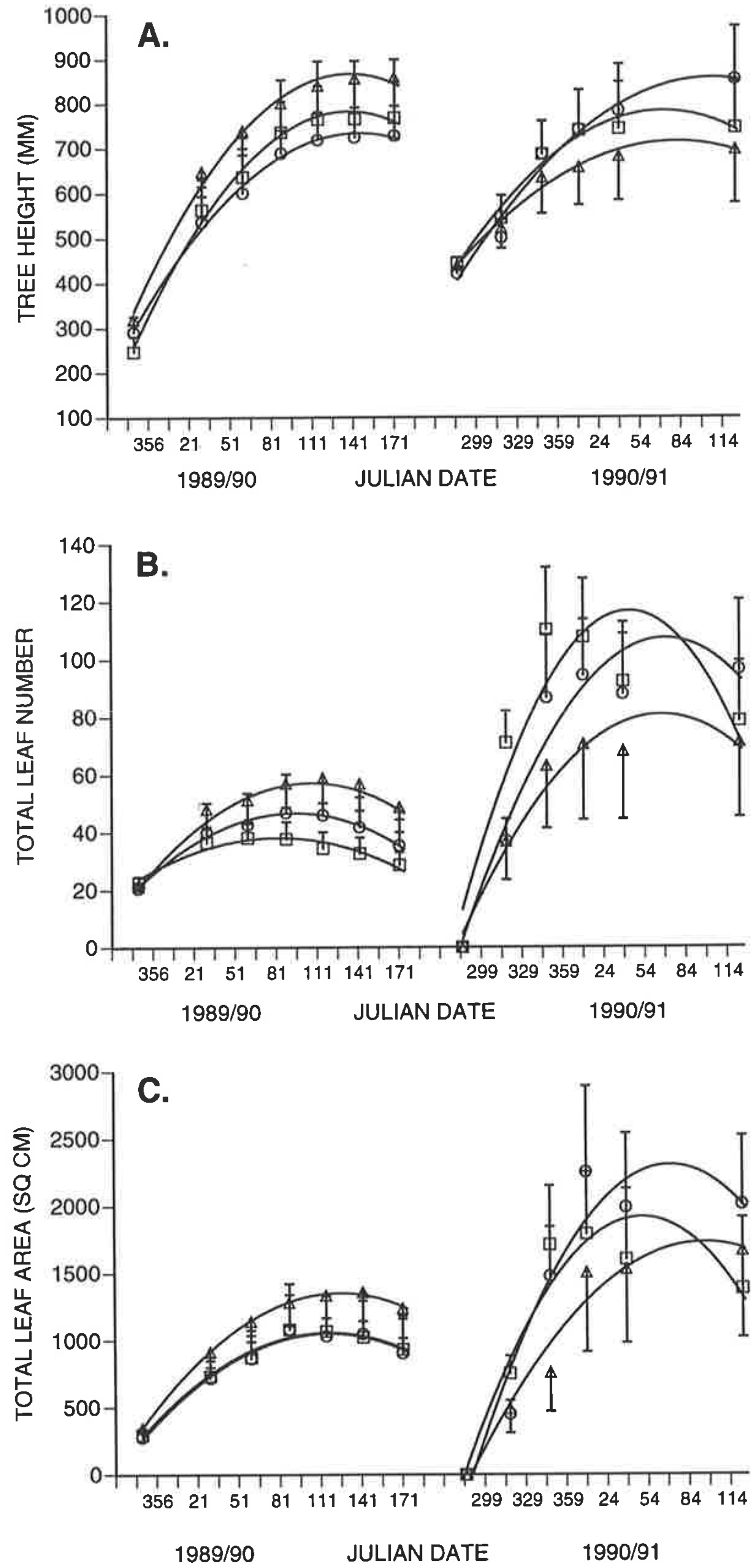


**Figure 5.6.** Relationship between tree parameters (mean ± standard error) and woolly apple aphid densities during Year 1 (1989-1990) and Year 2 (1990-1991) in Block B. (A) tree height, (B) total leaf number, (C) total leaf area. Solid lines represent predicted values. Symbols represent actual values.

No aphids □ Low infestation O High infestation Δ

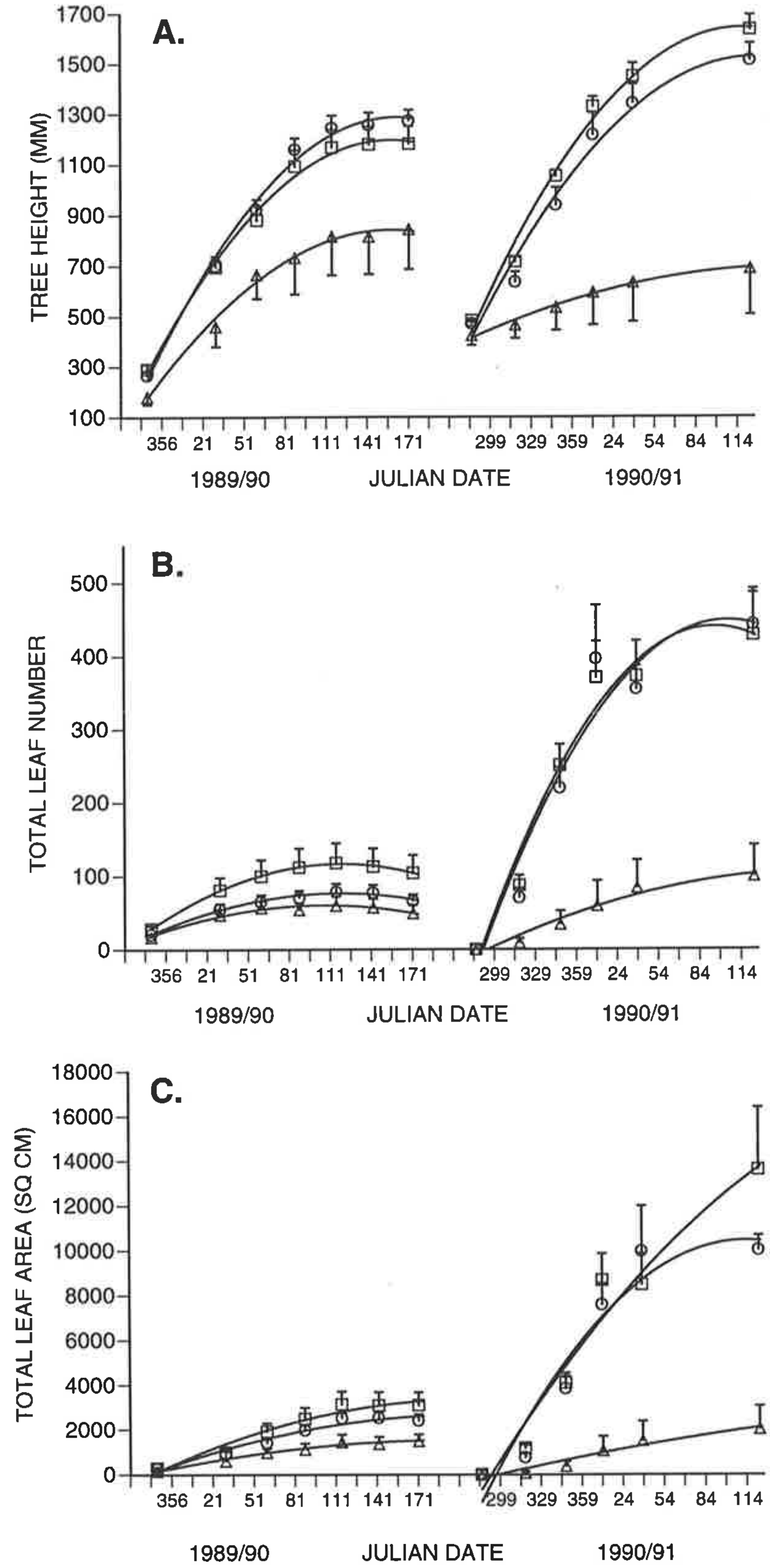


**Figure 5.7.** Relationship between tree parameters (mean ± standard error) and woolly apple aphid densities during Year 1 (1989-1990) and Year 2 (1990-1991) in Block C. (A) tree height, (B) total leaf number, (C) total leaf area. Solid lines represent predicted values. Symbols represent actual values.

No aphids □ Low infestation O High infestation Δ

**Table 5.5.** P values of the F statistic comparing the regression lines for total leaf number on trees infested with different densities of woolly apple aphid in three orchards, Year 1.

| | P value | | |
|---|---|---|---|
| Block | All | Control *versus* combined treatments | Low *versus* high |
| A | P>0.05 | P>0.05 | P>0.05 |
| B | P<0.01 | P<0.02 | P>0.05 |
| C | P<0.001 | P<0.001 | P>0.05 |

Leaf area was not significantly different among the three treatments at any time in Blocks A and B (Table 5.6). Mean leaf area in Block A rose after January, reached a peak in March (JD 60) and then declined for the remainder of the season (Figure 5.5C). Initially leaf area was similar in all treatments in this orchard, but by the last sampling date, the leaf area of uninfested trees was greater than that of the other treatments (Figure 5.5C). In Block B, leaf area was greatest in the high infestation treatment (Figure 5.6C) reflecting the trend seen for leaf number. In Block C, trees in the high infestation treatment had less leaf area than in the other treatments at the first sampling date and this difference increased with time (Figure 5.7C). After the second sampling date (late January) the leaf area of the uninfested trees was approximately twice that of trees with a high level of infestation. When comparing the regression lines, there were significant differences between the uninfested trees and the combined infested treatments as well as between the two levels of infestation (Table 5.6).

**Table 5.6.** P values of the F statistic comparing the regression lines for total leaf area of trees infested with different densities of woolly apple aphid in three orchards, Year 1.

| | P value | | |
|---|---|---|---|
| Block | All | Control *versus* combined treatments | Low *versus* high |
| A | P>0.05 | P>0.05 | P>0.05 |
| B | P>0.05 | P>0.05 | P>0.05 |
| C | P<0.001 | P<0.001 | P<0.001 |

#### 5.6.1.4. Aphid numbers

In all three orchards the uninfested trees remained free of aphids for the entire first season, in spite of infestations, sometimes at very high levels, in trees in nearby treatment plots. In Blocks A and B, colony numbers rose sharply in both infested treatments two months after infestation, reaching a peak in May (JD 140; Figure 5.8A,B). In March and April there was no difference in colony number between the two treatments, however, in the last two months there were more ($P<0.05$) colonies in the high infestation treatment. In Block B, there was a consistently greater number of colonies in the high infestation treatment (Figure 5.8B). This difference ($P<0.05$) was significant only at the onset of infestation at the end of March. In Block C, the highly infested trees had more colonies ($P<0.05$) than trees in the other treatment level after the infestation was established in late March (Figure 5.8C).

Aphid number, as estimated by colony area, (Appendix Four) differed significantly between treatments in April and May in Block A ($P<0.05$). At the end of the season, aphid numbers in the low infestation treatment continued to increase while numbers in the high infestation treatment dropped, resulting in no significant differences between the two levels (Figure 5.8D). Numbers in Blocks B and C were greatest in the high infestation for the last three sample dates ($P<0.05$). In Block B, aphid numbers in the low infestation treatment rose slowly throughout the season, and in the high infestation treatment increased rapidly in the last month (Figure 5.8E). Numbers remained low in the low infestation treatment of Block C for the entire season while in the high treatment they rose continuously (Figure 5.8F). For most of the season aphid numbers for the low infestation treatment were highest in Block B; Block C had the highest number in the high infestation treatment for two of the four sampling dates (Figure 5.8E, F). The initial number of aphids was similar within treatments for all orchards.

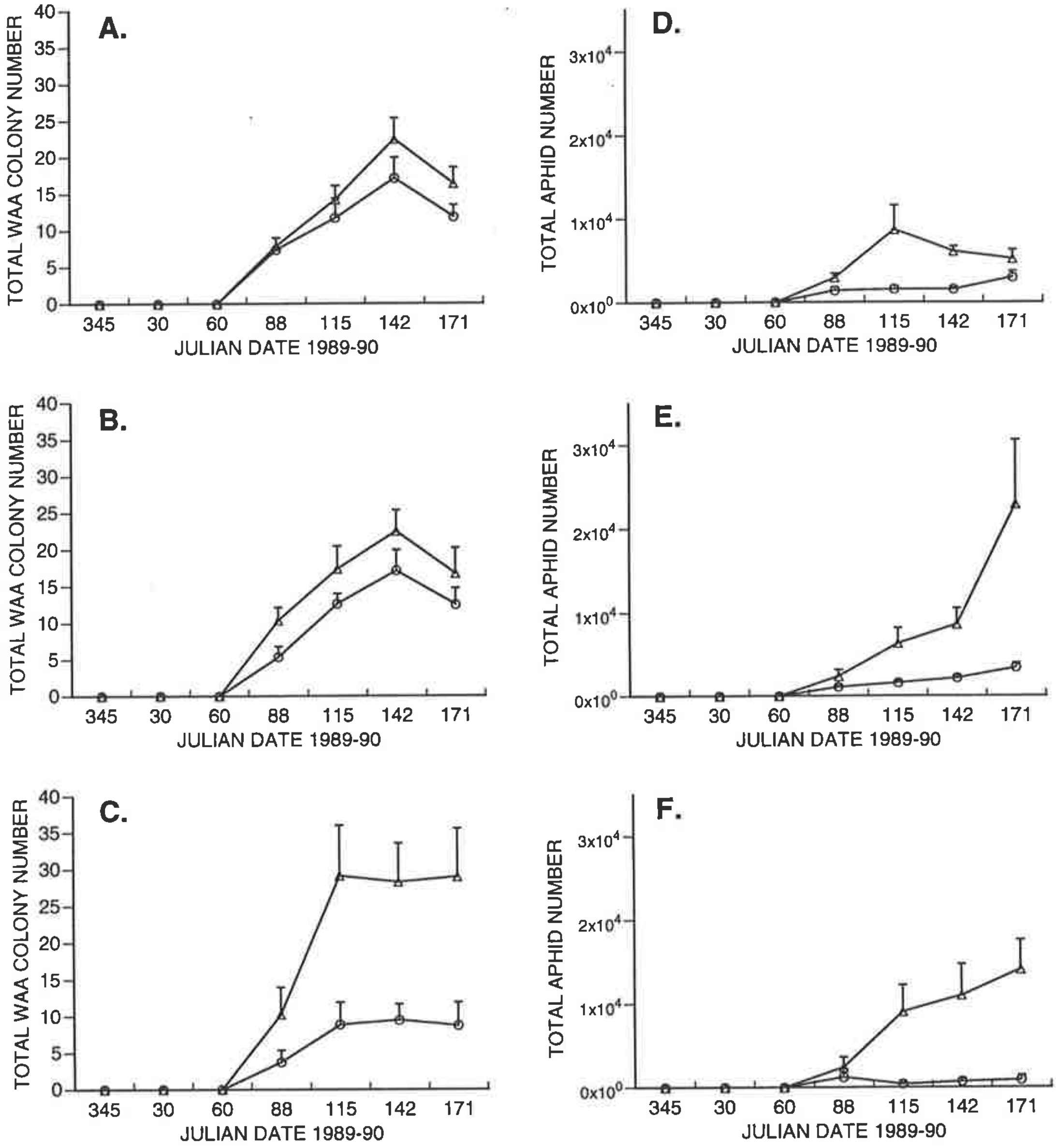


**Figure 5.8.** Number (mean ± standard error) of colonies and aphids on trees infested with different densities of woolly apple aphid in Year 1, 1989-1990. (A) number of colonies in Block A, (B) number of colonies in Block B, (C) number of colonies in Block C, (D) number of aphids in Block A, (E) number of aphids in Block B, (F) number of aphids in Block C. Number of aphids is estimated by the area of the colony (Appendix Four).

Low infestation O High infestation Δ

### 5.6.2. Year Two

#### 5.6.2.1. Biological overview

Throughout the winter, aphid colonies, with all life stages, were visible on the dormant trees. In early spring (September), when the trees were being pruned, there was a migration of first instars along the stem. Like the movements in the autumn, the majority of aphids were moving up the stem to cluster around the apical bud with relatively few descending. Any slight movement of the stem by wind or knocking dislodged the aphids. After pruning, the aphids were found at the highest point of the cut. This upward movement and consequent congregation continued until October. Aphids that were descending did not remain at the root area but were found wandering along the ground. *A. mali* was active in all three orchards throughout the spring and there was a large number of mummified aphids. By mid-spring (October) the crawlers were no longer found moving along the tree stems and nearly all the WAA colonies had disappeared from the infested treatment plots. At this point it was possible to see the amount of distortion that the plants had sustained through aphid feeding. This distortion was in the form of wrinkled and thickened bark around the entire stem. The areas of galling or splitting were not clearly defined, however, making it impossible to quantify the damage.

Uninfested trees broke dormancy almost three weeks earlier than infested trees. By October all the uninfested trees and approximately 95% of trees in the low infestation treatments had produced leaves. Some (34.8%) of the highly infested trees still had not broken dormancy by the middle of December (Figure 5.9). These trees eventually died (Figure 5.9) having produced no new leaves. There were more dead trees in Block A than in the other two blocks (62.5, 25, and 28.6% for Blocks A, B, and C respectively).

As the trees broke dormancy and began to grow it appeared that the bore water overspray from the previous season was still affecting trees in Block A.

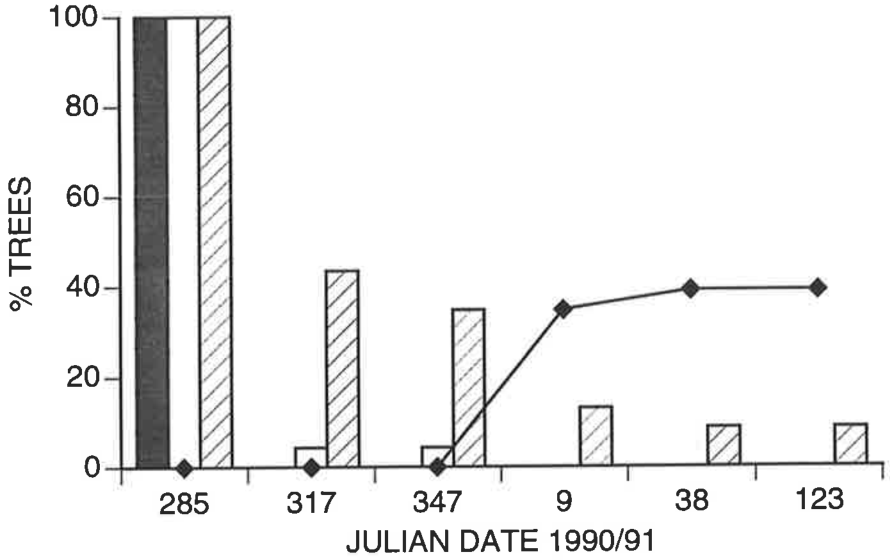


**Figure 5.9.** Percentage of trees that were dormant or dead in three blocks (combined) during Year 2 (1990-1991).

Dormant trees: No aphids ■ Low infestation □ High infestation ▨

Per cent dead trees in the high infestation treatments —◆—————◆—

Unfortunately the trees were again sprayed by the water from the lucerne paddock for three days before detection. This probably accounted for the continued poor growth of the trees in Block A for the remainder of the experiment. In spite of this, it was apparent that there were differences between treatments and measurements were continued. In Block B one tree was broken in half in late January and excluded from subsequent samples.

In April all trees were removed and the roots inspected for active WAA infestation and signs of galling as a result of previous infestation. No evidence of old or current infestations were noted in any tree from the three blocks.

#### 5.6.2.2. Tree growth

In the second year, the trees did not become much taller than their maximum height of the first season. There was a dramatic increase in the production of side shoots however, particularly in Block C. This will be considered in relation to leaf number and area in Subsection 5.6.2.3.

As in the first season, the uninfested trees in Block A were taller than the infested trees and appeared to show a greater rate of increase early in the season. The trees in the uninfested and low treatments increased by an average of 224 cm and 142 cm respectively, compared with an average increase of 15 cm in the high infestation treatment (Figure 5.5A). The overall comparison of regressions showed significant differences ($P<0.001$) between the uninfested trees and the combined infested trees as well as between the two levels of infestation (Table 5.7). In Block B there were no differences in tree height though the trees in the high infestation treatment were shorter than trees in the other treatments (Figure 5.6A; Table 5.7). The previous year the trees in this treatment were taller than the trees in the other treatments. Uninfested trees remained intermediate between the other two treatments after the first two sampling dates. In Block C, differences in height among the treatments were more obvious than in the other two blocks with height in the high infestation treatment

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less than in the other two treatments (Figure 5.7A). In comparing regression lines there were significant differences (P<0.001) between the uninfested trees and the combined infested trees as well as between the two levels of infestation (Table 5.7).

**Table 5.7.** P values of the F statistic comparing the regression lines for height of trees infested with different densities of woolly apple aphid in three orchards, Year 2.

| | P value | | |
|---|---|---|---|
| Block | All | Control *versus* combined treatments | Low *versus* high |
| A | P<0.001 | P<0.001 | P<0.001 |
| B | P>0.05 | P>0.05 | P>0.05 |
| C | P<0.001 | P<0.001 | P<0.001 |

### 5.6.2.3. Leaf and shoot growth

Total leaf number increased rapidly in the first two months in both the uninfested and low infestation trees in Block A (Figure 5.5B). From the second sampling date onwards, the highly infested trees had noticeably fewer leaves than the other treatments. The uninfested trees increased leaf number rapidly for two months before levelling off in January. These significant differences (P<0.001) were reflected when comparing the regression lines between the two levels of infestation, and between the uninfested trees and the combined levels of infestations (Table 5.8). In Block B, the uninfested trees had slightly more leaves than the trees in the other two treatments throughout the season (Figure 5.6B), but overall, differences in leaf number were not significant (Table 5.8). Leaf number increased rapidly in the uninfested and low infestation trees in Block C and continued to rise until the end of the experiment (Figure 5.7B). The trees in the high infestation treatment had fewer leaves than the trees in the other treatments throughout the season. When comparing the regression lines significant differences were seen between the combined levels of infestation and the uninfested trees as well as between the low and high infestation levels (Table 5.8).

Total leaf area mirrored leaf number in all three blocks (Figures 5.4C, 5.5C, and 5.6C) both in trend and significance. In Block A the differences in leaf area did not seem as marked as the increases in leaf number. In Block B, there were no differences in total leaf area among treatments throughout the season (Table 5.9). In both Blocks A and C there were significant differences ($P<0.001$) between the regression lines for the uninfested trees and the combined levels of infestation. These differences were also apparent in the comparison of the low and high treatments (Table 5.9).

**Table 5.8.** P values of the F statistic comparing the regression lines for total leaf number on trees infested with different densities of woolly apple aphid in three orchards, Year 2.

| | P value | | |
|---|---|---|---|
| Block | All | Control *versus* combined treatments | Low *versus* high |
| A | P<0.001 | P<0.001 | P<0.001 |
| B | P>0.05 | P>0.05 | P>0.05 |
| C | P<0.001 | P<0.001 | P<0.001 |

**Table 5.9.** P values of the F statistic comparing the regression lines for total leaf area of trees infested with different densities of woolly apple aphid in three orchards, Year 2.

| | P value | | |
|---|---|---|---|
| Block | All | Control *versus* combined treatments | Low *versus* high |
| A | P<0.001 | P<0.001 | P<0.001 |
| B | P>0.05 | P>0.05 | P>0.05 |
| C | P<0.001 | P<0.001 | P<0.001 |

In Block A there were no side shoots (feathers) produced on the trees in the high infestation treatment. There were more side shoots on the uninfested trees than the trees with a low level of aphids. This trend was also mirrored in the leaf number and leaf area. While there were side shoots in all treatment levels in Block B no differences were seen in shoot number, side shoot leaf number or area. In Block C, however, there were fewer side shoots, leaves and less leaf area in the highly infested trees than the trees in the other treatments. The results for these variables are presented in Appendix Seven.

## 5.7. DISCUSSION

Woolly apple aphid had a significant negative effect on the growth of its host both in the laboratory and in the field. Consistent trends of decreased height, leaf number and leaf area were observed in all experiments and in all but one of the orchard blocks (Tables A6.1-A6.3; A7.1-A7.6).

There were obvious differences between uninfested and infested trees under controlled laboratory conditions (Tables 5.1-5.3; Figures 5.1-5.3) and while differences could be seen between the two levels of infestation (Figures 5.1-5.3) they could not be detected statistically in all cases. Aphid densities in the low treatment achieved the same numbers as the high treatment before the end of the experiment. Lower temperatures would have lead to the two levels of infestation being different for a longer period of time perhaps leading to more observable differences in tree growth. This study was set, nevertheless, at temperatures that reflected the mean temperatures during the summer months (ca. 25°C) in the Mount Lofty Ranges of South Australia.

The laboratory study of Weber and Brown (1988) found an overall reduction in dry matter of stems and leaves after 16 weeks of infestation. The trends seen in Weber and Brown's (1988) study reflect those seen in this experiment with reductions of growth variables in infested trees compared with uninfested trees and little, if any, significant differences between levels of infestation. However, it is difficult to compare their results with this study since they did not quantify the impact the infestations had on tree height, leaf number or leaf area specifically, neither did they clearly separate the effects of the root and shoot populations.

The intrinsic rate of increase exhibited by the aphid, in all three laboratory experiments was very similar, within both the low and the high infestation treatments. In all cases the intrinsic rate of increase was between 29 and 35% (mean 33%) lower for the highly infested trees than the trees with a low infestation. This indicates a clear density dependence with a slower rate of increase when there is a higher initial number of aphids. This could be due to a 'feedback' mechanism where a host of decreasing nutritional value will adversely affect the development or reproduction of the insect feeding on them. For example, the fecundity of the rose-grain aphids (*Metopolophium dirhodum*, Wlk.) reared throughout their larval life on a host of low nutritional quality was lower than that of aphids reared on high quality plants (Grüber and Dixon 1988).

There were clear differences in tree growth between the three field blocks, irrespective of aphid infestation. This was most evident in the uninfested trees. The trees in Block C were much more vigorous than those in the other two orchards. This may have been due to the greater amount of top soil at the site, which had been used as a market garden in the past. This would provide a greater source of nutrients than the slightly poorer soil in the other site. The trees were also protected from the prevailing winds by a band of mature apricot trees to the east, though the effects of the wind could be still seen in their slanted growth. By contrast, the trees in Blocks A and B were subject to strong winds and grew on an obvious slant. Whether the overall decreased growth of trees in Blocks A and B, compared to the trees in Block C, was due to soil condition, wind conditions or a combination of these is difficult to determine. The trees in Block A also appeared to be affected by the overspray of the bore water from the adjacent lucerne pasture. They showed little growth in the first season and were much shorter than expected in the second season. Leaf production was also affected in the first season. In spite of the detrimental effects of the bore water, differences in tree growth were evident among the three treatments and, for this reason, the results of this block were left in the study.

In the first season there were little differences among treatments in any of the growth variables measured in Blocks A and B. However, there were differences between treatments for all variables in Block C. These differences were most marked when comparing the uninfested trees with the combined infested treatments (Tables 5.4 - 5.6). The highly infested trees in Block C were consistently shorter with fewer leaves and lower leaf area than those in the other two treatments. Reasons for this are unclear, however it may be related to the observation that these trees experienced the greatest growth in the first year. It is possible that this greater tree growth enabled the impact of the WAA infestations to be detected at an earlier stage. The differences among treatments in this orchard became more apparent in the second year.

In the second year of growth, differences among treatments were first apparent while the trees were breaking dormancy in the early spring. Almost 40% of the trees in the high infestation treatments died in the second season in spite of the absence of aphids during that year (Figure 5.9). Dormancy was delayed in both the low and high infestation treatments by up to two months. During the second season the trees in the low infestation treatments appeared to be less vigorous than the uninfested trees except in Block B. In Blocks A and C, the height of highly infested trees was 34% and 58% that of uninfested trees at the end of the experiment (Table 5.10). Tree height in the low infestation was also reduced compared to uninfested trees. Trees were affected more by high levels of infestation than low infestations, indicating that the effects were dependent on aphid density which is consistent with the findings of the laboratory growth experiments.

Changes in side shoot leaf numbers and leaf area of the field experiment mirrored the trends seen for total leaf number and area. Whether the WAA infestation affected the number of side shoots produced was outside the scope of this experiment and removing the first year laterals made determination of any interactions difficult. Nevertheless, there were definitely fewer side shoots in the high infestation treatments than in the other two treatments. This was especially apparent in Blocks A and C (Figure A7.1A-G).

**TABLE 5.10.** Percent decrease in height, leaf number and leaf area of trees infested with WAA compared to uninfested trees in Year 2.

| | | Date | | | | |
|---|---|---|---|---|---|---|
| **Variable** | **Density** | 13.XI.90 | 13.XII.90 | 09.I.91 | 07.II.91 | 03.V.91 |
| | | % decrease | % decrease | % decrease | % decrease | % decrease |
| **Block A** | | | | | | |
| height | low | 14.19 | 14.27 | 10.55 | 13.09 | 12.51 |
| | high | 25.05 | 31.87 | 33.18 | 33.78 | 34.16 |
| leaf number | low | 60.45 | 54.10 | 48.67 | 42.39 | 30.45 |
| | high | 89.20 | 90.07 | 93.19 | 94.03 | 92.90 |
| leaf area | low | 14.10 | 37.84 | 39.91 | 40.60 | 35.88 |
| | high | 25.05 | 93.39 | 94.84 | 95.72 | 94.33 |
| **Block B** | | | | | | |
| height | low | 8.05 | -0.22 | -0.36 | -5.52 | -14.81 |
| | high | 3.88 | 7.34 | 10.91 | 8.13 | 6.38 |
| leaf number | low | 47.70 | 21.30 | 12.20 | 4.65 | -23.04 |
| | high | 47.89 | 42.62 | 34.38 | 26.05 | 9.00 |
| leaf area | low | 39.95 | 13.55 | -25.81 | -24.37 | -45.61 |
| | high | 34.31 | 55.35 | 15.83 | 4.34 | -20.69 |
| **Block C** | | | | | | |
| height | low | 11.21 | 10.72 | 8.44 | 7.34 | 7.42 |
| | high | 34.80 | 49.22 | 55.22 | 56.30 | 57.87 |
| leaf number | low | 18.76 | 12.08 | -7.01 | 4.66 | -3.53 |
| | high | 89.52 | 86.26 | 84.01 | 77.45 | 76.71 |
| leaf area | low | 34.02 | 6.00 | 12.37 | -17.52 | 26.25 |
| | high | 92.81 | 90.79 | 87.56 | 82.10 | 85.02 |

While there were clear indications that WAA influenced the three variables presented here it was not possible to directly correlate aphid feeding to any one variable. Nor does it suggest that only one of the variables could be used to determine the effects of aphid density on tree vigour. Tree height, leaf number and area while inextricably linked are not totally dependent on each other. For example, tall trees may have a larger number of leaves or, conversely, have larger internodes resulting in the same number of leaves as a smaller tree with short internodes. A tree with a large number of small leaves will result in a comparable leaf area as a tree with few large leaves.

A decline in the leaf number on infested trees may be accounted for in part by the decreased growth of the tree. As a tree increases in height it forms new leaves at the stem tip and produces side shoots. In the laboratory experiment, however, no side shoots were produced and, without this additional source of leaves, and because few new leaves were formed on the main stem, any increase in leaf number was drastically curtailed. It is doubtful that the absence of side shoots was a result of aphid infestation as the uninfested trees also failed to produce shoots. Rather, the lack of side shoots can probably be attributed to the short duration of the experiment. Some reduction in leaf number can be accounted for by the loss of leaves at aphid feeding sites. This is especially noticeable in sites of high aphid numbers. Often the leaf bud was sufficiently distorted that no new leaf was produced either in the first season or the following season (personal observation).

The ratio between the roots and shoots (including the stem) of trees tends to be constant (Vyvyan 1957; Wareing 1970). Any disturbance to either region automatically results in a shift in tree growth patterns in order to compensate for the disturbance and regain the normal root/shoot ratio (Mika 1986; Oliveira and Priestley 1988). Pruning obviously results in a change in the root/shoot ratio and has been shown to result in a reduction in root growth and an increase in above ground growth (Young and Werner 1984; Head 1967). Prior to the initial infestation the trees were pruned to force the scion bud to break and form the main stem of the tree. This is the normal practice in the first year of growth and is usually done in the nursery. To break scion bud dormancy requires that nearly all of the original stem be removed. Pruning such a large proportion of the young tree would certainly have a very strong effect on initial growth and it is possible that this pruning early in the first season followed by increasing aphid numbers disrupted the return to the root/shoot ratio needed for normal tree growth. These stresses may have been exacerbated by the normal dormant pruning regime prior to the second season. While the trees may have sustained minimal damage in the first year due to aphid infestation they may have been unable to overcome the combined stresses of aphid infestation and the effects of pruning in the following season.

The results of the laboratory and field experiments indicate that there are differences in the way trees react to two types of infestation. The small potted trees reacted to what could be described as a catastrophic infestation - very high aphid numbers over a short period of time. The infested trees in the orchard were exhibiting the results of both a chronic (low infestation) as well a catastrophic (high) infestation. High numbers of aphids resulted in the death of the trees, though over a longer period of time in the field. The immediate effects (ie. in the first season) of aphid feeding could be seen in Block C in all three parameters (Figure 5.7). By the second season the differences were becoming more pronounced and the effects of chronic infestation could be seen, not only in reduced leaf number and area but in extended dormancy and in the death of the trees.

Not only can trees react to different levels of infestation - but they can also be influenced by the site or sites of infestation. This is an important point that should not be overlooked when determining the effects aphid infestations have on tree growth. Brown and Schmitt (1990) observed that root infestations had a negative effect on tree growth under field conditions. However, the results of their study present several problems in interpretation. All trees in the study had above-ground infestations and the control trees became infested both on the roots and the above-ground portion of the trees. These trees became infested within the first year and continued for the duration of the experiment. This makes it impossible to conclude that the observed effects were solely due to root infestations.

Exactly how aphid populations reduce tree vigour remains unclear and while physiological explanations for the decrease in tree growth were not investigated as such, they can be speculated on. A high load of aphids feeding will result in the loss of assimilate from the tree and if this drain is great enough, the result would be a reduction of overall growth (Miles 1989b; Balch 1952). Large populations of aphids feeding on sycamore and lime trees can reduce tree growth apparently by imposing a large nutrient drain on the tree (Dixon 1971a,b) which also affected the quantity of food stored for growth in the following year. Dixon (1971a) found that leaves of aphid infested sycamore trees were smaller, contained greater levels of nitrogen and were greener at leaf fall than the leaves of uninfested trees. He also noted that the distribution of stored products was radically altered by the presence of aphids and that this could affect tree growth in the following season. The apple trees in the present study had reduced leaf number and area in the second year, suggesting that the same types of pressures may have been exerted on these trees. The inability of some of the highly infested trees to break dormancy or produce leaves in the second year would also suggest that the reserves in these trees were severely depleted at the end of the first season and were insufficient in overcoming stresses experienced in the following year. Dixon (1971b) noted that lime trees infested with lime aphid (*Eucallipterus tiliae* (L)) showed normal growth in the above-ground portions of the tree, such as girth, height increment, leaf number and size, however the roots of these trees did not grow. Furthermore, in the second year of the study, when kept aphid-free the trees had the same number of leaves as uninfested trees but the leaves were 40% smaller. Studies on the interaction between the lime aphid and its host were also studied by Llewellyn (1972) who noted that the magnitude of the energy drain by aphids was such that it must have a profound effect on tree growth. While the lime aphid feeds on the leaves of the lime tree rather than the woody portions there could be similar effects on tree growth.

Kandiah (1979a) noted that in the spring, the carbohydrate levels of one year old apple seedlings dropped to 72% of the amount found in the tree when dormant. This depletion was then followed by a period of replenishment throughout the season so that by autumn the carbohydrate levels were 2.5 times that of the dormant levels. By determining the movement of labelled C throughout the year he also noted that spring assimilates provided almost no contribution to the roots. Leaves that were fed $^{14}C$ in the summer showed that there was an emphasis on downward movement of assimilates and that there was an indication that the autumn assimilates were contributing to root growth and storage (Kandiah 1979b). He suggested that carbohydrates in the root contributed substantially to the initial growth of the new shoots however he was not implying that the roots were acting in a specialised storage capacity. Rather, he felt that his findings indicated that the entire structure of the tree behaved as a storage unit for carbohydrates but that there was a possibility that reserves in the roots could make a major contribution to growth in specific regions at particular times during the season. Should this be the case then it would in part explain the poor growth of the apple trees in the second season of the present experiment even when there were no aphids on the trees. While not quantified, the root system of infested trees were noticeably smaller than those of uninfested trees. This reduced root system coupled with reduced reserves in other parts of the tree such as stem and shoots could have lead to the death of the trees.

In addition to the loss of assimilate it is possible that the ability of the heavily infested trees to take up moisture from the soil is impaired (Brown *et al.* 1991). In the laboratory experiments, the trees showed classic symptoms of water stress (leaf flagging) in spite of a sufficient supply of water and nutrients. Whether the trees experienced this difficulty in the field is difficult to determine. Certainly the highly infested trees did not exhibit the same symptoms of water stress as the potted trees but neither were they carrying the same load of aphids as the potted trees.

The results in the present study do not support any hypotheses that ignore the impact of above-ground populations of WAA. As a result of the difficulty in controlling root infestations more emphasis has been placed on their subsequent damage to the trees. More realistically, both areas of infestation should be investigated more closely - both individually and in combination. The present experiment was to have been part of a larger study: the other half was to study the effects of root infestation in the absence of aerial infestation on tree growth. Two attempts were made in the first year and one in the second to establish seedling apple trees in order to infest them with root colonies. Unfortunately the trees were either killed by inadvertent herbicide application or eaten by sheep escaping from a nearby paddock. After the third attempt the experiments were abandoned due to time restrictions, budget restraints and lack of available seedlings. Valuable information would also have been gained had the trees in the present experiment been older and grafted prior to planting and infestation. If the trees had been allowed to bear fruit information could have been gained on the effects of aphid infestation on fruit yield. However, budget constraints and land use commitments prevented the use of older nursery stock and the experiment from becoming a long term project.

The present study indicates that WAA infestations on the above-ground portion of the tree can have a significant negative effect on tree growth at a critical time in the tree's life - when it is a young seedling. Such effects, especially at low levels, may, of course, be masked by the effects of other insects or environmental conditions in the field as the tree grows. With the increased used of resistant rootstock (Carlson 1970; Ferree and Carlson 1987; Webster 1993) the importance of WAA infestations shift from the roots to the aerial portions of the tree. While laboratory or small plot experiments cannot mirror field conditions, they provide useful indicators of the potential impact of WAA on apple trees.

It would be dangerous to extrapolate the results from these two experiments to explain the interactions between WAA and mature apple trees in established orchards. A fully grown tree has much greater reserves than a seedling, both photosynthetically and nutritionally, from which to draw in times of stress (Oliveira and Priestley 1988). Reserves constitute 20 to 30% of the total dry matter in apple trees (Priestley 1970). Furthermore, as the tree become larger, the ratio of aphid numbers to variables such as leaf, root or stem area will shift in favour of the tree. In the first analysis, woolly apple aphid densities may well affect tree growth at crucial stages in the development of the orchard. Infestations that are not controlled in the early stages will have serious repercussions in the following seasons as the tree produces scaffolding and its form takes shape.

The first few years in a tree's life are unquestionably important. A poor start will result in a tree that is slow to mature and has reduced yields once into its bearing years. For example, a small reduction in trunk growth over a succession of years can lead to a significant dwarfing of the whole tree after several years (Mika 1986). A reduction in leaf number may also reduce fruit yield in more mature trees as the spur leaves - those that are necessary for fruit production - may be reduced or lost as well as the vegetative leaves. It has also been shown that the number of laterals on one-year-old trees is an important determinant of early production and yield (van Oosten 1978; Quinlan 1978). Van Oosten (1978) noted that the more laterals a tree had in its first year the higher the yield was in subsequent years. In the present study the uninfested trees had more laterals than the infested trees (Tables A7.7-A7.9).

The impact of WAA on young trees may have been less important in the past, when orchards, with large trees, typically had a lifespan from thirty to forty years (Luckwill 1969). However, more recently, there has been a dramatic change in orchard production and management (Oberhofer 1989). While the greatest changes have occurred in Europe and North America, research into new orchard systems has also occurred in New Zealand and Australia (McKenzie 1981; Jotic 1981). With the pressures of high running costs, demands for high fruit quality and lower returns orcharding has become much more of a business (Barritt 1989) and growers have to be more rigorous in the management of their orchards. As a result the modern orchard will tend to have smaller trees planted at a density ranging from 600 to 5000 trees/hectare or more (Jackson 1989, 1993; Gil-Albert 1993; Tustin *et al.* 1993) compared to past densities of 200 trees/hectare. Trees would be staked and grown to a system to maximise light interception, would set fruit within three years (Gil-Albert 1993), compared to five years and have a lifespan of less than twenty-five years (Goedegebure 1993) rather than thirty or forty years. Modern orchards are very expensive to establish (Luckwill 1978) which makes the health of the tree that much more important.

Greater emphasis should be put on the control of WAA in nursery stocks, young orchards and high density orchards. WAA can be more prevalent due to the absence of pesticides such as azinphos-methyl which would not be used on non-bearing trees. In a high density situation it can be easy to allow low infestations to go unchecked. Also there are no guarantees that nursery stocks will be aphid-free resulting not only in a source of future infestation but in trees that have already had their growth affected by WAA. The costs associated with a modern orchard are too great to take chances on the initial health and vigour of the young trees. Use of a systemic aphicide such as vamidothion would be highly advantageous in the absence of azinphos-methyl in the non-bearing years. By applying it early in the season it would give protection for several years and allow predators and parasitoids to increase in the years where it is not applied. Certainly more importance should be placed on the control of low and chronic above-ground infestations of WAA on young nonbearing trees now that that portion of the tree's life has increased compared to its total lifespan. Further research into the effects and mechanisms of chronic aphid infestations on the above-ground portions of the tree is needed to augment the studies on root infestations.

# CHAPTER SIX

# SEASONAL ACTIVITY OF WOOLLY APPLE APHID ON MATURE TREES

## 6.1. ABSTRACT

The spatial distribution and seasonal abundance of woolly apple aphid colonies (WAA) on mature apple trees in a sprayed and an unsprayed orchard were studied over a two year period. Ten trees in each orchard block were randomly chosen and sampled in the first year. In the second year, the first 10 infested trees per block were sampled. The trees were divided into four directional quadrants (N-E, E-S, S-W, W-N) and four horizontal strata (rootcollar, lower, middle and upper). At regular intervals the number, size and position of aphid colonies were recorded for each tree. In the first year similar colony numbers were recorded in sprayed and unsprayed trees, but differences became apparent in the second year. In both years more colonies were found in the south-west and south-east quadrants and in the upper strata of the trees from the onset of infestation.

In the first year seasonal movements of aphids were investigated using sticky tape traps placed at three heights. Sixteen trees were assigned to one of four trapping treatments; traps were placed in the upper and middle strata, the trunk and all three levels of the tree. Traps were changed fortnightly and the numbers of aphids counted. Aphid numbers in all levels of the tree showed the same seasonal trends with peak catches at approximately the same time. There did not appear to be any seasonal migration up or down the tree.

## 6.2. INTRODUCTION

Woolly apple aphid presents unique difficulties in sampling. Because it feeds on the woody tissues of the tree, where it is often found lodged within cracks or crevices, sampling must either be done *in situ* or destructively. Destructive sampling would require the removal of shoots which over the course of a season, would be impractical due to the amount of tissue removed. The physical characteristics of the colonies make non-destructive sampling extremely difficult. The flocculant exudate of aphids hides individuals within the colonies making a simple count of *in situ* individuals impossible. An estimate can be made by measuring the width and length of a colony (Appendix Four), but this is time consuming and impractical as a sampling method. Finally, the aphids' ability to colonise the root system of trees compounds the difficulty in accurately estimating populations within an orchard. This difficulty in estimating populations has been noted by others (Thompson 1934; Jancke 1939; Carnegie 1963; Croft and Hoyt 1983; Noppert *et al.* 1987). Evenhuis (1958, 1962) stated that it was only possible to determine the times that the aphid population in the orchard was either 'very high' or 'very low', giving an indication of when the populations were increasing or declining.

In South Australia, no work has been done on the spatial distribution and movement of woolly apple aphid on mature trees. Control measures by growers tend to be initiated by intuition rather than a clear understanding of the aphids' distribution on the tree. A sampling method is in use that measures the number and size of colonies on a set number of trees which varies with the size of the orchard being sampled (A. Granger, S. A. Department of Agriculture, personal communication; Table 6.1). This method was established by experience and a knowledge of what infestation level the growers would tolerate before using chemical control with or without the advice of a pest manager.

**Table 6.1.** WAA rating system used by S.A. Department of Agriculture.

| Rating | WAA infestation/tree |
|---|---|
| 1 | No colonies |
| 2 | A few pinpoint colonies only |
| † 3 | up to 10 small colonies (each <1 $cm^2$) |
| 4 | up to 30 colonies |
| 5 | 30 to 100 colonies present |
| 6 | 100 to 200 colonies |
| 7 | > 200 colonies |

Ratings 1-3 are commercially acceptable
† threshold used by SA Dept. of Agriculture

Closely allied to the seasonal change in colony numbers is the movement of the aphids. As colonies become larger the youngest aphids tend to leave the colony. Throughout the season, aphids can be seen moving along the branches or trunk of the tree (Schoene and Underhill 1935; Evenhuis 1958; Hoyt and Madsen 1960). Reports on the amount and direction of WAA movements appear to vary geographically. In Europe and North America it has been suggested that the aphids overwinter on or near the roots migrating to this area in the autumn (Theobald 1921; Jenser 1983; Staübli and Chapuis 1987) followed by a corresponding spring migration from the roots to the canopy of the tree. In California however, Madsen and Bailey (1958) stated that aphids from the roots move upwards throughout the season and that the peak of this movement is during the summer months; they did not discover any seasonal migration up or down the tree. Lohrenz (1911) suggested that the aphids did not wander unless it was hot in which case they moved to the roots. Froggart (1903) found that there was no set pattern to the wanderings of the aphids, whereas Patch (1912) stated that young aphids sought the roots after leaving the colonies and that adults moved from the roots only when the roots were destroyed. In New Zealand Dumbleton and Jeffreys (1938) did not find any definite autumn migration to the roots. Reports from Australia indicate that the aphid can be found on the canopy of the tree in the spring (Bengston 1960), but there is no evidence that the aphids move there from the root area. Asante et al. (1993) found that there were relatively large numbers of aphids on the trunk and branches during the winter and spring.

In view of the uncertainties generated by the wide range of opinions reported above, an experiment was initiated to determine whether there were seasonal migrations of the aphid between the roots and canopy. A strong upwards spring migration from the root area to the canopy of the tree could have important implications in the chemical control of the aphid. Not only could the timing of sprays be better established but target areas of the tree could be delineated.

The sampling system used by the Department of Agriculture appears to be generally effective, nevertheless it was decided to investigate the colony distribution on the tree more closely to see whether simplification of the sampling regime might be possible. The question of greatest interest was to determine whether the colonies were equally distributed on the tree. Initial investigations showed that there may have been some differences in the number of colonies at different levels. The distribution of colonies on the tree may be related to aspect (Southwood 1978). Workers have noted, anecdotaly, that woolly apple aphids tend to colonise the centre of the tree and watershoots growing out of the trunk (Newman 1924; Evenhuis 1958). Thompson (1934) stated that, in England, the aphid was particularly abundant on the underside and north-east sides of the branches. Asante *et al.* (1993) stated that woolly apple aphid showed a clear preference for the lower part of the canopy and trunk.

Information concerning the spatial and temporal distribution of the aphid and its colonies could give an indication of how best to sample the tree; for example, will random sampling of pre-designated portions of the tree provide unbiased estimates or is one area likely to be more heavily infested, and thus require stratified sampling? Knowledge of the temporal growth of colony numbers in relation to position on the tree will thus assist in more efficient timing and application of sprays.

## 6.3. MATERIALS AND METHODS

### 6.3.1. Colony distribution and number

Two orchard blocks of mature (approximately 15 years old) Granny Smith/MM106 trees were chosen from the orchards at the Lenswood Research Centre (South Australian Department of Agriculture). Lenswood is situated in the 'Adelaide Hills' region of the Southern Mount Lofty Ranges of South Australia and is the major apple growing area of the state with warm, dry summers and cool moderate winters.

The term, ‘block’ is used in this study to designate a group of trees within the main orchard of the Research Station. It is the term commonly used among orchardists to denote specific sites within their orchards. It has no statistical connotation unless specifically stated. In the statistical context each tree is considered the experimental unit.

Each orchard block had 65 trees (five rows of 13 trees) with the rows running north-south. The trees were pruned to form a modified open vase shape. One orchard had not been sprayed for over five years and the second orchard was under a routine spray schedule. The unsprayed block was flanked by two sprayed blocks and each block were buffered by a row of Jonathans on each side. Only the trees in the centre four rows of the unsprayed block were sampled to minimise the effects of spray drift and possible edge effects. Ten trees were randomly sampled from each orchard while the trees were dormant (August) and there was no evidence of aphid infestation.

The trees were divided into four horizontal strata - rootcollar, lower, middle, and upper (Figure 6.1A). The rootcollar was that part of the trunk adjacent to the ground, where the scion was grafted to the rootstock. The lower portion was from the rootcollar to the first scaffold limb (approximately 80 cm) and represented the trunk itself. The middle portion was from the first scaffold limbs to a height of 1.8 m and the upper portion was that part of the tree above 1.8 m. With the modified vase shape, the upper portion of the canopy represented approximately the same area as the middle portion of the tree. The trees were also divided into four directional quadrants - N-E, S-E, S-W, N-W (Figure 6.1B). Each quadrant covered that part of the trees between the major compass points; ie. N-E represented that quarter of the tree from due north to due east.

At fortnightly intervals the number, position and size of WAA colonies were recorded for each tree. Colony size was roughly estimated (length and width) as it was impossible to measure all colonies. Concurrently, a visual check for the presence or absence of the woolly apple aphid parasitoid, *Aphelinus mali*, was made in each sample tree.

In the first year 10 sticky pipe traps were placed throughout each orchard to capture alates, apterae and parasitoids. They were placed on the east and west sides of non-sampling trees. The traps consisted of a portion of white PVC drainpipe (40 cm length and 10 cm diameter) covered with a piece of clear plastic wrap (GladWrap™). A thin layer of TacGel™ (polybutene) was painted on the wrap to catch the transient aphids and parasitoids. The sticky wrap was changed fortnightly. The intent was to intercept, rather than attract, both aphids and parasites that were flying or being blown from tree to tree.

Sampling commenced two weeks after aphids were first caught on yellow sticky tape traps placed on adjacent, non-sample trees; it was continued until

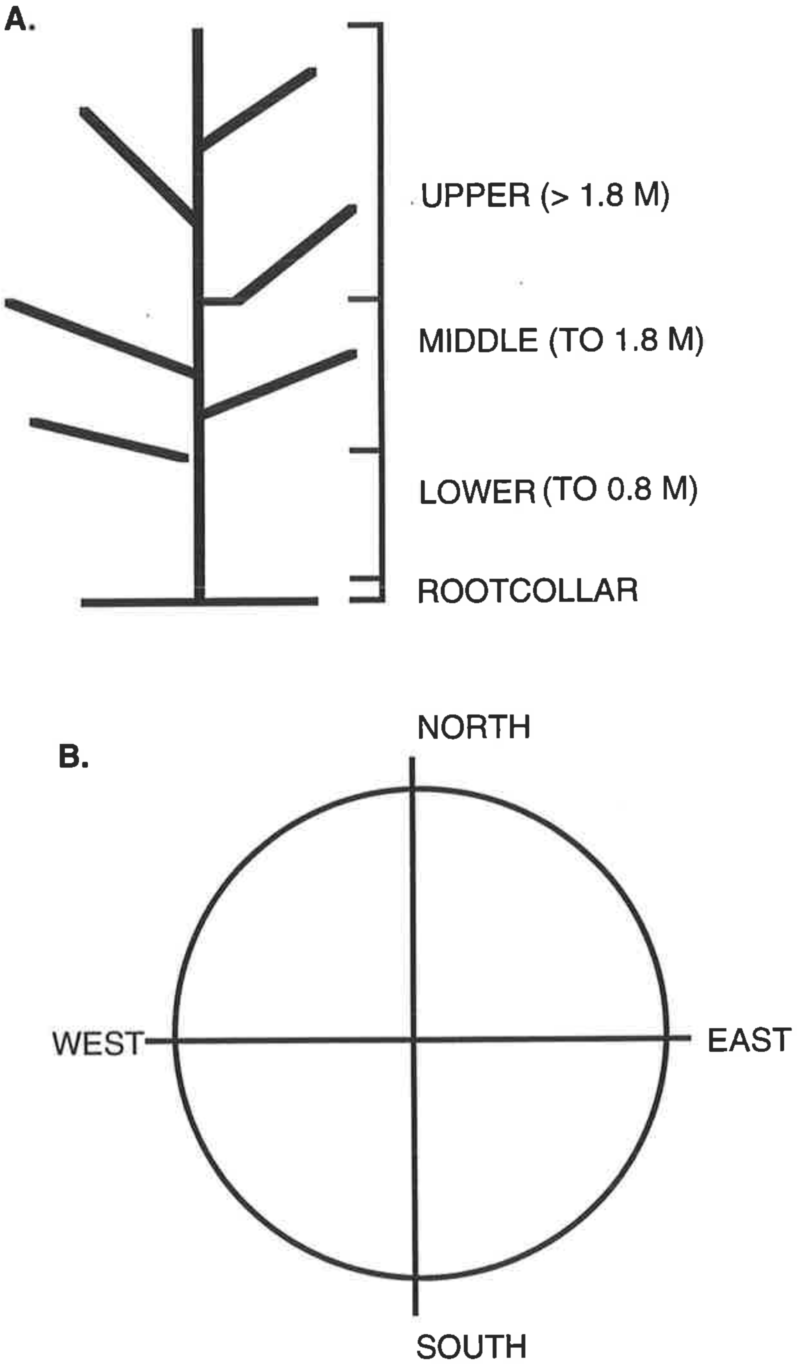
A.
UPPER (> 1.8 M)
MIDDLE (TO 1.8 M)
LOWER (TO 0.8 M)
ROOTCOLLAR
B.
NORTH
WEST
EAST
SOUTH

**Figure 6.1.** Tree divided into (A) height strata and (B) directional quadrants.

the number of colonies had dropped to zero for two consecutive samples. Data for one date in the unsprayed block were not collected (Julian date 68) due to rain. In mid-March (Julian date 73) an application of clorpyrifos (Lorsban®) was applied to the sprayed block specifically to control WAA.

The patchy distribution of infestation within the orchards lead to a modification of sampling in the second year of the study. All trees in the orchard blocks were checked for aphid colonies at weekly intervals and the first 10 infested trees in each block were chosen as the sample trees. This involved spending approximately 10 minutes per tree looking for aphid colonies, making sure that the entire tree was examined. The chosen trees were sampled for the rest of the season even if the initial infestation died out, as was the case in some trees. Trees were again divided into four directional quadrants but the four height divisions were reduced to three by the amalgamation of the rootcollar and lower sections. The sampling dates were increased to monthly intervals instead of fortnightly because of the length of time required to completely sample the trees. Due to the extremely low number of infested trees in the sprayed block after three months, it was decided to sample intensively six additional trees that had been infested the previous season.

Total colony numbers between the two adjacent blocks were compared by ANOVA (SAS 1985) for each sampling date. The expected frequency of colonies within each strata or quadrant was compared with the observed number of colonies and analysed by contingency tables (Sokal and Rohlf 1981). Analysis was based on the null hypothesis that there would be no difference in colony numbers among strata or quadrants. Trees that remained free of infestation throughout the year or had a total of less than four colonies were not included in the analysis. Data for all other trees in the block were pooled for the analysis on each date.

### 6.3.2. Seasonal movements of aphids

Sixteen trees were randomly chosen in each of the orchard blocks used in the above experiment. Each tree was assigned to one of four trapping regimes so that a total of four trees were assigned to each. The first treatment consisted of two sticky barrier traps around the tree trunk. One trap was placed approximately 10 cm above the rootcollar and the second trap was 15 to 20 cm below the first scaffold limbs. The traps were placed so that aphids travelling up the trunk would be caught by the lower trap and aphids travelling down the trunk from the canopy would be caught by the upper trap. A four cm wide trap was placed half-way between the two other traps to catch any aphids that managed to get through the first barriers. The second treatment consisted of four sticky barrier traps placed in the middle strata of the canopy (ca. 1.5 m). The third treatment placed four traps in the upper strata of the tree canopy (ca. 2.5 m). The four traps in these treatments were positioned at the main compass points to ensure equal coverage of the tree. The fourth treatment was the combination of the other three so that each tree in this group had a total of 10 traps.

The traps were made by wrapping a two centimetre wide strip of heavy cloth tape (Woton™) around the trunk or branch. This strip protected the tree and acted as a base for the traps that would be removed. A second strip of yellow cloth tape was placed over the first and a thin layer of Tac-Gel™ (polybutene) was applied so that the entire circumference of the branch was covered. Branches of similar circumference were chosen. The traps were first set out in September (1988) when the trees were dormant, with no aphid colonies visible, and changed at fortnightly intervals until early June (1989). Aphids caught on the traps were counted and developmental stages recorded. Any parasitoids or predators were also recorded.

Differences in trap size were minimised in the analysis by dividing the total number of aphids by the length of the trap and recorded as aphids per millimetre. For each sample date ANOVA (1985) was used to test for differences in the number aphids per millimetre among treatments. If there was no difference in aphid numbers among treatments, the data were pooled to compare the different strata. The number of aphids between the upper and middle strata of the tree were compared for each sample date by ANOVA (SAS 1985). Trap catches from the lower and upper traps of the trunk were compared in the same way. Orchard blocks were analysed separately because of the large differences in the number of aphids that were caught.

## 6.4. RESULTS

### 6.4.1. Colony distribution and number

In spite of the differences in colony numbers between the two years, the trends throughout the season were similar (Figure 6.2A-D). In the first year aphid colonies did not appear in the trees until late December/early January and peak infestations occurred in early March (Figure 6.2A). Infestation levels remained low throughout the season with five trees in the unsprayed block and two trees in the sprayed block remaining uninfested. Total colony number ranged from zero to 416 at the time of peak infestation. While not significant, there were more colonies in the sprayed block than the unsprayed block on all sample dates. Aphid infestation was also evident in the sprayed block at an earlier date (Julian date 25) than the unsprayed block (Julian date 40). Colony number fluctuated within individual trees throughout the season as the colonies became larger and amalgamated, died, or as new colonies were formed. Colony numbers declined in the sprayed block after the application of chlorpyrifos but within six weeks the population was beginning rise again (Figure 6.2A, B).

The observed number of colonies in quadrants differed significantly from the expected frequency (P<0.001) at each sample date in the first year.

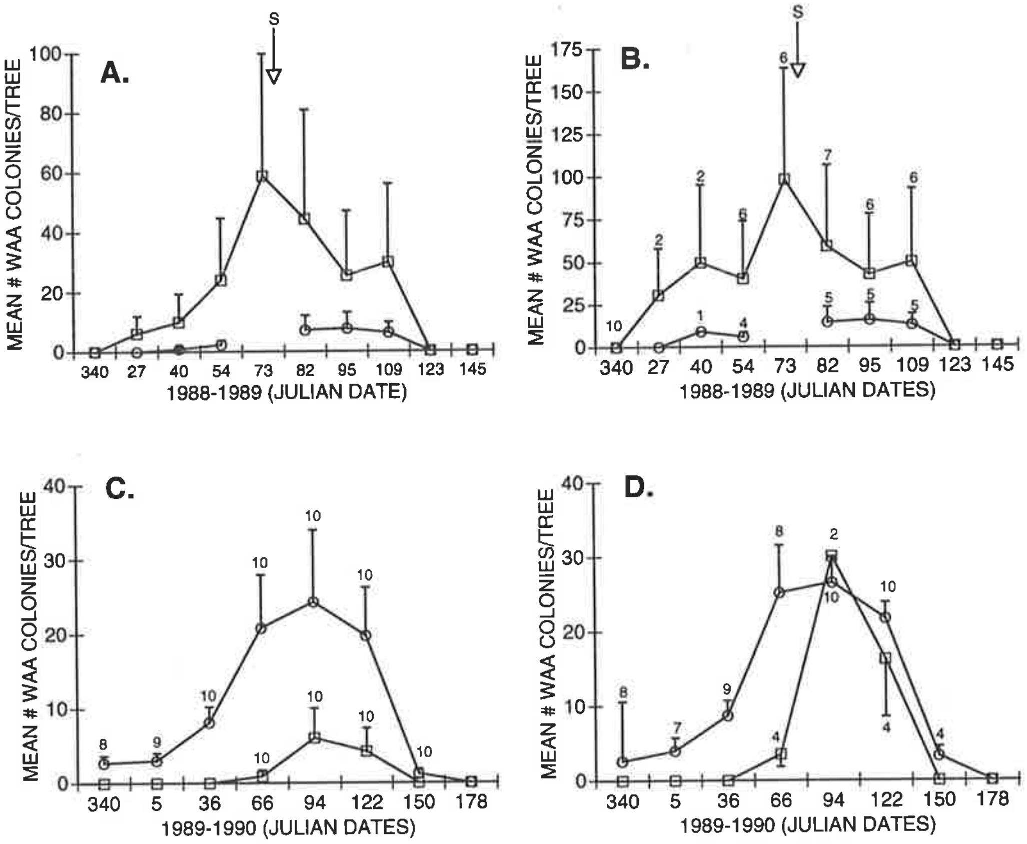

**Figure 6.2.** Total number (mean ± standard error) of WAA colonies on trees throughout the season.

(A) 1988-1989. All trees regardless of infestation are included. N=10 trees/block.
(B) 1988-1989 using only trees that were infested. Number of infested trees is indicated above symbol.
'S' = application of chlorpyrifos to sprayed orchard block.

(C) 1989-1990. All trees regardless of infestation are included.
(D) 1989-1990 using only infested trees. Number of infested trees is indicated above or near symbol.

Sprayed □ Unsprayed O

There were more colonies in the S-W and S-E quadrants than in the other two quadrants; a trend that was evident throughout the entire season (Figure 6.3A). The S-W quadrant consistently had the greatest percentage of the total colonies throughout the season. As with the directional quadrants, the observed colony number within strata differed significantly from the expected frequency ($P<0.001$). More than 50% of the total colonies were found in the upper portion of the tree canopy for all sample dates (Figure 6.3B). This tendency of WAA to colonise the upper portion of the tree was apparent from the beginning of the season until colony numbers dropped to zero (Figure 6.3B). As a corollary, there were very few colonies on the rootcollar or lower stratum on any date.

Colonies tended to remained smaller than 10 $mm^2$ but as the tree became more heavily infested the colonies merged, in some instances covering areas greater than 20 cm in length and encircling the shoot. On the most heavily infested trees, new growth was frequently encased in dense colonies. These large, dense colonies tended to be on the watershoots in the centre of the canopy regardless of the stratum. Once infestation was established the increase in colony number and size was rapid.

Parasitoid numbers remained low in both blocks all season with a maximum of nine caught in all sticky pipe traps (a total of 10 traps per orchard block). These were caught in April (Julian date 90-104) when colony numbers were on the decline. This low number was also seen in yellow sticky tape traps in adjacent non-sample trees. At the end of the season (May/June) however, more parasitoids were seen on the leaves than at any other time and parasitised colonies were also visible, though in very low numbers. At this time there were no parasitoids caught in the traps. Colonies removed throughout the season from non-sample trees in adjacent blocks showed no sign of parasitism.

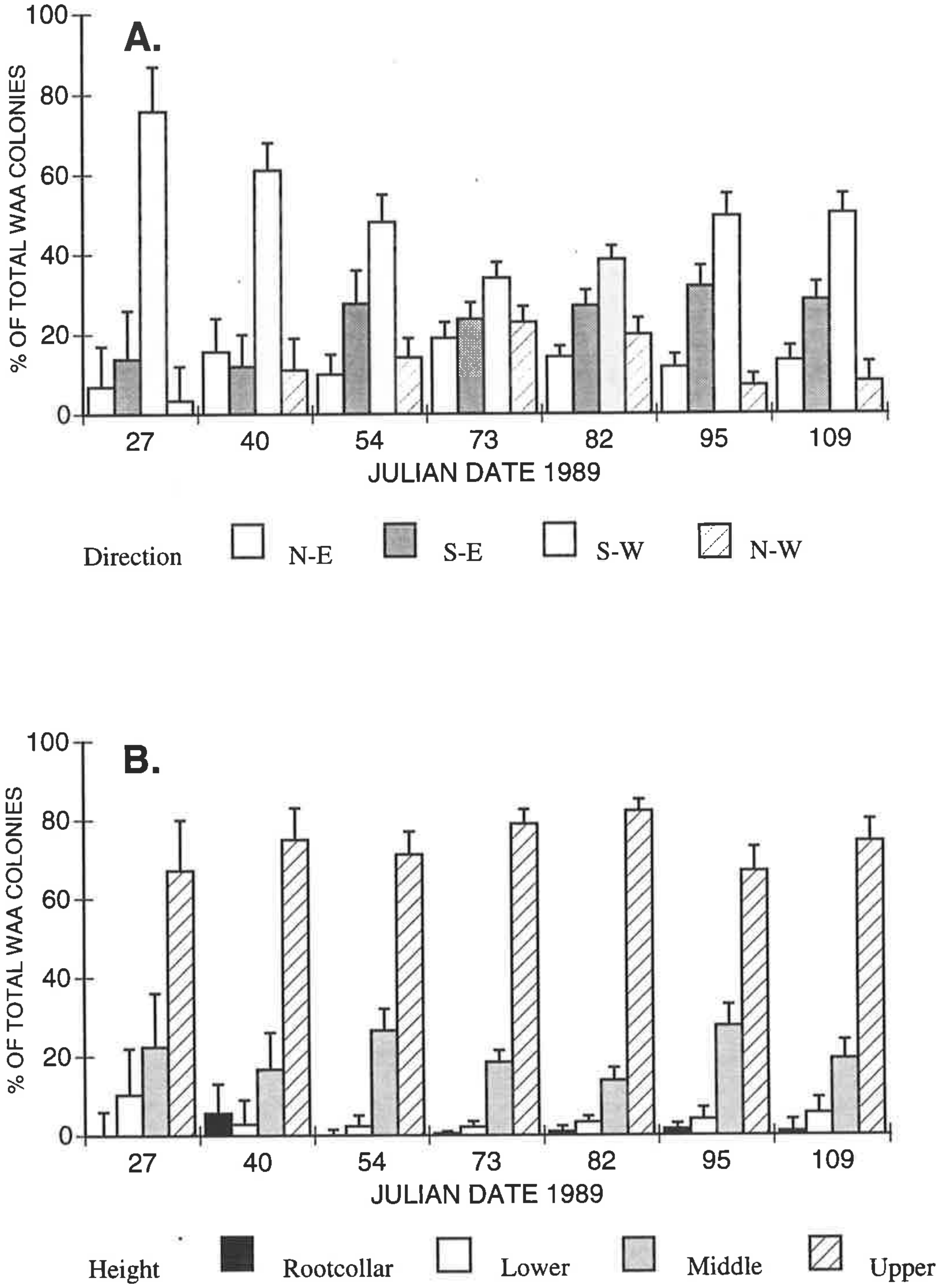

**Figure 6.3.** Percentage (mean ± standard error) of total WAA colonies counted in the first season (1989). Data for sprayed and unsprayed trees were pooled.
(A) directional quadrants.
(B) height strata of trees.

Similarly, the number of alatae and apterae caught in the pipe traps was very low throughout the season. No apterae were caught on the traps on any date but one. The exception was in the first two weeks of May where four first instars were caught (two each in the sprayed and unsprayed blocks). Peak numbers of alatae were caught in the first two weeks of April with three times as many alatae caught in the unsprayed than sprayed block (23 and 7 respectively). Numbers then declined so that by the middle of May no alatae were being trapped.

In the second year, there was a difference ($P<0.05$) in colony numbers between the sprayed and unsprayed orchard blocks which were analysed separately (Figure 6.2C, D). There were more infested trees in the unsprayed block than in the sprayed block. Aphid colonies appeared three months later in the sprayed block (early March) than in the unsprayed block. Thus, there were only three dates on which aphids were sampled in the sprayed block (Figure 6.2C, D). The infestation was very light with total colony numbers less than 70. The number of colonies in the unsprayed block in the second year were similar to those recorded for the first year. Peak numbers were recorded almost a month later (JD94) than in the first year (JD73), however this could have due to the different sampling regime used - once a month instead of fortnightly. The greatest difference could be seen in the number of colonies in the sprayed block which were much lower in the second season than in the first season. In spite of this the seasonal peak occurred at the same time as the unsprayed block.

The observed colony numbers in quadrants differed from the expected frequency ($P<0.001$) on all sampling dates. In contrast with the previous year, the proportion of colonies in the S-W and S-E decreased and the proportion in the N-E quadrants increased in both the sprayed and unsprayed blocks (Figure 6.4A, C). Nevertheless, the combined S-E and S-W quadrants still had the greatest percentage of colonies (over 50%) for all but one sampling date. The N-W quadrant of the trees continued to have the smallest numbers of colonies.

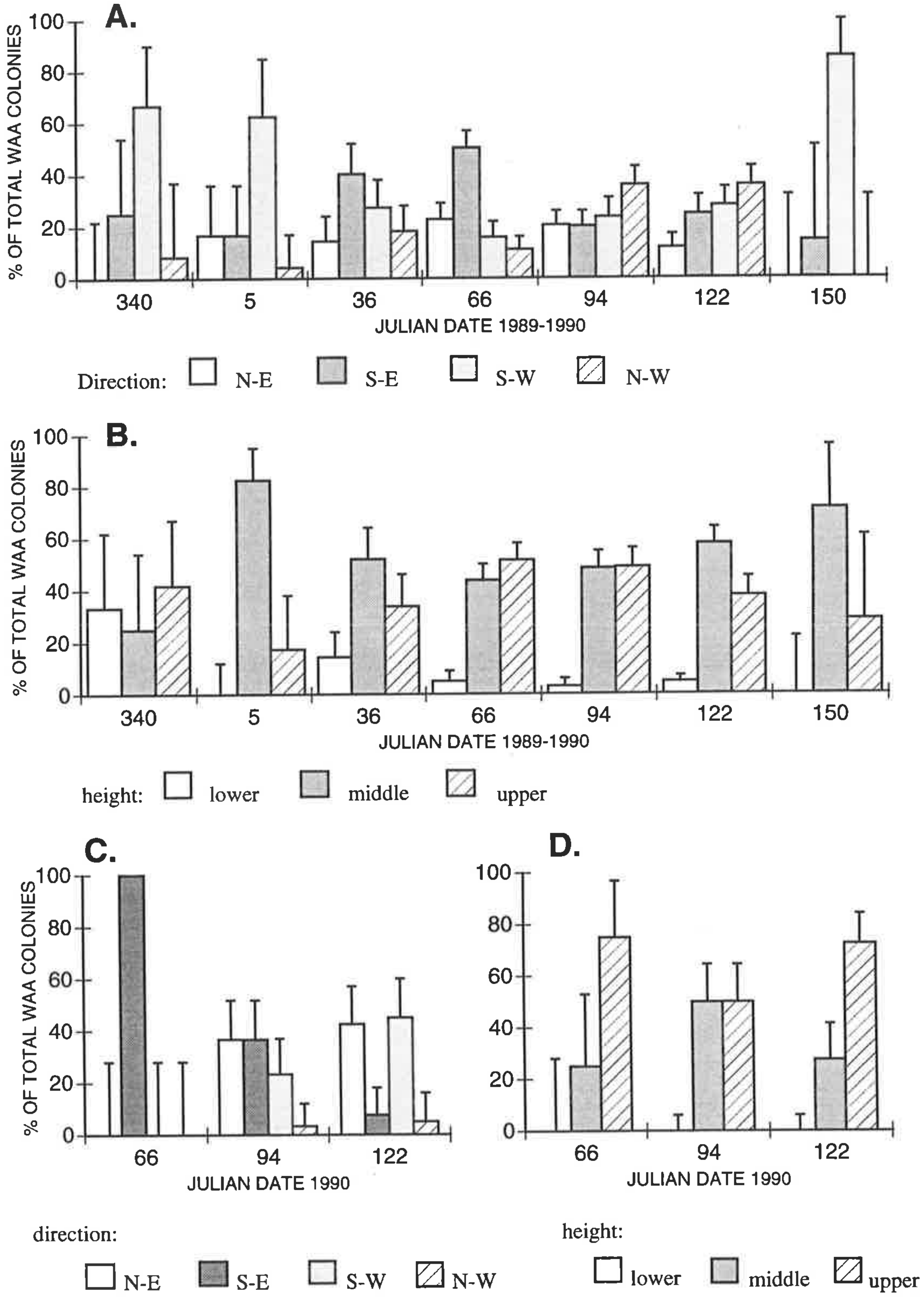

**Figure 6.4.** Percentage (mean ± standard error) of total WAA colonies counted in the second season (1989-1990).
(A) directional quadrants in the unsprayed block. (B) height strata in the unsprayed block.
(C) directional quadrants in the sprayed block. (D) height strata in the sprayed block.

As in the first season, there were very few colonies on the rootcollar and lower trunk. However, a greater proportion of colonies tended to be found in the middle stratum of the tree, particularly in the unsprayed block (Figure 6.4B, D).

### 6.4.2 Seasonal movements

The sticky tape trap catches showed trends similar to colony numbers throughout the first season (Figure 6.5A-D). In the sprayed orchard block, aphid numbers in the canopy traps peaked in late February and early March in the sprayed block (Figure 6.5C). In the unsprayed block, there were two peaks recorded for both upper and middle strata (Figure 6.5A). The first peak occurred in March in both strata with a two week difference between the two. Numbers in the middle stratum fell and then reached a second peak in the middle of April. This second peak was equal in magnitude to the first peak and declined more slowly. Aphid numbers in the upper stratum also experienced this double peak but the second peak was much smaller than the first and was followed by a sharp decline. There were no significant differences between the number of aphids (per millimetre) caught in traps that were placed in the middle and upper strata of the trees in either the sprayed or unsprayed block.

The first aphids were caught in low numbers in the trunk traps in the middle of December (JD349) in both the sprayed and unsprayed orchard blocks. There were slightly higher numbers caught in the upper trunk bands in the unsprayed block. This was reversed in the sprayed block with more aphids caught in the lower trunk traps. Aphid numbers reached their highest level on the trunk traps in mid-February in the sprayed block (Figure 6.7D) and late-March in the unsprayed block (Figure 6.7B). These peaks were approximately two to four weeks before peak colonies were seen in adjacent non-trap trees in the block. Only a very small number of aphids (less than one/sample period) were caught on the trunk trap placed between the lower and upper traps. There was also no difference in numbers between the lower and upper traps on the trunks. Aphids were caught in the canopy traps four to six weeks after the first aphids were caught in the trunks traps in both blocks.

The proportion of aphids moving up the trunk (expressed as a percentage of the total aphids caught in those two traps) was no higher in the spring than at other times during the season (Figure 6.6A, B). In the unsprayed block, approximately 50% of the aphids were moving up the trunk in six of the 10 sample dates (Figure 6.6A). In the sprayed block more than half of the aphids were moving up the tree for all but two of the 12 sample dates (Figure 6.6B).

Very few parasitoids and no predators were caught in any of the traps during the season. The greatest number were found on the upper trunk traps and the fewest were encountered on the traps from the canopy of the trees. Maximum trap catches did not exceed 10 parasitoids at any time. In spite of the low numbers there appeared to be two times during the season that the parasitoids were present; early to mid-October and at the end of March. A greater number were caught in the spring than in the autumn. No parasitoids were caught in either block from December to mid-March.

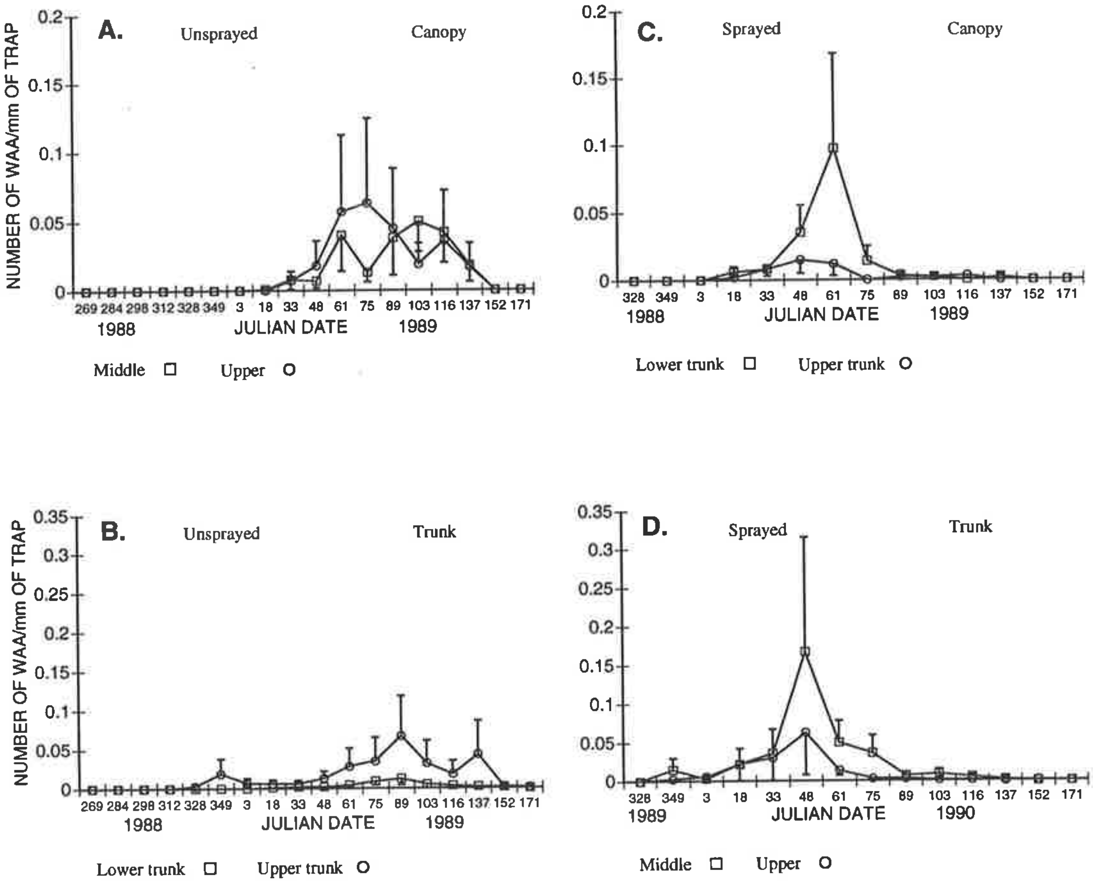

**Figure 6.5.** Mean (± standard error) number of WAA/mm tape caught on sticky tape traps.

(A) WAA caught in traps in the middle and upper strata of the tree in the unsprayed block.
(B) WAA caught in traps in the lower and upper portion of the trunk in the unsprayed block.

(C) WAA caught in traps in the middle and upper strata of the trees in the sprayed block.
(D) WAA caught in traps in the lower and upper portion of the trunk in the sprayed block.

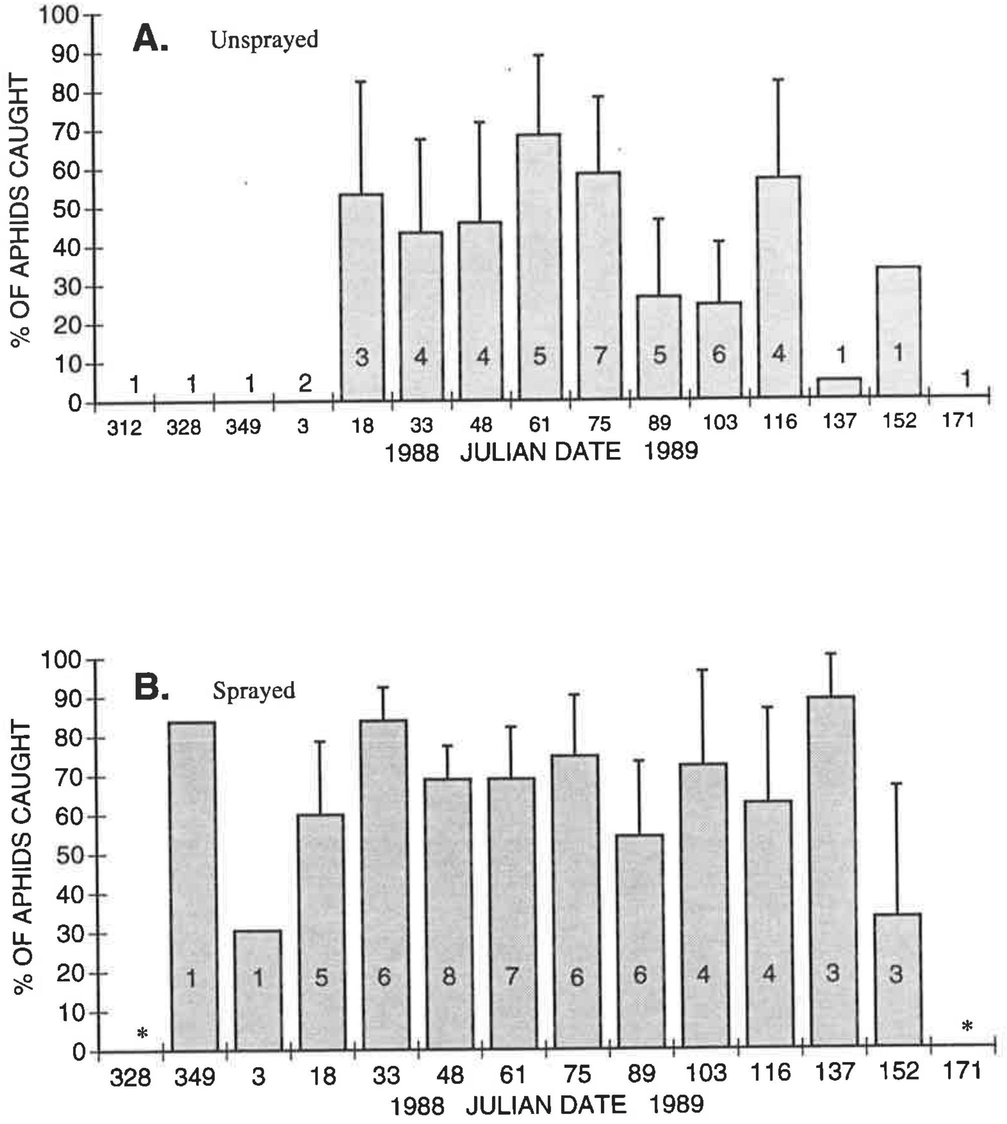

**Figure 6.6.** Aphids caught on the lower trunk traps expressed as a percentage of the total aphids caught on both the lower and upper trunk traps. Bars represent the means ± standard error for all trees that had aphids caught in traps. Number within the bar is the number of trees on which aphids were caught on the trunk traps (max = 8).

(A) unsprayed block (B) sprayed block

* Dates on which no aphids were caught in either trap.

## 6.5. DISCUSSION

Consistent trends in colony number were evident in both blocks over the course of the two year study. In the first year the seasonal peak was in early March (JD73) and approximately a month later in the second year. This difference is not substantial and could have been a reflection of the change in the sampling regime of the second year (sampling at monthly intervals rather than fortnightly) or by the cooler temperatures experienced that year. Aphid numbers caught in the sticky tape traps reflected the trends and peaks seen in colony numbers.

Colony numbers differed between the sprayed and unsprayed blocks, however this difference was significant only in the second year. The number of colonies in the unsprayed block were similar in both years, however colony numbers in the sprayed block were much lower in the second year. The number of infested trees was higher in the first year in the sprayed block and these trees were more heavily infested. Reasons for the difference between the two blocks in the second year are unclear. The spray schedule and general orchard management remained the same in both seasons. It is possible that other factors such as climate came into play.

There was no indication at the beginning of the seasons which trees were more likely to be infested. Trees that were infested in the first season were not always infested the second season, regardless of the degree of infestation in the first year. Some trees showed signs of infestation early in the season but did not remain infested. Reasons for this are unclear. Certainly there was no obvious sign of parasitism or predation to account for the sudden decline of aphid numbers. It may be that there were unseen physiological changes in the tree to make it less susceptible to aphid attack. Long term studies (ie: over the course of a number years) may elucidate this phenomenon and it is an area that should be researched further.

The low number and patchy distribution of WAA colonies within the orchard and on the trees made it difficult to detect statistically significant differences amongst strata or quadrants. It was clear however, that certain areas of the trees were favoured by the aphids. The preference of aphids for the upper portion of the trees has been noticed by other workers (A. Granger, S.A. Department of Agriculture; C. Bower, N.S.W. Department of Agriculture, personal communication). The high proportion of colonies in the upper part of the tree did not develop gradually but was apparent from the onset of infestation. The results of this study do not entirely agree with the findings of Asante *et al.* (1993). This could be due to differences in varieties tested, tree type, age or orchard management. Asante et al. (1993) used an unsprayed orchard of unpruned trees while the present study used trees that were pruned every year. It is difficult to directly compare the studies as the manner in which the trees were divided into horizontal strata were not the same. It is possible that the ‘middle’ portion of the trees (less than two meters) in Asante *et al.* (1993) corresponds to the ‘upper’ portion (greater than 1.8 meters) in the present study. What was evident in both studies, however, was that colony distribution was not random on the tree and that colony abundance was not equally distributed throughout the tree.

The null hypothesis that the frequencies would be equal in all quadrants or strata of the tree was clearly not the case. The S-E and S-W quadrants had the highest proportion of colonies. Since the combined southern quadrants represent that portion of the tree that gets the least amount of sun during the day, the greater occurrence of WAA on them is consistent with Thompson's (1934) observation that the aphids were most abundant on the north-east side of branches on trees in England - where the sun travels through the southern aspect. This relatively cooler aspect of the tree might also be an advantage to aphid survival in periods of high temperature. First instars, or crawlers, seek shade as the temperature rises and tended to concentrate on the north and north-east sides beneath trees or in shaded areas in North America (Schoene and Underhill 1935).

Contrary to the results found in Europe and North America, there did not appear to be any strong seasonal migration either up or down the trees. Aphid numbers were higher in the upper trunk traps in the unsprayed block indicating that the aphids were leaving the tree canopy. In the sprayed block, the reverse was true - there were more aphids caught in the lower trunk trap indicating that the aphids were going up towards the canopy. Aphid numbers caught in the two traps were not significantly different, however, so there could well have been an almost equal movement in either direction. The number of aphids caught in the traps in the first few weeks of sampling were similar, making it difficult to determine whether the delay between the catch in the trunk and canopy traps was a true indication of any sequence of aphid movement in the tree at the beginning of the season. The trap catches do however, indicate that the aphids probably overwinter in the canopy of the tree and do not migrate up the tree from the root area. Infestation of the tree canopy could, nevertheless, also be a result of first instars being blown from other trees as suggested by earlier work (Schoene and Underhill 1935; Dumbleton and Jeffreys 1938; Sproul 1981). This sort of haphazard immigration may account for the patchy distribution of aphids among trees within the orchard.

Parasitism and predation remained extremely low in both of these orchards for both years. It is possible that because the unsprayed block was between two sprayed blocks that it was impossible for the parasitoid to reach numbers high enough to be detected or to have any impact on the aphid population. Occasional, *ad hoc* observations of other orchard blocks in the area also indicated very low numbers of parasitoids or predators during the two years of study. *A. mali* numbers were at their highest after the application of chlorpyrifos, indicating that the parasitoid may not have been adversely affected by the pesticide. However, laboratory studies have shown that parasitoids, as larvae, pupae or adults are very susceptible to this pesticide (Chapter Four). Numbers remained so low throughout the season however, that no comparison of sprayed and unsprayed orchard blocks was possible.

All other orchard blocks within the Research Centre were subjected to conventional spray schedules. It was not possible to find an unsprayed orchard in the Adelaide Hills, such was the dependence on chemical control. Nor was it possible to locate an abandoned orchard in the area. Such an orchard would have made it difficult to compare results with a sprayed and managed (pruned) orchard. Tree growth is quite different in an unpruned tree and this does not accurately reflect what is found in a 'typical' orchard.

A greater number of parasitoids were caught on the tape traps than the PVC pipe traps. This difference was most noticeable in the spring, also in the unsprayed block where the greatest number were caught. The difference in trap catch may be explained by the use of yellow tapes in the tapes compared to the white of the pipe traps. *A. mali* is more attracted to yellow than white or green (Staübli and Chapuis 1987). Another important factor is that *A. mali* spends a large proportion of time walking along the branches and trunks of the tree, resting on the undersides of the leaves and relatively little time in flight, reducing the chances of being caught in hanging traps between trees. While the number of parasitoids was never very high it is interesting to note that the highest number was caught in the spring, suggesting that *A. mali* was entering the orchard either at the end of the first season or at the beginning of the second season. Both of these times would be when pesticide usage would be at its lowest level. This further suggests that given the right environment it would not be difficult to increase the numbers of parasitoids within an orchard. It would remain to be seen if the resulting increase in the parasitoid population would be effective in controlling the aphid population.

Whatever the reasons for colony distribution, it is an important consideration in sampling. While it is not expected that a pest manager or orchardist would sample trees in such an intensive manner, it is necessary to gain an understanding of colony distribution on the tree before deciding on an effective sampling program. To do this requires what would seem to be an excessively intensive sampling regime. This study also points out the danger of using a technique that involves pre-chosen 'sample' trees to be used throughout the season to monitor pest levels for the entire orchard. On the basis of the results shown here, such a regime could well have missed the onset of an infestation within the orchard.

There are several considerations to be taken into account when sampling. First, the patchy distribution of infestation throughout the orchard means that a significant proportion of the trees may not be infested over the entire season. Trees that may be infested in the beginning of the season may ‘spontaneously’ lose that infestation. Finally, trees that may have been heavily infested one year may not have any infestation the following year regardless of any control measures that may have been enacted. It is known that the cultivar Granny Smith, appears to be more susceptible to infestation than others (Chapter Two) but even with this cultivar infestation is patchy within the orchard. The present study had shown that aphids preferentially colonise specific parts of the tree, mainly the upper portion of the canopy and the east to south sides of the tree.

Armed with a better understanding of the spatial distribution of WAA colonies, a pest manager can limit their time to the more efficient sampling of the upper portions of the trees. This does not necessarily require the use of ladders, even when colonies are still small as the colonies are easily sighted with experience. With less time spent sampling the entire tree more trees could be used as ‘sample’ trees throughout the orchard thereby reducing the chances of missing an increase in infestation levels. The results also suggest that one might focus on the east and south sides of trees to greater advantage but until confirmed it would be safer to sample the entire upper portions.

The patchy distribution of WAA within an orchard such that only a certain portion of trees are infested suggests that an alternative control strategy might be possible. This would involve the use of selective spraying. It would be more time consuming, perhaps than the current method but could minimise the amount of pesticide application while maximising aphid kill. It would also have the benefit of minimising the harm to the beneficials in the orchard. Rather than utilising a set number of sample trees and checking all parts of the tree for colonies, the upper south to east portion could be checked in each tree. Trees that had more than the set threshold (ie: 15 small colonies) could be marked and individually sprayed or if there are more than 25% of the trees infested the entire orchard would be sprayed. Sampling in this manner could be done quite quickly by travelling on a motor bike or tractor and could be done in conjunction with another task such as checking the irrigation lines or mowing. Admittedly this is more time consuming than checking a set number of sample trees but it would catch all infestations and could reduce the amount of spray required. Whether it is cost efficient (both in time and money) to do several of these types of spray applications remains to be seen. By spraying selected trees only, the remaining unsprayed trees act as a reservoir for predators and parasitoids of WAA and other pests. The success of this type of more intensive sampling/spray regime depends on the growers' commitment to reducing sprays and increasing beneficials within his/her orchard.

As the trend towards high density, semi-dwarfing planting increases the distribution of WAA colonies within the orchard may change and this will affect the way sampling is carried out. The present orchards have trees whose branches do not interleave - they stand alone. This makes dispersal more difficult for the aphid and may account for the patchy distribution within the orchard. Exactly how the aphid disperses throughout the orchard is unclear but it seems likely that it is windborne as a first instar. Dispersal within a high density orchard, where the tree branches intertwine, will be much easier as the aphid can then walk from one tree to the next. This could result in a less patchy distribution within rows in the orchard or put another way, rows rather than trees could be the site of infestation. This type of orchard could make sampling and control easier. Trees tend to be pruned so that the main growth in within the row rather than into the orchard aisle so that sampling should be easier. Control could encompass spraying the entire row that has infested trees and this would be easier than spraying single trees throughout the orchard.

The application of chlorpyrifos specifically to control WAA near the end of the first season appeared to be only partially successful. Applied at the time of peak numbers of colonies, it reduced the infestation but not by a significant degree (Figure 6.3B). Aphid populations appear to decline naturally in late summer for reasons that are unclear (Bodenheimer 1947; Evenhuis 1958; Bonnemaison 1965; Noppert *et al* 1987). More effective control may have been achieved by spraying before the peak infestation occurred, around mid-February when the average number of colonies was below 20. Chlorpyrifos has been shown to be effective as a dormant or spring spray (Bower 1987), however, a second application was still required before the end of the season. This would imply that a single spray applied as the populations were beginning to rise (February) may achieve similar results and alleviate the necessity of an early season and autumn spray.

Factors such as the size of the predator and parasite populations play an important role in the orchard system and will become more important as the number of pesticide applications used against key pests such as codling moth are reduced. In the orchards used in the present study, there were negligible numbers of parasitoids or predators in both years of the study. Reasons for this are unclear but the number of pesticides used to control other pests may be implicated (Madsen and Morgan 1970; Staübli and Chapuis 1987; Dent 1991). Decisions still have to be made on the size and number of colonies that can be tolerated before control measures have to be effected. An efficient sampling method not only should give a better indication of the levels of infestation and need for spray application, but would be more cost effective. From this study it would appear that the most effective timing of pesticide application would be in the interval between the end of January and the beginning of March (Julian dates 30 and 60) when colony numbers were less than 25 per tree.

# CHAPTER SEVEN

# SUMMARY AND CONCLUSIONS

## 7.1. Summary

As indicated in the introduction to this thesis, the concern expressed to the South Australian Department of Agriculture by growers instigated this study into woolly apple aphid (WAA). The impression was strongly given that WAA was more prevalent in the orchards and was becoming increasingly difficult to control by chemical means. As indicated below, subsequent investigation showed that these concerns were real to the extent that populations of WAA can rise rapidly after insecticidal applications cease at the end of the season, WAA infestations have a debilitating effect on young trees, and that the insecticides used in local orchards do not allow the increase of *A. mali* (Chapters Three, Four, Five, Six).

Despite a tendency of previous studies of the interactions of WAA and the apple tree to stress the importance of root infestations (discussed further below), above-ground infestations by the insect has a negative effect on the growth of seedling trees (Chapters Five). High infestations can kill small trees within two years; low infestations can also reduce tree growth by a minimum of 10% in this period. The pathological effects of infestation cannot be correlated with specific phenological events in the life cycle of the tree, such as bud break or fruit set. Rather, the damage is pervasive, affecting characteristics such as leaf area and tree height. As well as this general damage, there is the specific damage to tissues of the new growth and to leaf axils. The debilitating effects of infestation are long lasting. Trees that have been infested in one season will show signs of that damage in the following season even after the infestation has been removed.

Thus, both immediate and chronic effects of WAA infestation on tree growth and vigour can have significant consequences. The growth rate of Cox/MM104 apple trees increases during the first nine years of life and thereafter declines (Dudney 1974). The effect of WAA on tree growth in early life may reduce overall tree size and, hence, long term productivity. The debilitating effects may cause trees to become more susceptible to diseases and other insect damage. For economic reasons, there is a trend by orchardists to plant trees with a shorter life span and earlier fruit bearing characteristics. The effects of above-ground WAA damage will likely be even more serious in trees with these characteristics. As a corollary, it is also likely that lower levels of infestation will lead to losses in productivity comparable with heavier infestations with the later bearing, longer lived varieties now in use.

A potential for the long term debilitating effects of WAA infestation has been identified. There is in consequence, a need for further studies that quantify the long term effects of this pest on the life time productivity of apple trees, particularly the impact of low level, chronic infestation over a number of growing seasons. There is increased interest in insect-plant interactions and the WAA-host interaction would provide a good model. This aphid has the advantage that, unlike other phytophagous insects or aphids, it does not directly influence the photosynthetic ability of the plant by destruction of the leaf tissue. There may be the possibility that there are negative feedbacks at work - ie: as the aphid feeds it reduces plant vigour and the plant then becomes a poor food source for the aphid thereby affecting the aphids ability to reproduce. These effects and fruit yield data could be used in a modelling system to study the effects of an insect on its host.

Root infestations, despite the use of resistant rootstocks still appears to be considered a greater problem than above-ground infestations. Such a conclusion was not tested directly in the present study and needs further re-examination.

Certainly, the cryptic nature of root infestations makes their chemical or biological control more difficult, nevertheless, WAA appears to be controlled quite successfully through resistant rootstocks (Northern Spy and the Malling-Merton series). A problem arises, however, with the use of seedling rootstock which is not resistant to WAA infestations. In these instances, especially, the effects of root infestations on tree vigour need to be more closely examined.

Woolly apple aphid clearly has a preference for the upper and portion of trees from the onset of infestation (Chapter Six), a characteristic that can be utilised by pest managers. There is also a preference by the aphid to colonise the southern aspect of the tree. These characteristics allow some simplification of sampling regimes which would permit faster estimates per tree and, therefore, a greater proportion of the orchard could be sampled. It is important, moreover, to sample a large portion of the orchard because of the patchy distribution of WAA infestation within orchards. If specific ('sample') trees or only a portion of the orchard are sampled throughout the season, infestations may develop unnoticed until they have reached unacceptable levels. With the trends towards high density orchards the aphid distribution throughout these orchards may change. This may lead to a further modification to the sampling regime employed.

The patchy distribution both within trees and among orchards limits the accuracy with which estimates of total woolly apple aphid populations can be made. Nevertheless, clear trends were established in the development and fate of populations that were monitored throughout the season. In the present study, populations peaked in the latter half of March to early April, although this timing could be expected to vary in different apple growing areas of the country in relation to prevailing climatic conditions. Differences may also occur in orchards within any one area, depending on microclimate, orchard practices and apple varieties. On the other hand, the similarity in population peaks in the two orchards studied here over two years suggests that certain regional decisions could be made concerning the control of WAA from a chemical perspective.

An application of chlorpyrifos at peak numbers (Mid-March) was not entirely successful and better control may well have been achieved had the spray been applied earlier in the season as colony numbers were beginning to rise. Careful monitoring of populations within orchards should lead to more accurate timing of sprays to an optimum time just as colony numbers begin to rise, allowing maintenance or re-establishment of a population that parasitoids are able to hold in check. Further monitoring of populations may eventually provide forecasts based on relatively simple degree-day models, but such a result would require a much larger sample size to be studied for a more extensive period of time over a greater number of sites than was possible in the present study. Application of sprays to only those trees that have sufficiently high infestations will provide refuges for the parasitoid thereby maximising its control.

WAA can be controlled by a variety of insecticides under laboratory conditions (Chapter Four) but such results do not necessarily reflect field conditions. For example, pirimor killed over 90% of aphid colonies under laboratory conditions but is known to give inadequate control in the field. The ultimate aim in orchard pest management is to reduce the amount of pesticides but it is likely that in the near future insecticides will continue to play an important rôle in pest control. With this in mind, it was of importance to determine the effects of commonly used insecticides on WAA; not only those that are used against it specifically, but those used against other pests (in particular codling moth). It was also important to gain insights into the effects these chemicals had, not only on WAA, but also on *A. mali*. These present studies indicate that the most effective chemical in controlling WAA may not be the best alternative from the point of view of maintaining the effectiveness of biological control. The results obtained also give some insight into the possible reasons of *A. mali's* poor performance.

Azinphos-methyl (APM), which is used to control codling moth and is the most commonly used pesticide in the orchards of South Australia, reduced WAA colonies by approximately 60% in the laboratory and it gives adequate control in the field. The disadvantage to APM is that application must cease prior to harvest with the result that WAA populations may then start to rise again. This has both advantages and disadvantages; while aphid levels may increase to unacceptable levels, parasitoid levels are also allowed to increase. The increase in parasitoids has the advantage of leaving the orchard with an overwintering population of *A. mali* that may control WAA early in the following season. The disadvantage lies in the rate at which WAA can increase at the end of the season and thereby produce a large overwintering population the following season.

If considered too high, aphid populations may be controlled with post-harvest sprays, but chemical measures to 'clear up' late infestations have the disadvantage of also destroying parasitoids, leaving an overwintering parasitoid population that may be too low to control WAA in the spring. There is also no clear indication that a high level of infestation at the end of one season will lead to high infestations the following year (Chapter Six). It would therefore be more effective to apply a spray early in the season - such as vamidothion - rather than at the end of the season. Clearly, it is important to prevent a cycle of pesticide application alternating with increases in aphid population in a way that is deleterious overall to control by natural enemies.

Other pesticides tested in the laboratory were also successful in killing WAA colonies; malathion, chlorpyrifos, endosulfan and vamidothion all killed more than 90% of colonies, although, there were few stages of the parasitoid that were not also killed by these insecticides (Chapter Four). With the exception of vamidothion, these insecticides are all used currently to control codling moth. There is much interest in fenoxycarb as an alternative to APM. Initial studies indicated that adult parasitoids exposed to this chemical were adversely affected, although it appeared that fenoxycarb had little effect on the parasitoids when treated prior to emergence (Chapter Four). These effects have not been tested at the field level. In particular, sub-lethal effects of insecticides on both parasitoid and aphid are important avenues of investigation, since many insects do not receive a lethal dose of insecticide.

There can be little doubt that one of the biggest limitations to the control of WAA by *A. mali* is the degree of chemical intervention in the orchards of South Australia (Chapter Two). More than 12 pesticides are used in South Australian orchards (Chapter Two) and pesticides are often being applied every 14 to 21 days (Wicks and Granger 1988). Of the 10 insecticides tested under laboratory conditions, six killed adult parasitoids within 24 hours and three others significantly decreased adult longevity when mummies were treated (Chapter Four). These findings, while still to be tested under semi-field and field conditions have serious implications for the effectiveness of *A. mali* while reliance remains on insecticides for WAA control. This scenario will change as there is now a concerted effort to reduce pesticides applications in South Australian orchards by 50% in the next five years. Some respite for beneficial insects should result, thus changing the ratio of insects found in orchards (Brown and Adler 1989).

The exact role of the parasitoid in controlling the aphid is still unclear. It may be that there are more factors involved in the poor performance of *A. mali* in controlling WAA in South Australia than its inability to survive the array of pesticides. Although the parasitoid has been described as one of the best examples of biological control (Chapter Three) it has also been suggested that it has been given credit for control that it does not necessarily exert (S. C. Hoyt, Washington State University personal communication). Predators such as coccinellids, syrphids and earwigs leave no trace of their presence after attacking colonies, whereas *A. mali* leaves a distinctive calling card in the mummified aphid. There is at least a strong possibility that *A. mali* plays no more than a minor role in controlling WAA infestations and that generalist predators have the potential to achieve a greater importance in controlling the pest. Further research into the interactions of generalist predators and the parasitoid and their effects on WAA populations would provide valuable insight into this area.

Even when seemingly well established, *A. mali* does not control WAA consistently and reasons for this are still not understood. Woolly apple aphid has several advantages over its parasitoid in surviving in orchards. Firstly, WAA has a lower temperature threshold, so it can increase in the cooler temperatures at the beginning of the season and therefore escape parasitoid control. Secondly, WAA can survive and more importantly, reproduce at both lower and higher temperatures than *A. mali* (Chapter Three); Asante *et al.* 1991; Asante and Danthanarayana 1992), thereby continuing to reproduce at times when the parasitoid cannot. Summer temperatures in South Australia can reach 30°C or more and at this point, *A. mali* longevity is substantially reduced, while WAA may remain relatively unaffected. The ability of WAA to survive and reproduce at a wider temperature range than its parasitoid gives it an advantage at both ends of the season.

Whatever the influences on the size of WAA populations, a decision must still be made on what levels of infestation are acceptable before intervention is initiated. At present, this is by no means a simple issue, as it concerns such disparate things as grower acceptance of infestation levels, which may be quite different from that decided on by pest managers, onset of infestation, levels of beneficial insects and level of damage that can be sustained by the trees. Ultimately the aim of this research and any further research into woolly apple aphid and its interactions within orchards is to determine such thresholds. Until we know what level of WAA infestation can be tolerated by trees before economic damage is exceeded we will not be sure if too much or too little control is being exerted. Given the chronic effects of WAA infestation, studies to determine the economic threshold must be long term and ultimately consider the yield of the effective lifetime of the tree. Until a threshold is established WAA will continue to be a sporadic but troublesome pest.

## 7.2. General Conclusions

1. WAA is considered a problem by the apple orchardists of the Adelaide Hills and the majority of them spray specifically for WAA.
2. There is a high degree of chemical intervention in the orchards of South Australia..
3. WAA has the ability to survive and reproduce at a wider temperature range than its parasitoid, giving it an advantage at both ends of the season but especially in the hot summers of South Australia.
4. The usefulness of monitoring WAA populations, especially on the upper portions and southern aspects of the trees has been demonstrated.
5. A serious effect of above-ground WAA infestations on apple seedlings during their first and second year of growth has been established.
6. The insecticides used within orchards to control WAA and key pests reduces the adult life span of *A. mali.*
7. A need for further research on the effects of chronic WAA above-ground infestation on the growth and productivity of apple trees has been shown.

# APPENDIX ONE

# A SURVEY OF APPLE GROWERS IN THE ADELAIDE HILLS REGION OF SOUTH AUSTRALIA

## WOOLLY APPLE APHID SURVEY 1987

A study of the woolly apple aphid is being conducted by Frances FitzGibbon, a postgraduate student at the Waite Institute. She is interested in studying the interactions between apple rootstocks, woolly apple aphids, and parasitic wasps, to look for ways to improve management of this pest. Following this questionnaire, selected orchards will be surveyed to determine the levels of woolly aphid infestation.

Instructions for completing the questionnaire.

For Yes/No questions, please circle the correct alternative and for the multiple choice questions, mark the correct alternative with an (X) or a (√). If your answer falls into the "other" category, please fill in the appropriate answer as completely as possible. Please be as precise as possible in answering questions 4, 6a, and 31a. The completed questionnaire can be returned in the enclosed envelope.

If you have any questions, or comments, please contact Frances FitzGibbon at the Waite Institute (372-2267). All information provided will be strictly confidential.

Your help in promptly completing this questionnaire is greatly appreciated.

## WOOLLY APPLE APHID SURVEY 1987

### A. GENERAL ORCHARD DESCRIPTION

1. Name: ____________________

2. Address: ____________________
____________________
____________________

3. Telephone number: ____________________

4. Where is the orchard located exactly? ____________________
____________________
____________________

5. How large is the orchard?........................

| | | |
|---|---|---|
| 1-5 | acres | ______ |
| 6-10 | acres | ______ |
| 11-15 | acres | ______ |
| 16-20 | acres | ______ |
| 21-25 | acres | ______ |
| over 25 | acres | ______ |

6. When standing at the top of the orchard, looking down, in which direction does the orchard slope? (if there is more than one slope in the orchard, use the predominant slope ..........................................

| | |
|---|---|
| N | ______ |
| NE | ______ |
| E | ______ |
| SE | ______ |
| S | ______ |
| SW | ______ |
| W | ______ |
| NW | ______ |

6a. If the orchard has more than one slope please describe the secondary one. (ie: in which direction do they slope, from top to bottom?).

________________________________________

________________________________________

________________________________________

7. Is the orchard subject to frost?.............................................. Y N

8. In general is the soil......................................... sandy ________
clay ________
loam ________
other ________
________________
________________

B. THE TREES

9. Which varieties are grown?....................... Red Delicious ________
Golden Delicious ________
Granny Smith ________
Jonathan ________
Others (specify) ________
________________
________________
________________
________________

10. Are any of the varieties a spur type?..............

| | | |
|---|---|---|
| Red Delicious | Y | N |
| Golden Delicious | Y | N |
| Granny Smith | Y | N |
| Jonathan | Y | N |
| Others (Specify) | Y | N |

________________
________________
________________

11. Is Northern Spy used as a rootstock? on.........

| | | |
|---|---|---|
| Red Delicious | Y | N |
| Golden Delicious | Y | N |
| Granny Smith | Y | N |
| Jonathan | Y | N |
| Others (specify) | Y | N |

12. Is one of the following rootstocks used?..........

| | | |
|---|---|---|
| MM106 | Y | N |
| M26 | Y | N |
| Others (specify) | Y | N |

13. How old are the trees?

| Red Delicious | Goldens | Grannies | Jonathans | Others |
|---|---|---|---|---|
| 1-5 yrs ______ | 1-5 yrs ______ | 1-5 yrs ______ | 1-5 yrs ______ | 1-5 yrs ______ |
| 6-10 ______ | 6-10 ______ | 6-10 ______ | 5-10 ______ | 5-10 ______ |
| 11-20 ______ | 11-20 ______ | 11-20 ______ | 11-20 ______ | 11-20 ______ |
| 20-40 ______ | 20-40 ______ | 20-40 ______ | 20-40 ______ | 20-40 ______ |
| >40 ______ | >40 ______ | >40 ______ | >40 ______ | >40 ______ |

14. What percentage of the orchard is planted to each variety?

| Red Delicious | Goldens | Grannies | Jonathans | Others |
|---|---|---|---|---|
| 1-5 yrs ______ | 1-5 yrs ______ | 1-5 yrs ______ | 1-5 yrs ______ | 1-5 yrs ______ |
| 6-10 ______ | 6-10 ______ | 6-10 ______ | 5-10 ______ | 5-10 ______ |
| 11-20 ______ | 11-20 ______ | 11-20 ______ | 11-20 ______ | 11-20 ______ |
| 20-40 ______ | 20-40 ______ | 20-40 ______ | 20-40 ______ | 20-40 ______ |
| >40 ______ | >40 ______ | >40 ______ | >40 ______ | >40 ______ |

15. What is the distance between trees/rows?
between trees ____________
between rows ____________

16. How are the trees trained?........................
central leader ________
trellis ________
open centre ________
vase shape ________
other (specify) ________
____________________
____________________

17. When are the trees pruned?........................
summer ____________
winter ____________
both ____________

18. What type of ground cover is used between the rows?........................................
clover __________
grass __________
clean cultivated __________
other (specify) __________
____________________
____________________

19. What type of ground cover is used between the trees?........................................
clover __________
grass __________
clean cultivated __________
herbicided __________
other (specify) __________
____________________
____________________

20. Do you have an irrigation system?....................................... Y N

21. If so, what type?..........................
overhead sprinklers __________
under tree sprinklers __________
drip irrigation __________

## C. SPRAY SCHEDULE

22. How many times per year do you spray for codling moth?..........................................

0-2 times ________
3-5 ________
> 5 ________

23. What sprays do you use for codling moth?......

azinphos-methyl ________
carbaryl ________
madison ________
other (specify) ________
________________
________________
________________

24. How many times per year do you spray for mites?

0-2 times ________
3-5 ________
> 5 ________

25. What sprays are used for mite control?........

peropal ________
neoron 500 ________
kelthane ________
torque ________
propargite ________
other (Specify) ________
________________
________________
________________

26. Do you spray winter oil?................................................ Y N

27. Do you spray for woolly aphid?...................................... Y N

28. If so what sprays are used?......................

vamidothion ________
azinphos-methyl ________
oil ________
dimethoate ________
metasystox ________
endosulphan ________
phosfone ________
other (specify) ________
________________
________________
________________

29. Is woolly aphid a problem in your orchard?............................ Y N

30. Is it increasing?........................................................ Y N

31. Are there any areas of the orchard where woolly aphid is more common?................................................................ Y N

31a. If yes, where in the orchard are they located?

______________________________________

______________________________________

______________________________________

32. Do you use dilute or concentrate spraying? Dilute Y N
Concentrate Y N

33. Do you keep.................................... weather records Y N
spray records Y N

34. Would you object to;

1. Having a temporary weather station placed in your orchard (consists of a shelter (6'h x 2'x 2') and a hygrothermograph to measure temperature and humidity). Y N

2. Samples being taken from the trees (small amounts of twigs and roots) throughout the year? Y N

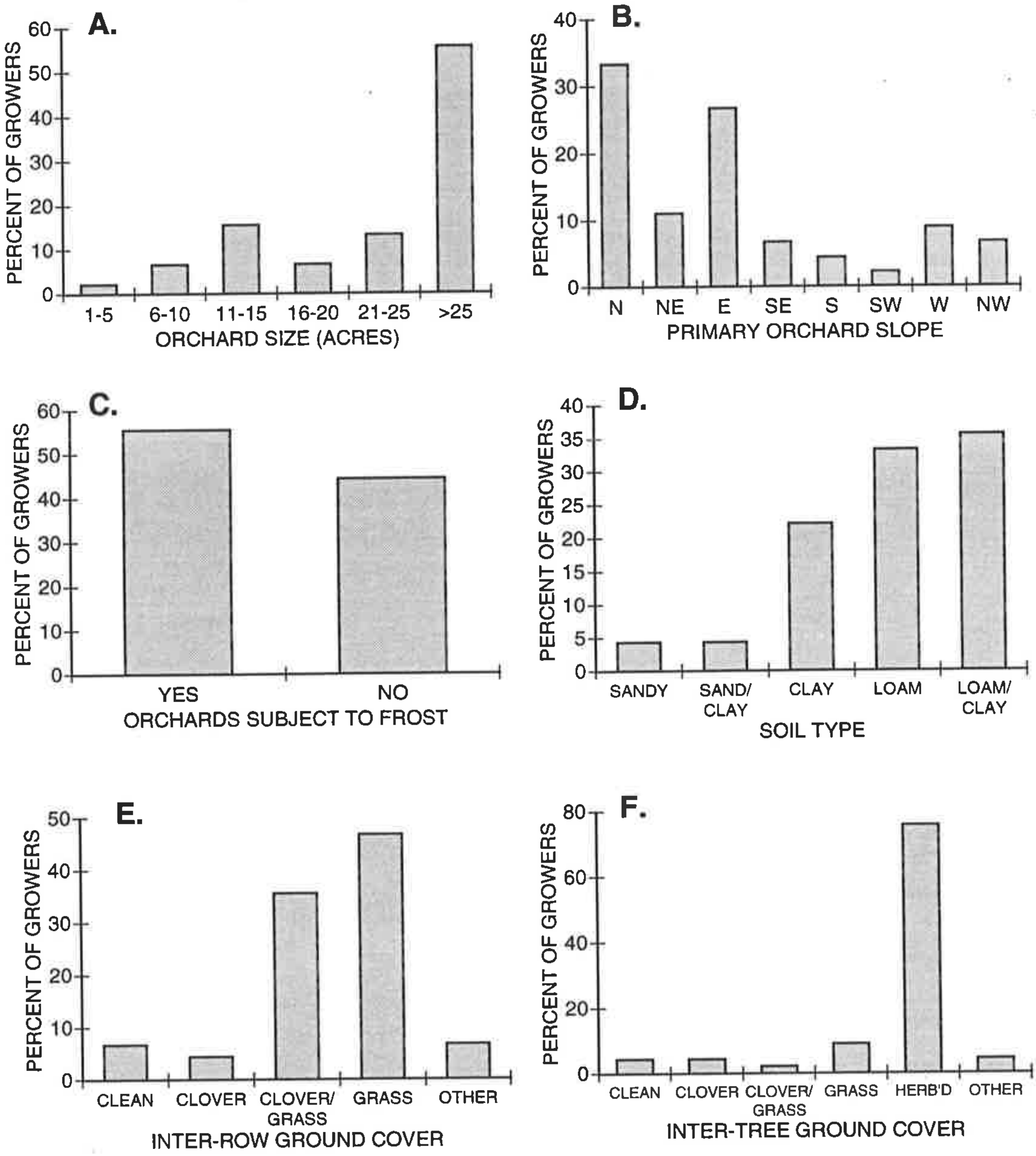

**Figure A1.1.** Per cent of growers' affirmative response to survey questions. (A) orchard size (acres), (B) primary slope in orchard, (C) whether the orchard is subject to frosts, (D) average type of soil throughout the orchard, (E) type of ground cover used in the alleys of the orchard, (F) type of ground cover used within the tree rows. 'Herb'd' = herbicide application.

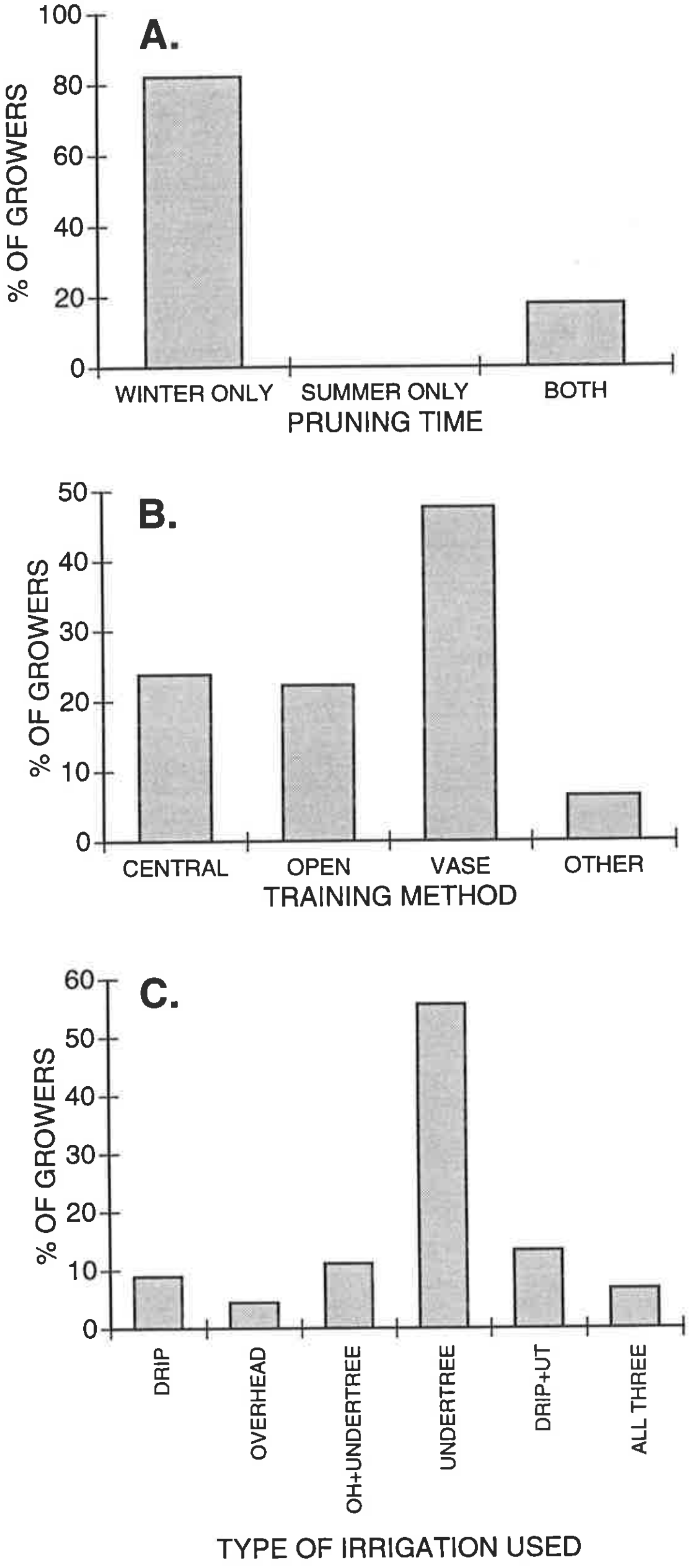

**Figure A1.2.** Per cent of growers' affirmative response to survey questions. (A) Pruning time, (B) the type of training method used in the orchard, (C) the type of irrigation used in the orchard. 'OH' = overhead sprinklers, 'UT' = undertree sprinklers.

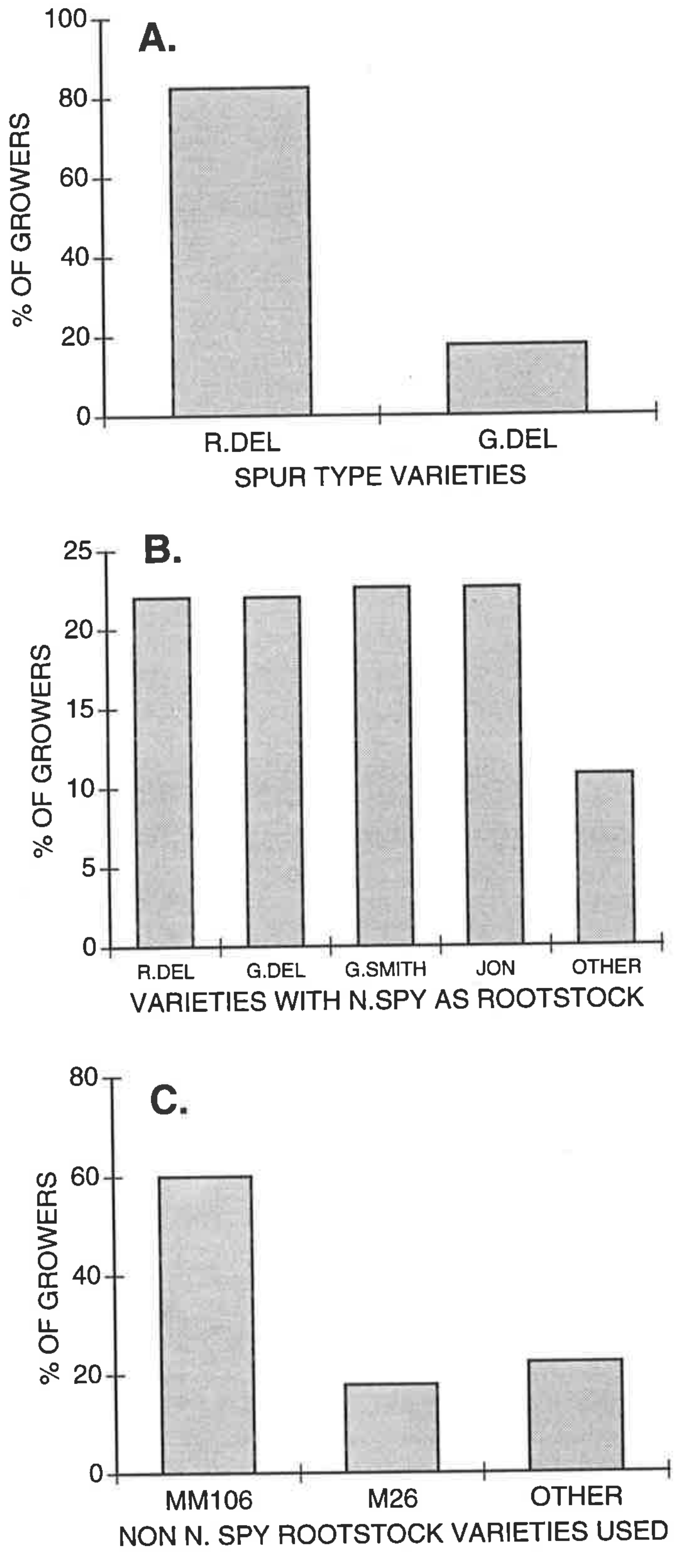

**Figure A1.3.** Per cent of growers' affirmative response to survey questions. (A) varieties that are spur type, (B) varieties that are grown on Northern Spy rootstock, (C) types of non-Northern Spy rootstock used in the orchard.

'R. Del' = Red Delicious, 'G. Del' = Golden Deliciuos, 'G. Smith' = Granny Smith, 'Jon' = Jonathan.

# APPENDIX TWO

## METHODS FOR GROWING APPLE SEEDLINGS UNDER SEMI-CONTROLLED CONDITIONS

A large source of apple seedlings was needed for the maintenance of a continuous culture of woolly apple aphid for experimental purposes. Local availability of seedlings throughout the year was insufficient to meet this demand and the cost of purchasing them, when available, would have been prohibitive. Therefore, a system of seed collection and growing seedlings was implemented to provide a continuous source of seedlings of similar age and physical properties.

Apple seed was obtained from Granny Smith cv apples collected during harvest (March) every year. Approximately 1000 seeds were cleaned of extraneous tissue, washed for 1 minute in a mild solution of mercuric chloride (0.1%) and rinsed in sterile, distilled water. They were left to air dry, then packed in plastic bags and stored until needed in a cool, dark place. A further 300 apples were collected and stored at 5±2°C for a minimum of 8 weeks to stratify before being used.

To provide a constant source of seedlings of similar age, a system of alternating the dried seeds with the partially stratified seeds was utilised. After being removed from the cold-stored apples, seeds were treated in the same way as the dried seeds prior to storage. One hundred seeds of both types were placed in separate plastic bags containing a mixture of sterilised U.C. (University of California) soil dampened with distilled water. The partially stratified seeds were prepared 1 month before they were needed while the unstratified seeds were prepared 2 to 3 months ahead. The longer the apples were held at 5°C, the more rapidly the seeds broke dormancy after being removed from the apples and placed in the soil.

When dormancy had broken and rootlets were visible, the seeds were transferred to flats containing sterilised recycled potting soil, covered with approximately two centimetres of soil/sand mixture and watered with a complete fertiliser mix (Aquasol™ 23N:4P:18K; 1.6gm/l) and a solution of fungicide (Captan®; 1gm/l water) to prevent 'damping off'. The flats were placed in a room with semi-controlled temperature (24-28°C) under a 16L:8D photoperiod regime. Four sodium vapour lamps, (General Electric Lumalux™) were placed 2 meters above the flats.

At the first true leaf stage, seedlings were transplanted to 10 cm plastic pots containing sterilised, recycled potting soil. They were fertilised with Aquasol™ complete fertiliser dissolved in water (1.6 gm/l). The pots were placed in large trays, subirrigated when necessary and fertilised at fortnightly intervals. The seedlings were kept under the same light and temperature conditions until required for experimental work. When seedlings became too large (over 45 cm) they were pruned back to 15 cm and used for rearing WAA stock cultures only.

Seedlings were sprayed with Bayleton® at a rate of 0.5 gm/l to prevent the spread of powdery mildew. Two spotted spider mites (TSM) (*Tetranychus urticae*) were controlled by monthly introductions of *Phytoseiulus persimilis* and *Typhlodromus occidentalis*. When a severe outbreak of TSM occurred, the plants were sprayed with Calibre® (0.5 gm/l) and Omite® (half rate of 0.25 gm/l) just prior to an extra introduction of predatory mites. All attempts were made to keep the plants as healthy as possible with the minimum use of pesticides or fungicides. Yellow sticky traps were placed in the room to control flying insects.

# APPENDIX THREE

# METHODS FOR REARING WOOLLY APPLE APHID AND *Aphelinus mali* IN THE LABORATORY

## A3.1. INTRODUCTION

Stock cultures of WAA were maintained in semi-controlled conditions to provide a source of aphids for experimental work. A consideration in the maintenance of a stock culture was the exclusion of different aphid biotypes. Sen Gupta (1969) reported the presence of a Northern Spy-resistant biotype (the Blackwood biotype) with slightly different developmental thresholds than the biotype found at the Waite Agricultural Research Institute or at the Lenswood Research Station. Therefore, it was of utmost importance to maintain a culture of aphids from the same area, whether it be from Lenswood or Adelaide. Aphids were chosen from the Lenswood site because it was not determined if the Blackwood biotype had been released into the orchards of the Waite Institute, resulting in a mixed population of aphids.

The culturing of woolly aphids has been described in anecdotal form by workers in the United States and more recently in Australia by Asante and Danthanarayana (1990). The former tend to be incomplete and the latter chose to use excised twigs to rear the aphids on rather than apple seedlings.

The method described here involves the use of Granny Smith seedlings grown under semi-controlled conditions (Appendix Two). Granny Smith is a cultivar highly susceptible to WAA (Sen Gupta 1969) and is commonly grown in this area (Chapter Two).

## A3.2. MATERIALS AND METHODS

Aphids were initially collected from a block of mature Granny Smith cv. apple trees located at the Lenswood Research Station (South Australia Department of Agriculture). Aphids were always collected in the spring when colonies were first visible and chosen from the same area for the remainder of the project. Shoots that had colonies were removed and transported to the Waite Institute, placed in vials of water and held at 20°±1°C, 16L:8D until ready to transfer to small apple seedlings.

The aphids were transferred to seedlings that were no more than 15 cm tall. Sections of excised twig with aphid colonies were placed along the central stem of the seedling, usually at the area where the leaf axil joins the stem. Aphids moved to the seedling as the twig desiccated. No effort was made to damage the seedling bark to induce them to settle in one place.

A glass lantern globe (Maxbilt Trading Co. Lantern Glass 285™, approximately 1 l capacity) and cellulose acetate collar were placed over the seedling, fitting flush with the top of the soil (Figure A3.1). The globe's upper opening was covered with fine gauze mesh (50 threads/cm) and held in place with a rubber band. The acetate collar had a series of holes cut into it which were covered with gauze to allow ventilation. The cages prevented the introduction of parasitoids or predators into

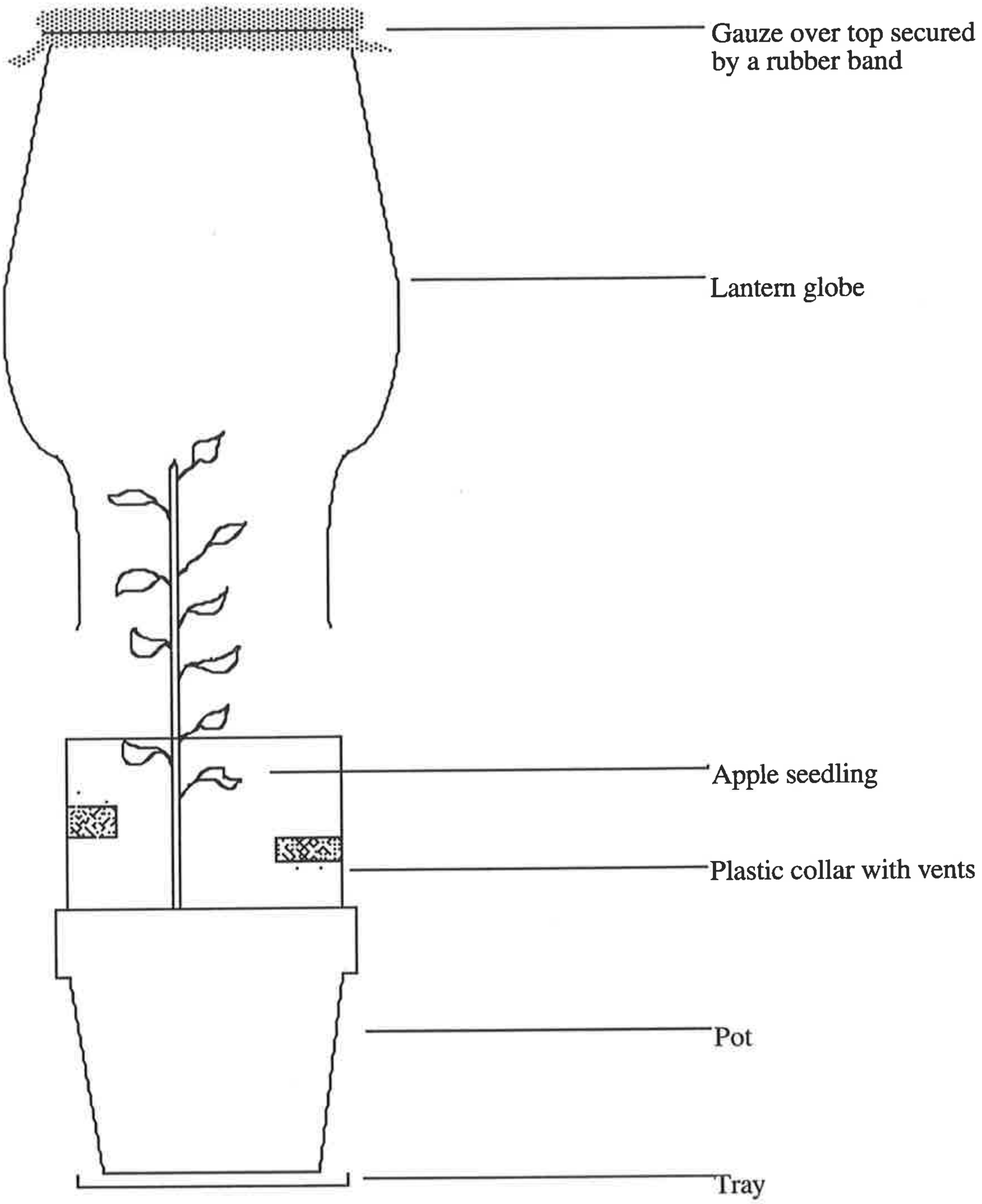
Gauze over top secured by a rubber band
Lantern globe
Apple seedling
Plastic collar with vents
Pot
Tray

**Figure A3.1.** Lantern globe cage used in rearing woolly apple aphid and *A. mali* under laboratory conditions.

the colonies or the infestation of 'clean' trees. When the parasitoid was not being reared in the same building, infested trees were left uncaged. The caged seedlings were kept at 23-28°C, 16L:8D, to prevent alate production. Lighting was provided by sodium vapour lamps (General Electric Lumalux™) placed two meters above the plants.

At 21 day intervals three to four stem portions of the infested trees were used to reinfest fresh seedlings. The number of trees infested varied with expected need.

In spite of the long daylength, alate production occurred when aphid numbers became high and the tree began to show signs of stress (excessive galling, leaf flagging). If left with a high number of aphids for more than three weeks the tree died. This necessitated putting the aphids on fresh seedlings at regular intervals. The 'old' trees were discarded as they were usually too distorted and weak to reuse even if all aphids were removed.

The trees were checked regularly for the presence of powdery mildew and two spotted mite which were controlled by applications of Bayleton® (0.5gm/l) and inundative releases of *Phytoseiulus persimilis*. No fungicide or insecticide was applied two weeks prior to any experiment. The trees were subirrigated as needed and fertilised (Aquasol™ 23N:4P:18K; 1.6 gm/l) at fortnightly intervals.

*Aphelinus mali* were collected from the Lenswood Research Centre and the Waite Institute orchards in the spring. Groups of 20 mummies were held in small glass petri dishes with a source of honey and water until adult emergence. Both males and females were transferred to caged infested seedlings. The parasitoids were left in the cages for 5 to 7 days. Mummies that were produced were removed to petri dishes and the cycle repeated.

## A.3.3. DISCUSSION

Rearing a continuous culture of WAA this way was simple and relatively inexpensive in time and resources. A source of apple seedlings was required for use in experiments, therefore there was no extra output producing seedlings for aphid rearing. Asante and Danthanarayana (1990) stated that there were no differences in developmental parameters of aphids reared on twigs compared to aphids collected from the field. However, their method requires a source of twigs throughout the year and the effect of dormant wood (in the winter) on the development of the aphids is unknown. Asante and Danthanarayana's system is short lived due to the limited resources available to the twig which may have an effect on the development of the colonies.

It was unnecessary to wound the plant in any way to encourage the aphids to settle as stated by other workers (Sen Gupta 1969; Asante and Danthanarayana 1990). They were as likely to settle somewhere else on the plant as on the wound site (personal observation). Wounding the tree increased the possibility of fungal infection. Aphids tended to wander down the length of the stem and settle in leaf axils and near the soil-stem interface. Once settled, they rarely moved again and colonies quickly formed. Older aphids wandered less than younger instars and settled nearer the site of the excised twig (personal observation).

*A. mali* could be easily reared in large numbers without losing a sex ratio that approximated that found in the field (Evenhuis 1958). The greatest difficulty was parasitisation of aphid colonies in other rooms and for this reason a parasitoid colony was not set up until needed. It was also easy to obtain parasitoids from the Waite Institute orchards as required for experiments. This ensured that genetic diversity was maintained.

# APPENDIX FOUR

# ESTIMATION OF THE NUMBER OF INDIVIDUALS WITHIN WOOLLY APPLE APHID COLONIES

## A4.1. ABSTRACT

The relationship between the physical dimensions of woolly apple aphid colonies and the number of individuals within the colonies was investigated to determine an easy and accurate method of estimating aphid numbers. There was a positive relationship between the length and width of the colony at the two widest parts and the number of individuals within the colony. This gave an estimate of the individuals of all stages.

## A4.2. INTRODUCTION

The waxy covering produced by woolly apple aphids precludes easy or accurate estimation of the number of individuals within a colony. Young aphids tend to cluster under the adults which also makes enumeration difficult (Lundie 1924).

It was necessary to find a method of obtaining an estimate of aphid numbers within colonies that was not only non-destructive (ie: remove the colony) but would minimise disturbing the aphids. When disturbed, adult aphids will tend to remove their stylets and wander over the tree (personal observation). Often, they fail to settle again and die. In studying the effects of aphid densities on tree growth (Chapter Five), an estimate of the number of aphids was required at each sampling date. The dimensions of colonies and the number of individuals was investigated to determine if there was a relationship between the two that could be successfully exploited.

## A4.3. MATERIALS AND METHODS

Three times throughout the growing season aphid colonies (total of 64 colonies) were removed from mature Granny Smith cv. trees. Colony size ranged from < 3 mm to > 20 mm in length. Each colony was put into a size class of small, medium or large which were arbitrarily determined. The shoots bearing the colonies were placed at an angle in petri dishes so that the colonies were not touching the bottom or sides of the dish. Length and width of the colony were measured. The width was measured with a piece of thread to take into account the depth of the colony. Length was measured by vernier callipers. Care was taken to ensure that the integrity of the wax covering was maintained during measurements. The shoots were then placed in a drying oven (ca. 60°C) for 15 minutes to force the aphids to retract their stylets and drop to the bottom of the petri dish. Once all the aphids had left the shoot, they were put in 70% ethanol and counted. The life stages were divided into two groups; adults plus fourth instars and first to third instars.

The data were analysed by regression (SAS 1985) with the dimensions of the colony as the independent variable and aphid number as the dependent variable. Various combinations of length and width were tried as the independent variable.

## A4.4. RESULTS AND DISCUSSION

Aphid numbers ranged widely within the three classes and there was a great deal of overlap. Some colonies that were classed as large had relatively few aphids and conversely small colonies could have a large number of individuals.

There was a positive linear relationship ($P<0.01$) between the product of the width and length of the colony and the number of aphids in the colony ($R^2 = 0.895$). The estimate of coefficient (b) was 0.939. To check whether the equation:

$$N = b(L*W) \tag{1}$$

gave a reasonable indication of aphid numbers, the estimated number of aphids was compared with the actual number in a sample of colonies of varying sizes chosen at random. Estimates of the aphid number in each colonies were made using the above coefficient and compared to the actual number of aphids. There was no significant difference ($P>0.05$, ANOVA) between the two.

The large variation within colonies may be accounted for in a number of ways. Young aphids hiding under the adults would have little effect on the dimensions of the colony. The age structure within the colony may also have affected the differences between the estimated and actual number of aphids. Differences between colonies mainly comprised of young aphids and those with a large number of adults may lead to differences in estimation. Highly accurate determination of aphid numbers within WAA colonies, other than the time consuming task of counting individuals, remains elusive, however, the method described here gives a reasonable estimate.

# APPENDIX FIVE

# LEAF AREA DETERMINATION OF APPLE LEAVES

## A5. 1. ABSTRACT

An accurate and simple method of estimating the area of apple leaves was determined. Leaf area estimates were compared for trees grown in the laboratory and field. A highly accurate estimate was obtained by taking the product of the longest point of the mid-vein and widest portion of the leaf and multiplying by a constant to form the equation, $A = b(L*W)$. The coefficient, b, had to be revised depending on the cultivar and conditions of growth.

## A5..2. INTRODUCTION

Studies have established the relationship between leaf area and shoot length (Barlow 1980; Johnson and Lasko 1985) in different cultivars. These studies have also investigated the relationship between leaf area and the dimensions of the leaf itself. Barlow (1980) stated that there was a close relationship between the product of the length of the midrib and the maximum breadth of the leaf to the leaf area as measured on an area meter.

An accurate method of estimating leaf area non-destructively was required in two experiments investigating tree growth (Chapter Five). It was decided to test and, if possible, improve the accuracy of the above method. It was also necessary to determine if the relationship was the same for trees of varying ages and growth conditions (laboratory and field).

## A5.3. MATERIALS AND METHODS

One hundred leaves of various sizes were taken from a group of three month old Granny Smith cv. apple trees. The trees were grown in a growth cabinet at 25±2°C and a photoperiod of 16L:8D. Each leaf was numbered and its length and greatest width measured before being removed. After removal the outline of the leaf was traced on paper to be measured at a later date. The leaves were then measured three times using a leaf area meter (Paton Electronic Planimeter™) to give a mean estimate of the leaf area. The area of the traced outline was measured three times on a Graphics Tablet™ (Apple) to obtain the mean. The mean areas estimated by the planimeter and Graphics Tablet were compared by ANOVA (SAS 1985) to see if there were any differences in the two techniques. The data were then analysed by regression (GLM, SAS 1985) with the mean area as the dependent variable and either length$^2$, width$^2$, or the product of the length and width as the independent variable. The combination that produced the closest fit was used in further estimates of the coefficient. The slope of the line of best fit was used as the coefficient in the equation.

To determine if the estimated coefficient could be used for any group of apple leaves, groups of leaves were removed from mature apple trees of different cultivars in the Alverstoke Orchard of the Waite Institute. The leaves were treated in the same way described above.

Subsamples of 5, 10, 16 and 20 of the initial 100 leaves were taken to determine the number of leaves required to get a good estimate of the coefficient required for each situation.

## A5.4. RESULTS AND DISCUSSION

There was no difference ($P>0.05$) between the two types of leaf area measurement (Planimeter and Graphics Tablet) and the latter was chosen as the preferred method because of its ease of use.

There was a positive linear relationship ($P<0.0001$) between the mean leaf area and the three dependent variables of length$^2$, width$^2$ and the product of length and width with $R^2$ values of 0.987, 0.990 and 0.999 respectively. The estimate of coefficient (0.667) was chosen from the regression of mean area and L*W to form the equation:

$$A = b(L*W) \qquad (1)$$

where A is leaf area, L is leaf length, W is the greatest width and b is the coefficient determined by the regression.

Areas of the mature trees of different cultivars were significantly different ($P<0.01$) from estimated areas using the coefficient from the laboratory trees indicating that a coefficient had to be estimated for each group of trees, whether different cultivars or growing conditions. Subsamples of the initial 100 leaves showed no difference in estimates from the largest sample. Thus, an accurate estimate of leaf area could be easily obtained by taking samples of between 20 and 50 leaves from the population of trees to be measured and estimating a coefficient to be used in Equation (1).

The estimated coefficient used in determining the leaf area in the laboratory experiment in Chapter Five was 0.667 and in the field experiment, 0.712. Leaves used to gain the estimate were taken from adjacent non-sample trees.

# APPENDIX SIX

# RELATIONSHIPS BETWEEN WOOLLY APPLE APHID INFESTATION AND TREE GROWTH UNDER LABORATORY CONDITIONS

**Table A6.1.** Mean (± standard error) of height, total leaf number, total leaf area of trees infested with three densities of WAA (Experiment 1). Mean (± standard error) of total colony and aphid number are also included.

| | | **Weeks of infestation** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 2 | | 4 | | 6 | |
| **Variable** | **Density** | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| height (mm) | no aphids | 10 | 198.50 ± 18.91 | 10 | 457.70 ± 18.43 | 10 | 625.00 ± 25.38 | 10 | 765.70 ± 31.33 |
| | low | 10 | 192.10 ± 16.30 | 10 | 418.40 ± 22.89 | 10 | 535.40 ± 31.84 | 10 | 674.00 ± 43.42 |
| | high | 10 | 183.90 ± 17.87 | 10 | 369.00 ± 46.26 | 10 | 479.60 ± 56.02 | 10 | 540.20 ± 66.69 |
| leaf number | no aphids | 10 | 12.90 ± 0.57 | 10 | 39.10 ± 3.39 | 10 | 51.30 ± 3.34 | 10 | 50.50 ± 4.06 |
| | low | 10 | 13.90 ± 0.72 | 10 | 34.20 ± 2.94 | 10 | 41 70 ± 4.29 | 10 | 46.00 ± 5.34 |
| | high | 10 | 13.50 ± 7.20 | 10 | 27.30 ± 4.19 | 10 | 29.50 ± 2.84 | 10 | 33.30 ± 3.20 |
| leaf area ($cm^2$) | no aphids | 10 | 196.56 ± 16.08 | 10 | 524.56 ± 29.73 | 10 | 719.81 ± 48.28 | 10 | 801.49 ± 50.00 |
| | low | 10 | 193.32 ± 20.20 | 10 | 406.10 ± 37.83 | 10 | 534.74 ± 43.57 | 10 | 608.90 ± 47.49 |
| | high | 10 | 213.88 ± 24.31 | 10 | 390.30 ± 52.78 | 10 | 477.39 ± 63.57 | 10 | 513.21 ± 66.12 |
| colony number | no aphids | 10 | 0 ± 0 h | 10 | 0 ± 0 h | 10 | 0 ± 0 h | 10 | 0 ± 0 h |
| | low | 10 | 0 ± 0 h | 10 | 4.50 ± 0.78 h | 10 | 3.90 ± 0.46 hi | 10 | 2.80 ± 0.44 i |
| | high | 10 | 0 ± 0 h | 10 | 11.70 ± 0.93 i | 10 | 6.40 ± 0.86 i | 10 | 5.20 ± 0.94 i |
| WAA number | no aphids | 10 | 0 ± 0 j | 10 | 0 ± 0 j | 10 | 0 ± 0 j | 10 | 0 ± 0 j |
| | low | 10 | 0 ± 0 j | 10 | 114.65 ± 16.98 j | 10 | 1165.62 ± 205.96 jk | 10 | 9011.02 ± 1435.11 k |
| | high | 10 | 0 ± 0 j | 10 | 491.16 ± 38.81 k | 10 | 2823.57 ± 323.96 k | 10 | 9127.93 ± 794.21 k |

For colony number and WAA number: Means within dates, followed by the same letter are not significantly different (P>0.05).
Unless otherwise indicated, means were compared by ANOVA and Tukey's (HSD) test.

**Table A6.2.** Mean (± standard error) of height, total leaf number, and total leaf area of trees infested with three densities of WAA (Experiment 2). Means (± standard error) of total colony and aphid number are also included.

| | | Weeks of infestation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 2 | | 4 | | 6 | | 8 | |
| **Variable** | **Density** | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| height (mm) | no aphids | 10 | 151.90 ± 9.34 | 10 | 303.80 ± 26.56 | 10 | 464.60 ± 33.55 | 10 | 594.90 ± 38.47 | 10 | 717.30 ± 43.81 |
| | low | 10 | 147.00 ± 9.08 | 10 | 323.30 ± 21.93 | 10 | 479.30 ± 29.24 | 10 | 613.10 ± 31.69 | 10 | 624.50 ± 31.83 |
| | high | 10 | 147.00 ± 8.35 | 10 | 319.30 ± 28.83 | 10 | 438.70 ± 44.03 | 10 | 505.40 ± 45.68 | 10 | 516.70 ± 41.18 |
| leaf number | no aphids | 10 | 13.00 ± 0.52 | 10 | 21.50 ± 0.92 | 10 | 29.50 ± 1.85 | 10 | 34.70 ± 1.98 | 10 | 40.20 ± 2.82 |
| | low | 10 | 13.00 ± 0.50 | 10 | 21.60 ± 0.81 | 10 | 29.40 ± 1.40 | 10 | 33.00 ± 1.94 | 10 | 32.40 ± 1.30 |
| | high | 10 | 13.30 ± 0.42 | 10 | 23.90 ± 1.39 | 10 | 29.20 ± 1.27 | 10 | 31.30 ± 1.43 | 10 | 31.10 ± 1.21 |
| leaf area (cm$^2$) | no aphids | 10 | 170.29 ± 14.98 | 10 | 360.61 ± 24.52 | 10 | 537.66 ± 27.66 | 10 | 704.15 ± 37.68 | 10 | 834.25 ± 35.80 |
| | low | 10 | 165.53 ± 10.66 | 10 | 321.58 ± 23.96 | 10 | 553.98 ± 68.66 | 10 | 736.31 ± 86.15 | 10 | 548.41 ± 4.69 |
| | high | 10 | 171.65 ± 10.47 | 10 | 371.69 ± 33.49 | 10 | 501.74 ± 39.62 | 10 | 554.03 ± 43.78 | 10 | -- |
| colony number | no aphids | 10 | 0.0 ± 0.0 e | 10 | 0.0 ± 0.0 e | 10 | 0.0 ± 0.0 e | 10 | 0.00 ± 0.0 e | 10 | 0 ± 0 |
| | low | 10 | 0.0 ± 0.0 e | 10 | 5.50 ± 0.5 f | 10 | 5.20 ± 0.7 f | 10 | 14.30 ± 2.5 e | 10 | 0.90 ± 0.5 |
| | high | 10 | 0.0 ± 0.0 e | 10 | 11.50 ± 0.9 g | 10 | 5.60 ± 0.6 f | 10 | 6.40 ± 1.4 e | 10 | 0 ± 0 |
| WAA number | no aphids | 10 | 0.0 ± 0.0 h | 10 | 0.0 ± 0.0 h | 10 | 0.0 ± 0.0 h | 10 | 0.0 ± 0.0 h | 10 | 0.0 ± 0.0 |
| | low | 10 | 0.0 ± 0.0 h | 10 | 141.03 ± 7.7 i | 10 | 2098.48 ± 254.6 hi | 10 | 9557.14 ± 623.1 i | 10 | 2494.55 ± 1290.1 |
| | high | 10 | 0.0 ± 0.0 h | 10 | 634.23 ± 59.2 j | 10 | 2948.18 ± 313.8 ij | 10 | 9185.30 ± 585.6 i | 10 | 0.0 ± 0.0 |

For colony number and WAA number: Means within dates, followed by the same letter are not significantly different (P> 0.05).
Unless otherwise indicated, means were compared by ANOVA and Tukey's (HSD).

**Table A6.3.** Mean (± standard error) of height, total leaf number and total leaf area of trees infested with three densities of WAA (Experiment 3). Mean (± standard error) of total colony and aphid number are also included.

| | | Weeks of infestation | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 2 | | 4 | | 6 | | 8 | |
| **Variable** | **Density** | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| height (mm) | no aphids | 10 | 87.92 ± 8.92 | 10 | 265.20 ± 16.54 | 10 | 408.40 ± 23.86 | 10 | 537.20 ± 32.04 | 10 | 637.70 ± 44.84 |
| | low | 10 | 101.90 ± 12.17 | 10 | 300.00 ± 24.36 | 9 | 423.89 ± 31.71 | 9 | 523.56 ± 39.59 | 9 | 558.89 ± 52.08 |
| | high | 10 | 91.00 ± 7.74 | 10 | 264.70 ± 22.85 | 10 | 353.50 ± 25.17 | 10 | 428.60 ± 22.19 | 10 | 421.50 ± 29.10 |
| leaf number | no aphids | 10 | 9.30 ± 0.54 | 10 | 20.9 ± 1.72 | 10 | 27.50 ± 1.97 | 10 | 32.20 ± 2.56 | 10 | 35.30 ± 3.19 |
| | low | 10 | 10.10 ± 0.32 | 10 | 23.6 ± 3.24 | 9 | 27.22 ± 2.43 | 9 | 27.20 ± 4.19 | 9 | 29.67 ± 3.61 |
| | high | 10 | 9.90 ± 0.43 | 10 | 20.0 ± 1.46 | 10 | 24.30 ± 1.30 | 10 | 25.40 ± 1.42 | 10 | 25.00 ± 1.48 |
| leaf area (cm$^2$) | no aphids | 10 | 79.46 ± 8.0 | 10 | 296.89 ± 40.47 | 10 | 510.21 ± 66.22 | 10 | 700.54 ± 54.60 | 10 | 860.11 ± 79.16 |
| | low | 10 | 90.66 ± 8.8 | 10 | 408.72 ± 47.42 | 9 | 551.22 ± 45.62 | 9 | 633.27 ± 69.69 | 9 | 748.58 ± 89.25 |
| | high | 10 | 82.60 ± 7.3 | 10 | 334.57 ± 29.67 | 10 | 467.59 ± 33.90 | 10 | 505.21 ± 36.58 | 10 | -- |
| colony number | no aphids | 10 | 0.0 ± 0.0 f | 10 | 0.0 ± 0.0 f | 10 | 0.0 ± 0.0 f | 10 | 0.0 ± 0.0 f | 10 | 0.0 ± 0.0 |
| | low | 10 | 0.0 ± 0.0 f | 10 | 5.2 ± .39 g | 9 | 5.6 ± .96 fg | 9 | 6.20 ± 2.03 f | 9 | 2.89 ± 0.84 |
| | high | 10 | 0.0 ± 0.0 f | 10 | 11.5 ± .96 h | 10 | 7.8 ± .93 g | 10 | 6.10 ± 1.74 f | 10 | 0.0 ± 0.0 |
| WAA number | no aphids | 10 | 0.0 ± 0.0 i | 10 | 0.0 ± 0.0 i | 10 | 0.0 ± 0.0 i | 10 | 0.0 ± 0.0 i | 10 | 0.0 ± 0.0 |
| | low | 10 | 0.0 ± 0.0 i | 10 | 72.70 ± 11.7 i | 9 | 763.78 ± 97.6 i | 9 | 5156.44 ± 296.9 j | 9 | 7624.22 ± 1175.6 |
| | high | 10 | 0.0 ± 0.0 i | 10 | 331.20 ± 41.0 j | 10 | 2075.00 ± 153.9 j | 10 | 6746.50 ± 443.4 j | 10 | 0.0 ± 0.0 |

For colony number and WAA number: Means within dates, followed by the same letter are not significantly different (P> 0.05). Unless otherwise indicated, means were compared by ANOVA and Tukey's (HSD) test.

**Table A6.4.** Statistical parameters for height, total leaf number and total leaf area of trees infested with three densitites of WAA (Experiment 1).

| Variable | Density | $R^2$ | Intercept | t | $t^2$ |
|---|---|---|---|---|---|
| height | no aphids a | 0.89 | 201.7 | 19.71 | -0.1513 |
| | low b | 0.77 | 198.7 | 15.86 | -0.1119 |
| | high b | 0.42 | 185.1 | 15.1 | -0.1588 |
| leaf number | no aphids a | 0.72 | 12.95 | 2.339 | -0.0344 |
| | low b | 0.52 | 14.38 | 1.599 | -0.0204 |
| | high c | 0.35 | 14.16 | 0.976 | -0.0128 |
| leaf area | no aphids a | 0.79 | 19751 | 2755 | -31.42 |
| | low b | 0.63 | 19480 | 1725 | -17.68 |
| | high b | 0.30 | 21578 | 1457 | -17.93 |

Density levels within variables, followed by the same letter are not significantly different (P>0.05).
Regression lines were compared using an overall test for coincidental regressions.

**Table A6.5.** Statistical parameters for height, total leaf number and total leaf area of trees infested with three densitites of WAA (Experiment 2).

| Variable | Density | $R^2$ | Intercept | t | $t^2$ |
|---|---|---|---|---|---|
| height | no aphids a | 0.80 | 149.3 | 11.98 | -0.0326 |
| | low b | 0.83 | 138.2 | 16.08 | -0.1283 |
| | high c | 0.60 | 146.8 | 14.26 | -0.1366 |
| leaf number | no aphids a | 0.75 | 13.00 | 0.662 | -0.0032 |
| | low b | 0.78 | 12.61 | 0.820 | -0.0082 |
| | high b | 0.77 | 13.62 | 0.813 | -0.0090 |
| leaf area | no aphids a | 0.87 | 16838 | 1461 | -4.77 |
| | low b | 0.58 | 13479 | 2004 | -17.41 |
| | high b | 0.65 | 17125 | 1703 | -18.85 |

Density levels within variables, followed by the same letter are not significantly different (P>0.05).
Regression lines were compared using an overall test for coincidental regressions.

**Table A6.6.** Statistical parameters for height, total leaf number and total leaf area of trees infested with three densitites of WAA (Experiment 3).

| Variable | Density | $R^2$ | Intercept | t | t2 |
|---|---|---|---|---|---|
| height | no aphids a | 0.84 | 88.96 | 13.23 | -0.0612 |
| | low b | 0.74 | 104.2 | 15.29 | -0.1279 |
| | high c | 0.76 | 93.26 | 13.55 | -0.1368 |
| leaf number | no aphids a | 0.65 | 9.68 | 0.838 | -0.0069 |
| | low b | 0.43 | 11.29 | 0.841 | -0.0095 |
| | high b | 0.68 | 10.34 | 0.748 | -0.0088 |
| leaf area | no aphids a | 0.73 | 7663 | 1687 | -5.055 |
| | low b | 0.66 | 11056 | 2085 | -17.74 |
| | high b | 0.77 | 8378 | 2149 | -27.34 |

Density levels within variables, followed by the same letter are not significantly different ($P>0.05$).
Regression lines were compared using an overall test for coincidental regressions.

**Table A6.7.** Statistical parameters and estimated intrinsic rate of increase for aphid numbers on trees infested with two densitites of WAA.

| Variable | Density | $R^2$ | Intercept | t | Rate of increase |
|---|---|---|---|---|---|
| Experiment 1 | low | 0.91 | 2.42 | 0.158 | 0.158 |
| | high | 0.93 | 4.79 | 0.104 | 0.104 |
| Experiment 2 | low | 0.96 | 3.01 | 0.150 | 0.150 |
| | high | 0.93 | 5.12 | 0.096 | 0.097 |
| Experiment 3 | low | 0.95 | 2.05 | 0.156 | 0.156 |
| | high | 0.93 | 4.31 | 0.11 | 0.110 |

Density levels within variables, followed by the same letter are not significantly different ($P>0.05$).
Regresion lines were compared using an overall test for coincidental regressions.

# APPENDIX SEVEN

# RELATIONSHIPS BETWEEN WOOLLY APPLE APHID INFESTATION AND TREE GROWTH IN THE FIELD

**Table A7.1.** Mean (± standard error) of height, total leaf number and total leaf area of trees infested with three densities of WAA. Block A, Year 1. Mean (± standard error) of total colony and aphid number are also included.

| Variable | Density | Date | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 11.XII.89 | | 30.I.90 | | 01.III.90 | | 29.III.90 | | 25.IV.90 | | 22.V.90 | | 20.VI.90 | |
| | | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ±(SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) |
| height (mm) | no aphids | 8 | 316.63<br>(37.24) | 8 | 556.25<br>(70.58) | 8 | 596.88<br>(78.47) | 8 | 600.75<br>(85.84) | 8 | 611.25<br>(89.02) | 8 | 614.38<br>(88.48) | 8 | 614.38<br>(88.48) |
| | low | 8 | 281.13<br>(21.81) | 8 | 508.13<br>(34.52) | 8 | 562.50<br>(46.89) | 8 | 580.63<br>(56.78) | 8 | 583.75<br>(57.46) | 8 | 589.38<br>(60.64) | 8 | 589.38<br>(60.64) |
| | high | 8 | 243.63<br>(25.96) | 8 | 536.25<br>(53.83) | 8 | 559.38<br>(57.55) | 8 | 560.00<br>(57.27) | 8 | 563.13<br>(57.13) | 8 | 574.38<br>(58.08) | 8 | 574.38<br>(58.08) |
| leaf number | no aphids | 8 | 26.50<br>(3.83) | 8 | 40.88<br>(6.45) | 8 | 41.25<br>(7.68) | 8 | 39.88<br>(7.34) | 8 | 38.00<br>(7.24) | 8 | 34.25<br>(6.29) | 8 | 27.38<br>(5.03) |
| | low | 8 | 20.88<br>(2.57) | 8 | 37.25<br>(4.18) | 8 | 36.13<br>(5.63) | 8 | 32.88<br>(6.49) | 8 | 32.50<br>(7.25) | 8 | 29.88<br>(5.07) | 8 | 23.63<br>(5.36) |
| | high | 8 | 19.00<br>(1.83) | 8 | 39.00<br>(7.51) | 8 | 40.13<br>(8.15) | 8 | 34.13<br>(6.43) | 8 | 33.25<br>(5.00) | 8 | 28.38<br>(4.94) | 8 | 17.38<br>(3.67) |
| leaf area ($cm^2$) | no aphids | 8 | 319.99<br>(43.02) | 8 | 588.47<br>(102.99) | 8 | 620.26<br>(126.77) | 8 | 662.99<br>(189.45) | 8 | 596.45<br>(176.99) | 8 | 534.56<br>(151.69) | 8 | 459.92<br>(138.81) |
| | low | 8 | 277.24<br>(19.52) | 8 | 560.21<br>(94.29) | 8 | 553.12<br>(87.87) | 8 | 483.48<br>(105.88) | 8 | 528.45<br>(111.27) | 8 | 440.13<br>(102.38) | 8 | 363.85<br>(106.72) |
| | high | 8 | 238.88<br>(27.16) | 8 | 540.32<br>(96.93) | 8 | 486.44<br>(104.37) | 8 | 408.20<br>(62.04) | 8 | 429.49 c<br>(85.29) | 8 | 310.74<br>(42.80) | 8 | 150.79<br>(30.70) |
| colony number | no aphids | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0 a<br>(0.0) | 8 | 0.0 a<br>(0.0) | 8 | 0.0 a<br>(0.0) | 8 | 0.0 a<br>(0.0) |
| | low | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 7.38 b<br>(1.65) | 8 | 11.75 b<br>(2.64) | 8 | 17.13 a<br>(2.85) | 8 | 11.88 a<br>(1.63) |
| | high | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 7.88 b<br>(1.09) | 8 | 14.25 b<br>(1.87) | 8 | 22.5 b<br>(2.89) | 8 | 16.38 b<br>(2.25) |
| WAA number (colony area) | no aphids | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0 c<br>(0.0) | 8 | 0.0 c<br>(0.0) | 8 | 0.0 c<br>(0.0) | 8 | 0.0 c<br>(0.0) |
| | low | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 1376.75 d<br>(406.70) | 8 | 1549.88 c<br>(343.79) | 8 | 1549.63 d<br>(183.10) | 8 | 2973.00 d<br>(740.11) |
| | high | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 2921.75 d<br>(518.82) | 8 | 8674.50 d<br>(2930.52) | 8 | 6082.88 e<br>(592.40) | 8 | 5184.33 d<br>(1096.10) |

For colony number and WAA number: means were compared by ANOVA and Tukey's (HSD) test. Means within dates, followed by the same letter are not significantly different ($P>0.05$). All other variables were compared by overall comparison of regressions (Chapter Five).

**Table A7.2.** Mean (± standard error) of height, total leaf number and total leaf area of trees infested with three densities of WAA. Block B, Year 1. Mean (± standard error) of total colony and aphid number are also included.

| | | Date | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | Density | 11.XII.89 | | 30.I.90 | | 01.III.90 | | 29.III.90 | | 25.IV.90 | | 22.V.90 | | 20.VI.90 | |
| | | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) |
| height (mm) | no aphids | 8 | 247.00<br>(34.76) | 8 | 563.75<br>(67.89) | 8 | 636.88<br>(87.63) | 8 | 735.38<br>(117.92) | 8 | 765.00<br>(130.38) | 8 | 765.50<br>(130.68) | 8 | 768.50<br>(131.08) |
| | low | 8 | 291.63<br>(35.07) | 8 | 536.88<br>(78.98) | 8 | 601.38<br>(98.15) | 8 | 690.00<br>(115.37) | 8 | 719.38<br>(120.94) | 8 | 724.63<br>(121.06) | 8 | 728.75<br>(121.72) |
| | high | 8 | 321.38<br>(12.24) | 8 | 649.38<br>(55.97) | 8 | 740.63<br>(54.88) | 8 | 802.38<br>(61.38) | 8 | 841.88<br>(60.71) | 8 | 857.50<br>(65.84) | 8 | 860.00<br>(65.16) |
| leaf number | no aphids | 8 | 22.63<br>(2.15) | 8 | 36.63<br>(4.68) | 8 | 38.25<br>(4.83) | 8 | 37.75<br>(6.06) | 8 | 34.5<br>(5.62) | 8 | 32.63<br>(5.55) | 8 | 28.63<br>(4.48) |
| | low | 8 | 20.75<br>(2.19) | 8 | 40.13<br>(10.14) | 8 | 42.88<br>(10.78) | 8 | 47.00<br>(13.30) | 8 | 46.13<br>(11.36) | 8 | 41.88<br>(10.27) | 8 | 35.50<br>(8.88) |
| | high | 8 | 21.75<br>(1.69) | 8 | 48.38<br>(6.32) | 8 | 51.63<br>(7.55) | 8 | 57.25<br>(8.48) | 8 | 59.00<br>(8.72) | 8 | 57.00<br>(9.54) | 8 | 48.88<br>(8.83) |
| leaf area ($cm^2$) | no aphids | 8 | 297.54<br>(45.33) | 8 | 733.29<br>(117.42) | 8 | 872.99<br>(165.00) | 8 | 1083.29<br>(257.66) | 8 | 1067.78<br>(271.66) | 8 | 1022.18<br>(272.29) | 8 | 932.55<br>(249.70) |
| | low | 8 | 280.35<br>(46.94) | 8 | 703.15<br>(157.38) | 8 | 865.87<br>(209.31) | 8 | 1086.55<br>(336.26) | 8 | 1036.63<br>(293.98) | 8 | 1049.11<br>(293.18) | 8 | 905.15<br>(254.66) |
| | high | 8 | 347.96<br>(23.14) | 8 | 918.11<br>(118.77) | 8 | 1145.04<br>(152.81) | 8 | 1286.28<br>(178.11) | 8 | 1339.65<br>(173.27) | 8 | 1362.53<br>(217.11) | 8 | 1240.20<br>(223.07) |
| colony number | no aphids | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0 a<br>(0.0) | 8 | 0.0 a<br>(0.0) | 8 | 0.0 a<br>(0.0) | 8 | 0.0 a<br>(0.0) |
| | low | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 5.38 b<br>(1.44) | 8 | 12.63 b<br>(1.38) | 8 | 20.75 b<br>(2.76) | 8 | 12.5 b<br>(2.24) |
| | high | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 10.38 c<br>(1.76) | 8 | 17.38 b<br>(3.08) | 8 | 29.88 b<br>(4.24) | 8 | 16.75 b<br>(3.45) |
| WAA number (colony area) | no aphids | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0 d<br>(0.0) | 8 | 0.0 d<br>(0.0) | 8 | 0.0 d<br>(0.0) | 8 | 0.0 d<br>(0.0) |
| | low | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 1098.25 de<br>(214.89) | 8 | 1588.00 d<br>(272.45) | 8 | 2169.50 d<br>(288.71) | 8 | 3433.63 d<br>(594.11) |
| | high | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 2370.13 e<br>(798.84) | 8 | 6312.50 d<br>(1858.61) | 8 | 8592.63 e<br>(1942.59) | 8 | 22898.00 e<br>(7675.83) |

For colony number and WAA number: means were compared by ANOVA and Tukey's (HSD) test. Means within dates, followed by the same letter are not significantly different (P>0.05). All other variables were compared by overall comparison of regressions (Chapter Five).

**Table A7.3.** Mean (± standard error) of height, total leaf number and total leaf area of trees infested with three densities of WAA. Block C, Year 1. Mean (± standard error) of total colony and aphid number are also included.

| Variable | Density | Date | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 11.XII.89 | | 30.I.90 | | 01.III.90 | | 29.III.90 | | 25.IV.90 | | 22.V.90 | | 20.VI.90 | |
| | | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) | N | Mean ± (SE) |
| height (mm) | no aphids | 8 | 291.50<br>(19.01) | 8 | 696.88<br>(42.43) | 8 | 882.38<br>(55.76) | 8 | 1094.13<br>(64.03) | 8 | 1168.75<br>(71.51) | 8 | 1180.75<br>(71.14) | 8 | 1183.75<br>(70.99) |
| | low | 8 | 268.00<br>(14.23) | 8 | 703.75<br>(21.87) | 8 | 925.63<br>(39.41) | 8 | 1162.13<br>(43.97) | 8 | 1247.50<br>(47.64) | 8 | 1260.00<br>(47.63) | 8 | 1276.88<br>(41.54) |
| | high | 8 | 182.38<br>(30.89) | 8 | 462.86<br>(81.03) | 8 | 668.57<br>(97.65) | 8 | 735.57<br>(147.21) | 8 | 817.86<br>(153.37) | 8 | 817.14<br>(149.62) | 8 | 846.43<br>(158.77) |
| leaf number | no aphids | 8 | 28.25<br>(4.07) | 8 | 81.13<br>(17.02) | 8 | 99.75<br>(22.27) | 8 | 112.13<br>(25.26) | 8 | 118.00<br>(26.52) | 8 | 112.63<br>(24.96) | 8 | 104.13<br>(24.23) |
| | low | 8 | 20.75<br>(1.07) | 8 | 55.25<br>(7.39) | 8 | 65.38<br>(9.03) | 8 | 70.88<br>(9.42) | 8 | 78.75<br>(10.76) | 8 | 77.38<br>(10.17) | 8 | 66.00<br>(8.65) |
| | high | 8 | 17.63<br>(2.69) | 8 | 47.71<br>(7.36) | 8 | 57.29<br>(8.17) | 8 | 54.43<br>(9.08) | 8 | 60.29<br>(11.89) | 8 | 57.29<br>(11.53) | 8 | 49.57<br>(11.06) |
| leaf area ($cm^2$) | no aphids | 8 | 292.32<br>(24.04) | 8 | 995.75<br>(249.93) | 8 | 1919.50<br>(377.87) | 8 | 2502.12<br>(486.41) | 8 | 3142.38<br>(570.80) | 8 | 3090.02<br>(602.57) | 8 | 3100.59<br>(580.83) |
| | low | 8 | 272.18<br>(24.28) | 8 | 938.42<br>(90.16) | 8 | 1429.88<br>(141.13) | 8 | 1990.21<br>(201.74) | 8 | 2530.25<br>(245.74) | 8 | 2545.72<br>(263.36) | 8 | 2421.87<br>(270.46) |
| | high | 8 | 161.95<br>(27.18) | 8 | 615.50<br>(122.17) | 8 | 996.54<br>(166.80) | 8 | 1137.37<br>(254.23) | 8 | 1459.14<br>(323.94) | 8 | 1382.20<br>(294.28) | 8 | 1504.01<br>(285.06) |
| colony number | no aphids | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0 a<br>(0.0) | 8 | 0.0 a<br>(0.0) | 8 | 0.0 a<br>(0.0) | 8 | 0.0 a<br>(0.0) |
| | low | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 3.75 ab<br>(1.61 | 8 | 8.88 a<br>(3.06) | 8 | 9.50 a<br>(2.16) | 8 | 8.75 a<br>(3.15) |
| | high | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 10.29 b<br>(3.69) | 8 | 29.14 b<br>(6.88) | 8 | 28.29 b<br>(5.28) | 8 | 29.00 b<br>(6.61) |
| WAA number (colony area) | no aphids | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0 c<br>(0.0) | 8 | 0.0 c<br>(0.0) | 8 | 0.0 c<br>(0.0) | 8 | 0.0 c<br>(0.0) |
| | low | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 1233.38 c<br>(969.63) | 8 | 400.63 c<br>(185.99) | 8 | 719.25 c<br>(334.30) | 8 | 11.63 c<br>(531.91) |
| | high | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 0.0<br>(0.0) | 8 | 2405.86 c<br>(1253.52) | 8 | 9070.14 d<br>(3204.08) | 8 | 11020.86 d<br>(3737.90) | 8 | 14099.57 d<br>(3552.96) |

For colony number and WAA number: means were compared by ANOVA and Tukey's (HSD) test. Means within dates, followed by the same letter are not significantly different (P>0.05)
All other variables were compared by overall comparison of regressions (Chapter Five).

**TABLE A7.4.** Mean (± standard error) of height, total leaf number and total leaf area of trees infested with three densities of WAA. Block A, Year 2.

| | | **Date** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Density** | 15.X.90 | | 13.XI.90 | | 13.XII.90 | | 09.I.91 | | 07.II.91 | | 03.V.91 | |
| | | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| height (mm) | no aphids | 8 | 449.38<br>(33.20) | 8 | 583.38<br>(61.12) | 8 | 651.88<br>(85.69 | 8 | 667.13<br>(91.24) | 8 | 673.13<br>(92.45) | 8 | 673.25<br>(91.38) |
| | low | 8 | 447.50<br>(14.61) | 8 | 500.63<br>(33.92) | 8 | 558.88<br>(55.93) | 8 | 596.75<br>(72.99) | 8 | 585.00<br>(63.43) | 8 | 589.00<br>(60.24) |
| | high | 8 | 428.50<br>(18.65) | 8 | 437.25<br>(18.87) | 8 | 444.13<br>(20.59) | 8 | 445.75<br>(21.06) | 8 | 445.75<br>(21.06) | 8 | 443.25<br>(20.45) |
| leaf number | no aphids | 8 | 0.0<br>(0.0) | 8 | 71.75<br>(11.74) | 8 | 93.13<br>(20.69) | 8 | 93.75<br>(24.53) | 8 | 83.75<br>(16.92) | 8 | 65.25<br>(21.69) |
| | low | 8 | 0.0<br>(0.0) | 8 | 28.38<br>(3.52) | 8 | 42.75<br>(10.18) | 8 | 45.63<br>(10.82) | 8 | 48.25<br>(12.88) | 8 | 45.38<br>(14.94) |
| | high | 8 | 0.0<br>(0.0) | 8 | 7.75<br>(3.10) | 8 | 9.25<br>(3.80) | 8 | 6.68<br>(4.2) | 8 | 5.00<br>(3.32) | 8 | 4.63<br>(3.27) |
| leaf area ($cm^2$) | no aphids | 8 | 0.0<br>(0.0) | 8 | 703.93<br>(139.13) | 8 | 1122.08<br>(302.11) | 8 | 1184.76<br>(422.16) | 8 | 1408.10<br>(455.42) | 8 | 882.55<br>(103.66) |
| | low | 8 | 0.0<br>(0.0) | 8 | 408.08<br>(95.68) | 8 | 697.53<br>(212.90) | 8 | 908.89<br>(259.79) | 8 | 836.37<br>(300.40) | 8 | 565.91<br>(170.49) |
| | high | 8 | 0.0<br>(0.0) | 8 | 50.56<br>(28.11) | 8 | 74.16<br>(43.31) | 8 | 77.99<br>(51.44) | 8 | 60.34<br>(39.80) | 8 | 50.08<br>(33.03) |

**Table A7.5.** Mean (± standard error) of height, total leaf number and total leaf area of trees infested with three densities of WAA. Block B, Year 2.

| | | Date | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Density** | 15.X.90 | | 13.XI.90 | | 13.XII.90 | | 09.I.91 | | 07.II.91 | | 03.V.91 | |
| | | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| height (mm) | no aphids | 8 | 443.00<br>(14.06) | 8 | 545.13<br>(49.78) | 8 | 686.63<br>(74.18) | 8 | 740.00<br>(91.21) | 8 | 743.57<br>(105.72) | 8 | 745.29<br>(105.33) |
| | low | 8 | 421.00<br>(14.02) | 8 | 501.25<br>(39.39) | 8 | 688.13<br>(72.99) | 8 | 742.63<br>(88.08) | 8 | 784.63<br>(103.86) | 8 | 855.63<br>(117.96) |
| | high | 8 | 439.13<br>(11.58) | 8 | 524.00<br>(46.43) | 8 | 636.25<br>(81.72) | 8 | 659.25<br>(85.28) | 8 | 683.13<br>(98.90) | 8 | 697.75<br>(119.60) |
| leaf number | no aphids | 8 | 0.0<br>(0.0) | 8 | 71.00<br>(10.99) | 8 | 110.25<br>(21.60) | 8 | 107.63<br>(20.29) | 8 | 92.29<br>(20.60) | 8 | 78.43<br>(20.95) |
| | low | 8 | 0.0<br>(0.0) | 8 | 37.13<br>(7.64) | 8 | 86.75<br>(21.85) | 8 | 94.50<br>(19.30) | 8 | 88.00<br>(20.89) | 8 | 96.50<br>(24.10 |
| | high | 8 | 0.0<br>(0.0) | 8 | 37.00<br>(13.49) | 8 | 63.25<br>(21.81) | 8 | 70.63<br>(26.30) | 8 | 68.25<br>(23.74) | 8 | 71.38<br>(26.25) |
| leaf area ($cm^2$) | no aphids | 8 | 0.0<br>(0.0) | 8 | 750.76<br>(134.52) | 8 | 1711.23<br>(439.62) | 8 | 1791.63<br>(462.14) | 8 | 1601.97<br>(526.24) | 8 | 1382.91<br>(533.53) |
| | low | 8 | 0.0<br>(0.0) | 8 | 450.85<br>(101.06) | 8 | 1479.45<br>(367.45) | 8 | 2254.12<br>(636.10) | 8 | 1992.39<br>(547.74) | 8 | 2013.64<br>(508.09) |
| | high | 8 | 0.0<br>(0.0) | 8 | 493.21<br>(184.63) | 8 | 764.01<br>(292.45) | 8 | 1507.99<br>(594.48) | 8 | 1532.51<br>(552.71) | 8 | 1668.99<br>(647.50) |

**Table A7.6.** Mean (± standard error) of height, total leaf number and total leaf area of trees infested with three densities of WAA. Block C, Year 2.

| Variable | Density | Date | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 15.X.90 | | 13.XI.90 | | 13.XII.90 | | 09.I.91 | | 07.II.91 | | 03.V.91 | |
| | | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| height (mm) | no aphids | 8 | 483.75<br>(4.89) | 8 | 717.13<br>(18.72) | 8 | 1056.38<br>(23.61) | 8 | 1332.50<br>(38.91) | 8 | 1453.00<br>(51.64) | 8 | 1637.88<br>(58.12) |
| | low | 8 | 473.13<br>(5.17) | 8 | 637.38<br>(39.66) | 8 | 943.13<br>(68.96) | 8 | 1220.00<br>(87.82) | 8 | 1346.38<br>(74.79) | 8 | 1516.38<br>(66.99) |
| | high | 8 | 425.71<br>(40.58) | 8 | 467.57<br>(54.32) | 8 | 538.43<br>(91.94) | 8 | 596.71<br>(130.84) | 8 | 635.00<br>(157.19) | 8 | 690.00<br>(184.62) |
| leaf number | no aphids | 8 | 0.0<br>(0.0) | 8 | 87.25<br>(13.93) | 8 | 250.50<br>(28.56) | 8 | 370.75<br>(49.42) | 8 | 372.50<br>(48.51) | 8 | 428.13<br>(58.12) |
| | low | 8 | 0.0<br>(0.0) | 8 | 70.88<br>(12.66) | 8 | 220.25<br>(27.34) | 8 | 396.75<br>(72.62) | 8 | 355.13<br>(31.84) | 8 | 443.25<br>(48.23) |
| | high | 8 | 0.0<br>(0.0) | 8 | 9.14<br>(5.91) | 8 | 34.43<br>(17.62) | 8 | 59.29<br>(33.73) | 8 | 84.00<br>(37.09) | 8 | 99.71<br>(41.52) |
| leaf area ($cm^2$) | no aphids | 8 | 0.0<br>(0.0) | 8 | 1182.63<br>(145.12) | 8 | 4099.39<br>(451.51) | 8 | 8667.54<br>(1185.59) | 8 | 8486.39<br>(1381.55) | 8 | 13601.37<br>(2772.04) |
| | low | 8 | 0.0<br>(0.0) | 8 | 780.26<br>(163.10) | 8 | 3853.30<br>(555.32) | 8 | 7595.33<br>(946.96) | 8 | 9972.77<br>(2014.82) | 8 | 10030.51<br>(638.55) |
| | high | 8 | 0.0<br>(0.0) | 8 | 85.05<br>(56.77) | 8 | 377.44<br>(223.06) | 8 | 1078.01<br>(5.88) | 8 | 1519.12<br>(139.33) | 8 | 2037.65<br>(1022.65) |

**Table A7.7.** Mean (± standard error) of total side shoot number, total side shoot leaf number and total side shoot leaf area of trees infested with three densities of WAA. Block A, Year 2.

| | | Date | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Density** | 15.X.90 | | 13.XI.90 | | 13.XII.90 | | 09.I.91 | | 07.II.91 | | 03.V.91 | |
| | | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| side shoot number | no aphids | 8 | 0.00<br>(0.00) | 8 | 1.75<br>(0.73) | 8 | 2.75<br>(1.00) | 8 | 2.88<br>(1.01) | 8 | 2.75<br>(1.05) | 8 | 3.13<br>(0.90) |
| | low | 8 | 0.00<br>(0.00) | 8 | 0.75<br>(0.41) | 8 | 0.75<br>(0.37) | 8 | 1.00<br>(0.38) | 8 | 1.00<br>(0.38) | 8 | 1.13<br>(0.30) |
| | high | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) |
| leaf number | no aphids | 8 | 0.00<br>(0.00) | 8 | 20.13<br>(8.19) | 8 | 42.38<br>(16.65) | 8 | 42.75<br>(20.15) | 8 | 34.63<br>(12.50) | 8 | 41.38<br>(16.47) |
| | low | 8 | 0.00<br>(0.00) | 8 | 9.25<br>(5.23) | 8 | 16.50<br>(8.67) | 8 | 21.38<br>(10.85) | 8 | 23.13<br>(12.12) | 8 | 23.38<br>(11.13) |
| | high | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) |
| leaf area ($cm^2$) | no aphids | 8 | 0.00<br>(0.00) | 8 | 218.36<br>(89.10) | 8 | 527.77<br>(217.86) | 8 | 568.53<br>(307.56) | 8 | 771.55<br>(349.25) | 8 | 537.32<br>(285.23) |
| | low | 8 | 0.00<br>(0.00) | 8 | 171.77<br>(100.53) | 8 | 298.61<br>(161.43) | 8 | 561.79<br>(264.74) | 8 | 472.33<br>(281.67) | 8 | 212.71<br>(90.06) |
| | high | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) | 8 | 0.00<br>(0.00) |

**Table A7.8.** Mean (± standard error) of total side shoot number, total side shoot leaf number and total side shoot leaf area of tree infested with three densities of WAA. Block B, Year 2.

| | | **Date** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Density** | 15.X.90 | | 13.XI.90 | | 13.XII.90 | | 09.I.91 | | 07.II.91 | | 03.V.91 | |
| | | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| side shoot number | no aphids | 8 | 0.00<br>(0.00) | 8 | 2.63<br>(0.71) | 8 | 3.88<br>(1.16) | 8 | 4.13<br>(0.92) | 8 | 3.57<br>(1.11) | 8 | 4.00<br>(1.05) |
| | low | 8 | 0.00<br>(0.00) | 8 | 1.50<br>(0.68) | 8 | 3.13<br>(0.90) | 8 | 3.50<br>(1.09) | 8 | 3.25<br>(1.00) | 8 | 3.50<br>(0.97) |
| | high | 8 | 0.00<br>(0.00) | 8 | 1.00<br>(0.46) | 8 | 2.00<br>(0.95) | 8 | 2.00<br>(0.67) | 8 | 2.25<br>(0.98) | 8 | 2.63<br>(1.13) |
| leaf number | no aphids | 8 | 0.00<br>(0.00) | 8 | 27.63<br>(7.78) | 8 | 59.38<br>(18.78) | 8 | 60.63<br>(16.02) | 8 | 50.71<br>(18.55) | 8 | 46.00<br>(16.30) |
| | low | 8 | 0.00<br>(0.00) | 8 | 12.5<br>(5.65) | 8 | 57.50<br>(20.45) | 8 | 60.75<br>(18.89) | 8 | 57.63<br>(18.18) | 8 | 63.63<br>(19.12) |
| | high | 8 | 0.00<br>(0.00) | 8 | 16.5<br>(7.83) | 8 | 41.00<br>(17.44) | 8 | 50.13<br>(22.66) | 8 | 44.75<br>(18.88) | 8 | 47.38<br>(18.96) |
| leaf area (cm$^2$) | no aphids | 8 | 0.00<br>(0.00) | 8 | 306.73<br>(105.04) | 8 | 917.47<br>(344.32) | 8 | 944.77<br>(340.21) | 8 | 846.53<br>(398.98) | 8 | 739.19<br>(385.55) |
| | low | 8 | 0.00<br>(0.00) | 8 | 82.08<br>(33.61) | 8 | 844.81<br>(296.98) | 8 | 1505.83<br>(592.58) | 8 | 1259.49<br>(425.25) | 8 | 1182.99<br>(375.54) |
| | high | 8 | 0.00<br>(0.00) | 8 | 205.31<br>(113.96) | 8 | 455.41<br>(247.83) | 8 | 1031.09<br>(482.58) | 8 | 968.69<br>(419.32) | 8 | 1109.02<br>(450.63) |

**Table A7.9.** Means (± standard error) of total side shoot number, total side shoot leaf number and total side shoot leaf area of trees infested with three densities of WAA. Block C, Year 2.

| | | **Date** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Density** | 15.X.90 | | 13.XI.90 | | 13.XII.90 | | 09.I.91 | | 07.II.91 | | 03.V.91 | |
| | | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE | N | Mean ± SE |
| side shoot number | no aphids | 8 | 0.00<br>(0.00) | 8 | 3.50<br>(1.20) | 8 | 12.25<br>(1.61) | 8 | 12.25<br>(1.29) | 8 | 12.75<br>(1.21) | 8 | 14.13<br>(1.45) |
| | low | 8 | 0.00<br>(0.00) | 8 | 2.00<br>(0.63) | 8 | 13.75<br>(4.14) | 8 | 14.75<br>(2.98) | 8 | 12.75<br>(1.33) | 8 | 15.25<br>(1.98) |
| | high | 8 | 0.00<br>(0.00) | 7 | 0.29<br>(0.18) | 7 | 1.00<br>(0.58) | 7 | 3.14<br>(1.42) | 7 | 3.43<br>(1.38) | 7 | 4.14<br>(1.58) |
| leaf number | no aphids | 8 | 0.00<br>(0.00) | 8 | 42.25<br>(13.67) | 8 | 199.13<br>(28.24) | 8 | 308.13<br>(48.38) | 8 | 309.50<br>(47.77) | 8 | 359.50<br>(58.83) |
| | low | 8 | 0.00<br>(0.00) | 8 | 23.75<br>(8.43) | 8 | 161.88<br>(25.37) | 8 | 337.00<br>(69.63) | 8 | 293.13<br>(32.31) | 8 | 376.63<br>(47.36) |
| | high | 8 | 0.00<br>(0.00) | 7 | 3.43<br>(2.31) | 7 | 20.43<br>(10.68) | 7 | 43.00<br>(23.27) | 7 | 67.71<br>(27.30) | 7 | 78.86<br>(31.22) |
| leaf area ($cm^2$) | no aphids | 8 | 0.00<br>(0.00) | 8 | 481.89<br>(136.53) | 8 | 2938.15<br>(512.45) | 8 | 6977.82<br>(1158.95) | 8 | 6652.94<br>(1390.17) | 8 | 11201.40<br>(2768.80) |
| | low | 8 | 0.00<br>(0.00) | 8 | 283.60<br>(93.61) | 8 | 2744.59<br>(427.57) | 8 | 6173.52<br>(850.50) | 8 | 8427.83<br>(1967.53) | 8 | 8103.43<br>(570.79) |
| | high | 8 | 0.00<br>(0.00) | 7 | 35.41<br>(28.83) | 7 | 132.50<br>(81.17) | 7 | 691.84<br>(362.96) | 7 | 1093.21<br>(561.26) | 7 | 1491.43<br>(685.46) |

**Table A7.10.** Statistical parameters for height, total leaf number and total leaf area of trees infested with three densitites of WAA. Block A, Year 1.

| Variable | Density | $R^2$ | Intercept | t | $t^2$ |
|---|---|---|---|---|---|
| height | no aphids a | 0.15 | 338.10 | 4.300 | -0.0155 |
| | low a | 0.34 | 297.60 | 4.390 | -0.0155 |
| | high a | 0.32 | 275.20 | 4.803 | -0.0178 |
| leaf number | no aphids a | 0.07 | 27.55 | 0.296 | -0.0016 |
| | low a | 0.10 | 22.70 | 0.280 | -0.0015 |
| | high a | 0.19 | 20.78 | 0.398 | -0.0022 |
| leaf area | no aphids a | 0.04 | 33350 | 594.0 | -2.822 |
| | low a | 0.01 | 30691 | 491.3 | -2.471 |
| | high a | 0.26 | 26946 | 532.4 | -3.139 |

Density levels within variables, followed by the same letter are not significantly different ($P>0.05$).
Regresion lines were compared using an overall test for coincidental regressions.

**Table A7.11.** Statistical parameters for height, total leaf number and total leaf area of trees infested with three densitites of WAA. Block B, Year 1.

| Variable | Density | $R^2$ | Intercept | t | $t^2$ |
|---|---|---|---|---|---|
| height | no aphids a | 0.25 | 256.70 | 6.665 | -0.0211 |
| | low a | 0.20 | 296.11 | 5.304 | -0.0161 |
| | high a | 0.57 | 335.97 | 6.705 | -0.0212 |
| leaf number | no aphids a | 0.11 | 23.82 | 0.281 | -0.0014 |
| | low b | 0.05 | 21.13 | 0.447 | -0.0020 |
| | high b | 0.22 | 22.43 | 0.576 | -0.0023 |
| leaf area | no aphids a | 0.14 | 28465 | 1130 | -4.123 |
| | low a | 0.11 | 26056 | 1155 | -4.218 |
| | high a | 0.34 | 34548 | 1387 | -4.777 |

Density levels within variables, followed by the same letter are not significantly different ($P>0.05$).
Regresion lines were compared using an overall test for coincidental regressions.

**Table A7.12.** Statistical parameters for height, total leaf number and total leaf area of trees infested with three densitites of WAA. Block C, Year 1.

| Variable | Density | $R^2$ | Intercept | t | $t^2$ |
|---|---|---|---|---|---|
| height | no aphids a | 0.78 | 275.54 | 10.36 | -0.0291 |
| | low ab | 0.91 | 247.43 | 11.42 | -0.0313 |
| | high ac | 0.34 | 174.90 | 7.483 | -0.0209 |
| leaf number | no aphids a | 0.17 | 28.33 | 1.274 | -0.0046 |
| | low b | 0.37 | 20.70 | 0.812 | -0.0030 |
| | high b | 0.24 | 118.78 | 0.659 | -0.0026 |
| leaf area | no aphids a | 0.39 | 10956 | 2827 | -6.155 |
| | low b | 0.67 | 14376 | 2207 | -4.828 |
| | high c | 0.38 | 13172 | 1283 | -2.929 |

Density levels within variables, followed by the same letter are not significantly different (P>0.05).
Regresion lines were compared using an overall test for coincidental regressions.

**Table A7.13.** Statistical parameters for height, total leaf number and total leaf area of trees infested with three densitites of WAA. Block A, Year 2.

| Variable | Density | $R^2$ | Intercept | t | $t^2$ |
|---|---|---|---|---|---|
| height | no aphids a | 0.09 | 468.71 | 3.308 | -0.0115 |
| | low b | 0.08 | 446.68 | 2.165 | -0.0073 |
| | high c | -0.03 | 429.23 | 0.2763 | -0.0010 |
| leaf number | no aphids a | 0.23 | 14.25 | 1.453 | -0.0060 |
| | low b | 0.24 | 3.842 | 0.715 | -0.0025 |
| | high c | 0.00 | 2.380 | 0.096 | -0.0004 |
| leaf area | no aphids a | 0.17 | 4281 | 2168 | -8.660 |
| | low b | 0.21 | 915.9 | 1454 | -5.839 |
| | high c | 0.01 | 971.9 | 115.3 | -0.480 |

Density levels within variables, followed by the same letter are not significantly different (P>0.05).
Regresion lines were compared using an overall test for coincidental regressions.

**Table A7.14.** Statistical parameters for height, total leaf number and total leaf area of trees infested with three densitites of WAA. Block B, Year 2.

| Variable | Density | $R^2$ | Intercept | t | t2 |
|---|---|---|---|---|---|
| height | no aphids a | 0.21 | 437.70 | 4.595 | -0.0153 |
| | low a | 0.30 | 404.86 | 4.852 | -0.0130 |
| | high a | 0.12 | 438.56 | 3.455 | -0.0108 |
| leaf number | no aphids a | 0.33 | 12.85 | 1.693 | -0.0069 |
| | low a | 0.32 | 2.318 | 0.408 | -0.0047 |
| | high a | 0.14 | 4.447 | 1.046 | -0.0036 |
| leaf area | no aphids a | 0.23 | 4374 | 2892 | -11.14 |
| | low a | 0.30 | -1481 | 3261 | -10.83 |
| | high a | 0.17 | -6491 | 2048 | -5.837 |

Density levels within variables, followed by the same letter are not significantly different (P>0.05).
Regresion lines were compared using an overall test for coincidental regressions.

**Table A7.15.** Statistical parameters for height, total leaf number and total leaf area of trees infested with three densitites of WAA. Block C, Year 2.

| Variable | Density | $R^2$ | Intercept | t | t2 |
|---|---|---|---|---|---|
| height | no aphids a | 0.93 | 436.80 | 12.20 | -0.0308 |
| | low b | 0.81 | 412.23 | 10.75 | -0.0258 |
| | high c | 0.04 | 413.42 | 2.420 | -0.0051 |
| leaf number | no aphids a | 0.67 | -21.23 | 5.283 | -0.0151 |
| | low b | 0.65 | -27.79 | 5.167 | -0.0140 |
| | high c | 0.18 | -8.095 | 0.903 | -0.0018 |
| leaf area | no aphids a | 0.59 | -68976 | 9744 | -13.15 |
| | low d | 0.65 | -124878 | 12087 | -31.20 |
| | high c | 0.15 | -18164 | 1429 | -1.491 |

Density levels within variables, followed by the same letter are not significantly different (P>0.05).
Regresion lines were compared using an overall test for coincidental regressions.

**Table A7.16.** P values of the F statistic comparing the slopes of the regression lines for side shoot numbers of trees infested with different densities of woolly apple aphid in three blocks, Year 2.

| | P value | | |
|---|---|---|---|
| Block | All | Control *versus* combined treatments | Low *versus* high |
| A | - | - | - |
| B | P>0.05 | P>0.05 | P>0.05 |
| C | P<0.001 | P>0.05 | P<0.001 |

**Table A7.17.** P values of the F statistic comparing the slopes of the regression lines for side shoot leaf number of trees infested with different densities of woolly apple aphid in three blocks, Year 2.

| | P value | | |
|---|---|---|---|
| Block | All | Control *versus* combined treatments | Low *versus* high |
| A | - | - | - |
| B | P>0.05 | P>0.05 | P>0.05 |
| C | P<0.001 | P<0.005 | P<0.001 |

**Table A7.18.** P values of the F statistic comparing the slopes of the regression lines for side shoot leaf area of trees infested with different densities of woolly apple aphid in three blocks, Year 2.

| | P value | | |
|---|---|---|---|
| Block | All | Control *versus* combined treatments | Low *versus* high |
| A | - | - | - |
| B | P>0.05 | P>0.05 | P>0.05 |
| C | P<0.001 | P<0.002 | P<0.001 |

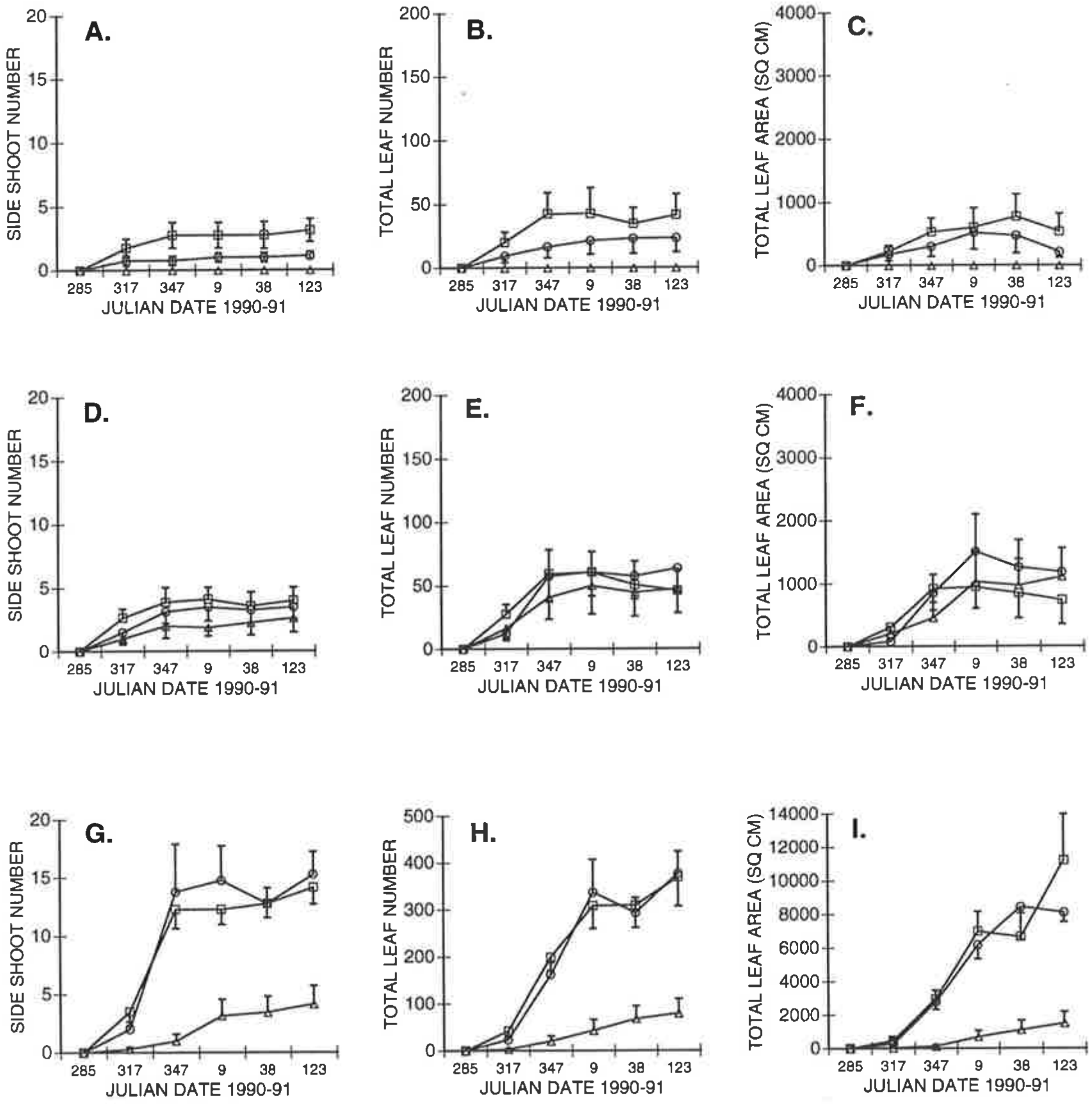

**Figure A7.1.** Relationships between tree parameters (mean ± standard error) and woolly apple aphid infestation during Year 2 (1990-1991). (A) side shoot number, (B) total leaf number, (C) total leaf area in Block A. (D) side shoot number, (E) total leaf number, (F) total leaf area in Block B. (G) side shoot number, (H) total leaf number, (I) total leaf area in Block C.

No aphids □ Low infestation ○ High infestation △

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Errata and Addenda to Woolly Apple Aphid: Interactions Within an Orchard System
Frances FitzGibbon

## Chapter Two

Page 6, Section 2.1, line 7 -
replace 'growers' with 'respondents'

Page 8, Section 2.3, second paragraph -
add - 'A total of 63 growers were chosen from the list provided by the Apple and Pear Growers' Association. This comprised 75 per cent of the commercial orchards in the region.'

Page 15, Section 2.4, Figure 2.2B -
replace 'AMP' with 'APM'
Figure 2.2 legend, first line -
replace 'od' with 'of'; add 'APM = azinphos-methyl'

Page 16, Figure 2.3 legend -
add 'APM = azinphos-methyl'

## Chapter Three

Page 27, Section 3.3.1, line 3 -
replace '20' with '15' and '40' with '30'
line 11 -
replace 'replicated' with 'repeated'
at end of paragraph; add 'Developmental rates, lower threshold temperatures and thermal constants were determined in the same manner as set out in Section 3.3.3.'

Page 27, Section 3.3.2.1, line 15 -
add 'Differences between the total developmental times of males and females were compared by ANOVA (SAS 1993). If there was no significant difference between the sexes the data were pooled in subsequent analyses.'

Page 27, Section 3.3.2.1, line 28 -
replace 'replicated' with 'repeated'

Page 28, Section 3.3.2.3, line 12 -
after 'dilute honey' - add '(4:1, honey :water)'

Page 30, Section 3.4, Table 3.1 -
replace legend with 'The duration (mean ± standard error) in days of the life stages (nymphal instars) of WAA reared on small apple seedlings at three constant temperatures. The values for Assante *et al.* (1991) are presented in square brackets. N is the number of nymphs in that stage.

Page 31, Section 3.4.2, second paragraph, line 2 -
replace '1M:1.8F' with '1M:5.8F'

Page 30, Section 3.4.1, Figure 3.1 legend -
add '(N=53)'

Page 32, Section 3.4.2, Table 3.3 legend -
replace 'three' with 'four'

Page 34, Section 3.5, second paragraph, line 3 -
replace '1M:.8F' with '1M:1.8F'

## Chapter Four

Page 40, Section 4.3.1, second paragraph, lines 1 and 2 -
remove lines and replace with 'Trees were randomly placed in groups of five and assigned to one of 11 sprays; nine pesticides (Table 4.1), water plus a wetting agent, and a water control.'

Line 3 -
after '...4.1)'. add ' Fenoxycarb was not used because it is an insect growth regulator used against lepidopterous pests rather than aphids.

Page 42, Section 4.3.2.1, last line -
replace 'replicated' with 'repeated'.

Page 42, Section 4.3.3.2, first paragraph, line 7 -
replace 'replicated' with 'repeated'

second paragraph, line 2 -
add, after '...Table 4.1' ' and a water control.'

Page 43, Section 4.3.2.3, first paragraph, line 4 -
add at end of line '(Figure 4.5).'

Page 48, Section 4.4.2.1, line 1 -
delete 'parasitised'

## Chapter Five

Page 83, Section 5.5.2, line 1 -
replace '108' with 'one hundred and eight,'

## Chapter Six

Page 118, Section 6.3.1, paragraph 3, line 6 -
replace 'four' with 'three'

Page 121, Section 6.3.1, line 3 -
replace 'clorpyrifos' with 'chlorpyrifos'

Page 122, Section 6.3.2, line 2 -
after '...the above experiment' add 'These trees were not used as sample trees in the experiment described in the previous section.'

Page 129, Section 6.4.2, paragraph 2, line 6 -
replace 'Figure 6.7D' with 'Figure 6.5D'
line 7 -
replace 'Figure 6.7B' with 'Figure 6.6B'

**References**

Page 199 -
replace 'Froggart' with 'Froggatt'

Pages 195, 198, 22, 206 and 209 -
replace 'Entomologica Experimentalis et Applicata' with 'Entomologia Experimentalis et Applicata'

Page 196 -
under Bonnemaison, L.
replace 'Annales de la Societe Entomologie de France' with 'Annales de la Societe Entomologique de France'