

**A PLANT HEALTH MANAGEMENT SYSTEM FOR APHIDIDAE ON
LETTUCE UNDER VARIABLE SHADEHOUSE CONDITIONS IN
THE CENTRAL FREE STATE, SOUTH AFRICA**

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DECLARATION OF INDEPENDENT WORK

DECLARATION WITH REGARD TO INDEPENDENT WORK

I, Rudolph Johannes Pretorius, identity number [REDACTED] and student number 9901671, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree MAGISTER TECHNOLOGIAE: AGRICULTURAL MANAGEMENT, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfilment (or partial fulfilment) of the requirements for the attainment of any qualification.

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What makes things baffling is their degree of complexity, not their sheer size; a star is simpler than an insect.

~Martin Rees, 1999 (Scientific American)

SUMMARY

Aphids (Hemiptera: Aphididae) are amongst the most destructive insects in agricultural crop production systems. This reputation stems from their complex life cycles which are mostly linked to a parthenogenetic mode of reproduction, allowing them to reach immense population sizes within a short period of time. They are also notorious as important and efficient vectors of several plant viral diseases. Their short fecund life cycles allow them to be pests on crops with a short growth period, e.g. lettuce (*Lactuca sativa* L.). It is common practice to provide this crop with some degree of protection from environmental extremes on the South African Highveld. Shadehouses are popular in this regard, but aphids are small enough to find their way into these structures, and their presence on lettuce is discouraged due to phytosanitary issues. In addition, the excessive use of insecticides is criticized due to the negative influence on human health, and because aphids can rapidly develop resistance. This necessitates the use of alternative control options in order to suppress aphid numbers. Biological control is popular in this regard and the use of predatory ladybirds (Coleoptera: Coccinellidae) is a popular choice.

This study investigated the aphid and coccinellid species complex encountered under varying shadehouse conditions on cultivated head lettuce in the central Free State Province (South Africa). Their seasonality was also examined, along with variations in their population size throughout a one-year period. Finally, the impact of varying aphid populations on some physical characteristics of head lettuce was examined, and recommendations for aphid control (using naturally occurring coccinellid predators) were made. Two shadehouse structures were evaluated during this study. One was fully covered with shade netting and designed to exclude the pugnacious ant, *Anoplolepis custodiens* (Hymenoptera: Formicidae), while the other was partially covered with shade netting (on the roof area) allowing access to the ants. Six cycles of head lettuce were planted and sampled four times during each cycle. These were scheduled to monitor the seedling, vegetative and heading stage of lettuce.

Four important aphid species were recorded on the lettuce, namely *Acyrtosiphon lactucae*, *Nasonovia ribisnigri*, *Myzus persicae* and *Macrosiphum euphorbiae*. Both structures harboured similar aphid and coccinellid species, but their population dynamics differed. *A. lactucae* dominated in the absence of *A. custodiens* in the fully covered structure (whole study), while *N. ribisnigri* dominated in the partially covered structure in the presence of these ants during the warmer months (December – January). *M. euphorbiae* replaced this species as the dominant species in the absence of *A. custodiens* (April – September). *M. persicae* occurred during the winter (May – August) in the fully covered structure. Promising coccinellid predators were *Hippodamia variegata* and *Scymnus* sp. 1, and to a lesser extent, *Exochomus flavipes* and *Cheilomenes lunata*. However, the fully covered structure hampered the entrance of the larger adult coccinellid species, resulting in their lower occurrence. Aphid and coccinellid activity peaked during the summer months (October – January), and the fully covered structure attained the highest aphid infestation levels and coccinellid larval numbers during this time. On the other hand, aphid numbers were higher in the partially covered structure during the cooler months of the year (April – July) and this structure also harboured more adult coccinellids. In most cases, aphid infestation levels did not affect the amount of leaves formed. However, symptomatic damage in terms of head weight reduction did occur under severe infestation levels. Specific environmental conditions within a shadehouse structure concurrently contributed to this reduction, with less favourable conditions accelerating this condition.

Results from this study have shown that even though the type of shadehouse structure does not influence the insect species complex found on lettuce, it does have an influence on detrimental and beneficial insect population dynamics. Aphid species infesting lettuce have been identified, along with coccinellid predators that could potentially be used in their control. Both types of structures had advantages and disadvantages, and therefore, decisions concerning shadehouses should not be focused on which type of structure to use, but rather which type of structure to use during different seasons of the year.

OPSOMMING

Plantluise (Hemiptera: Aphididae) is van die mees vernielsugtigste insekte in landboukundige gewasproduksie-stelsels. Hierdie reputasie spruit voort uit hul komplekse lewensiklusse wat meestal aan 'n partogenetiese mode van reproduksie gekoppel is, wat dit vir hul moontlik maak om binne 'n kort tydperk enorme populasies te bereik. Hulle is ook berug as belangrike en doeltreffende vektore van verskeie plant virale siektes. Hul kort vrugbare lewensiklusse laat hul toe om plaë van gewasse met 'n vinnige groeiseisoen te wees, bv. op blaarslaai (*Lactuca sativa* L.). Dit is algemene praktyk om hierdie gewas tot in 'n mate te beskerm van omgewings-uiterstes op die Suid-Afrikaanse Hoëveld. Skaduhuise is gewild in hierdie opsig, maar plantluise is klein genoeg om toegang tot hierdie strukture te verkry en hul teenwoordigheid op blaarslaai is nie gewens as gevolg van fitosanitêre kwessies. Bykomend tot dit, word die oormaat gebruik van insekdoders baie gekritiseer as gevolg van hul negatiewe impak op menslike gesondheid, en ook omdat plantluise maklik weerstand kan ontwikkel. Dit het die gebruik van alternatiewe beheer opsies genoodsaak om plantluis getalle te beheer. Biologiese beheer is gewild in hierdie opsig en die gebruik van predatoriese lieweheersbesies (Coleoptera: Coccinellidae) is 'n gewilde keuse.

Hierdie studie het die plantluis en lieweheersbesie spesie kompleks wat op blaarslaai aangetref word onder verskillende skaduhuis toestande in die sentrale Vrystaat Provinsie (Suid-Afrika), ondersoek. Hul seasonaliteit was ook vasgestel, tesame met variasies in hul populasie-groottes oor 'n periode van een jaar. Uiteindelik was die impak van wisselende plantluis populasies op sekere fisiese eienskappe van blaarslaai ook ondersoek, en aanbevelings vir plantluis beheer (deur gebruik te maak van natuurlik teenwoordige predatore) is gemaak. Gedurende die studie is twee skaduhuis strukture vergelyk. Een was ten volle toegespan met skadunet en ontwerp om die veglustige mier, *Anoplolepis custodiens* (Hymenoptera: Formicidae) uit te hou, terwyl die ander een gedeeltelik toegespan was met skadunet (op die dak-area) en toeganklik vir die miere was. Ses siklusse blaarslaai was geplant en monsters is vier keer geneem

gedurende elke siklus. Hierdie is geskeduleer om die saailing, vegetatiewe en krop stadium van die blaarslaai te monitor.

Vier belangrike plantluis spesies is waargeneem op die blaarslaai, naamlik *Acyrtosiphon lactucae*, *Nasonovia ribisnigri*, *Myzus persicae* en *Macrosiphum euphorbiae*. Beide strukture het soortgelyke plantluis en lieweheersbesie spesies gehuisves, maar met verskille in hul populasie dinamika. *A. lactucae* het in die afwesigheid van *A. custodiens* in die ten volle toegespande struktuur (hele studie) gedomineer, terwyl *N. ribisnigri* dominant was in die gedeeltelik toegespande struktuur in die teenwoordigheid van hierdie miere gedurende die warmer maande (Desember – Januarie). *M. euphorbiae* het hierdie spesie as dominante spesie vervang in die afwesigheid van *A. custodiens* (April – September). *M. persicae* het voorgekom gedurende die winter (Mei – Augustus) in die ten volle toegespande struktuur. Die belowendste lieweheersbesie predatore was *Hippodamia variegata* en *Scymnus* sp. 1, en tot in 'n mindere mate, *Exochomus flavipes* en *Cheilomenes lunata*. Die ten volle toegespande struktuur het egter die toegang van die groter volwasse lieweheersbesie spesies verhinder, wat tot gevolg gehad het dat hulle minder hier waargeneem is. Plantluis en lieweheersbesie se aktiwiteit het gedurende die somer maande hul piek bereik (Oktober – Januarie), en die ten volle toegespande struktuur het die hoogste plantluis infestasië-vlakke en lieweheersbesie larwale getalle gedurende hierdie tydperk gehad. Aan die ander kant, was plantluis getalle hoër in die gedeeltelik toegespande struktuur gedurende die koeler maande van die jaar (April – Julie) en die struktuur het ook meer volwasse lieweheersbesies gehuisves. In die meeste gevalle, het plantluis infestasië-vlakke nie die hoeveelheid blare wat gevorm is, beïnvloed nie. Daar het wel simptomatiese skade in terme van gewigsverlies van die kropslaaikoppe voorgekom tydens hoë infestasië-vlakke. Spesifieke omgewings-toestande binne 'n skaduhuis struktuur was bydraend tot die vermindering, waar minder gunstige toestande die kondisie versnel het.

Die resultate van die studie het aangetoon dat alhoewel die tipe skaduhuis struktuur nie die insek spesie kompleks wat op die blaarslaai gevind word beïnvloed nie, dit wel 'n invloed het op die nadelige en voordelige insek populasie dinamika. Plantluis spesies wat blaarslaai infesteer is geïdentifiseer, tesame met die lieweheersbesies predatore wat potensiaal toon vir hul beheer. Beide tipe strukture het voor- en nadele getoon, en daarom moet die besluite wat geneem word ten opsigte van die skaduhuis nie gefokus word op die tipe struktuur wat gebruik moet word nie, maar wel op watter tipe struktuur gebruik gaan word gedurende verskillende seisoene van die jaar.

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CHAPTER 1

THEORETICAL FRAMEWORK

1.1 ORIGIN OF CULTIVATED LETTUCE (*Lactuca sativa* L.)

The cultivation of lettuce (*Lactuca sativa* L.) may have originated in the Middle East, based on the fact that wild lettuce species are to be found in the area (Ryder, 2002). Here it most probably originated from *Lactuca serriola* L. (De Vries, 1997). However, the first recorded history on the cultivation of lettuce has its origin in ancient Egypt (2500 BC) where paintings of this crop are to be found on tomb walls (Harlan, 1986; Keimer, 1986), depicting thick stemmed lettuces with narrow, long, pointed leaves (Ryder, 1999). Ancient Greece and Rome also cultivated lettuce (where it existed in a variety of forms) from where it was introduced into Northern and Western Europe. Here the cultivation of well-known types (Latin, Butterhead and Crisphead) subsequently took place. Lettuce was distributed from these areas to the New World (most probably with Columbus in 1494), and it also found its way into China where it was first noted in the 5th century (Ryder, 2002).

1.2 CLASSIFICATION AND COMMON USES OF LETTUCE

1.2.1 Classification

Lettuce is a well-known and popular leaf vegetable of which both the leaves and stalks are consumed and the seeds are used in the production of oil (De Vries, 1997). Since its domestication from a weedy species, lettuce has become the most commonly consumed salad plant, embellishing the diets of millions of people throughout the world. Its popularity is demonstrated by the fact that several forms of this species have been developed to suite different needs and tastes, and it is used in a variety of food dishes. This leafy vegetable is placed in the large plant family, Asteraceae, along with about a hundred other species in the genus (Lindqvist, 1960). *L. sativa* is currently arranged in seven edible varieties namely Butterhead, Cos, Latin, Stalk, Cutting, Crisphead and Oilseed lettuce (Rodenburg, 1960; De Vries, 1997). Each of these groups has its

own unique characteristics, but Crisphead- or head lettuce is the most widely cultivated variety.

1.2.2 Uses and production

Lettuce is one of the few members of the plant family Asteraceae actually cultivated for food. Other well-known edible members of this family include artichoke and endive, and there are even records of people in Japan utilizing Chrysanthemum flowers as a source of food (Ukiya *et al.*, 2002). Though the energy food value of lettuce is low, it contains vitamins A, B and C, while the seeds contain vitamin E (De Vries, 1997; Rubatzky & Yamaguchi, 1997). Along with its low calorie value, lettuce is viewed as being beneficial for human consumption (average intake of 110 grams per week) (Magee *et al.*, 2005), and with the increasing awareness towards a healthy lifestyle, the importance of this crop may increase even further. The extensive production of this crop on a global scale (Figure 1.1) further emphasizes its popularity.

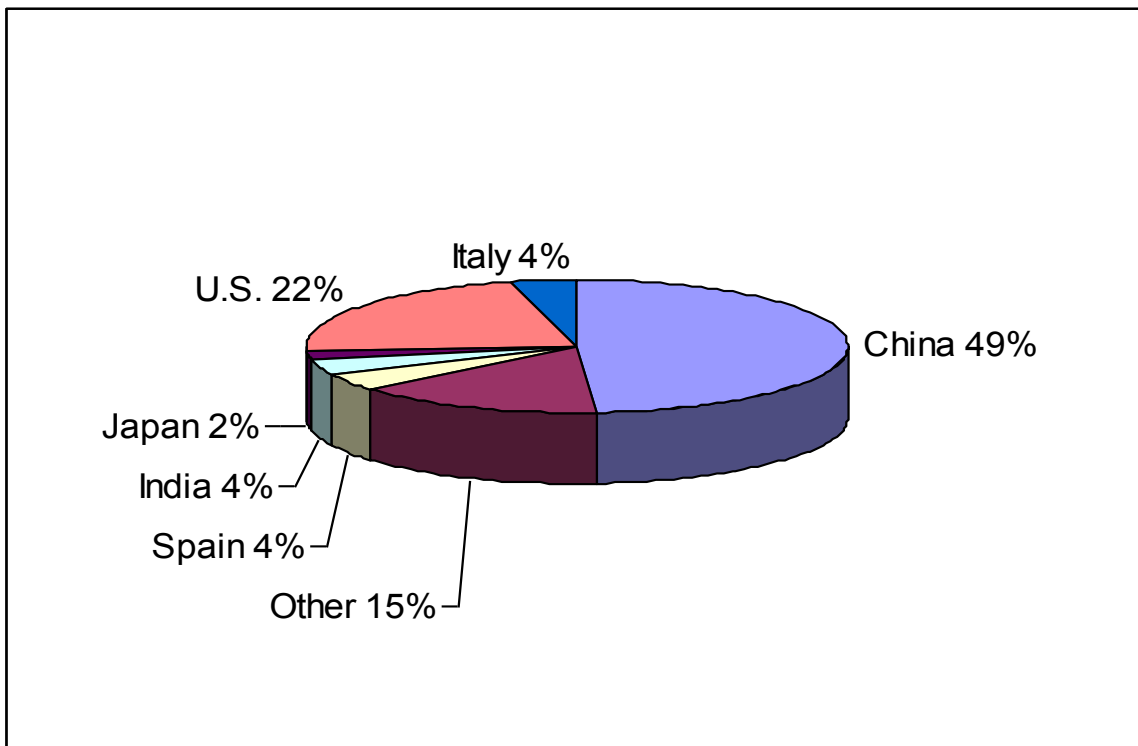


Figure 1.1: Average world lettuce production for 2003-05 (Adapted from FAO, 5/2006).

Similar to developed countries, production of lettuce in South Africa is also extensive, with sales taking place throughout the year on the local markets. When evaluating the figures of the major fresh produce markets, the average sale of lettuce amounts to $2.4 \times 10^3 \text{ t-month}^{-1}$ (average: August-December 2006) (South Africa. National Department of Agriculture, 2007). The highest yields are usually obtained during the April seeding-date, while summer months (November to December) deliver poor yields in warmer parts of the country (Bester *et al.*, 1989a). It is therefore necessary to constantly review lettuce cultivars in order to identify the most suitable ones for a particular production area, and also to use structures which would limit yield loss during these unfavourable periods. To cope with this, lettuce is sometimes produced in shade net structures and plastic tunnels.

1.3 CROP PRODUCTION UNDER PROTECTIVE STRUCTURES

1.3.1 Overview

Worldwide, there is great variation in crop yields from year to year due to factors such as floods, drought, wind and hail damage, insect and disease damage, as well as temperature extremes. To cope with these problems, some producers prefer to make use of plastic tunnels, glasshouses or shade net structures to protect their crop, and to ensure higher yields (Figure 1.2).

1.3.2 Introduction to glasshouses and plastic tunnels

The total world surface area covered with plastic tunnels and glasshouses (hereafter referred to as greenhouses) amount to 300 000 ha, of which 105 000 ha is devoted to the production of ornamentals, and the remaining 195 000 ha is used for vegetable production (Van Lenteren, 2000). In general, a glasshouse can be defined as a sturdy structure, used in areas with temperature extremes to produce crops year-round on a commercial basis. Glass is used as the principal covering material and the costs of erecting and operating such a structure can be high, since climate and humidity control is usually necessary (Stork, 2001). For this reason, glasshouses (in most cases) only lend themselves to the production of high value crops, or for use in scientific studies.

Plastic tunnels on the other hand are cheaper to erect (using a steel frame to which special tunnel plastic is attached), but the operating cost might be just as high, because they also need heating and cooling during periods of temperature extremes (Stork, 2001). These structures are more commonly used in South Africa for the commercial production of certain high value crops i.e. tomatoes, cucumbers, peppers, ornamentals, etc. Many factors will have an influence on the choice of a covering material for greenhouses, including initial investment, local climate, and maintenance costs (Papadopoulos & Hao, 1997).



Figure 1.2: Most important protective structures used in the production of certain crops. Shadehouse structure (top left), plastic tunnel (top right) and glasshouse (bottom).

1.3.3 Advantages of greenhouse crop production

These structures enable growers to supply their produce during so-called 'off seasons' when the specific crop may not be readily available (Hewett, 2006). When operated correctly, they also provide a more favourable growing environment for the crop, resulting in increased production (Cemek *et al.*, 2006), which in turn will enable the producer to provide a crop of higher quality and predictability (Giacomelli & Roberts, 1993). Another advantage of greenhouses is demonstrated by the fact that a large quantity of produce can be grown on a relatively small surface area. This is well demonstrated by agricultural production in the Netherlands. From the 0.5% (about 10 000 ha) surface area covered by glasshouses, about 20% of the total value of agricultural products is realized (Van Lenteren, 2000). Also, greenhouses enable crops to be grown in areas generally not suited for production (e.g. growing lettuce in desert areas as illustrated by Glenn *et al.* [1984]).

The protection provided by plastic tunnels has been shown to limit necessary preparation and marketing loss of lettuce, due to a more attractive and cleaner product as opposed to field-grown lettuce (Rader & Karlsson, 2006). Abak *et al.* (1994) have also shown that the head weight of lettuce can be increased by as much as 66% in tunnels, compared to lettuce grown in the open field. The use of additional light sources will also improve head weight in lettuce grown in active cultivated greenhouses (Ito, 1989). All of these advantages can be linked to the protection of the crop from extreme temperatures, pests, diseases, rain, wind and hail, and the improved artificial climate created (if operated correctly). Various scientific tools and technical equipment that are available in this modern age, has transformed greenhouse operation into a science based activity (Singh *et al.*, 2006), with a wide array of research activities on all aspects of crop production and production techniques, leading to improved crop production.

1.3.4 Problems associated with greenhouse crop production

There is, however, also a wide range of problems associated with greenhouse crop production. The artificial climate created within these structures can be

favourable towards the development of certain diseases as illustrated by Fletcher (1984). Some diseases that are of no particular importance in open field cultivation can even become a serious problem in a greenhouse setup. There is evidence that diseases such as grey mould (caused by *Botrytis cinerea*) prefer conditions of poor ventilation and dampness – just as those found in plastic tunnels and glasshouses (Fletcher, 1984). Certain fungal pathogens (e.g. *Rhizoctonia solani*) also favor development in greenhouses (Wareing *et al.*, 1986). Vegetables and ornamentals grown under greenhouse conditions are, for example, more prone to attack by powdery mildews (Van Lenteren, 2000).

Above described structures are also renowned for the greenhouse effect they create, and subsequent cooling is necessary in order to provide a more favourable growing environment to the crop (Giacomelli & Roberts, 1993) in warmer areas or during periods of high temperatures. Cooling is achieved by using different techniques, ranging from natural ventilation to evaporative cooling systems, shading, roof sprinkling, zone cooling and heat pumps (Sang-Woon & Yong-Cheol, 2002) – all of which contributes to the higher costs of operating greenhouses (active protected cultivation). The risk of active protected cultivation is crop losses when malfunctioning of the system occurs. In some cases, producers do not heat or cool plastic tunnels (passive protected cultivation) with the result that a big temperature fluctuation is obtained, compared to ambient temperatures. This can lead to changes in the tissue composition of some plants, resulting in an unmarketable or inferior product. This is well demonstrated by Gent (2005), who found that nighttime temperatures in unheated tunnels were 1 – 2°C warmer than ambient temperatures, while daytime temperatures were up to 10°C warmer compared to ambient temperatures. Rader & Karlsson (2006) also observed a noticeable difference between soil temperatures inside and outside unventilated plastic tunnels. Soil temperatures were higher inside the tunnel throughout the whole season. In lettuce, such conditions can lead to bolting (rapid stem elongation and leaf twisting), resulting in an unmarketable product (Rader & Karlsson, 2006). The closed environment is also favourable towards the development of high humidity levels, because moisture produced by

the plants during the process of transpiration has no means of escape when these structures are kept closed during adverse weather conditions.

1.4 LETTUCE PRODUCTION IN SHADEHOUSE STRUCTURES

1.4.1 General description of shadehouse structures

Another popular way of providing protection to cultivated crops is the application of shade cover in the form of shade netting. In its simplest form, this method entails the use of shade netting, of which several densities are available from local companies such as Knittex[®] and Alnet[®], attached to a structure usually consisting of wooden poles and supporting wires and cables or to a plastic tunnel steel frame.

1.4.2 Advantages of lettuce production in shadehouse structures

Growing crops under the protection of shade netting in South Africa is increasing in popularity, mainly because it is the cheapest system available (compared to greenhouses), and because it is relatively easier to construct (Stork, 2001). Furthermore, shadehouse structures are preferred above greenhouses, because they allow for better air movement through the structure and thus cooling (especially important for the regions in South Africa that experience high summer temperatures), which in turn eliminates the greenhouse effect to a large extent. The fact that the crop is provided with some shade (dependant on the density of the shade netting), also contributes to the attractiveness of this method of cultivation in the warmer parts of the country. Despite the fact that various methods for cooling greenhouses are also available (Davies, 2005), there is still the issue of high energy costs and the high cost of erecting and maintaining these structures, resulting in the attractiveness of shadehouse structures. In warmer countries, it is known that lettuce can not be cultivated in tunnels during the hot summer months if additional cooling is not provided, because of the high temperatures they attain (Sang-Woon & Yong-Cheol, 2002). Gimenez *et al.* (2002), showed that there is a noteworthy increase in specific leaf area of cabbage, lettuce and spinach crops under direct cover, resulting in a rise of quality in these products. This was brought about by the specific microclimate

created by the protection of the crop which altered the air and soil temperature and creating a more uniform growing environment.

Harris (1992), mentioned that the provision of some shade to lettuce plants produced during warmer months is essential, because it is originally a cool weather crop, with the optimum temperatures for growth ranging from 23°C during the day, to 7°C during the night. Temperature plays an important role in the successful cultivation of lettuce. There is, however, limited information available on the influence that a protective cover (in the form of shading) has on the productivity of lettuce. It must be born in mind that lettuce is also a leafy vegetable with a relatively large leaf surface area, and shading this plant is preferable in warmer regions in order to avoid excessive transpiration. It has been shown that transpiration will decrease linearly with a decrease in irradiance (Anandacoomaraswamy *et al.*, 2000). Other sources (e.g. Watson *et al.*, 2002) proved that the effect of shading (using shade netting) can have a significant effect on the quality of some fruits and this may also hold true for leafy vegetables. This is due to the provision of a more stable environment in which the direct negative influence of environmental factors such as hail, frost, direct sunlight, strong winds, and direct falling rain, are largely eliminated. In support of this, Bester *et al.* (1989b) has found that the higher yields obtained from lettuce cultivated under a 30% shade netting during the cooler months in South Africa, is directly related to the protection from frost.

Environmental conditions can have a dramatic influence on the metabolism of lettuce leaves, resulting in altered leave composition, which in turn will affect the nutritional value of the crop. It was found that these changes were directly related to changes in light intensity and ambient temperature (Gent, 2005). The severity of tipburn occurring on field grown lettuce increases with an increase in irradiation (Wissemeier & Zühlke, 2002), while sunburn is another problem associated with high light intensity which eliminates the possible beneficial effects of direct sun exposure (Woolf & Ferguson, 2000). Furthermore, bolting, bitterness, and poor heading of lettuce are well-known disorders which seem to

increase with an increase in ambient temperatures (Rader & Karlsson, 2006). Temperatures beyond 32°C may result in thermodormancy which can also contribute to crop losses. On the other hand, high levels of irradiation and temperature aren't the only threat to lettuce, as it is known that frost can be a serious problem prior to harvesting of mature lettuce heads, causing the heads to decay and making them susceptible to attack by diseases (Stork *et al.*, 2001). Therefore a shade cover to the crop can only be of benefit in warmer parts of the country experiencing frost during winter months.

1.4.3 Possible problems with lettuce when using shade netting

A possible problem associated with shading of lettuce is the higher nitrate levels which may accumulate in the leaves as indicated by Ysart *et al.* (1999). He found that long hours of sunlight produced a crop with lower nitrate content. Despite the increase in production due to the protection from frost discussed above, Bester *et al.* (1989b) also found that a shade cover did not drastically increase lettuce production in certain cultivars if measured against field-grown lettuce in general.

The level of light transmission to the crop will vary when using different densities of shade netting. This in turn will alter the microclimate in terms of air temperature, humidity and leaf temperature, as was found by Papadopoulos & Hao (1997). This makes it difficult (and sometimes even impossible) to create the same environmental conditions within a shadehouse. This is also true for shadehouse structures situated at different localities. Factors which will inevitably determine the microclimate in shadehouse structures is the latitude, area covered by the structure, orientation of the structure, canopy area within the structure, bare soil surface area, structural design, season, etc. (Singh *et al.*, 2006). To further complicate the matter, some producers who use shadehouse structures, tend to only partially cover the structure with shade netting. This is attained by leaving one or more sides uncovered for reasons including improved air circulation, ease of production, ease of movement for labourers, etc. On the other hand, some producers tend to fully cover the shadehouse structure on all

sides in order to create more uniform growing conditions and limit the free movement of pests.

1.5 PEST PROBLEMS ARISING FROM THE USE OF GREENHOUSES AND SHADEHOUSE STRUCTURES

It is known that greenhouses in cooler regions can have detrimental pest outbreaks year round as a result of the continuous production of crops made possible by artificial climate control, which in turn improves the survival and developmental rate of certain insect pests. Greenhouses in warmer regions are also faced with insect pest problems, because these structures are opened to encourage air movement during the hottest time of the day, while labourer movement in and out of the structure also occurs. This allows insects to enter the structure unhindered and establish themselves on the monocrop environment (Van Lenteren, 2000), where they can multiply under the more favourable conditions.

Although it is clear that insect pests can have access to greenhouses, it is controllable, because the covering material is a solid material and the only access is through ventilating flaps, doors, damaged areas, etc. However, shadehouse structures are covered with a woven product that does not restrict the movement of smaller insect pest species. If the shadehouse structure is only partially covered, even bigger insect pest species (e.g. many Lepidoptera species) have free access to the crop within the structure. Although some studies focusing on the use of insect-proof screens have been conducted to test their efficiency in restricting access of insect pests (Martin *et al.*, 2006), little seems to be known about the effect that shade netting will have on the movement of insect pests. The shade netting may act as a barrier to bigger insects, not only to harmful species, but also to beneficial species. This information can prove valuable to producers of lettuce which cultivate their crop in shadehouse structures, as lettuce is prone to attack to a wide array of insect pests. The decision over whether or not to use shade netting as a physical barrier to insect

pests, therefore requires some degree of knowledge of the insect pests that can be expected on lettuce.

1.6 COMMON PESTS OF LETTUCE

A study conducted by Peacock & Norton (1990) in the U.K., found that more than ten percent loss of organically grown vegetables can be attributed directly to insect pests. These results were, however, for organically cultivated produce and it has been found by Young *et al.* (2005) that vegetables cultivated in this manner will show increased attack by insect pests. Although the majority of lettuce produced for commercial purposes may not be grown organically, it can be expected that phytophagous insect pests will exert a big influence on production capabilities. Insect pests which regularly cause problems to cultivated lettuce fall into the following main orders: Orthoptera, Hemiptera, Diptera, Coleoptera, and Lepidoptera. Brief accounts on the most problematic organisms (family and/or species) within each of these orders are provided separately.

1.6.1 Orthoptera

This order of insects is well-known due to the damage that certain types of locusts and grasshoppers cause to cultivated crops, especially when they form large swarms which migrate over several thousands of kilometers (e.g. the family Acrididae). The damage caused can be related to the polyphagous feeding habits of these insects, which will devour most plants that prove edible. Lettuce certainly falls into this category, and it has even been reported that some grasshoppers require a water-soluble substance, found in romaine lettuce, for completion of larval growth (Kreasky, 2003). Another strong argument in support of this statement is illustrated by the regular use of lettuce for rearing grasshoppers and locusts for experimental purposes (e.g. as performed by Barbara & Capinera [2003], Sword [2003], and Capinera & Froeba [2007], to name but a few). It can be presumed that lettuce seedlings will be most vulnerable to attack, which could lead to significant losses should these insects occur in large numbers. Damage during the seedling stage will be detrimental as the growing point might be injured, or total destruction of the plant may occur.

Other families in the order that will feed on lettuce include Tettigoniidae (Sword, 2005) and Pyrgomorphidae (Figure 1.3). In general, however, it can be presumed that the damage these insects cause will be of insignificant value (except when the plant is totally devoured or the growth point is damaged in the seedling stage).



Figure 1.3: Pyrgomorphidae grasshopper feeding on lettuce seedling (Roodevallei, Free State Province).

Crickets in the genus *Gryllus* can also occasionally damage lettuce seedlings. These insects tend to feed at night and hide in soil cracks, ditches and even weeds (stressing the importance of good sanitation practices) during the day, making detection difficult. These insects can, however, be controlled by using baits (University of California, 1992), and in general they are considered a minor pest of cultivated lettuce.

1.6.2 Diptera

The larvae of leafminer flies are well-known for the characteristic tunnels they create in leaves when feeding between the upper and lower epidermal layers of the leaf. The consequence is a reduction in the photosynthetic capabilities of the plant, unattractive and unmarketable heads, and provision of entry to pathogenic organisms into the plant (University of California, 1992). Some well-known species that are of economic importance in lettuce production include *Liriomyza huidobrensis*, *L. trifolii*, *L. langei* and *L. sativae* (Diptera: Agromyzidae).

The species *L. huidobrensis* (commonly known as the pea leafminer) attacks a wide variety of crops, both under open field cultivation, and in greenhouse production (Ode & Heinz, 2002). They seem to be common in large lettuce producing areas, e.g. in California (U.S.A.) and Italy (Jackson *et al.*, 2004; Masetti *et al.*, 2006). A wide range of control options against these leafminers have been reviewed (Civelek & Yoldaş, 2003; Liu, 2003; Weintraub & Horowitz, 1997).

1.6.3 Coleoptera

When one consults any entomology textbook (e.g. Triplehorn & Johnson [2005]), it immediately becomes evident that beetles as a group include a wide array of phytophagous species of which many are polyphagous. Because of this, only mention will be made of the most important groups that commonly cause damage to lettuce stands.

When in the seedling stage, lettuce is vulnerable to attack by flea beetles (Coleoptera: Chrysomelidae) which tend to cause small excavations or pits on the underside of the leaves and even stunting or death of the seedling. In older plants, these insects tend to feed on the older wrapper (outer) leaves, but do not cause economic damage (University of California, 1992). The feeding scars left by these insects, may result in undesirable alterations to the crops' appearance (Kays, 1999).

Tenebrionidae species cause damage to cultivated lettuce in both the larval- and adult stages. The adults will chew the seedlings or feed on the foliage, while the larvae feed on the seedlings (University of California, 1992). The larvae of click beetles (Coleoptera: Elateridae) can also damage lettuce seedlings. Some species bore into the stem of the plant while others tend to feed on the roots (Watt, 1986; University of California, 1992).

1.6.4 Lepidoptera

A wide array of lepidopteran pests seems to favour lettuce as a host plant, which, besides the Hemiptera, can be considered the most important insect pests of cultivated lettuce (University of California, 1992). A family renowned for the damage they cause is the Noctuidae (University of California, 1992). Some species in the family that can cause serious problems to lettuce include *Trichoplusia ni*, *Spodoptera exigua*, *Heliothis zea*, *Helicoverpa armigera* and *Helicoverpa punctigera*, respectively known as cabbage loopers, beet army worms, and bollworms. The damage they cause is due to their habit of boring into mature heads and primarily contaminating the crop with their frass, rendering these products unsuitable for marketing.

High population densities of these insects can also damage seedling stands, resulting in slow growth and inhibition of uniform maturing (University of California, 1992). *S. exigua* and *H. zea* larvae feed on the crown and may sometimes kill the growing point of seedlings (University of California, 1992). Plants between the seedling and heading stage can, however, tolerate some damage without loss of yield or quality from these insect pests (University of California, 1992). It has been found by Sethi *et al.* (1999), that *T. ni* and *S. exigua* may show different preferences in terms of feeding sites on the plant, and for this reason it may be that the combined impact of these two species occurring together can be detrimental. Numerous studies have been the focus of potential control agents (other than chemical control) against these pests, demonstrating the importance of this family in commercial lettuce production. These studies include susceptibility of larval instars to *Bacillus thuringiensis* (Bell &

Creighton, 1968; Herbert & Harper, 1985), effect of viral infections (Lowe & Paschke, 1968; Ali *et al.*, 2002) and the influence of noxious plant chemicals (e.g. lettuce latex) on the feeding behavior of larvae (Dussourd, 2003).

Another well-known lepidopteran pest of cultivated lettuce is cutworms (also Noctuidae). Cutworms include several species that damage the seedlings by clipping the stem just below or near the soil surface (University of California, 1992). The damage they cause can be extensive, because these insects can destroy several seedlings during just one night, removing most of the stand (Figure 1.4).



Figure 1.4: Cutworm damage (open spaces in rows) to lettuce stand (Roodevallei, Free State Province).

Cutworms also tend to feed on the leaves after emergence and this will have a negative influence on the appearance of the product (Kays, 1999), and it will also limit the production capabilities of the crop. Cutworms also occasionally bore into

the head of lettuce plants where they cause damage similar to that described for cabbage loopers and beet army worms (University of California, 1992).

Other known lepidopteran pests include members from the family Tortricidae, of which the species *Clepsis spectrana* has been found feeding on lettuce (Alford, 1976). The tobacco budworm, *Heliothis virescens* (Lepidoptera: Noctuidae), is a well-known pest of cotton in the USA and may infest the border areas of lettuce fields planted near cotton (University of California, 1992). Its control by means of genetic modified crops has been stressed by Kirsten (1999). Numerous other species of *Spodoptera* have also been found to feed on lettuce in the United States (e.g. *S. ornithogalli*, *S. praefica* and *S. frugiperda*). The imported cabbageworm (*Pieris rapae*) occasionally feed on lettuce seedlings in California (USA), but economic damage is uncommon (University of California, 1992).

1.6.5 Hemiptera

The order is characterized by insects possessing piercing-sucking mouthparts that may cause damage to lettuce by either reducing the production capabilities of the crop, or by transmitting certain diseases. The family Aleyrodidae (whitefly) is renowned for their high reproduction capabilities, especially in greenhouses, where they can transmit several diseases, and is considered a major agricultural pest, causing considerable crop loss (Bellotti & Arias, 2001). A common pest species, the greenhouse whitefly (*Trialeurodes vaporariorum*), is one of the major pests associated with greenhouse production and trials have been conducted for the biological control of this species (Feng *et al.*, 2004) in order to reduce the use of harmful chemicals in its control. Another well-known species is *Bemisia tabaci*, known to transmit Lettuce infectious yellows virus which can devastate lettuce crops (University of California, 1992). Studies have been conducted to determine the parameters necessary for transmission of this virus (Ng *et al.*, 2004), but it seems as if the disease is still considered to be important in lettuce production.

The family Miridae also includes several species that can damage lettuce stands. Chemical control of these insects is complicated by their feeding habits (between

the leaves of head lettuce) and various alternative control measures have proved to be successful, e.g. the use of trap crops (Rämert *et al.*, 2001). The hemipteran insect pest known to cause the most damage to cultivated lettuce through their feeding habits, is undoubtedly aphids (Aphididae) (Tatchell, 2007). Lettuce apart, aphids can be regarded as the most serious pests of agricultural crops in temperate climatic zones of the world (Minks & Harrewijn, 1987).

1.7 APHIDS

1.7.1 Aphid origin, classification, and general characteristics

To better understand the role that aphids fulfill as pests in modern agriculture, it is necessary to consider their evolutionary history and the main forces responsible for their evolution. The evolutionary history of aphids from its primitive ancestors dates back 280 million years to the Carboniferous period (Heie, 1967). It is accepted that the first true aphidoids first appeared in the Early to Late Jurassic period of Eurasia some 170-150 million years ago (Grimaldi & Engel, 2005), but the fore wing of one species (*Triassoaphis cubitus*) is known from the Triassic period (Heie, 1987a). In addition, a later discovery yielded a still older, and very primitive, aphid from the Triassic period (Shcherbakov & Wegierek, 1991). The Cretaceous period yielded more fossils than the Triassic period, and some of the recent aphid families also came into existence during this period (Heie, 1987a). This turnover in the aphid fauna could have been the result of the transition of the era of gymnosperms to the era of angiosperms (Heie, 1987a; Shaposhnikov, 1987). The Tertiary period yielded still more fossil aphids than the Cretaceous period. Aphids from the Tertiary period display a higher degree of similarity with the modern aphid fauna (with exceptions notable during the Eocene), compared to the aphid fauna of the Cretaceous period, with some genera still in existence in the present (Heie, 1987a; Heie, 2001).

Currently there are approximately 4 700 known species of aphids in the world (Remaudière & Remaudière, 1997). Aphids are soft-bodied insects of the order Hemiptera (traditionally known as the Homoptera) and are placed in the sub-order Sternorrhyncha (and within the superfamily Aphidoidea) along with the

scale insects (superfamily Coccoidea), whiteflies (superfamily Aleyrodoidea) and psyllids (superfamily Psylloidea) (Blackman & Eastop, 2000). The superfamily Aphidoidea includes the families Adelgidae (conifer woolly aphids), Phylloxeridae (phylloxera) and Aphididae (aphids) (Nieto Nafria & Mier Durante, 1998). A general characteristic of the Sternorrhyncha is the mouthparts or rostrum (used for sap-sucking) which has its base between the fore-coxae (Gullan & Cranston, 2000) and is deflected back along the ventral surface of the body when at rest or not in use (opisthorhynchous mouthparts). Other characteristics of Sternorrhyncha include relatively well-developed antennae and tarsi that are 1- or 2-segmented (Blackman & Eastop, 2000). Aphids use their rostrums as piercing-sucking organs to feed on their primary diet which is the phloem sap of plants (Gullan & Cranston, 2000). Aphids in particular can be readily recognized on the basis of the following characteristics: presence of siphunculi, five- or six-segmented antennae, two-segmented tarsi with a shorter first segment, and a cauda or tail (depicted in Appendix 7). Some of these features might have been modified, secondarily lost, or reduced in certain species (Blackman & Eastop, 2007).

1.7.2 Aphid life cycles

Aphids exhibit complex variations in their life cycles – a feature that sets them apart from other Hemiptera (Blackman & Eastop, 2007). Aphids usually have four instars and the time needed to reach adulthood is dependant on certain factors, such as host plant quality, temperature, weight at birth, and whether or not it is a winged (alate) or wingless (apterous) individual (Dixon, 1987b). Only the most important life cycle characteristics will be dealt with here.

Most known aphid species are capable of both sexual and asexual reproduction with a period between the sexual reproductions in which several generations multiply by means of asexual reproduction (parthenogenesis) (Dixon, 1987a). This mode of reproduction is commonly referred to as cyclical parthenogenesis (Blackman & Eastop, 2000) and aphids which interrupt their parthenogenetic reproduction to perform sexual reproduction in this way are referred to as being

holocyclic (See Figures 1.5, 1.6 & 1.7 for representations of these types of life cycles).

Under certain favourable conditions the sexual phase of some aphid species has been completely lost (known as anholocycly) and reproduction takes place through continuous parthenogenesis throughout the year (Blackman & Eastop, 2000). Only a small fraction of all known aphid species appears to be wholly dependant on parthenogenetic reproduction (anholocyclic), but none of the species has secondarily lost this mode of reproduction (Blackman & Eastop, 2000). Anholocyclic species are usually found where the geographical occupation of an aphid extent beyond that of its primary hosts (Williams & Dixon, 2007). Greenhouse conditions can also result in anholocyclic life cycles in aphid species which might usually be holocyclic under normal circumstances. The parthenogenetic mode of reproduction results in rapid multiplication (Dixon, 1987b) and enables aphids to exploit short-lived resources (Stadler & Dixon, 2005). Parthenogenesis in aphids is not that different from sexual reproduction, the only difference being the absence of meiosis (Dixon, 1987a). In all aphids, parthenogenetic reproduction is combined with viviparity. Here the development of a nymph starts when its mother is still an embryo and this means that the embryo inside an adult parthenogenetic female, carry embryos themselves. The result of such 'telescoping' is a shortening of generation time, overlapping generations, increased reproductive potential, and an increased rate of development of resistance against pesticides (Blackman & Eastop, 2000).

The success of aphids to survive and reproduce relies on their ability to disperse to other hosts. Aphids have overcome this problem through an ingenious method. Two female morphs are responsible, one wingless and highly fecund, while the other is winged, much more active and less fecund. A young colony will consist out of the wingless morphs which feed intensively in order to produce as much young as possible. With the growth of the colony, winged forms will appear which will migrate to new hosts where they will initiate new colonies. The fecundity of the wingless morphs will ensure that enough winged forms are

produced to permit survival of some individuals, even if mortality proves to be high in the alates (Blackman & Eastop, 2000). This ability to disperse has enabled aphids to rapidly exploit new habitats in which they can reproduce and feed (Robert, 1987).

Furthermore, some aphid species migrate between a primary host (used for sexual reproduction) and a secondary host (used for parthenogenetic reproduction), a feature known as host alteration or heteroecy (Blackman & Eastop, 2000). This phenomenon is made possible by the division of function between the wingless and winged morphs as discussed in the preceding paragraph. Host alternating aphid species are described as being heteroecious, while those that don't alternate between a primary and secondary hosts, are known as monoecious or autoecious (Williams & Dixon, 2007). In holocyclic host alternating aphid species (heteroecious species), eggs produced on the primary host by a sexual generation during the previous season will hatch into a highly fecund, parthenogenetic, wingless female (winged in some rare cases), known as the Fundatrix (or stem mother) (Costa, 2006) which is morphologically different from the later parthenogenetic females (Blackman & Eastop, 2000). Asexual reproduction on the primary host then takes place due to obligate parthenogenesis (Wilson *et al.*, 2003) with fundatrigenia being produced. Asexual reproduction then involves the fundatrix and the fundatrigenia to produce spring migrants. These winged individuals will disperse from the primary host to the secondary host where they will start their own colonies through parthenogenetic reproduction. A return migration to the primary from the secondary host usually takes place towards the end of the season (by aphids known as immigrants). These returning immigrant alates are usually morphologically different from the alates which initially dispersed from the primary to the secondary host. In the case of the Aphidinae, the returning migrants will be males and gynoparae females. Egg-laying, mating females (also known as oviparae) will then be produced by the gynoparae females. Mating between the oviparae females and males takes place, and eggs are laid which overwinters (from which a fundatrix will eventually emerge again) (see Figure 1.5 for representation of this life cycle).

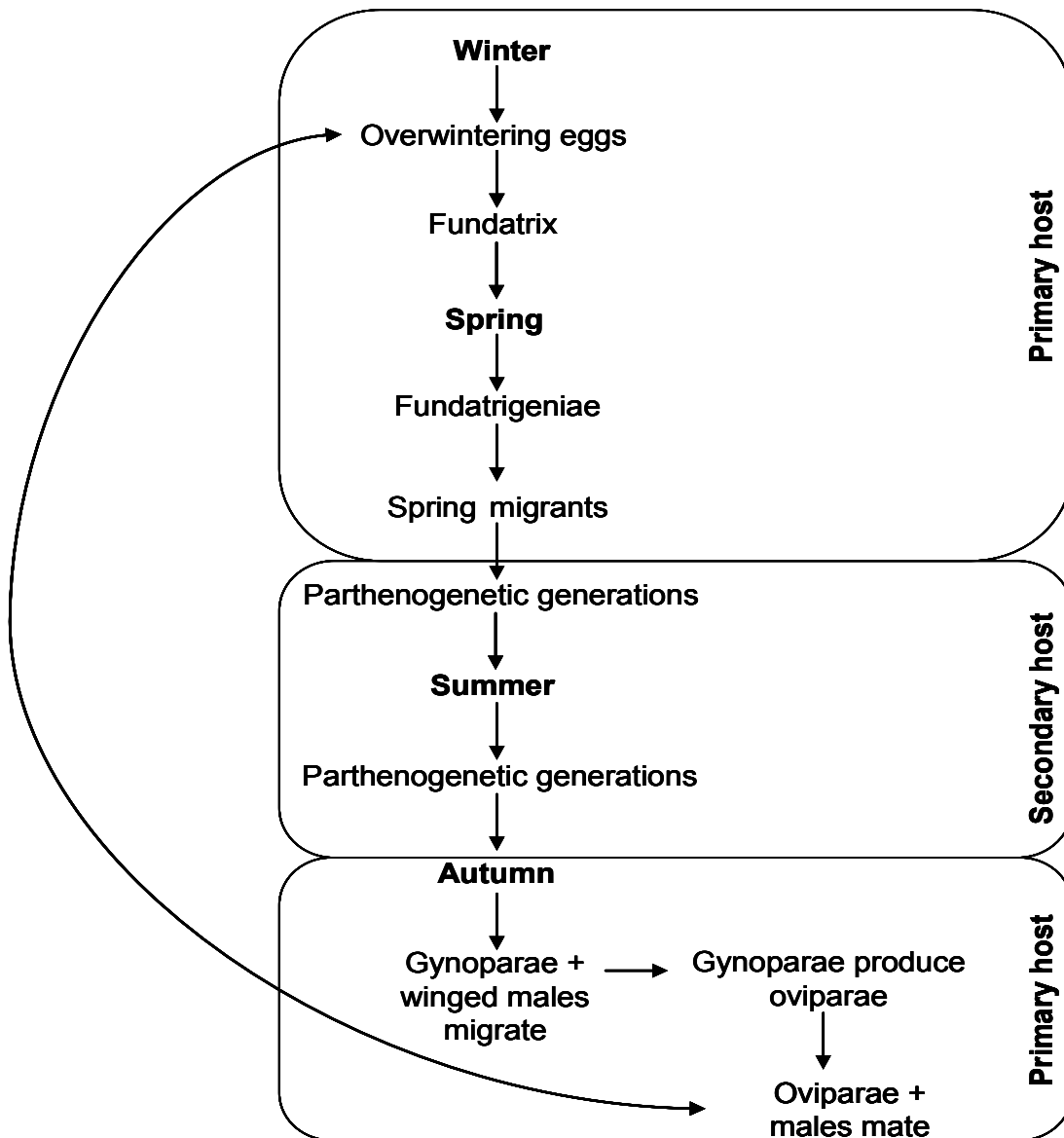


Figure 1.5: Generalized representation of a holocyclic heteroecious aphid life cycle.

In certain cases (as with the Anoeciinae, Eriosomatinae, Hormaphidinae) only females return to the primary hosts (also known as sexuparae) where wingless sexual morphs of both sexes are produced which will mate to lay eggs (Figure 1.6). The production of the sexual generation is reported to be dependant on the temperature, daylength and host plant nutrition (Kawada, 1987).

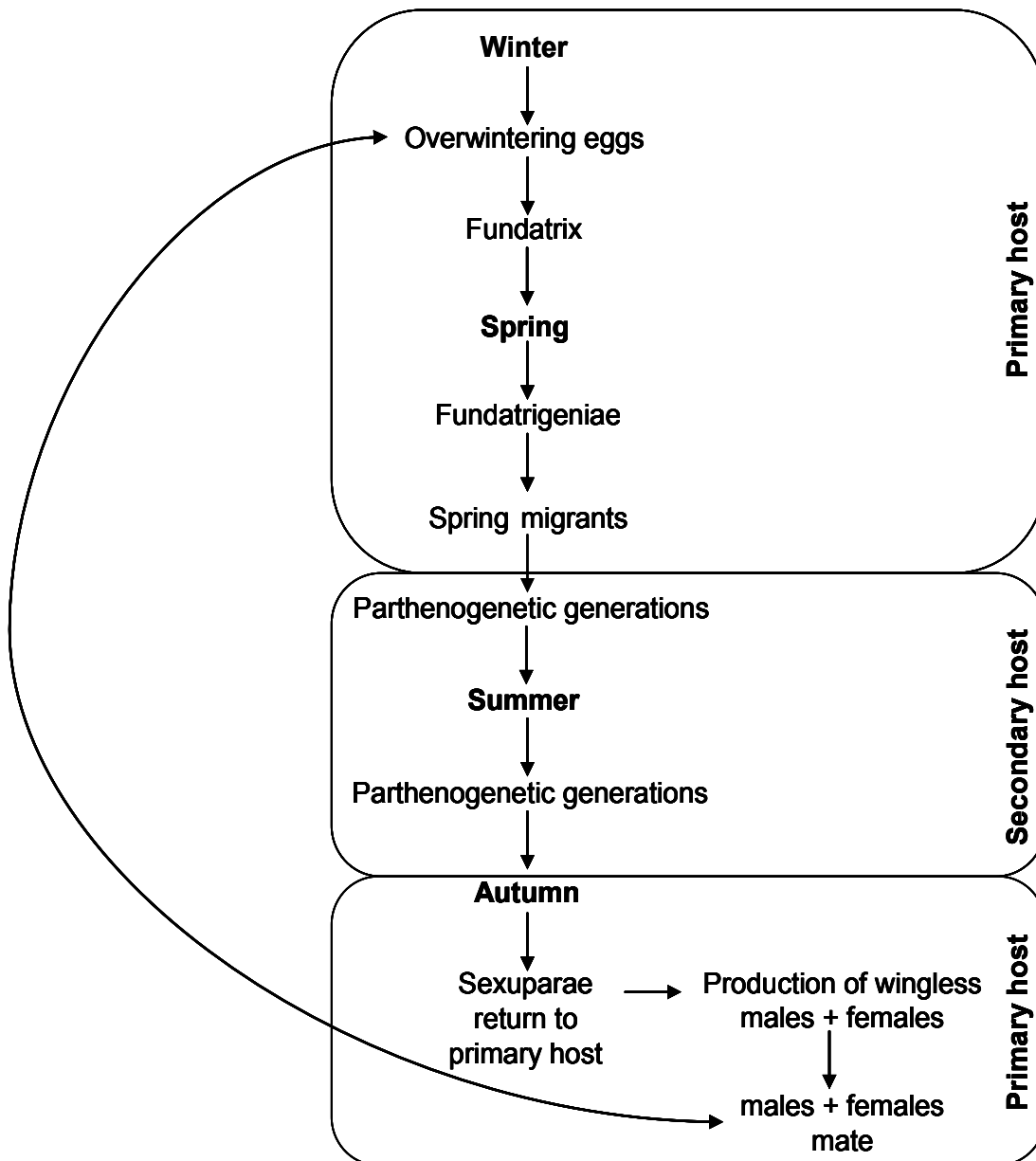


Figure 1.6: Generalized representation of an alternate holocyclic heteroecious aphid life cycle present in certain aphid species in the subfamilies Anoeciinae, Eriosomatinae and Hormaphidinae.

Host alteration appears to have fulfilled a major role in the exploitation of herbaceous plants by aphids (Blackman & Eastop, 2000). As mentioned above, some species are monoecious (or autoecious). In these species the occurrence of host alteration (heteroecy) has been lost altogether and these aphids complete

their entire life cycle on herbaceous plants. These aphids can also be either holocyclic or anholocyclic (Williams & Dixon, 2007) (see Figure 1.7 for a representation of a holocyclic monoecious life cycle present among certain aphid species). In the monoecious holocyclic species, the alatae are rather uniform in appearance, while some differences are present in the different generations of the apterous forms (Miyazaki, 1987). The fundatrices of monoecious species are also more similar to the other morphs. The males of monoecious species may be either winged or wingless, primarily because they do not need to disperse to new hosts (Miyazaki, 1987).

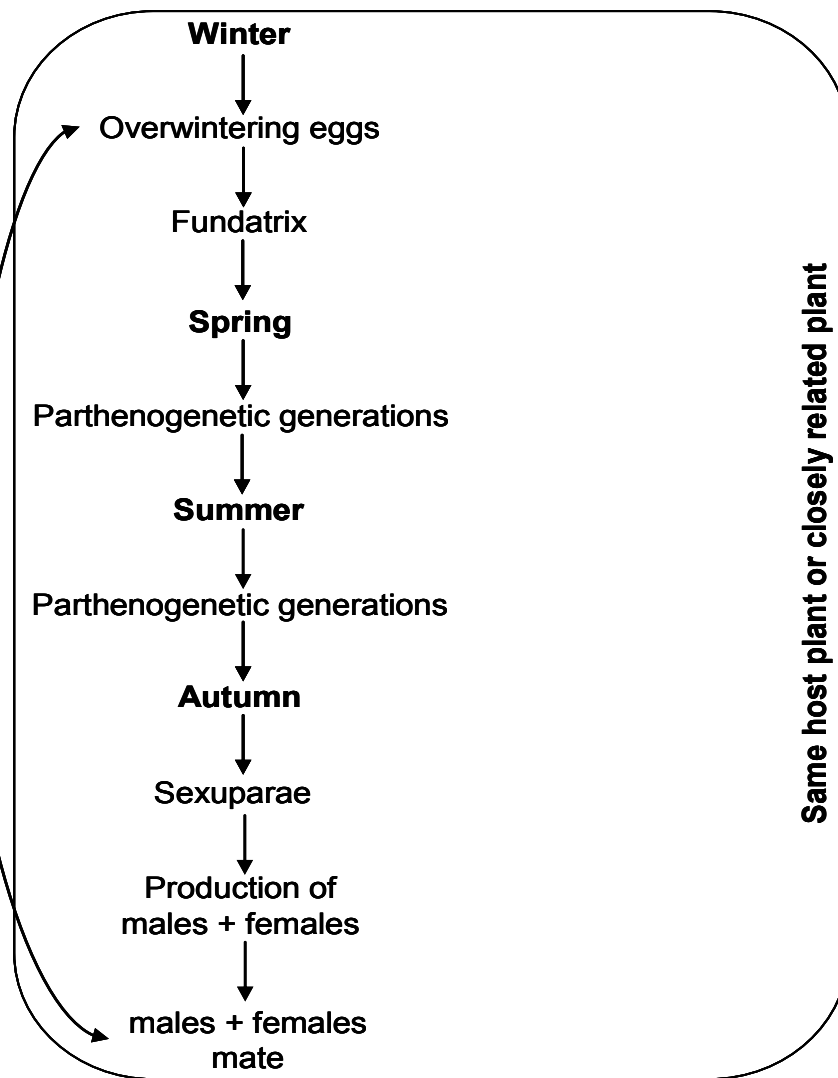


Figure 1.7: Generalized representation of a holocyclic monoecious aphid life cycle.

1.7.3 Host preference and feeding behaviour

Many of the Cretaceous aphids had proboscises which were up to twice the length of their bodies, a characteristic believed to be related to feeding on host plants with thick, rough bark (similar to those found on conifers) (Grimaldi & Engel, 2005). It is known that the origin of the Aphidoidea took place prior to that of the angiosperms and a now extinct group of gymnosperms might have acted as the original hosts for these insects (in temperate or subtropical climates [Shaposhnikov, 1987]), from which they eventually migrated to Coniferae and related families of woody angiosperms (Blackman & Eastop, 2000). In support of this view, it is known that most of the older aphid groups within the Aphididae have primitive associations with certain families of woody angiosperms. If the host record of any genera of aphid is studied, it soon becomes evident that most aphid species and genera prefer particular plant families as hosts (Blackman & Eastop, 2000). It is true that the primary- and secondary hosts of aphids are, in most cases, rather distinct, but they retain their specificity (Blackman & Eastop, 2000). The primary hosts of heteroecious aphids are dicotyledons and the secondary hosts are dicotyledons or monocotyledons (Shaposhnikov, 1987). Aphids in general appear to be monophagous, although certain exceptions to the rule do exist (e.g. *Aphis fabae* and *Myzus persicae*) (Dixon, 1987c).

Feeding on the phloem sap (principle food of aphids) of host plants is made possible by a specialized rostrum (Figure 1.8), which is a long segmented organ specially adapted for sucking (Heie, 1987b). The segmented area of this organ is the labium which acts as a sheath around the piercing stylets which consists of two mandibles and two maxillae (Triplehorn & Johnson, 2005). This forms a stylet-bundle that contains the food canal (used for food uptake) and a salivary canal (used for secretion of saliva) lying in a groove in the labium (Gullan & Cranston, 2000). The mandibular stylets enclose the maxillary pair which is tightly interlocked by a series of ridges and grooves to prevent leakage of fluids whilst feeding takes place. Each of the mandibles contains a central duct with a pair of dendrites that most probably serve as proprioceptors to monitor stylet movement and positioning on the host tissue (Powell *et al.*, 1999). There might

be some differences in the morphology of the stylet tips among the different stages of development and morphs of aphids (De Zoeten, 1968).



Figure 1.8: An aphid (*Myzus persicae*) rostrum viewed under a light microscope (Image taken from: Pest and Diseases Image Library, Bugwood.org).

Aphid host-finding and initiation of feeding is more complex than it may seem and usually involves a sequence of events. Winged or alate individuals of heteroecious species are most commonly responsible for host-finding and acceptance, but monoecious species also undertake flights from the host plant to other plants of the same species (in response to factors such as predators and parasitoids, change in host plant quality, crowding, etc.) (Klingauf, 1987). The events that may lead to host-finding and initiation of feeding, include pre-alighting behavior, plant contact and assessment of surface cues before stylet insertion, probing of the epidermis, penetration, stylet pathway activity, sieve element puncture and salivation, and phloem acceptance followed by sustained ingestion

(Klingauf, 1987; Powell *et al.*, 2006). A list of factors which determines host selection is given by Klingauf (1987) and include light, gravity, host shape-, colour- and odour, plants growing in the immediate vicinity, spacing of the host plant, weeds and bare soil around and in the vicinity of the host plant, wind speed, barometric pressure, phloem sap, quantity of food available, surface texture and structure of host, and chemical composition of the host's surface.

1.7.4 Importance of aphids on lettuce cultivated under protective structures

Aphids are considered one of the most serious agricultural pests (Hooks & Fereres, 2006) and the homogenous crop habitats found in agro-ecosystems contribute largely to this status (Dixon, 1987a). The uniform habitat results in higher predictability and improved chances for survival and reproduction for these insect pests. Twenty-one aphid species are known to utilize lettuce as a host (refer to Blackman & Eastop [2000] for a list of these species). It is susceptible to attack by aphids throughout its whole growth period, especially if grown in areas with mild winters or in greenhouses. The damage aphids can cause on lettuce is two fold, they can either directly damage plants through their feeding habits, or they can indirectly impair plants through the transmission of viruses (Hooks & Fereres, 2006). Based on this, Irwin *et al.* (2007) have mentioned that aphids can be placed in one of four categories, namely transient non-vectors (aphid species incapable of forming colonies on the crop or transmitting viruses), transient vectors (aphids incapable of forming colonies on the crop, but capable of transmitting viruses), colonizing non-vectors (aphid species capable of colonizing the crop, but incapable of transmitting viruses), and colonizing vectors (aphids that are capable of both forming colonies on the crop and transmit diseases (refer to Chapter 6 for a further discussion on these different categories of aphid pests).

Aphids are the most common vectors of plant viruses and are known to transmit about 50% of the 600 viruses associated with invertebrate vectors (Hull, 2002). Examples of viral diseases transmitted by aphids to lettuce, and which is worth

mentioning here, is Lettuce mosaic virus (LMV) and Beet western yellows virus (BWYV). Such devastating diseases have been the focus of several studies (e.g. Walkey & Pink [1990] and Nebreda *et al.* [2004]) in order to identify the most common species of aphid vectors and means of transmission. Although aphids are not the sole vectors of viral diseases (as shown by Wisler & Duffus, 2000), they are undoubtedly important, and many studies have focused on breeding resistant cultivars to aphid-borne viruses (Liu & McCreight, 2006). Other control options have also been reviewed against aphid vectors such as the use of ultralow oxygen treatments to lower the occurrence of aphid vectors (Liu, 2005) and the use of entomopathogenic fungi (Parker *et al.*, 2002). These studies have been developed in order to minimize the use of pesticides on crops, not only due to the concerns it harbours to consumers, but also due to the development of resistance against pesticides. The development of such resistance can mostly be traced to the excessive use of pesticides which exert selective pressure on treated populations (Foster *et al.*, 2000).

To overcome the problems associated with resistance to pesticides and environmental contamination, the integrated approach to pest management was formulated (Kogan, 1998) which entails the combination of different control strategies to manage pest problems (refer to Chapter 6 for a detailed discussion of these strategies).

1.8 THE INTEGRATED PEST MANAGEMENT (IPM) CONCEPT

1.8.1 Overview

IPM implies the integration of various control methods and approaches into the pest management program, and is a holistic approach which takes into consideration the ecology of the environment and the influence that management actions can have upon the environment. In fact, the IPM concept was formalized by entomologists in the late 1950's and early 1960's in response to the environmental damage caused by the widespread and indiscriminate use of pesticides (Ali Ahmed, 2002). Van Emden (2007) mentioned that the main driving force behind IPM stemmed from pesticide mismanagement in the 1940s to

1950s. Contrary to common belief, IPM is not aimed at the total elimination of pesticides, but rather to restrict its use to more strategic situations (Gouws *et al.*, 2001). Moreover, IPM aims at managing a pest problem, rather than the total elimination thereof (Dent, 1991). The integrated approach can therefore be viewed as a means to lessen the dangers to humans, animals, plants and the environment caused through the indiscriminate use of harmful pesticides, and strives towards the prevention of resistance against pesticides in pest populations and the encouragement of biological control. Integrated pest management lends itself perfectly to the employment of a sustainable pest control program and it will provide producers with a long term control strategy and improve cost savings. It is stated by Van Emden (1996) that this tactic is effective on both small- and large scale areas, and it is commonly accepted that this is the way to go as far as pest control in the future is concerned.

IPM provides the producer with a tool to reduce pest populations and keep them at levels below those causing economic damage (Powell & Webster, 2004). Producers are aware of the advantages of an integrated approach to pest management, and lettuce producers (e.g. in Victoria, Australia) are already combining several control strategies to ensure efficient pest (and in particular aphid) control, whilst shifting away from the conventional use of broad-spectrum insecticides (Cole & Horne, 2006) which can be costly and ineffective when pest populations are high (Walkey & Pink, 1990). Control strategies that can be employed into the integrated pest management system are discussed in Chapter 6. Controlling pests through the use of natural enemies (biological control) is a preferred tactic in many of these systems. An advantage is the ability to combine it with other control strategies in order to achieve more reliable pest control (e.g. as demonstrated by Ali Ahmed, 2002).

1.8.2 Implications for biological control in shadehouse structures

Biological control is basically the use of an organism (also referred to as a natural enemy) to control a pest species. The natural enemies used may be generalists (feeding on a wide range of hosts) or specialists (feeding on a particular species

or family of hosts) in feeding habits. Specialist species are usually imported from the country of origin of the pest to be controlled, whilst the generalist species are usually native (Castañé *et al.*, 2004). Three general approaches to biological control is recognized, namely importation (also known as classical biological control), augmentation, and the conservation of natural enemies.

Classical biological control occurs when the natural enemies are imported into a geographical unit where they did not previously occur (Powell & Pell, 2007). The opposite is true for augmentation, namely the natural enemies already occur in the system, but they are too few to have any real impact on aphid numbers. They are therefore reared and released *en masse* to control aphid populations (Powell & Pell, 2007). Releasing biological control agents (inoculative and inundative releases) into greenhouses could result in improved pest control (timing of release in proportion to aphid populations is very crucial), because of the closed environment which will slow their escape. This will be much more difficult to achieve in a shadehouse structure (especially in a partially covered structure), because of the free movement it permits. Rearing and releasing adult Coccinellidae, the most common natural enemy of aphids, can also turn out to be an expensive exercise and is not practiced on a big scale in South Africa.

For these reasons it might be easier to permit natural occurring enemies to colonize shadehouse structures in response to pest populations – a phenomenon known as conservation biological control, whereby natural occurring coccinellid predators are encouraged to populate the crop infested with aphids. This is achieved by modifying the environment or pesticide application procedures (Eilenberg *et al.*, 2001). Encouraging natural enemies to move in from the adjacent habitat is key to the success of this strategy. To accommodate this, producers can make use of more selective insecticides in their IPM programme which will allow beneficial insects to colonize the crop (Cole & Horne, 2006). Attempts to use selective pesticides, and only when necessary, is central to the concept of sustainable agriculture. Another possibility is the manipulation of the physical habitat to make it more attractive to the predators (Brewer & Elliot, 2004;

Powell & Pell, 2007) (refer to Chapter 6 for a full discussion on possible methods). Conservation biological control is currently viewed as one of the most important components in an integrated pest management programme in which natural enemies are deployed against pest species.

1.8.3 Biological control of aphids

Several insect orders harbour aphid predators which might act as biological control agents, but the most important families include Syrphidae and Cecidomyiidae (Diptera), Anthocoridae (Hemiptera), Chrysopidae (Neuroptera) and Coccinellidae (Coleoptera) (Ali Ahmed, 2002). Collectively, these insects are known as Aphidophaga (aphid feeders) (Völkl *et al.*, 2007). The success of these insects in controlling aphid populations is directly related to their capability to locate their prey, and to their ferocity once the prey has been found (Völkl *et al.*, 2007). In general, aphid predators may not be important in regulating aphid populations in the long-term, but they are important in regulating aphid densities during certain seasons and in certain areas (Powell & Pell, 2007).

Often, some of the above-mentioned insect families are used together to compliment each other in pest control, or they might be used in collaboration with other biological control agents (e.g. with entomopathogenic nematodes as illustrated by Powell & Webster, 2004, or entomopathogenic fungi as illustrated by Steinkraus *et al.*, 2002). Using different biocontrol agents in collaboration with each other is usually preferred for controlling aphids under controlled environments, because they prove difficult to eradicate under these conditions (Gullino *et al.*, 1999). As far as the insect biocontrol agents are concerned, the family Coccinellidae is viewed as one of the most important control agents linked to biological control and is considered an important predator of aphids.

1.9 COCCINELLIDAE AS BIOLOGICAL CONTROL AGENTS OF APHIDS

Most species of ladybirds (Coleoptera: Coccinellidae) are predaceous (phytophagous and fungivorous species are known), with both the larvae and adults feeding primarily on aphids (Völkl *et al.*, 2007). The larvae are elongate

and somewhat flattened with tubercles or spines on their bodies (Triplehorn & Johnson, 2005) and are often observed foraging in aphid colonies (Figure 1.9).



Figure 1.9: Coccinellidae larva feeding on aphids on a lettuce plant (Roodevallei, Free State Province).

According to Völkl *et al.* (2007), the foraging behavior of larval Coccinellidae is influenced by four factors. The first of these is a tendency for larvae to move to the top of the host plant due to negative geotaxis. Aphid colonies lower down the plant thus have a greater chance of survival, compared to colonies found at the top of the host plant. Secondly, the size of the host plant and the architecture of the plant play an important role. Larvae are more successful in simplified habitats than in diverse ones. Thirdly, the structure of the leaf is important, because hairs, wax covers, etc., may hinder the movement of the larvae. Lastly, larval searching behavior is altered after they have encountered prey. When an aphid colony is encountered they will switch to an area-restricted feeding behavior. Despite this,

coccinellid larvae play an important role in bringing down aphid numbers, and as such, play an integral role in the biological control of aphids in any integrated pest management programme.

Coccinellidae not only prey on aphids, but also on other harmful pests such as scale insects and mites (Triplehorn & Johnson, 2005). The fact that both the mature and immature insects feed on the same pest species (and have high consumption rates) has made them a favorite in many biological pest control programmes. Indeed, this trait is not common among insects displaying complete metamorphosis and one possible explanation might be the abundance and vulnerability (in most cases) of their prey. Coccinellids are regularly used in biological control programmes and some species have even been imported from other countries to control certain insect pests. A popular example is the species *Rodolia cardinalis*, which have been imported to control the Australian bug, *Icerya purchasi* (Skaife, 1979) in South Africa.

However, as is the case with most biological control programmes, the outcomes are uncertain and influenced by various factors. In some cases the larger coccinellid species will consume smaller species (Hindayana *et al.*, 2001), leading to a decrease in biological control efficiency. Introduced exotic ladybirds may also displace native coccinellids (Snyder *et al.*, 2004), or the other way round. During prey scarcity, coccinellid eggs may be subject to cannibalism by other species of Coccinellidae (Cottrell, 2004), resulting in lower predator numbers in the field and necessitating costly augmentation strategies. In addition to this, the efficacy of coccinellid predators is also determined by their host-finding ability and host preferences. It was illustrated by Garcia & O'Neil (2000), that attack rates of Coccinellidae on aphids is positively related to prey density, and the search rates is inversely related to prey densities. They also found that the leaf area of the crop played an important role in attack rates and, in addition, the size of the host plant is also important in this regard (Völkl *et al.*, 2007). In addition, Elliot *et al.* (2000) has illustrated that the search rate of coccinellids is also influenced by air temperature and time of day. The number of

predators present is also important for the effectiveness of control, because low predator populations usually don't have any real impact on aphid numbers (Flint & Dreistadt, 2005). The efficiency of these beetles is difficult to determine due to their generalist feeding habits and high mobility. These factors are very important and will ultimately determine the success or failure of Coccinellidae predators employed against aphids in protective structures.

Coccinellid predators are diverse in terms of dietary preference and specificity (Sloggett & Majerus, 2000). Certain coccinellid species are unable to complete their development on certain aphid species (Michaud, 2000), and even if they are able to complete their development on an aphid species, control might not be satisfactory (Omkar & Mishra, 2005). Preference for certain aphid species above other species can be the result of such factors as morphology, behavior, and chemical constitution of the aphids. Evolutionary convergence between aphid and coccinellid species may also influence prey preference. In a comparative study on the efficacy between a native species of Coccinellidae and an introduced one, it was found that the native coccinellid species were better adapted in searching efficacy, reproduction and feeding on an indigenous aphid species (Berthiaume *et al.*, 2007). For this reason it will be better to make use of natural control by native Coccinellidae species (if they accept the aphid prey) in shadehouse structures, especially in partially covered shadehouse structures.

1.10 OBJECTIVES OF THE STUDY

With this information as background, the study aimed at determining the following:

- ☛ Occurrence and seasonality of the different aphid pest species, and their associated naturally occurring Coccinellidae predatory guilds, occurring on shadehouse cultivated lettuce in the central Free State.
- ☛ The influence of different shadehouse structures and climatic factors on the occurrence and the population dynamics of these aphid species and their associated Coccinellidae predators on such lettuce.

- The influence of varying aphid populations and shadehouse structures on some physical characteristics of head lettuce.
- Implications for a plant health management system for aphids on lettuce under variable shadehouse conditions in the central Free State.

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CHAPTER 2

MATERIALS AND METHODS

2.1 STUDY AREA AND TIME FRAME

The study was conducted on the farm Roodevallei, situated between the towns of Brandfort and Bultfontein (28°25'S 26°13'E) in the central Free State Province, South Africa (Appendix 1). The trial took place from 01 December 2005 until 07 December 2006. The farm is situated on the highveld which is dominated by grassland vegetation (O'Connor & Bredenkamp, 1997) and the area also experiences frost, as well as periodic hail storms and summer rainfall. The soil type chosen for the trial area is a deep red sandy-loam soil with good drainage capability, which still allows for good water holding capacity. This is essential, because waterlogging occurring on heavier soils may cause root diseases and other disorders (Niederwieser, 2001).

2.2 TRIAL DESIGN

Two shadehouse structures were constructed, using treated wooden poles and supporting cables onto which grey shade netting (with a 25% shade factor) was attached as cover for the lettuce crop against hail storms and temperature extremes (direct sunlight and frost). The shadehouse structures were of the flat-roofed design which is meant to keep material costs lower, whilst still allowing adequate protection to the crop. Each structure had a dimension of 12 x 18 meters, resulting in a total surface area of 216 m² per structure. This gave a total trial area of 432 m². One of the structures is referred to as a fully covered shadehouse, because shade netting was used to cover the top as well as the sides of the structure (Figure 2.1). The shade netting on the sides were buried to a depth of 300mm in the soil to restrict the movement of soil-dwelling invertebrates and other animals (birds, small mammals, reptiles, etc). This shade netting was placed at a 45° angle to deflect strong winds in order to prevent damage to the structure during storms.

To further restrict the movement of specifically soil dwelling invertebrates (and especially the pugnacious ant species, *Anoplolepis custodiens*), plastic sheeting was attached to the border poles of the fully covered shadehouse structure to a height of 300mm (Figure 2.1). This alone would not deter ants from entering the structure, due to the flexible arolium found between the tarsal claws of Hymenoptera which are perfectly adapted to attach to smooth surfaces (Federle *et al.*, 2001). Therefore, the plastic was treated with a sticky substance (known as Plantex[®]) to entrap ants attempting to enter the structure. A thin band of Plantex[®] was also painted onto each wooden pole in the fully covered shadehouse at a height of approximately 400mm from the soil level in order to deter ants from gaining access to the structure via the roof. This is the same principal as placing controlled-release chlorpyrifos bands around the trunks of orchard trees in order to prevent pugnacious ants (*A. custodiens*) from reaching homopteran pest species (James *et al.*, 1998). The technique of using a sticky substance as a physical barrier does not only apply to insects and has been employed to restrict the movement of other arthropod pests, for instance mites (Acari: Tetranychidae) into fruit trees (Takano-Lee & Hoddle, 2002).

The second structure is referred to as a partially covered shadehouse structure, because only the roof area was covered with shade netting (using the same grey shade netting), while the sides were left open to allow easy access in and out of the structure for all invertebrates and other organisms (Figure 2.1). No plastic sheeting was attached to the border poles and Plantex[®] was not used in this structure. Each of the two shadehouse structures contained eight planting blocks (visible in Figure 2.1), each block with a dimension of 1 x 15 meters. This specific dimension was chosen in order to ease planting, scouting, and harvesting of the lettuce plants. Each planting block contained three rows of head lettuce with a total of 153 plants per block, resulting in a total of 1 224 plants per structure (153 plants/block x 8 planting blocks). Each plant was assigned an accession number which was identical in both structures (Appendix 2). To differentiate between the lettuce plants in the two structures, each structure was given a color

code, namely blue for the fully covered shadehouse structure and white for the partially covered shadehouse structure.

2.3 SEEDLINGS AND CULTIVARS

A seedling nursery outside Bloemfontein (Free State Province) supplied the head lettuce seedlings used during the trials. Seedlings were obtained from the nursery four weeks after germination during the warmer months of October to March, and six weeks after germination during the cooler months of April to September. Tropical Emperor (a Hygrotech[®] cultivar) was planted in both structures during the warmer months due to better resistance to bolting (Jenni & Dubuc, 2003) and Del Oro (also a Hygrotech[®] cultivar) was planted in both structures during the cooler months due to its tolerance towards colder conditions. Only healthy looking seedlings with a minimum of three healthy leaves and a height of approximately 3-4 cm (Stork *et al.*, 2001) were used for the trial.

2.4 PLANTING CYCLES AND TECHNIQUES

A total of six replicates were planted during the trial (Table 2.1). Running the trial over a full year period was required in order to establish aphid movement and population dynamics as they occur throughout the different seasons, and because some species could exhibit heteroecy and be absent during certain times (Blackman & Eastop, 2000).

From the table it is also clear that roughly 60 days (or two months) were allowed for each planting cycle after which the plants were harvested by hand. Following harvesting, a full day was allowed before planting the next cycle during which time all dead leaves and other organic material were removed from the planting blocks. This was done with the focus on preventing the spread of fungal pathogens such as downy mildew (*Bremia lactucae*) (Stork *et al.*, 2001; Carisse & Pillion, 2002). Combating the spread of powdery mildew (*Erysiphe cichoracearum*) which occurs under moderate temperature- and humidity levels (Stork *et al.*, 2001) were also targeted by this procedure.

In order to reduce the occurrence of lepidopteran pests (specifically cutworms), all weeds (which could act as a source of infestation) were controlled just after harvesting by cultivating the planting blocks with three-tinned forks (Speight, 1983). The seedlings were transplanted directly into the soil by hand, at a spacing of 30 x 25 cm which correlates with the recommended plant spacing of 25 cm between plants by Harris (1992) and 45 x 30 cm as recommended by Bester *et al.* (1989). The planting blocks were watered thoroughly directly after transplantation in order to ensure the survival of the seedlings. During the first four weeks after transplanting, weeds were controlled by removing them by hand in order to avoid competition for water and nutrients, and the spread of diseases such as Septoria leaf spot (*Septoria lactucae*). After this four week period, the lettuce plants were well established and weeds were left to grow in order to keep disturbances to insect activities to a minimum. All of these procedures were performed simultaneously in both structures to ensure uniformity. No herbicides or fungicides were applied during the trial.

2.5 IRRIGATION AND FERTIGATION

To accommodate the plant spacing used during the trial, dripper-line irrigation was installed with a spacing of 30cm between the drippers, and three rows of dripper line per planting block (one line for each row of head lettuce). An overhead irrigation system was avoided, because of the precipitation effect created by this type of system. Direct water contact, such as rain/precipitation is known to affect certain insect communities negatively, as demonstrated by Norris *et al.* (2002) and aphid colonies may be no exception. In addition, falling water will wash off honeydew (Dik *et al.*, 1991), which could have an adverse affect on ant foraging behavior. The lettuce in both structures were irrigated simultaneously, and irrigation scheduling was determined according to the needs of the plant by visually monitoring soil, atmospheric, and plant moisture conditions each day (Jovanovic *et al.*, 1999). Irrigation was usually performed once daily (twice during very warm periods), and no irrigation took place during rainy conditions when the soil was already saturated with water. Irrigation was scheduled to take place either early in the mornings and/or late in the afternoon.

Water soluble fertilizers were diluted into one of the irrigation water tanks and supplied to the plants directly through the irrigation system (a process known as fertigation). The fertilizers used in the trial were Hygroponic[®] (containing N, P, K, S, & Mg) and Calcium-nitrate. Unlike irrigation, fertigation was performed only once weekly (simultaneously in both structures) as the area used for the trial was never planted with other crops in the past and therefore not depleted of nutrients. The fertilizers were directly mixed with water into the tank used for this purpose, and the electrical conductivity (EC) and pH were quantified using handheld probes (Appendix 3). The Appendix indicates that a total of twenty six fertigations took place for the duration of the trial. The EC values ranged from 2.2 to 2.9, whilst the pH varied between 6.7 and 7.6, which are in line with a recommended pH of 7.0 and EC of between 1.5 and 2.5 (Harris, 1992). During rainy conditions, fertigation was not performed in order to avoid drowning of the plants (especially seedlings) and wastage of fertilizer.

2.6 RECORDING OF TEMPERATURE AND RAINFALL DATA

Temperatures within each shadehouse structure were recorded daily using pre-calibrated maximum-minimum thermometers (supplied by Dicla Farm and Seeds[®]) attached to the centre pole of each structure. The thermometers were so placed as to avoid direct sunlight and rain falling on them. Temperature readings (minimum and maximum temperatures) were taken every 24 hours between 08:00 and 10:00 in the morning, after which the thermometers were reset to record the next set of temperatures. The recorded temperatures for both structures during the trial period are shown in Appendix 4. A standard rain gauge was applied to measure the rainfall and this data was also recorded (Appendix 5). The rain gauge was positioned in an area clear from any obstructions between the two structures. Data was recorded each morning (between 08:00 and 10:00) after rain have fallen the previous day and/or night.

2.7 SAMPLE TECHNIQUES

Plants were randomly selected for the trial using a 'cross' sampling procedure. Strings were spanned and drawn between the four corner poles of the

shadehouse structure, creating a cross, and the plants falling on these string lines were chosen for the trial. This method differed slightly from previous aphid population studies on lettuce (Parker *et al.*, 2002), but it had the added advantage of a complete representation of plants in all areas of the shadehouse structure (borders and centre). Cultivated areas have the tendency of creating an edge effect, whereby insect populations increase or decrease near the borders, as demonstrated by Dauber & Wolters (2004) in a study with ant communities. The cross sample method therefore largely excluded the risk of skew results due to the creation of such an edge effect.

A total of twenty four plants in each structure were selected with the cross sample method (Table 2.2), and these same plants were sampled throughout the trial period during each of the six planting cycles (Table 2.1). The same plants (using the accession numbers) were monitored in both structures. For example, if az 02 were monitored in the fully covered shadehouse structure, then az 02 would also be monitored in the partially covered shadehouse structure and the color codes, namely blue (fully covered shadehouse structure) and white (partially covered shadehouse structure) served to distinguish between the plants in the two structures. If a certain plant died from disease, insect damage, or any other reason during the course of a specific planting cycle, the next plant in the same row was chosen to continue the trial. For example, if the plant az 02 died – az 03 was chosen for sampling and if az 03 also died, az 04 was chosen. Plants were sampled four times (sample occasions) during each planting cycle. The specific sample dates are provided in Table 2.3 indicating that samples were conducted in such a manner as to cover all three growth stages (seedling, vegetative and heading) of the lettuce plant (Figure 2.2).

Seedling development encompasses germination and seedling emergence above soil level (3-7 days after germination), and it ends in the three- to four-leaf stage. Vegetative growth is the period in which the stem lengthens and new leaves are formed at the growing point. Soon the lengthening of the stem ceases, but new leaves are still being formed. This will lead to leaves only unfolding

partially, signaling the start of the heading stage (\pm 5 weeks after seedling development). During heading, the rosette leaves grow more upright. The margins of new leaves forming in the centre of the rosette will become entrapped against the upright leaves and in this way a round head will form into the characteristic crisp head of head lettuce (University of California, 1992).

The following data were collected from each plant during sampling:

- planting cycle,
- date of sampling,
- time of sampling,
- growth stage of plant,
- weight of plant (only measured at end of each planting cycle),
- number of living leaves present,
- number of living leaves infested with aphids,
- number of aphids present,
- number of Coccinellidae adults present,
- number of Coccinellidae larvae present,
- absence or presence of the ant, *A. custodiens*, in partially covered shadehouse structure,
- whether or not samples of Aphididae, Formicidae and Coccinellidae were collected, and
- sample number/s of collected specimens.

The exact procedures that were followed in monitoring the above-mentioned is explained in the following chapters. Plants were sampled in the morning, beginning at 09:00 during August – January and 10:00 during February – July. The reason for this was to allow ambient temperature to rise to an adequate level for diurnal predators to become active, especially members of the family Coccinellidae which prefer to feed during the daylight hours (Pfannenstiel & Yeorgan, 2002). The only insecticide used during the trial was cutworm bait (Kombat[®]) in order to control cutworms during the first two weeks after

transplanting to prevent the loss of too many seedlings. The bait had no direct influence on aphids or their associated predators and did not influence the trial. Cutworm bait was only used during cycles in which the larvae were present and damaged more than ten seedlings per planting block.

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Figure 2.1: Fully covered shadehouse structure with shade netting used to cover the sides of structure (top), plastic sheeting attached to border poles of fully covered shadehouse structure (middle), and partially covered structure showing absence of shade netting at sides of structure (bottom) (Roodevallei, Free State Province).



Figure 2.2: The three lettuce growth stages in which the plants were sampled. Seedling stage (top), vegetative stage (middle), and heading stage (bottom).

Table 2.1: Lettuce planting cycles and cultivars used in each (Roodevallei, Free State Province).

Planting cycle	Cultivar	Planting date	Harvesting date
Dec - Jan	Tropical Emperor	01 Dec 2005	30 & 31 Jan 2006
Feb - Mar	Tropical Emperor	02 Feb 2006	29 & 30 Mar 2006
Apr – May	Del Oro	30 Mar 2006	29 & 30 May 2006
Jun – Jul	Del Oro	01 Jun 2006	28 & 29 Jul 2006
Aug - Sep	Del Oro	01 Aug 2006	29 & 30 Sep 2006
Oct - Nov	Tropical Emperor	01 Oct 2006	07 & 08 Dec 2006

Table 2.2: Accession numbers of sampled lettuce plants and planting blocks in which each were located (Roodevallei, Free State Province).

Planting block	Plant number
A	az 02
A	ay 51
B	bx 06
B	by 08
B	bx 45
B	bz 42
C	cy 15
C	cz 17
C	cx 39
C	cy 37
D	dx 21
D	dy 30
E	ex 24
E	ex 30
F	fx 16
F	fz 13
F	fx 36
F	fy 38
G	gx 09
G	gy 07
G	gy 45
G	gz 46
H	hx 02
H	hx 50

Table 2.3: Sample dates during the six lettuce planting cycles (Roodevallei, Free State Province).

Planting cycle	Sample dates	Sample number
1	15 Dec 2005	1
	30 & 31 Dec 2005	2
	13 & 14 Jan 2006	3
	22 & 23 Jan 2006	4
2	17 Feb 2006	5
	03 Mar 2006	6
	17 & 18 Mar 2006	7
	29 & 30 Mar 2006	8
3	15 Apr 2006	9
	28 & 29 Apr 2006	10
	15 & 16 May 2006	11
	29 & 30 May 2006	12
4	17 Jun 2006	13
	03 Jul 2006	14
	14 & 15 Jul 2006	15
	28 & 29 Jul 2006	16
5	19 Aug 2006	17
	02 Sep 2006	18
	15 & 16 Sep 2006	19
	29 & 30 Sep 2006	20
6	20 Oct 2006	21
	10 & 11 Nov 2006	22
	24 & 25 Nov 2006	23
	07 & 08 Dec 2006	24

CHAPTER 3

OCCURRENCE AND SEASONALITY OF APHIDIDAE AND COCCINELLIDAE SPECIES ASSOCIATED WITH LETTUCE, CULTIVATED UNDER VARIABLE SHADEHOUSE CONDITIONS IN THE CENTRAL FREE STATE (SOUTH AFRICA)

Abstract

Introduction: Consumer demands have resulted in the year-round production of lettuce in certain regions of South Africa. However, the sequential planting of this crop could put it at risk to attack by several aphid species throughout the year, complicating control strategies. Knowledge over the seasonality and aphid species complex found on the crop is therefore necessary in order to execute preventative and control actions. It is also vital in order to determine the peak abundance of their natural enemies.

Methods: Lettuce was planted throughout a one-year period (December 2005 to November 2006) in two different shadehouse structures. The one structure was partially covered with shade netting, permitting free movement of insect pests and the native pugnacious ant, *Anoplolepis custodiens*. The other structure was fully covered with shade netting into which the movement of ants were excluded. Aphid and coccinellid samples were collected from these. The species and seasonality of the different aphids (Hemiptera: Aphididae) and coccinellids (Coleoptera: Coccinellidae) was then determined. The similarity in occurrence for each individual aphid and coccinellid species between the two structures was also determined, along with the insect species which were positively associated with each other within each individual structure.

Results: A total of nine aphid species and five coccinellid species were observed during the trial. Both structures harboured similar aphid and coccinellid species, with minor exceptions. The aphid species *Acyrtosiphon lactucae*, *Macrosiphum euphorbiae* and *Aphis* sp. 1 showed no seasonality and were present throughout the trial period in both structures. The aphid, *Nasonovia ribisnigri*, also exhibited similar occurrence in both structures, but only during the warmer months of

December – January. *Myzus persicae* was only observed in the fully covered structures during the cooler months of the year. The remaining aphid species had a low occurrence. The coccinellids *Hippodamia variegata* and *Scymnus* sp. 1 showed a significant similar occurrence in both structures, with no seasonality observed. Other species such as *Cheilomenes lunata* and *Exochomus flavipes* were observed less frequently. Similarities in the occurrence of different species in each particular structure, is also discussed.

Conclusions: Shade netting did not impede the movement of aphids and coccinellids. The most important aphid species associated with lettuce production in the central Free State (Southern Africa) are *A. lactucae*, *N. ribisnigri*, *M. euphorbiae* and *M. persicae*. The most commonly observed coccinellid predators were *H. variegata*, *Scymnus* sp. 1, and various larvae of this family. *E. flavipes* and *C. lunata* could prove to be potential candidates in biological control, but their bigger size limits their movement into the fully covered structure. Some of the aphid species are able to co-exist on lettuce plants and collectively lead to damage. The more numerous coccinellids and their larvae also had a similar occurrence intensity to several of the aphid pest species, which could indicate preference for these species.

Key words: Aphid diversity; Seasonal distribution; Natural enemy diversity

3.1 INTRODUCTION

Lettuce (*Lactuca sativa* L.) is viewed as one of the most important salad vegetables cultivated for human consumption (Harris, 1992). Its popularity stems from several factors, including health benefits (Nicolle *et al.*, 2004), freshness associated with its characteristic texture, and minimal preparation requirements (Rico *et al.*, 2007). The importance of lettuce in human nutrition is well illustrated by its extensive use in fast-food outlets and restaurant industries, while it is also sold in a variety of forms and packaging options from which consumers can choose (Stork *et al.*, 2001). This popularity, coupled to the fact that higher prices can be obtained during the so-called 'off-seasons', encourages producers to deliver their produce throughout most of the year, as lettuce is primarily intended for the fresh-market, with long-term storage not a preferred option (Hammig & Mittelhammer, 1980).

To enable year-round production, some producers have reverted to the production of lettuce using protective structures (plastic tunnels, shadehouses, etc.), which provide added protection against adverse weather conditions. Shadehouses are of particular interest to producers on the South African Highveld, as they provide some protection from direct sunlight during hot summer months, and frost during winter months. However, they offer limited protection against pest infestations, especially if the structure is only partially covered with shade netting. Aphids are renowned pests of cultivated lettuce, with a worldwide total of twenty-one species recorded from the crop (Blackman & Eastop, 2000), and five species recorded from lettuce in the sub-Saharan region (Millar, 1994). Because lettuce is exotic to South Africa, it can be expected that most of these aphid species would be exotic, and have translocated through the distribution of the crop or by some other means. Indeed, it is generally accepted that most of the pest aphid species encountered by entomologists on economically important crops are alien invaders (Blackman & Eastop, 2000).

Seasonal variance in temperature, rainfall, daylength, etc., could result in some of these aphid species exhibiting seasonal cycles regarding their behaviour and

development (Hardie & Vaz Nunes, 2001; Williams & Dixon, 2007). These have been intensively studied for various aphid species, especially certain pest species (e.g. Lees, 1959; Dixon, 1987; Phillips *et al.*, 2000). Temperature plays a specific important role in this regard, and can influence aphid population development dramatically, which could ultimately determine the presence or absence of a species. An extreme example of this is a study conducted by Hullé *et al.* (2003), who found that increasing temperatures experienced on the Sub-antarctic Islands will accelerate the rate of aphid development which in turn, could increase their capacity to spread to other areas where they presently do not occur. Different aphid species also vary in their ability to thrive across different temperature ranges, which may differ from hot to cold conditions (Dixon, 1987). Aphids are capable of avoiding unfavourable climatic conditions by employing a range of survival tactics (i.e. host alteration, aestivation, hibernation, or by remaining in the egg stage for a longer period) (Dixon, 1985). The result of this would be that certain species pose a threat during certain times of the year, while they are absent during others.

The species composition and seasonal occurrence of lettuce aphid pest species will therefore largely be determined by seasonal variances. Nebreda *et al.* (2004) noted that there was a difference in aphid species recorded from the spring crop and the autumn crop of lettuce, while some species occurred throughout both seasons. This would imply that the sequential planting of lettuce could put it at risk to attack by various aphid species, which could complicate control. Reasons include differences in preferred feeding sites on the plant in different species (Parker *et al.*, 2002), or different species acting as vectors for different viral diseases (Li *et al.*, 2001; Nebreda *et al.*, 2004; Zhu *et al.*, 2006). Knowledge of the aphid species complex throughout the different seasons will enable producers to predict periods of higher risk in order to take preventative measures.

In the Free State Province, information on the different aphid species occurring on lettuce throughout the year is limited. It is likely that aphid seasonality will in

turn also influence the coccinellid predator species complex encountered on the crop. Lettuce has a short growing season (Parker *et al.*, 2002), and after transplantation, aphids must infest the crop, and it is only after infestation that coccinellid predators will follow (Wissinger, 1997). Predators therefore have a limited time window in which they can feed and reproduce, compared to predators feeding on aphids found on perennials (Brown, 2004). The efficiency of coccinellids to locate their hosts in such cases is crucial, and will ultimately determine the predator species complex. Pest densities also play an important role in aphid predator presence or absence (Bianchi & Van der Werf, 2004).

The objectives of this study were to identify the aphid species which colonize lettuce cultivated under variable shadehouse conditions in the central Free State (South Africa), along with their natural occurring coccinellid predators. The seasonality (absence-presence) of these insects was also determined in order to more accurately predict future infestations which would enable producers to make the necessary proactive management decisions. Comparisons between the two structures for the same species were conducted in order to determine the degree of similarity in their occurrence between the different structures. A comparison between the different species was also conducted in each structure in order to determine which species were positively associated with one another.

3.2 MATERIALS AND METHODS (Refer to Chapter 2)

Area of research and time frame: Refer to Chapter 2 (section 2.1)

Trial design and experimental layout: Refer to Chapter 2 (section 2.2, 2.3, 2.4 & 2.7)

Aphids: Aphid samples were collected from lettuce plantings throughout a one-year period (December 2005 – November 2006) over a total of six planting cycles. Samples were taken at roughly 14 day intervals (four sampling occasions were conducted during each planting cycle). Aphids were collected directly from the plants after which they were stored in specimen vials containing 70% ethanol

absolute. The former were collected from the plant in such a manner as to attain the most accurate representation of the species present during a particular sampling occasion. Mature apterous individuals were collected from each observed colony and alatae were also taken (when present). Single aphid specimens (when colonies were absent) were also collected. For reference purposes, the samples were numbered in accordance to the accession numbers assigned to the plants from which they were collected (refer to Chapter 2). Samples were stored in a reference collection containing information on plant accession numbers, date of collection, and planting cycle. Subsequently, aphids were visually sorted (using a stereo microscope) up to morphospecies level, from which a few selected samples of each morphospecies were dispatched for species identification. Identification was carried out by the Biosystematics Division of the Agricultural Research Council (ARC) in Pretoria (South Africa). Microscope slides of apterae and alatae (where possible) of the different species recorded were prepared in order to ease subsequent identification.

Predators: Coccinellid predators were only collected from the plants that were sampled for aphids. The reason for this was to only collect predators that were directly associated with the specific aphid species present on the sampled plants. Coccinellidae larval counts were also made for each plant sampled, but identification up to species level was not conducted and they were sampled as a unit (indicated as larvae). Specimens were preserved, labeled and stored in the same manner as described for aphid specimens, and identification was performed by the Department of Zoology & Entomology at the University of the Free State (South Africa).

Collection of rainfall and temperature data: Refer to Chapter 2 (section 2.6).

Statistical analysis: Sørensen's coefficient of similarity ($C_s = 2j/(a+b)$), as described by Southwood (1978), was used to test the degree of similarity in aphid and coccinellid species composition between the two structures. In this coefficient j = number of aphid and coccinellid species common to both

structures, and a and b = number of species observed in each structure individually. The coefficient ranges in value between 0 and 1, where 0 indicates no similarity in species occurrence, while 1 indicates perfect similarity in species occurrence. In this chapter, values above 0.50 were considered to indicate a similarity in species occurrence. A slightly modified version of Sørensen's coefficient of similarity ($C_s=2a/(2a+b+c)$), as described by Romesburg (2004), was applied to determine the absence-presence factor (and therefore the similarity/dissimilarity in occurrence) for each species of aphid and coccinellid, between the two structures. In this coefficient a = number of times in which a species occurred simultaneously in both structures, b = number of times in which the species only occurred in the fully covered structure, while it was absent from the partially covered structure, and c = number of times in which the species only occurred in the partially covered structure, while it was absent from the fully covered structure. The coefficient was employed in order to identify similarities/dissimilarities in occurrence between the two structures for each aphid and coccinellid species individually, in terms of a) occurrence during the whole trial period, b) occurrence during the different growth stages of the crop (which were the four sampling occasions of each planting cycle), and c) occurrence during the different planting cycles. The different aphid and coccinellid species occurring within each individual structure were also compared by means of this coefficient, in order to test for similarities/dissimilarities in their occurrences.

3.3 RESULTS

Aphid and coccinellid species composition: During the trial, a total of nine aphid and five predatory coccinellid species (excluding larvae) were identified on the lettuce (Table 3.1). Eight aphid and four coccinellid species (excluding larvae) were observed in the fully covered structure, and six aphid and five coccinellid species (excluding larvae) were observed in the partially covered structure (Table 3.1). Sørensen's similarity coefficient revealed a high value ($C_s = 0.800$) when the similarity in species composition between the two structures were measured, indicating a great overlap of species, and thus a similarity in species

composition between the two structures. Despite the similarity, some aphid species (*Myzus persicae*, *Rhopalosiphum nymphaeae*, *Rhopalosiphum rufiabdominalis* and *Aphis craccivora*) were only observed in one of the two structures (Table 3.1). A species in the genus *Harmonia* (*Harmonia* sp. 1) was the only coccinellid which did not occur in both structures (Table 3.1).

Aphid seasonality and occurrence similarities between the structures:

Certain aphid species displayed definite patterns of seasonality, while others occurred throughout the year (Table 3.1). *Macrosiphum euphorbiae*, *Acyrtosiphon lactucae* and a species in the genus *Aphis* (*Aphis* sp. 1), were present during all six planting cycles in the fully covered structure (Figure 3.1). In the partially covered structure, *A. lactucae* was also present during all six planting cycles (Figure 3.2). The result was a similarity in occurrence ($C_s > 0.50$) between the two structures during the whole trial period (Table 3.2A), throughout all planting cycles (Table 3.2B) and for all growth stages of the crop (Table 3.2C). *M. euphorbiae* was absent during planting cycles 1 & 6 in the partially covered structure (Figure 3.2), resulting in zero values when comparing its similarity in occurrence between the structures during these periods (Table 3.2B). However, this species had a similar presence in both structures ($C_s > 0.50$) during the remaining planting cycles (Table 3.2B), giving rise to its overall similarity in occurrence between the two structures for the whole trial period (Table 3.2A). This species was also present in both structures during most growth stages of the crop (Table 3.2C). *Aphis* sp. 1 was absent during planting cycle 6 in the partially covered structure (Figure 3.2), but it had a similar occurrence ($C_s > 0.50$) during planting cycles 1, 2, 3 & 5 (Table 3.2B), explaining the similarity in occurrence between the two structures over the whole trial period (Table 3.2A). This species was also present in both structures during most growth stages of the crop, except the heading stage (Table 3.2C).

Nasonovia ribisnigri exhibited seasonality in both structures by only occurring simultaneously in both structures during planting cycle 1 (Table 3.2B), but it was also observed on two other separate occasions (March & September) in the

partially covered structure (Figures 3.2). It did not infest the crop during the seedling stage (Table 3.2C) and the subsequent absence of *N. ribisnigri* from the fully covered structure (Figure 3.1) resulted in a lack of occurrence similarity for the remaining planting cycles (Table 3.2B). *M. persicae* was only observed during middle May to middle August in the fully covered structure (Figure 3.1). The aphid species, *R. rufiabdominalis*, *R. nymphaeae* and *Aphis pseudocardui* were all observed during a single occasion in the fully covered structure (Figure 3.1). *A. craccivora* and *Aphis pseudocardui* was also observed only on a single occasion in the partially covered structure (Figure 3.2).

Coccinellid seasonality and occurrence similarities between the structures:

A species in the genus *Scymnus* (*Scymnus* sp. 1) showed no seasonality in both structures (Figures 3.1 & 3.2). Similar occurrence in both structures during most planting cycles, except for planting cycle 3 (Table 3.2B), yielded a similarity in its occurrence ($C_s > 0.50$) for the whole trial period of this species (Table 3.2A). It was also the only coccinellid present in both structures during all the growth stages of the crop (Table 3.2C). *Hippodamia variegata* was only absent from the beginning of June to the end of September in the fully covered structure (Figure 3.1). In the partially covered structure this species was present throughout the year, except during planting cycle 2 (Figure 3.2). The similarity in occurrence ($C_s > 0.50$) for *H. variegata* in both structures over the whole trial period (Table 3.2A) can be ascribed to this species occurring simultaneously in both structures during planting cycles 1, 3 & 6 (Table 3.2B). The species also exhibited a similar occurrence in both structures during most growth stages of the crop (Table 3.2C). In the fully covered structure, the coccinellids *Exochomus flavipes* and *Cheilomenes lunata* were only observed during the warmer months of November and December (Figure 3.1). However, they were observed more often in the partially covered structure, where *E. flavipes* was recorded during the first (December – January) and fourth (June -July) planting cycles. *C. lunata* was noted on four different occasions throughout the year in this structure (Figure 3.2). There was an absence of coccinellid larvae from the end of March up until middle October in both structures (Figures 3.1 & 3.2), but during their

presence they were significantly similarly present ($C_s > 0.50$) in both structures during planting cycles 1 and 6 (Table 3.2B), and during most growth stages of the crop (Table 3.2C). *Harmonia* sp. 1 was only observed once (April) in the partially covered structure (Figure 3.2).

Inter-species association within each structure: *A. lactucae* displayed a similarity in occurrence with *M. euphorbiae*, *Aphis* sp. 1, *H. variegata*, and *Scymnus* sp. 1 in both structures. There was also similarity with the coccinellid larvae in the fully covered structure, but not in the partially covered structure (Tables 3.3 & 3.4). *M. euphorbiae* and *Aphis* sp. 1 also had a similarity in occurrence with the coccinellid *Scymnus* sp. 1 in both structures (Tables 3.3 & 3.4), and *M. euphorbiae* also had a similar occurrence to *H. variegata* and *Aphis* sp. 1 in the partially covered structure (Table 3.4). The coccinellids *C. lunata* and *E. flavipes* occurred simultaneously in the fully covered structure, while *H. variegata* had a similar occurrence pattern to the coccinellid larvae in the same structure (Table 3.3). The coccinellid larvae also had a similar occurrence to *N. ribisnigri* in the fully covered structure (Table 3.3). The only coccinellids which positively correlated with each other in the partially covered structure were *H. variegata* and *Scymnus* sp. 1 (Table 3.4). Zero values in terms of the comparison of certain species with others can be explained by the total absence of one of these species from the particular structure.

Presence of *Anoplolepis custodiens*: These ants were only observed during the warmer months of the trial in the partially covered structure (excluded from the fully covered structure) and the surrounding trial area (September – March).

3.4 DISCUSSION

Similarity in aphid species composition between the structures is not surprising, because the fully covered structure was not expected to restrict the movement of these small insects. Lopes *et al.* (2003) found that anti-aphid screens (opening width: 0.24 - 0.40 mm) was the only screen material through which all the tested aphids could not pass. The pores found in the agricultural shade netting industry

are larger ($\pm 2 \times 7$ mm) to permit 'breathing' and air circulation. In addition, the aphids may also have been present during transplantation (being transported from the seedling nursery), or they may have entered the fully covered structure through the movement of people (Kelliher, 1994) from the adjacent area. It was also not surprising to find that there was almost no difference in the coccinellid species composition between the two structures, because coccinellids would find their way into fully covered structures through small openings. It must be kept in mind that the objective of this section of the study was to measure the absence and presence of different aphid and coccinellid species occurring on lettuce throughout the different seasons, with no reference to their population dynamics. If this was considered (as is the case in Chapter 4), it is clear that certain aphid and/or coccinellid species prefer a certain structure within which they reach larger population densities.

Five of the twenty-one aphid species recorded worldwide from lettuce by Blackman and Eastop (2000), were also observed during our study. These were *A. lactucae*, *A. craccivora*, *M. euphorbiae*, *M. persicae* and *N. ribisnigri*. All are introduced species, and pests of cultivated crops (Blackman & Eastop, 2000). *A. lactucae* is probably a specialist on plants in the genus *Lactuca* where they are reported to be monoecious holocyclic (Blackman & Eastop, 2000). However, the permanent presence of this species (in apterous form) in both structures during the trial could suggest anholocycly. Possible explanations include the absence of the trigger that indicates environmental change, partial anholocycly where certain anholocyclic races or clones can exist (Blackman & Eastop, 2000) within a species which usually demonstrates holocycly, or the hibernation of viviparous females which are able to reproduce (albeit at a reduced rate) if the winter temperatures are not too severe (Miyazaki, 1987). Its permanent presence in both structures and in all growth stages of the crop makes this an important aphid pest species in the region with regard to variable shadehouse structures.

M. euphorbiae is reported to be heteroecious holocyclic in parts of the U.S.A., but anholocyclic in Europe and elsewhere (Blackman & Eastop, 2000). This was the

case during the trial, where it was present throughout the whole trial period (anholocyclic) in the fully covered structure. It would seem as if the milder winter temperatures which never dropped far below 0°C for extended periods of time (Figure 3.3) played a role in this regard. The absence of this species from the partially covered structure during the spring and summer months (planting cycles 1 & 6) could be attributed to the presence of the pugnacious ant, *Anoplolepis custodiens*. This species has the potential to colonize lettuce plantings from a very early stage up until harvest in both types of structures, especially during autumn to spring.

N. ribisnigri colonizes lettuce as a secondary host (Blackman & Eastop, 2000). It is heteroecious holocyclic between *Ribes* spp. (which were present in the vicinity of the trial area) and its secondary hosts, explaining why this particular species exhibited seasonality and why there was only such a similarity in occurrence between the two structures during the first planting cycle. The reason why this species did not colonize lettuce during the seedling stage can be a function of its feeding preferences where it feeds at the heart of the plant, being protected by the older wrapper leaves from the foraging pugnacious ants. The notorious *M. persicae* is a heteroecious holocyclic species in temperate areas of the world, but it may be anholocyclic during winter months on secondary hosts when peach, its primary host, is scarce or absent, and where the climate permits active individuals to survive throughout the winter months (Blackman & Eastop, 2000; Williams & Dixon, 2007). This was observed in the fully covered structure with this species only occurring during the cooler months, indicating that *M. persicae* will utilize lettuce as a secondary host if it is available during cooler months. The total absence of this species from the partially covered structure is unclear. *A. craccivora* is reported to be a pest of crops in warmer areas of the world (Blackman & Eastop, 2007). The species is anholocyclic (although sexual morphs have been recorded in some cases) and its presence on a single occasion in the partially covered structure cannot be explained. A possibility why this species did not form observable colonies could have been the higher presence of the coccinellid *C. lunata* in the partially covered structure. Even

though these two species were not observed to occur concurrently, the possibility cannot be excluded, as *C. lunata* is reported to be an important predator of *A. craccivora* (Ofuya, 1995).

Irwin *et al.*, (2007) mentioned that no more than four or five aphid species are apt to infest a certain crop in a specific area of the world. However, many other species which do not feed on the particular crop (and therefore pose no threat) can land on the crop, with up to 40 – 80 different species during a single season. Aphids will land indiscriminately on a plant and a brief probing of the epidermis would usually determine re-take-off (from non-hosts), or permanent settlement (on potential hosts), followed by reproduction (Robert, 1987). The species, *A. pseudocardui*, *R. rufiabdominalis*, *R. nymphaeae* and *Aphis* sp. 1, resorts within this category by being collected only as alates with no observable colonies formed. *A. pseudocardui*, *R. rufiabdominalis* and *R. nymphaeae* were also observed less frequently. These species aren't known pests of cultivated lettuce (Millar, 1994; Blackman & Eastop, 2000), and are therefore of limited concern to lettuce producers. As *Aphis* sp. 1 could not be identified up to species level, defining its importance to lettuce production is problematic. If this species is a transient vector, non-persistent viruses could be spread rapidly as these insects alight, probe, and fly to the next plant in search of a suitable host. However, no indication of viral diseases were observed during the trial, and this coupled to the fact that *Aphis* sp. 1 was observed for most periods of the year in both structures, and also during most of the growth stages of the plant with no damage symptoms, suggests no real threat to lettuce production in the area.

Skaife (1979) mentioned that species of the genus *Scymnus* are important predators of mites. However, they could also attack aphids and are considered aphidophagous (Magro & Hemptinne, 1999) as shown by numerous authors (e.g. Iperiti, 1999; Brown *et al.*, 2003; Resende *et al.*, 2006). This would explain their consistent presence in both structures and all growth stages of the crop. However, the *Scymnus* species observed during the trial was the smallest coccinellids observed and it is doubtful whether they would feed on adult aphids.

It seems more likely that these predators will feed on aphid nymphs (Brown *et al.*, 2003), that is if they prey on aphids at all. Hodek & Honek (1996) also mentioned that *Scymnus* spp. have specialized mouthparts which could limit the size of their prey, while small Coccinellidae species are reported to feed on small aphids (Völkl *et al.*, 2007). Their impact on aphid colonies might therefore be limited. *H. variegata* was an important natural enemy of aphids occurring on lettuce during the trial. This species is known to be among the most abundant coccinellids occurring in certain aphid-related studies (e.g. Aslan & Uygun, 2005; Athanasios *et al.*, 2006), and have been used in biological control (El Habi *et al.*, 2000). The absence of this species in the fully covered structure from June to the end of September could have been related to its lower numbers in the field due to cooler conditions, and the relatively larger size of this coccinellid which could restrict its access into this structure. *H. variegata* was observed more frequently in the partially covered structure, but its absence from the structure during February to March (in which time it also showed a low occurrence in the fully covered structure) could be ascribed to the relatively higher rainfall experienced during this period (Figure 3.4). Despite these differences, the overall similarity in occurrence of this species between the structures renders it an attractive option for biological control in both types of structures and during most growth stages of the crop.

The possible reason for *E. flavipes* and *C. lunata* only occurring during the summer months in the fully covered structure, may be related to higher prey densities in this structure (refer to Chapter 4). Higher numbers of these beetles in the warmer months will inevitably lead to some of them finding their way into the fully covered structure in search of prey and this has led to the similarity in occurrence between these two species during this period in the fully covered structure. The fact that *C. lunata* was observed on a more frequent basis in the partially covered structure might be related to the size of this predator. In terms of physical size it was the largest of all the coccinellid species observed during the trial and would therefore be less likely to access the fully covered structure. The absence of coccinellid larvae during the cooler months is a function of these

insects not reproducing during these periods. This genus, *Harmonia*, is known to feed on *M. euphorbiae* (Snyder *et al.*, 2004), which would explain their presence during the particular period, but the single observation made for this species suggests a low occurrence of this coccinellid in the area.

The similarity in time of occurrence between *A. lactucae*, *M. euphorbiae* and *Aphis* sp. 1, indicates that these aphid species can co-exist and collectively lead to damage in lettuce stands. Co-occurrence of certain aphid species with certain predatory coccinellid species could also indicate that these coccinellids prefer these aphids as prey. If this is the case, then *H. variegata*, *Scymnus* sp. 1 and the coccinellid larvae would prey on *A. lactucae* and *M. euphorbiae*. In addition, *Scymnus* sp. 1 would feed on *Aphis* sp. 1, while the coccinellid larvae would prey on *N. ribisnigri*. The fact that the coccinellid larvae observed were larger and had a similarity in time of occurrence with *H. variegata* in the fully covered structure, suggests them to be the larvae of this species. A similarity in time of occurrence between *H. variegata* and *Scymnus* sp. 1 has been witnessed in previous studies as well (e.g. Aslan & Uygun, 2005).

3.5 CONCLUSIONS

Shade netting did not impede the movement of aphids, and to a lesser degree, their coccinellid predators. Several non-significant aphid species were collected from the crop, but the most important aphid species associated with lettuce production in the central Free State (South Africa) is, *A. lactucae*, *N. ribisnigri*, *M. euphorbiae* and *M. persicae*. Seasonality was observed for *N. ribisnigri* and *M. persicae*, with the first-mentioned thriving in summer months in the presence of *A. custodiens*, while the latter occurred during the winter months. *A. lactucae* and *M. euphorbiae* showed no seasonality, but the presence of *A. custodiens* can restrict the presence of *M. euphorbiae* in the partially covered structure. The most commonly observed coccinellid predators were *H. variegata*, *Scymnus* sp. 1, and various larvae of this family. However, more precise studies are required to determine the prey preferences of the smaller *Scymnus* sp. 1. The species, *E. flavipes* and *C. lunata*, could prove to be potential candidates in

biological control, but their bigger size limits their movement into the fully covered structure. Some of the aphid species are able to co-exist on lettuce and can collectively lead to damage. The more abundant coccinellids and their larvae also occurred concomitant to several of the aphid pest species, which could indicate prey preference for these species.

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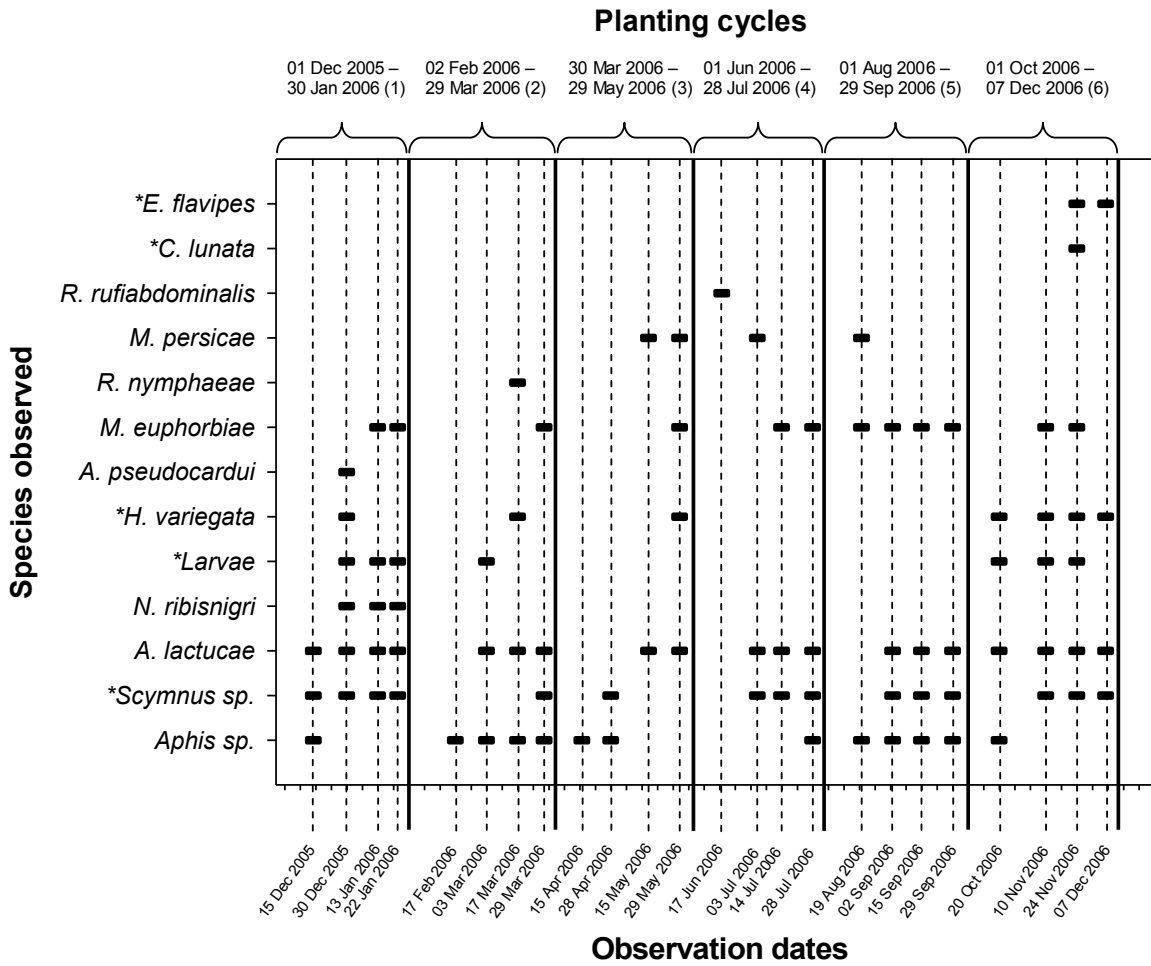
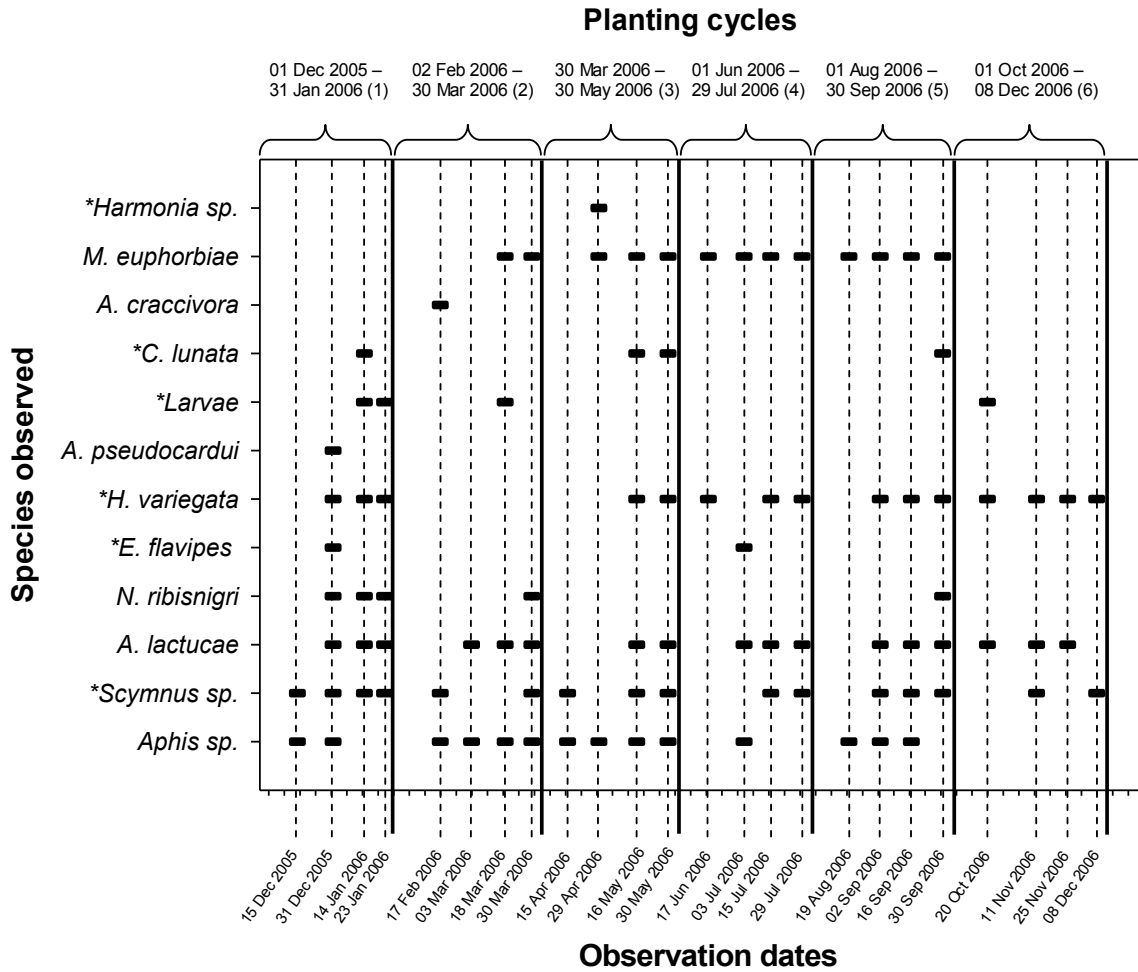
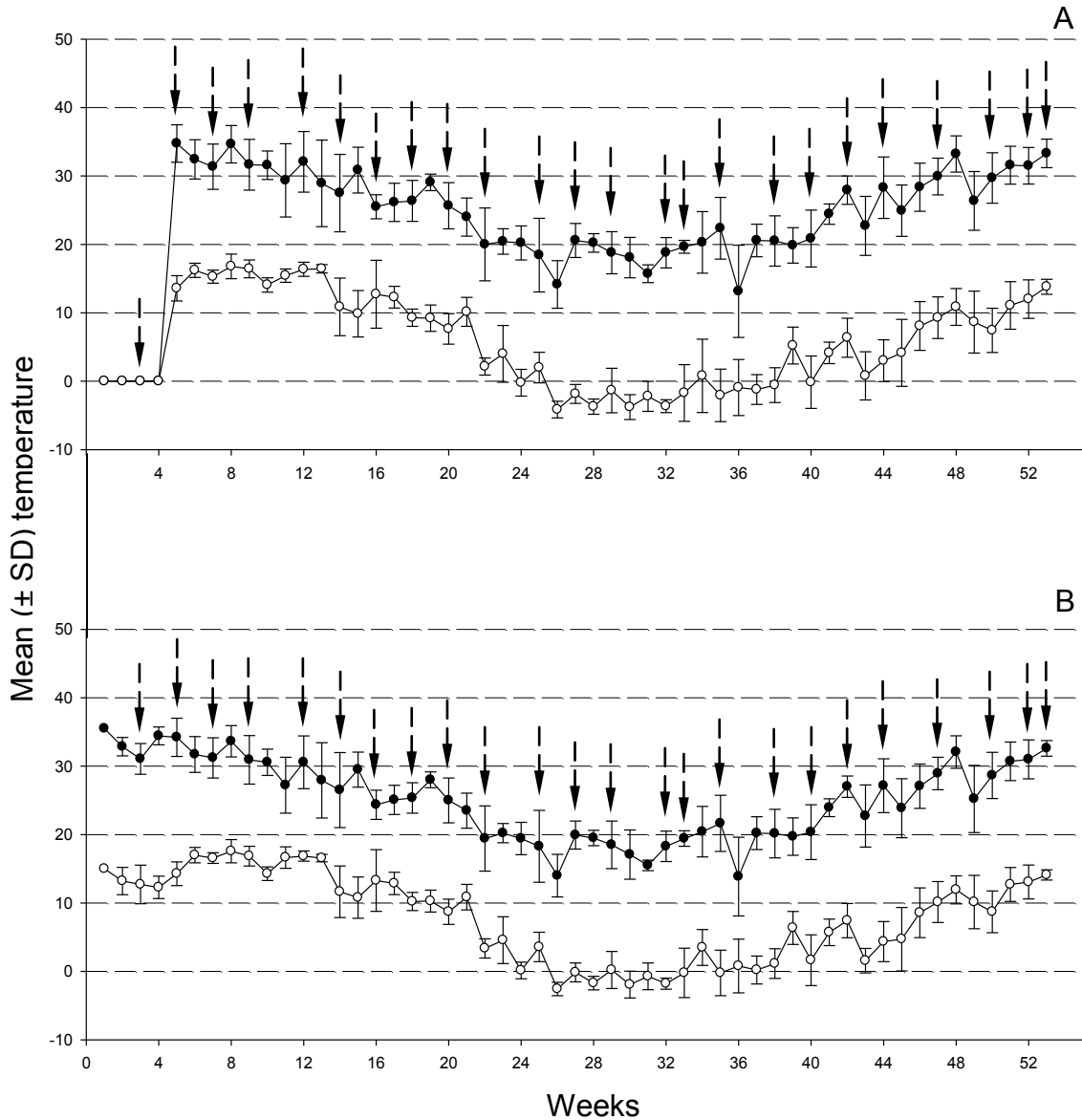


Figure 3.1: Seasonality and occurrence of aphid and coccinellid species observed on lettuce in the fully covered structure during the trial (Roodevallei, Free State Province).



* = Predatory Coccinellids

Figure 3.2: Seasonality and occurrence of aphid and coccinellid species observed on lettuce in the partially covered structure during the trial (Roodevallei, Free State Province).



Solid circles = maximum temperatures. Open circles = minimum temperatures

Figure 3.3: Weekly mean (\pm SD) maximum and minimum temperatures recorded from fully covered shadehouse structure (A) and partially covered shadehouse structure (B) (Roodevallei, Free State Province). Arrows indicate weeks in which sampling were conducted.

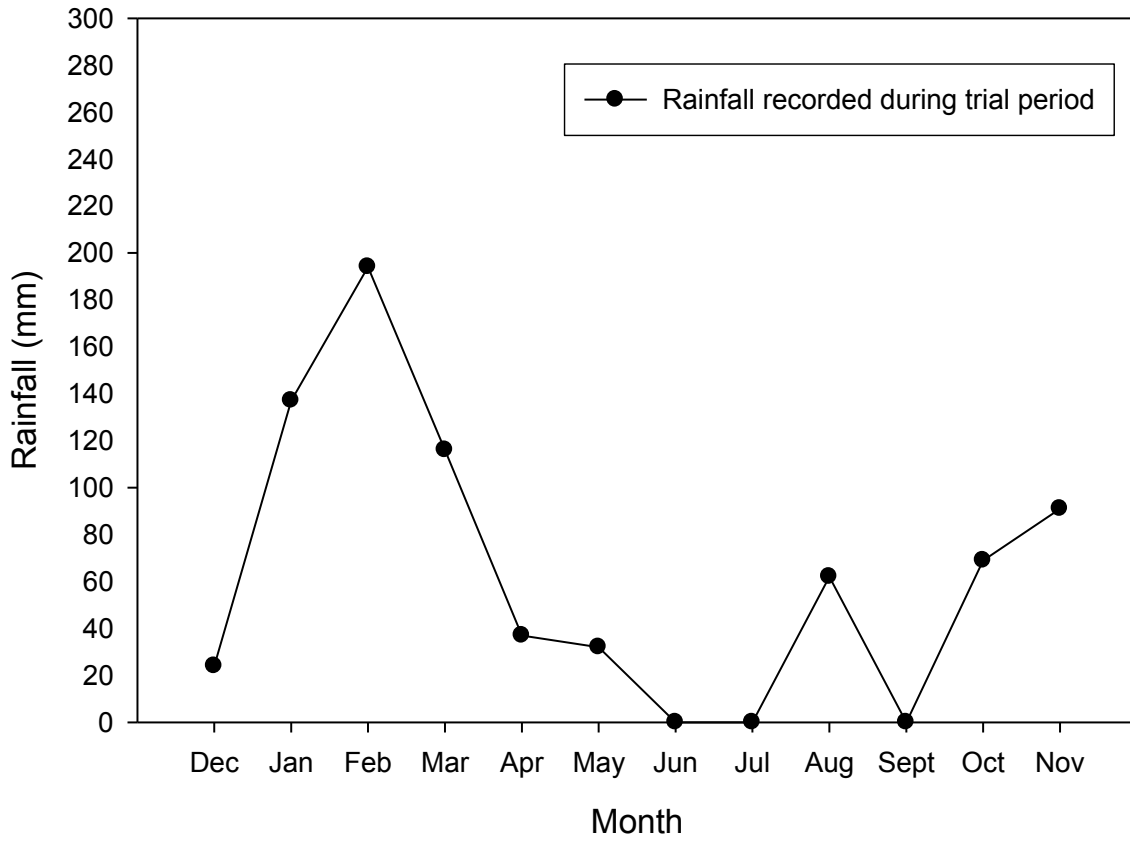


Figure 3.4: Rainfall measurements obtained during trial period (Roodevallei, Free State Province).

Table 3.1: Aphididae and Coccinellidae species observed from both shadehouse structures during the trial (Roodevallei, Free State Province).

Observed aphid species	Structure type	
	Fully covered	Partially covered
<i>Acyrtosiphon lactucae</i> (Passerini)	P	P
<i>Aphis craccivora</i> (Koch)*	A	P
<i>Aphis pseudocardui</i> (Theobald)*	P	P
<i>Aphis</i> sp. 1	P	P
<i>Macrosiphum euphorbiae</i> (Thomas)	P	P
<i>Myzus persicae</i> (Sulzer)*	P	A
<i>Nasonovia ribisnigri</i> (Mosley)*	P	P
<i>Rhopalosiphum nymphaeae</i> (Linnaeus)*	P	A
<i>Rhopalosiphum rufiabdominalis</i> (Sasaki)*	P	A

Observed predator species	Structure type	
	Fully covered	Partially covered
<i>Cheilomenes lunata</i> (Fabricius)*	P	P
<i>Exochomus flavipes</i> (Thunberg)	P	P
<i>Harmonia</i> sp. 1*	A	P
<i>Hippodamia variegata</i> (Goeze)	P	P
<i>Scymnus</i> sp. 1	P	P
Various larvae*	P	P

P = Present, A = Absent

*Seasonality observed for these particular species

Table 3.2: Sørensen’s coefficient of similarity used to determine similarity in occurrence for each individual species between the two shadehouse structures during the whole trial period, individual planting cycles and growth stages of the crop (Roodevallei, Free State Province).

	A: Whole trial period	B: Planting cycles						C: Growth stages			
	FC vs. PC	1	2	3	4	5	6	SS	EVG	LVG	HS
<i>lactuc</i>	0.944*	0.857*	1.000*	1.000*	1.000*	1.000*	0.857*	0.667*	1.000*	1.000*	0.909*
<i>crac</i>	-	-	-	-	-	-	-	-	-	-	-
<i>pseu</i>	1.000*	1.000*	-	-	-	-	-	-	1.000*	-	-
<i>aphis</i>	0.741*	0.667*	1.000*	0.667*	-	0.857*	-	0.889*	0.750*	0.800*	0.400
<i>euph</i>	0.640*	-	0.667*	0.500*	0.667*	1.000*	-	0.667*	0.400	0.500*	0.889*
<i>pers</i>	-	-	-	-	-	-	-	-	-	-	-
<i>ribis</i>	0.750*	1.000*	-	-	-	-	-	-	1.000*	1.000*	0.500*
<i>nymph</i>	-	-	-	-	-	-	-	-	-	-	-
<i>rufia</i>	-	-	-	-	-	-	-	-	-	-	-
<i>lun</i>	-	-	-	-	-	-	-	-	-	-	-
<i>flavi</i>	-	-	-	-	-	-	-	-	-	-	-
<i>harm</i>	-	-	-	-	-	-	-	-	-	-	-
<i>varie</i>	0.545*	0.500*	-	0.667*	-	-	1.000*	0.667*	0.800*	0.286	0.571*
<i>scym</i>	0.774*	1.000*	0.667*	-	0.800*	1.000*	0.800*	0.500*	0.750*	0.750*	0.909*
<i>lar</i>	0.545*	0.800*	-	-	-	-	0.500*	1.000*	-	0.500*	1.000*

lactuc = *A. lactucae*, *crac* = *A. craccivora*, *pseu* = *A. pseudocardui*, *euph* = *M. euphorbiae*, *pers* = *M. persicae*, *ribis* = *N. ribisnigri*, *nymph* = *R. nymphaeae*, *rufia* = *R. rufiabdominalis*, *aphis* = *Aphis* sp. 1, *lun* = *C. lunata*, *flavi* = *E. flavipes*, *harm* = *Harmonia* sp. 1, *varie* = *H. variegata*, *scym* = *Scymnus* sp. 1, *lar* = Coccinellid larvae, FC = Fully Covered Structure, PC = Partially Covered Structure, SS = Seedling Stage, EVG = Early Vegetative Growth, LVG = Late Vegetative Growth, HS = Heading Stage

* = Similarity in species occurrence

Table 3.3: Sørensen's coefficient of similarity between aphid and predatory coccinellid species associated with lettuce in the fully covered shadehouse structure (Roodevallei, Free State Province).

	lactuc	crac	pseu	euph	pers	ribis	nymph	rufia	aphis	lun	flavi	harm	varie	scym
crac	-													
pseu	0.100	-												
euph	0.710*	-	-											
pers	0.261	-	-	0.250										
ribis	0.273	-	0.500*	0.267	-									
nypm	0.100	-	-	-	-	-								
rufia	-	-	-	-	-	-	-							
aphis	0.563*	-	-	0.480	0.118	-	0.143	-						
lun	0.100	-	-	0.154	-	-	-	-	-					
flavi	0.190	-	-	0.143	-	-	-	-	-	0.667*				
harm	-	-	-	-	-	-	-	-	-	-	-			
varie	0.538*	-	0.250	0.316	0.182	0.200	0.250	-	0.200	0.250	0.444	-		
scym	0.824*	-	0.125	0.741*	0.105	0.333	-	-	0.500*	0.125	0.235	-	0.364	
lar	0.538*	-	0.250	0.421	-	0.600*	-	-	0.200	0.250	0.222	-	0.571*	0.455

lactuc = *A. lactucae*, crac = *A. craccivora*, pseu = *A. pseudocardui*, euph = *M. euphorbiae*, pers = *M. persicae*, ribis = *N. ribisnigri*, nypm = *R. nymphaeae*, rufia = *R. rufiabdominalis*, aphis = *Aphis* sp. 1, lun = *C. lunata*, flavi = *E. flavipes*, harm = *Harmonia* sp. 1, varie = *H. variegata*, scym = *Scymnus* sp. 1, lar = Coccinellid larvae

* = Similarity in species occurrence

Table 3.4: Sørensen's coefficient of similarity between aphid and predatory coccinellid species associated with lettuce in the partially covered shadehouse structure (Roodevallei, Free State Province).

	lactuc	crac	pseu	euph	pers	ribis	nymp	rufia	aphis	lun	flavi	harm	varie	scym
crac	-													
pseu	0.111	-												
euph	0.667*	-	-											
pers	-	-	-	-										
ribis	0.455	-	0.333	0.222	-									
nymp	-	-	-	-	-	-								
rufia	-	-	-	-	-	-	-							
aphis	0.581*	0.133	0.133	0.667*	-	0.211	-	-						
lun	0.381	-	-	0.353	-	0.444	-	-	0.222					
flavi	0.211	-	0.667*	0.133	-	0.286	-	-	0.250	-				
harm	-	-	-	0.143	-	-	-	-	0.133	-	-			
varie	0.813*	-	0.125	0.571*	-	0.400	-	-	0.345	0.421	0.118	-		
scym	0.727*	0.118	0.118	0.552*	-	0.476	-	-	0.600*	0.400	0.111	-	0.770*	
lar	0.381	-	-	0.118	-	0.444	-	-	0.111	0.250	-	-	0.316	0.200

lactuc = *A. lactucae*, crac = *A. craccivora*, pseu = *A. pseudocardui*, euph = *M. euphorbiae*, pers = *M. persicae*, ribis = *N. ribisnigri*, nymp = *R. nymphaeae*, rufia = *R. rufiabdominalis*, aphis = *Aphis* sp. 1, lun = *C. lunata*, flavi = *E. flavipes*, harm = *Harmonia* sp. 1, varie = *H. variegata*, scym = *Scymnus* sp. 1, lar = Coccinellid larvae

* = Similarity in species occurrence

CHAPTER 4

THE INFLUENCE OF CERTAIN BIOTIC AND ABIOTIC FACTORS ON THE POPULATION DYNAMICS OF IMPORTANT LETTUCE APHID PEST SPECIES AND THEIR NATURAL ENEMIES UNDER VARIABLE SHADEHOUSE CONDITIONS

Abstract

Introduction: Head lettuce is most commonly grown in South Africa and is cultivated under both open-field and protective structure conditions. When cultivated under protection, this crop is subject to aphid infestations throughout the year, with *Acyrtosiphum lactucae*, *Macrosiphum euphorbiae*, *Myzus persicae* and *Nasonovia ribisnigri* as the most common species. Both density-dependant and density-independent factors regulate the population size of these aphids throughout the different seasons. A study was conducted to determine the influence of certain abiotic- (temperature and rainfall) and biotic (Coccinellidae and Formicidae) factors on the population dynamics of these aphids throughout a one-year period (short term) under varying shadehouse conditions.

Methods: Six cycles of lettuce were planted (December 2005 – November 2006) under two different types of shadehouse structures in the central Free State, South Africa. One of the structures (fully covered structure) was designed to physically exclude the pugnacious ant, *Anoplolepis custodiens*, while the other did not have these restrictions (partially covered structure). Also, the fully covered structure was covered on all sides with shade netting. Aphid and coccinellid populations were assessed four times during each of the six planting cycles. Depending on the estimation of aphid numbers, the plants were placed in a specific aphid infestation class, while predator abundance was determined on the basis of their exact numbers. Maximum and minimum temperature data were collected daily, along with the rainfall measurements.

Results: Variations in aphid population size followed the same trend in both structures over time (time x structure interaction; Wilks' lambda = $P > 0.05$)

during planting cycles (PC) 2 (Feb 06 – Mar 06), 3 (Apr 06 – May 06), 4 (Jun 06 – Jul 06) and 5 (Aug 06 – Sep 06), but not during PC 1 (Dec 05 – Jan 06) and 6 (Oct 06 – Nov 06). Mean overall aphid infestation levels varied significantly ($P < 0.05$) between the two structures during PC 3 and 4 (more aphids in partially covered structure), and during PC 1 and 6 (more aphids in fully covered structure). Time had a significant effect on aphid population development/decline during all planting cycles (time effect; $P < 0.01$). The prevalence of the four important aphid species throughout the year was also determined. Variations in coccinellid numbers followed the same trend in both structures over time only during PC 2. Mean overall coccinellid numbers didn't differ statistically between the two structures during this time ($P > 0.05$). PC 1 and 6 differed in mean overall predator counts between the two structures (more predators in the fully covered structure). Planting cycles 3, 4, and 5 also differed in the mean overall number of coccinellids between the two structures (higher numbers in the partially covered structure). Coccinellid population growth over time was significant (time effect; $P < 0.01$) during all planting cycles, except for PC 2. The prevalence of different coccinellid species (and their larvae) throughout the year was also determined. Correlations between aphid and coccinellid numbers, and correlations between these organisms and certain abiotic factors are also discussed.

Conclusions: Insect and coccinellid activity on shadehouse cultivated lettuce will reach its highest levels during the warmer spring and summer months, and its lowest levels during winter months. The fully covered structure will harbour more aphids and coccinellid larvae during the summer months, while the reversed situation is true for the winter months (although larvae are then absent). The design of the structure will impact the microclimate of a structure, and also determine which aphid and coccinellid species (and life stages) will show the highest abundance during specific seasons of the year.

Key words: *A. lactucae*; *N. ribisnigri*; *M. euphorbiae*; *M. persicae*; Development; Temperature range; Rainfall; Ants; Coccinellidae; Interactions

4.1 INTRODUCTION

Production of lettuce is increasing in South Africa with head lettuce (also known as Crisphead or Iceberg lettuce) featuring as the most commonly cultivated cultivar (Stork *et al.*, 2001). It is grown under conditions ranging from open-field cultivation to protective cultivation. Just as in the case with open-field cultivation, head lettuce grown under protective structures (and particularly in shadehouse structures) in the central Free State, is prone to attack by various aphid species (see Chapter 3). The most notable of these are *Acyrtosiphon lactucae*, *Macrosiphum euphorbiae*, *Nasonovia ribisnigri* and *Myzus persicae*. All are recognized pests of cultivated lettuce in several other parts of the world as well (Blackman & Eastop, 2000; Palumbo *et al.*, 2000; Parker *et al.*, 2002), and their presence renders the lettuce heads unattractive, which, due to phytosanitary issues surrounding pest infestations, could lead to rejection of the crop in certain cases.

The success of aphids as phytophagous pests is the result of several unique physiological and morphological adaptations (Heie, 1987). It is for example known that aphid pest species have a shorter developmental time in which more energy is invested into reproduction (Llewellyn & Mohamed, 1982). The implication of this is that these species can reach high population levels in the minimum period of time (Heie, 1987; Dixon, 1998), even on short-season crops such as lettuce. The rate of aphid population growth is, however, governed by certain biotic and abiotic regulating factors. Density-independent abiotic factors such as temperature, humidity, host plant quality, rainfall, etc., all play an important role in aphid development and generation time, as well as in the production of sexual morphs (Dixon, 1971; Webb & Moran, 1978; Kawada, 1987; Walgenbach *et al.*, 1988; Acreman & Dixon, 1989; Bale, 1991; Satar & Yokomi, 2002; Bayhan *et al.*, 2005; Diaz *et al.*, 2007). Most aphid species display optimal development within a certain temperature range (Berg, 1984; Aldyhim & Khalil, 1993; Satar & Yokomi, 2002; Bayhan *et al.*, 2005;), while extreme high, as well as extreme low temperatures, can be detrimental (Chun-Sen *et al.*, 2004; Diaz & Fereres, 2005; Kuo *et al.*, 2006; Ghulam *et al.*, 2007).

Aphid colonies (i.e. high population numbers) have a biotic consequence in that they attract other arthropods, which, in turn, exert an influence on their population dynamics. These usually include predators, parasitoids, and honeydew-seeking insects. Parasitoids include parasitic wasps from several specialized families, while predators from several orders are known (Skaife, 1979). The best studied predators are the ladybird beetles (Coleoptera: Coccinellidae), which has widely been reported to affect the rate of aphid population growth and abundance (e.g. Kindlmann *et al.*, 2007). However, there is an extensive debate over the real impact that these insects exert on aphid populations. For instance, studies have shown that predators only have an impact during periods in which aphid population growth is slowed (Mackauer & Way, 1976; Snyder & Ives, 2003) or have declined from higher numbers (Dixon, 1971). Whatever their impact may be, coccinellid predators respond to aphid presence by showing increased abundance on crops infested by these pests, and by reproducing in the vicinity of aphid colonies (Donaldson *et al.*, 2007). Adult beetles deposit eggs in areas on the plant that would provide sufficient prey to the developing immature stages which themselves can have a larval period extending over several aphid generations (Kindlmann *et al.*, 2007). Synchronization with aphid populations might therefore be crucial for coccinellid predators to have any real impact on aphid populations, especially on short-season crops.

Aphid honeydew-seeking insects include ants (Formicidae) in the subfamilies *Dolichoderinae*, *Formicinae* and a few species of *Myrmicinae* (*Myrmica* and *Tetramorium*) (Kunkel *et al.*, 1985). The interaction between ants and aphids usually provides the classical example of mutualism (Way, 1963; Sudd, 1987; Flatt & Weisser, 2000; Stadler & Dixon, 2005), but ants do not always tend aphids. They are known to remove some of the aphids in certain cases, or they might even prey on them (Way, 1963; Kawada, 1987; Stadler & Dixon, 1999). Higher ant densities on crops infested with homopteran pests could also influence natural enemy abundance negatively, which could, in turn, lead to an increase in pest numbers (James *et al.*, 1999).

Studies on certain abiotic (rainfall and temperature) and biotic (presence/absence of coccinellids) factors influencing the abundance of aphid pest species, can help predict time windows in which larger populations can be expected. This part of the study is therefore aimed at investigating the impact of these factors on the population dynamics of the four prominent aphid pest species occurring on shadehouse cultivated lettuce, over a one-year period (short term) in the presence and absence of the pugnacious ant, *Anoplolepis custodiens*.

4.2 MATERIALS AND METHODS (Refer to Chapter 2)

Area of research and time frame: Refer to Chapter 2 (section 2.1)

Trial design and experimental layout: Refer to Chapter 2 (section 2.2, 2.3, 2.4 & 2.7)

Aphid sampling procedure: Aphid populations in both structures were assessed in the field by carefully probing for their presence on the pre-selected sample plants, followed by enumeration of the aphids (nymphs, winged and wingless adults combined). Subsequently, the aphid infestation level was expressed numerically for each plant individually by placing it in a specific infestation class (Table 4.1) according to the time of sampling (e.g. seedling stage, early vegetative growth, late vegetative growth, or heading stage). This is similar to the procedure followed by Parker *et al.* (2002). The exercise was repeated four times (referred to as the four sampling occasions/periods) during the course of each of the six planting cycles. The first three sampling occasions in each planting cycle were non-destructive to the plants, but during the last sampling occasion of each planting cycle, plants were physically removed from the soil and visually examined, a process referred to as the whole plant destructive sampling method. As it was impossible to identify the aphid species in the field, and to determine the population dynamics for each species individually, aphid numbers were pooled into a single unit in order to determine the aphid infestation class. However, in order to establish which species were present and also dominant during a particular sampling occasion, aphid samples were

collected from each plant that was sampled. The number of samples collected for each of the four important aphid pest species (*M. persicae*, *M. euphorbiae*, *N. ribisnigri* and *A. lactucae*) were quantified, while those of the economically less important aphid species were pooled (indicated as 'other'). The sampling dates for each of the six planting cycles are shown in Table 2.3 (Chapter 2), while Table 2.1 shows the cultivar and planting/harvesting dates for each of the six planting cycles.

Predator sampling procedure: Because predator numbers were lower than those of the aphids, they were not classed, but fully enumerated during each sampling occasion and numbers were not estimated. All predators observed were identified to species level in the field. Larvae were sampled as a single unit because of their high abundance during certain planting cycles and also because of the difficulty in identifying them up to species level in the field.

***Anoplolepis custodiens*:** Due to disturbances in their natural occurrence on account of weed removal and shadehouse construction, the presence of pugnacious ants had to be encouraged in the immediate vicinity of the partially covered structure. This was accomplished by placing large stones at most of the perimeter poles of the structure, two months prior to the initiation of the trial. The ants did establish nests under some of the stones and their absence/presence within the structure was noted during the individual sampling occasions. Disturbances to the nest areas were avoided for the duration of the trial.

Collection of rainfall and temperature data: Refer to Chapter 2 (section 2.6)

Statistical analysis: Aphid and coccinellid population dynamics were noted over four sampling occasions during each planting cycle, and therefore the data were analyzed using repeated measures analysis (MANOVA). GLMProc, with the repeated option, was used to complete the MANOVA (SAS, 2004). Pearson's correlation was employed for comparing aphid and coccinellid populations with

rainfall and temperature data, and also to compare aphid populations with those of the coccinellid predators (SAS, 2004).

4.3 RESULTS

Mean aphid infestation levels: Variations in aphid population size over time showed a similar trend in both structures during planting cycles (henceforth referred to as PC) 2 (time x structure interaction; Wilks' lambda = 0.98, $F = 0.31$, $P = 0.8214$; Figure 4.1C), 3 (Wilks' lambda = 0.84, $F = 2.81$, $P = 0.0506$; Figure 4.1E), 4 (Wilks' lambda = 0.84, $F = 2.79$, $P < 0.0518$; Figure 4.1G), and 5 (Wilks' lambda = 0.90, $F = 1.61$, $P = 0.2012$; Figure 4.1I). The two structures also did not differ statistically from each other in mean overall aphid infestation levels during PC 2 ($F = 0.06$, $df = 1$, $P = 0.8132$) and 5 ($F = 1.26$, $df = 1$, $P = 0.2668$). PC 5 was the only occasion in which mean infestation levels attained a value of more than 1 in the partially covered structure (mean \pm SD = 2.5417 ± 1.021). However, mean overall aphid infestation levels varied significantly between the two structures during PC 3 ($F = 7.67$, $df = 1$, $P = 0.0081$) and PC 4 ($F = 4.96$, $df = 1$, $P = 0.0308$), although remaining relatively low (peaking at 0.9167 ± 0.2823 during PC 3, and 0.7917 ± 0.4149 during PC 4). The partially covered structure mostly attained higher aphid infestation levels here. Variations in aphid population size over time did not show a similar trend between the structures during planting cycles 1 (Wilks' lambda = 0.34, $F = 28.68$, $P < 0.0001$; Figure 4.1A) and 6 (Wilks' lambda = 0.31, $F = 33.06$, $P < 0.0001$; Figure 4.1K) due to higher infestations in the fully covered structure. Both these planting cycles also differed significantly in overall mean aphid infestation levels between the two structures ($F = 89.31$, $df = 1$, $P < 0.0001$ & $F = 114.93$, $df = 1$, $P < 0.0001$, respectively). The highest aphid infestation class (mean \pm SD) was observed in the fully covered structure during PC 1 (4.4167 ± 1.139). Time had a significant effect on aphid population development/decline during all planting cycles, and in all cases, highly significant values (time effect; $P < 0.01$) were obtained.

Aphid species abundance: A total of $n = 674$ aphid specimens were collected during the trial, 59.35% ($n = 400$) from the fully covered structure and 40.65%

($n = 274$) from the partially covered structure (Table 4.2). *A. lactucae* represented the most samples collected during PC 1 ($n = 87$; 67.97%), PC 2 ($n = 9$; 47.37%), PC 3 ($n = 27$; 58.70%), PC 4 ($n = 18$; 54.55%) and PC 6 ($n = 58$; 96.67%) in the fully covered structure (Figure 4.2A). It was only dominant during PC 6 in the partially covered structure, representing 100% of the collected samples ($n = 11$), and also during PC 2 ($n = 9$; 60%) if the economically unimportant species are not considered (Figure 4.2B). *N. ribisnigri* was the second most collected species in the fully covered structure during PC 1 ($n = 32$; 25%), but the most collected species in the partially covered structure during this time ($n = 32$; 68.09%) (Figure 4.2). It was absent from the fully covered structure during subsequent planting cycles. Even though *M. euphorbiae* had the most samples collected only during PC 5 ($n = 52$; 45.61%) in the fully covered structure (present during all planting cycles), it had the most samples collected during PC 3 ($n = 32$; 69.57%), PC 4 ($n = 29$; 80.56%) and PC 5 ($n = 86$; 80.37%) in the partially covered structure (Figure 4.2). *M. persicae* was only observed during PC 3 to PC 5 in the fully covered structure with few samples collected (maximum of $n = 3$) (Figure 4.2A). Non-significant aphid species (indicated as 'other') were present in both structures (Figure 4.2), but are not considered due to their non-pest status on lettuce.

Mean predator counts: Variations in coccinellid numbers showed a similar trend in both structures over time during PC 2 (time x structure interaction; Wilks' lambda = 0.89, $F = 1.79$, $P = 0.1632$; Figure 4.1D). Mean overall coccinellid numbers also did not differ statistically between the two structures ($F = 0.41$, $df = 1$, $P = 0.5270$) during this time, and their numbers (mean \pm SD) stayed consistently low (maximum of 0.2083 ± 1.021 predators/plant in the partially covered structure and 0.2500 ± 0.6079 predators/plant in the fully covered structure). Variations in coccinellid numbers over time did not show a similar trend between the structures during PC 1 (Wilks' lambda = 0.66, $F = 7.53$, $P = 0.0004$; Figure 4.1B), PC 3 (Wilks' lambda = 0.78, $F = 4.04$, $P = 0.0128$; Figure 4.1F), PC 4 (Wilks' lambda = 0.70, $F = 6.20$, $P = 0.0013$; Figure 4.1H), PC 5 (Wilks' lambda = 0.80, $F = 3.72$, $P = 0.0182$; Figure 4.1J), and PC 6 (Wilks'

lambda = 0.38, $F = 24.25$, $P < 0.0001$; Figure 4.1L). PC 1 and PC 6 were the only two occasions during which mean coccinellid numbers reached an average of above 1 predator/plant. Both these planting cycles differed significantly in mean overall predator counts between the fully covered and partially covered structures ($F = 64.49$, $df = 1$, $P < 0.0001$ and $F = 12.94$, $df = 1$, $P = 0.0008$, respectively), due to more predators in the fully covered structure. The third sampling period of PC 6 (fully covered structure) had the highest coccinellid count for the trial (14.917 ± 8.328 predators/plant), but (as with the aphids) their numbers plunged dramatically from sampling period three to sampling period four. Planting cycles 3 ($F = 12.76$, $df = 1$, $P = 0.0008$), 4 ($F = 12.20$, $df = 1$, $P = 0.0011$), and 5 ($F = 9.80$, $df = 1$, $P = 0.0030$) also differed in the mean overall number of coccinellids between the two structures, but due to higher numbers in the partially covered structure (same situation as with aphid infestations). Coccinellid population growth over time was significant (time effect; $P < 0.01$) during all planting cycles, except during PC 2 (Wilks' lambda = 0.91, $F = 1.39$, $P = 0.2598$) when predator numbers stayed relatively constant.

Coccinellid species abundance: A total of 908 coccinellid specimens (adults and larvae) were observed during the trial (Table 4.2). Of these, $n = 659$ (72.58%) were observed in the fully covered structure ($n = 126$ [19.12%] as adults, and $n = 533$ [80.88%] as larvae), and $n = 249$ (27.42%) from the partially covered structure ($n = 173$ [69.48%] as adults, and $n = 76$ [30.52%] as larvae). High predator numbers in the fully covered structure during PC 1 and PC 6 can be ascribed to more coccinellid larvae (Figure 4.4). Adult coccinellid numbers were higher in the partially covered structure during PC 1 ($n = 23$ vs. $n = 43$), PC 3 ($n = 3$ vs. $n = 27$), PC 4 ($n = 3$ vs. $n = 26$) and PC 5 ($n = 6$ vs. $n = 33$). *Scymnus* sp. 1 and *Hippodamia variegata* were substantially the most abundant coccinellids observed in both structures (Figure 4.3). Other coccinellid species also showed occasional higher abundance, with *Cheilomenes lunata* (PC 1: partially covered structure) and *Exochomus flavipes* (PC 6: fully covered structure) the most noticeable in this regard (Figure 4.3).

Relationship between aphid and coccinellid population dynamics: PC 1 showed a positive correlation ($P < 0.05$) between aphid infestation levels and coccinellid numbers, but during PC 2, this was only the case during the last sampling occasion ($r = 0.67331$, $P < 0.0001$). During PC 4, predator numbers stayed low in the fully covered structure, but a correlation was witnessed during the third sampling period ($r = 0.31708$, $P = 0.0281$). Only the third sampling period of PC 6 showed a significant correlation between aphid and predator numbers ($r = 0.72912$, $P < 0.0001$).

Correlation between abiotic factors with aphid and coccinellid populations: Rainfall measured during each planting cycle is depicted in Table 4.3. Rainfall did not correlate with either aphid ($r = 0.17657$, $P = 0.4092$), or predator ($r = -0.02037$, $P = 0.9247$) numbers in the fully covered structure, nor with aphid ($r = -0.24343$, $P = 0.2517$) and predator ($r = 0.10722$, $P = 0.6180$) numbers in the partially covered structure during the entire trial. Acknowledging these results as an indication that their population numbers are not influenced by rainfall, would be a mistake. During PC 2, rainfall was exceptionally high (mean \pm SD = 77.500 ± 57.797), while aphid and predator numbers were low for that time of year. Recorded mean (\pm SD) maximum and minimum temperatures are also depicted in Table 4.3. Mean maximum temperatures never differed statistically ($P > 0.05$) between the two structures, but the fully covered structure had a consistently higher maximum temperature range (Table 4.3). Maximum temperature correlated with both aphid ($r = 0.50635$, $P = 0.0137$) and predator ($r = 0.41379$, $P = 0.0497$) numbers in the fully covered structures, but only with predator numbers in the partially covered structure ($r = 0.40880$, $P = 0.0473$). Differences in mean minimum temperatures between the structures were recorded during PC 4 and PC 5 (Table 4.3). The fully covered structure had a lower temperature range throughout the trial. In both structures, no correlations ($P > 0.05$) were observed between minimum temperatures and aphid and predator numbers.

4.4 DISCUSSION

It is normal for aphid and coccinellid numbers to increase during the warmer months of the year (Campbell *et al.*, 1974), explaining the correlation observed with maximum temperatures. Similarly, Aheer *et al.* (2007) also observed a correlation between maximum temperatures and aphid populations with wheat-infesting aphids. The higher numbers of both aphids and coccinellids, also explains why coccinellid and aphid numbers correlated with each other during the whole of PC 1. However, differences in the overall aphid infestation levels, and dissimilarities in aphid population growth trends between the two structures over the four sampling occasions of PC 1 and 6, suggests that something altered their population growth during these two periods. This is evident from higher aphid populations in the fully covered structure which reached the highest infestation levels during PC 1, while infestation levels remained low in the partially covered structure. Frazer *et al.* (1981) also reported a significant increase (five times higher) of cage-covered aphid populations as opposed to non-covered populations on alfalfa. Donaldson *et al.* (2007) made a similar observation with *Aphis glycines* on soybeans, when caged aphid populations increased with 500% to near 1000% their original densities. Rainfall and temperature were most likely not responsible for these differences, because neither maximum nor minimum temperatures differed significantly between the two structures during these two planting cycles, a phenomenon also observed by Frazer *et al.* (1981) who measured and compared the temperature in field cages. Furthermore, it is doubtful whether host plant quality played a role, because fertigation was similar for both structures. However, due to plant and soil analysis not being conducted, this it is not absolutely certain. The differences were probably a function of certain density-dependant responses to aphid presence, most likely the effects of crowding, the prevalence of different life stages of natural enemies, and the presence of pugnacious ants (particularly *A. custodiens*) in the partially covered structure.

Crowding usually results in the dispersal of winged morphs due to competition and lowered food quality (Donaldson *et al.*, 2007). It is plausible to suggest that

most of the dispersing aphids in the fully covered structure returned to the plants within the structure after being disorientated by the shade netting covering the sides. Here they would reproduce and contribute to the infestation levels (Donaldson *et al.*, 2007). Dispersing aphids in the partially covered structure were not hindered in this way. Higher aphid numbers in the fully covered structure in turn led to the positive responses displayed by the coccinellids (also observed by Rondon *et al.*, 2005; Donaldson *et al.*, 2007), mainly due an increase in their larvae. Adult coccinellids breed during the warmer months, and even though they were less abundant in the fully covered structure during most planting cycles, some adults would have eventually found their way into the structure in order to reproduce. More prey would sustain more predators, and larvae are apt to survive under such crowded conditions where they can become more abundant than the adults (Donaldson *et al.*, 2007). More larvae in the fully covered structure were therefore responsible for the observed difference in overall coccinellid numbers during these two planting cycles, and also for the different trend in coccinellid population development over time between the two structures. Palumbo (2002b) found that coccinellid larvae with an abundance of 3 predators/plant did not give satisfactory control to aphid populations explaining why aphid populations kept increasing in the fully covered structure during PC 1, despite the higher larval numbers. Another possibility for the lack of control might have been poor synchronization with aphid populations. Ladybird beetle larvae were more successful in controlling aphid populations during PC 6 in the fully covered structure where their numbers correlated with those of their prey during the third sampling period, leading to the sharp decline in aphid numbers. A similar situation was observed by Fox *et al.* (2004). As expected, coccinellid numbers also declined as a consequence of fewer prey, because prey abundance directly influences survival, growth and reproduction of these insects.

The question then arises why fewer larvae were present in the partially covered structure during these planting cycles? This can be contributed to the presence of *A. custodiens* (Figures 4.2 & 4.3). In addition to the dispersing of alates from this structure, these ants could have decreased aphid numbers either through

predation, disturbance, or a combination of both (Way, 1963; Buckley, 1987; Nelson *et al.*, 2004). This in turn would have led to less prey available to sustain the coccinellid larvae. However, more controlled studies are required to establish exactly how these ants influence the aphid species under discussion before a definite conclusion can be made. A myriad of factors could have influenced the behaviour of the ants towards the aphids on the lettuce crop, e.g. protein needs vs. carbohydrate needs, drought stress, colony size, species present, effect of host quality on honeydew, distance of colonies from ant nests, etc. (Itioka & Inoue, 1996; Sakata, 1999; Stadler & Dixon, 2005). Their behaviour might therefore differ annually depending on the status of such factors. It was witnessed that nearby colonies of the same ant species attended honeydew-producing insects (Aphididae and Membracidae), indicating that these ants do indeed collect honeydew (Skaife, 1979). However, upon closer investigation, it was confirmed that the ants from the trial area carried lettuce pest aphids into their nests, although it was not determined if these aphids were alive or not. Despite this, it is strongly suspected that *A. custodiens* were also responsible for smaller aphid colonies, highlighting the fact that their presence must not always be regarded as a negative factor. However, these aggressive ants would also attack/remove eggs and larvae of coccinellids which is a negative factor, and which could also have led to lower coccinellid larval numbers. Adult ladybird beetles are better adapted to withstand attack from ants, and can also readily take to the air when threatened. This, coupled to the difficulty of entering the fully covered structure due to their relatively larger size (refer to Chapter 3), explains why significantly more adult coccinellids were observed in the partially covered structure during planting cycles 3, 4 and 5, and also why the variation in their numbers differed significantly between the two structures over time during these planting cycles.

Aphid and coccinellid numbers would be lower during the cooler months of the year (Legrand *et al.*, 2004), explaining the lack of correlation between minimum temperatures and these organisms. A similar trend in aphid population development over the four sampling occasions of PC 2 to 5 was observed in both

structures. This is especially true for PC 2 and PC 5 in which there was a significant similarity in overall infestation levels. Both these planting cycles experienced favourable and constant temperature ranges for aphid development in both structures, but infestation levels were low during PC 2. Thus, temperature could not have been responsible, but rather the high rainfall experienced during this time. Leite *et al.* (2007) also report that rainfall can negatively impact aphid populations in the field with their studies on *Aphis gossypii*, strengthening this assumption. The higher rainfall also negatively impacted coccinellid numbers, resulting in a situation where their numbers stayed consistently low and similar throughout all the sampling occasions of this planting cycle. The correlation observed between aphids and predators here during the last sampling period, was probably a consequence of low numbers observed for both organisms, rather than a linear increase in their numbers, and is therefore a mere coincidence. Higher aphid numbers (which were similar in both structures) during PC 5 (where it also reached its highest level in the partially covered structure) were the result of spring populations of aphids developing rapidly in both structures, especially since natural predators were still lacking or were not yet abundant (Gutierrez *et al.*, 1980), as was indeed the case with the pugnacious ants and coccinellids.

Despite aphid population growth/decline following the same trend during PC 3 and PC 4 over time in both structures, something must have been responsible for the overall significant higher aphid populations in the partially covered structure, which is opposite to the observations made during the warmer months of PC 1 and PC 6. This is ascribed to the absence of *A. custodiens* and the coccinellid larvae which were present during PC 1 and PC 6, demonstrating the potential of this structure to result in increased infestation levels. *A. custodiens* would have retreated to their subterranean nests for the winter, while there would be little or no reproduction of coccinellids during this time. Adult coccinellids were relatively more abundant in the partially covered structure during these two planting cycles, also presumably due to the absence and/or lower occurrence of the pugnacious ants and the subsequent availability of more prey. The fact that the partially

covered structure was warmer than the fully covered structure during the winter months (and especially during PC 4), could also account for higher overall aphid and adult coccinellid numbers in this structure during these two planting cycles.

During PC 1, the dominant *N. ribisnigri* in the partially covered structure had a better chance of escaping ant and coccinellid predation, due to their habit of feeding at the heart of the plant (Liu, 2004). This would have made them less susceptible to attack compared to the dominant *A. lactucae* in the fully covered structure which leads a more exposed existence where it feeds on the older wrapper (outer) leaves (personal observations). The overall dominance of *A. lactucae* in the fully covered structure suggests that the occupation of specific niches could give rise to certain species dominating a habitat in response to small changes in the agro-ecosystem, in this case the absence of *A. custodiens*. These aphids are also able to better withstand the more humid conditions experienced within the fully covered structure due to a waxy coating covering their bodies. The high rainfall during PC 2 therefore allowed populations of this species to survive in both structures, due to reduced foraging of coccinellids and ants. The marginally higher and lower temperatures experienced in this structure, could also be an indication of their ability to better withstand such conditions. The absence of *N. ribisnigri* during the cooler months could be related to their heteroecious holocyclic lifestyle, and their optimum developmental temperature range which has been recorded to be around 18°C - 21°C in the field (Palumbo, 2002a). This was consistent with the average temperature experienced during PC 1 in the partially covered structure ($\pm 23^{\circ}\text{C}$). Another study by Parker *et al.* (2002) also indicated this species to be dominant only during the summer months on lettuce. *M. euphorbiae* exhibited more tolerance to the low autumn and winter temperatures (anholocyclic in the region) and took up the available niche in the partially covered structure where it became dominant from PC 3 to 5. During PC 3 and PC 4, mean maximum temperatures fluctuated between 18.87°C and 21.15°C in the partially covered structure, while the mean minimum temperature was observed at -0.31°C. Barlow (1962) found that the threshold for development of this species lies at -0.03°C, while a linear developmental curve is found

between 5°C and 25°C, explaining why this species were dominant during these planting cycles. Another possibility for their superiority in this structure in particular might be the absence (or lower presence) of natural occurring enemies (coccinellid larvae and ants) during these two planting cycles. Karley *et al.* (2003) proved that the numbers of this species can be reduced by up to 68% in the presence of abundant predators. The appearance of *M. persicae* in the fully covered structure during the cooler months of the third planting cycle is related to the life history of this species (see discussion in Chapter 3), rather than an avoidance of the high temperatures experienced during the summer months. Indeed, studies have shown that population growth of *M. persicae* is only negatively influenced at 31.6°C– 42.3°C (Tamaki *et al.*, 1980), a temperature range only exceeded once during PC 1. Despite this, they are well-known pests in regions experiencing mild temperatures and can therefore withstand low temperatures (Leite *et al.*, 2002).

Both *H. variegata* and *Scymnus* sp. 1 were the predominant predators throughout the trial, while other species (*C. lunata* and *E. flavipes*) only became abundant during the warmer months when aphid populations were higher. However, the impact of these coccinellids might be significant during these times and require further investigation into their suitability as biocontrol agents of aphid pests.

4.5 CONCLUSIONS

Insect and coccinellid activity on shadehouse cultivated lettuce will reach its highest levels during the warmer spring and summer months, and its lowest levels during winter months. A fully covered shadehouse structure into which *A. custodiens* is denied access, and into which adult coccinellids find it harder to access the structure due to their larger body size, favour aphid population development during the warmer months. Also the design of the structure makes it difficult for alatae to leave the structure, contributing to population growth. Absence of pugnacious ants in this structure, coupled to a higher humidity level and both a lower and higher temperature range than the partially covered

structure, seemed to favour the aphid *A. lactucae*. The absence of these ants also allowed more coccinellid larvae to survive due to the availability of more prey, as well as the fact that the ants could not kill or remove the coccinellid larvae as such. The holocyclic *N. ribisnigri* was better adapted to survive the presence of this ant in the partially covered structure due to their cryptic feeding habits, but was replaced by the anholocyclic *M. euphorbiae* during the cooler months. A partially covered structure will in turn experience higher aphid infestation levels during the winter months (but not nearly as high as those of a fully covered structure in the summer months), in part due to the absence of pugnacious ants, and in part due to a more tolerable temperature range. Some predators (i.e. *H. variegata* and *Scymnus* sp. 1) were constantly associated with the aphid pest species, while others (i.e. *C. lunata* and *E. flavipes*) only showed increased abundance during periods in which aphid populations peaked.

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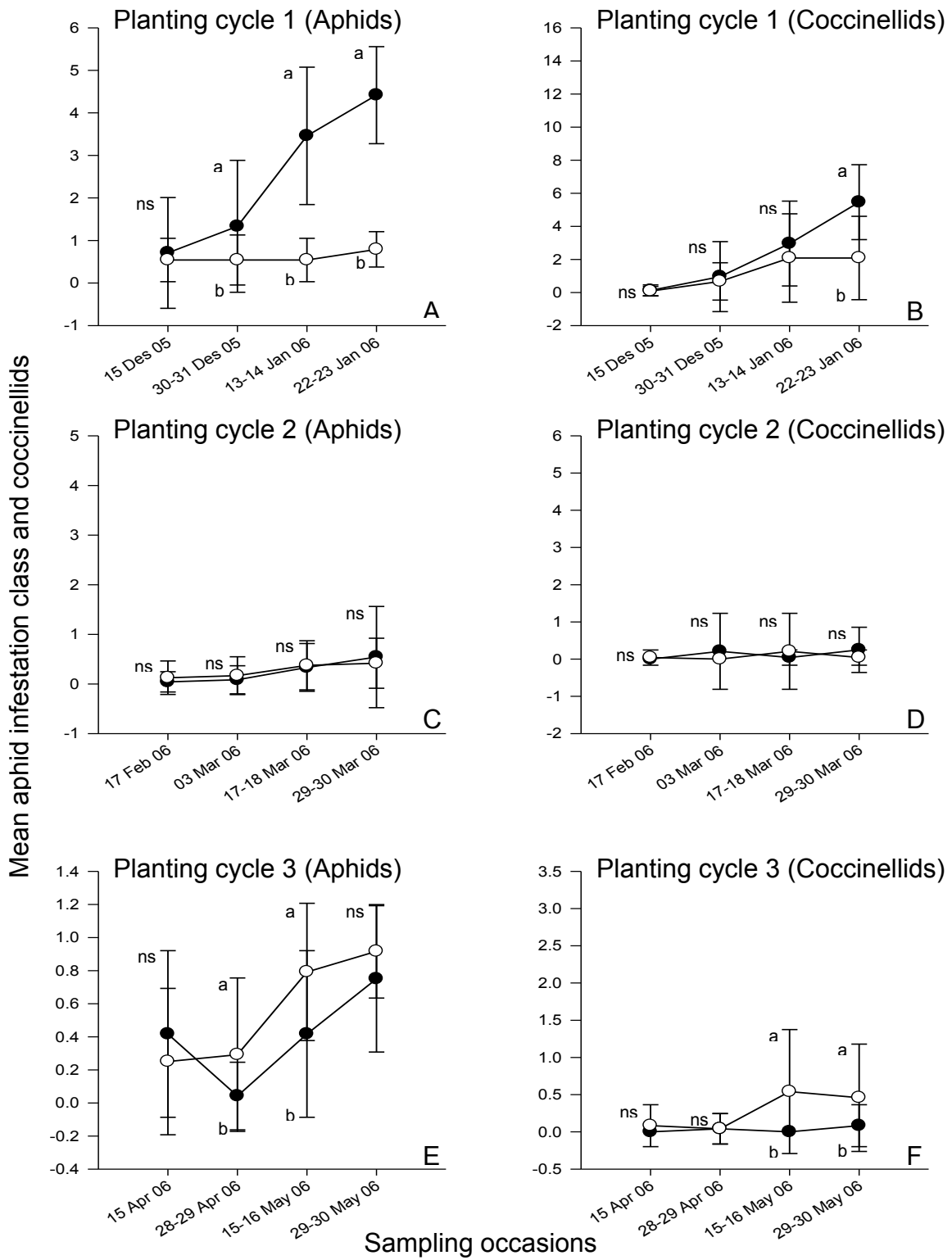
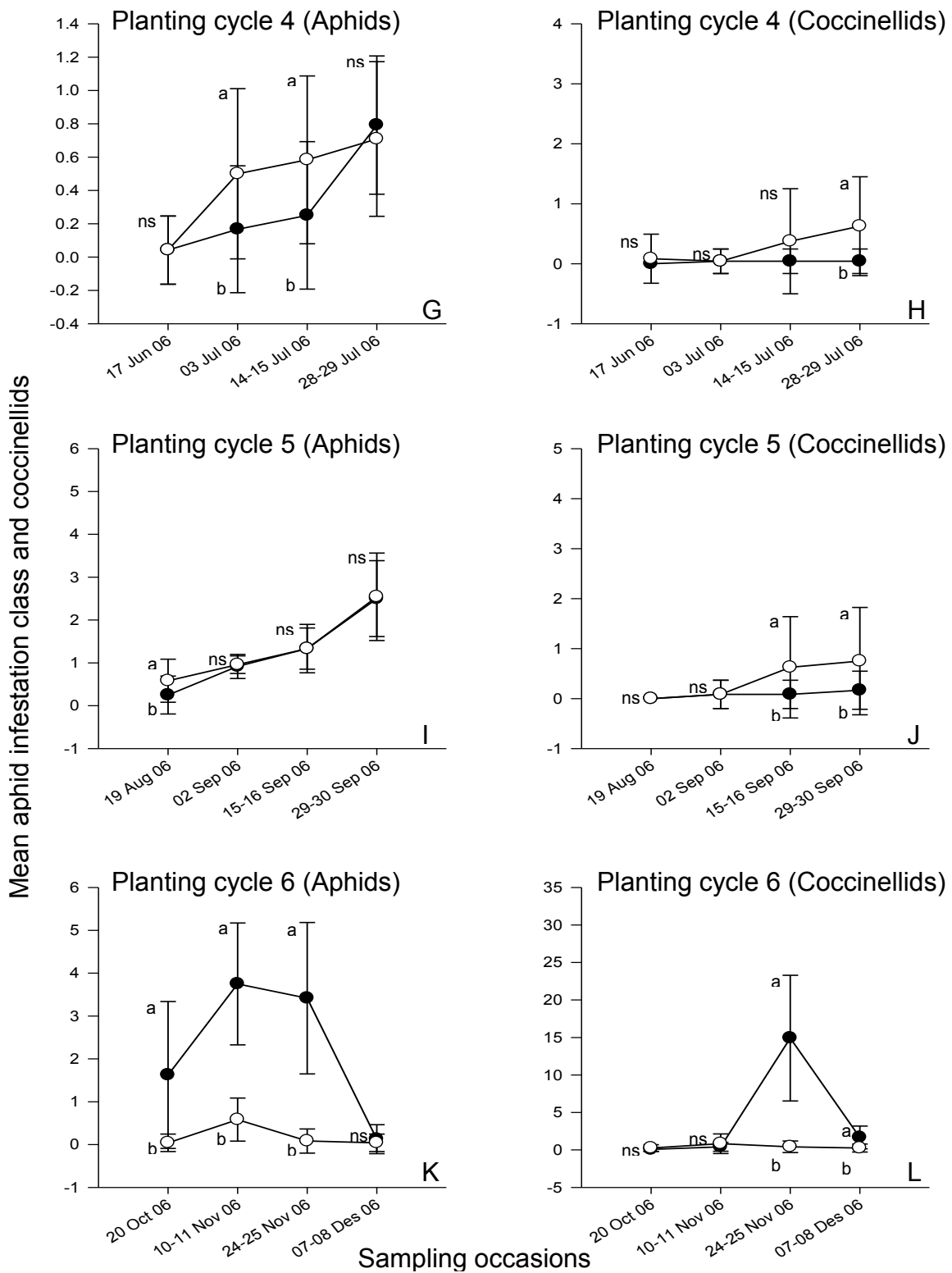


Figure 4.1: Continued on next page.



Closed circles = fully covered structure; Open circles = partially covered structure. ns = non significant. Means followed by different letters on the same sampling date are significantly different at $P < 0.05$.

Figure 4.1 (continued from previous page): Aphid infestation classes and predator numbers (mean \pm SD) observed during each of the six individual planting cycles (Roodevallei, Free State Province).

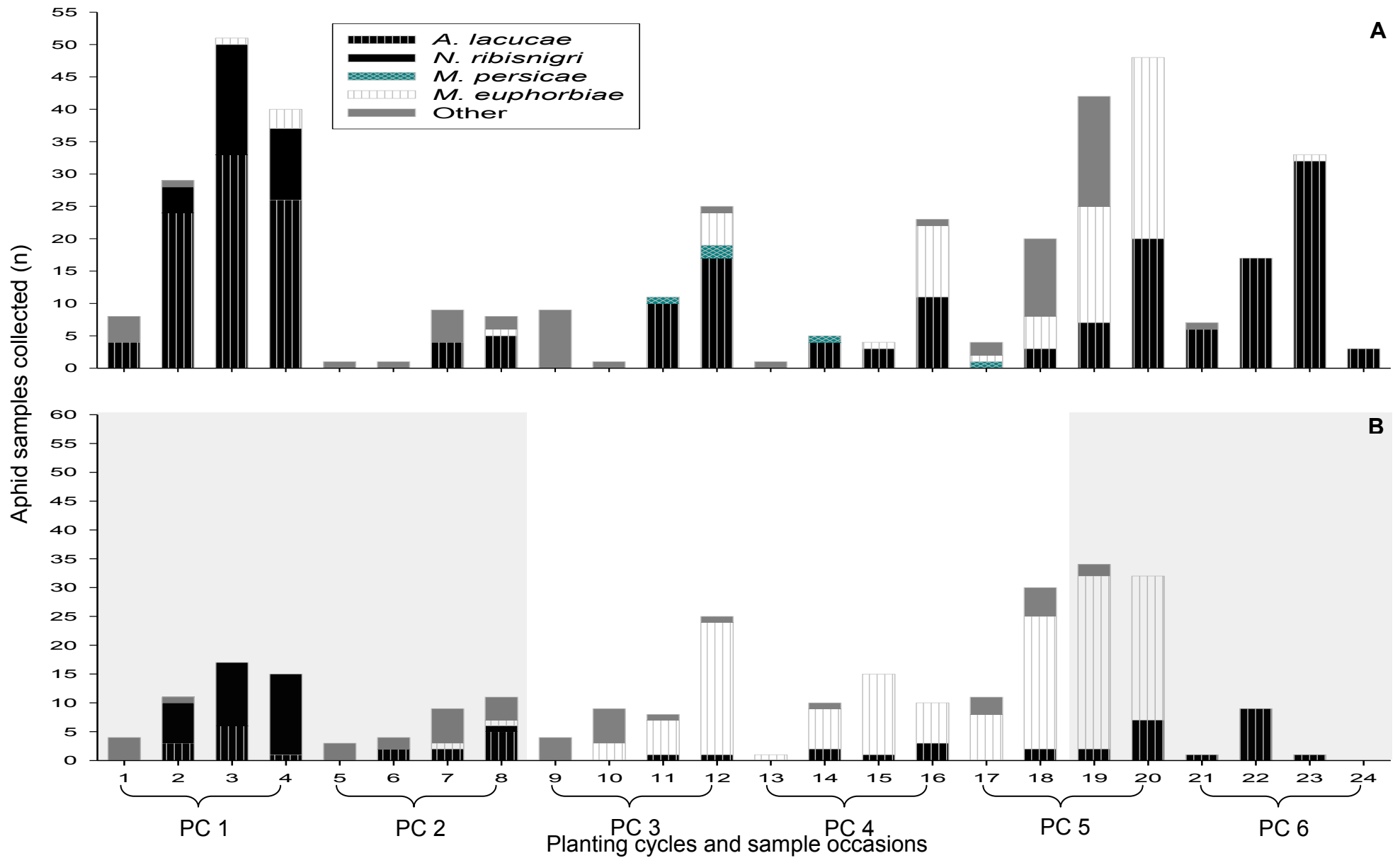


Figure 4.2: Species and number of aphid samples collected from fully covered structure (A), and partially covered structure (B) (Roodevallei, Free State Province). Shaded areas indicate the presence of *A. custodiens*.

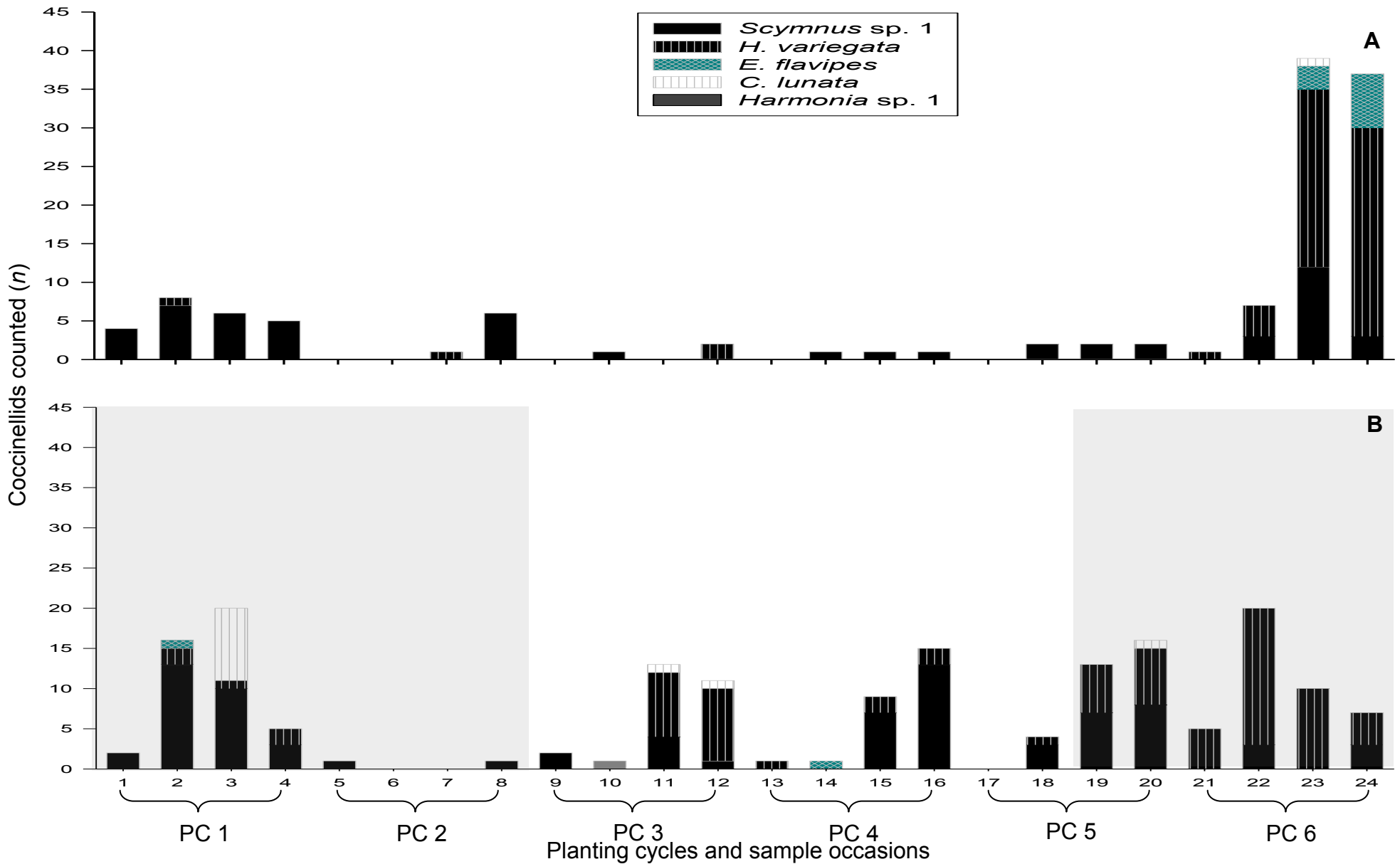


Figure 4.3: Species and number of coccinellid predators counted from fully covered structure (A), and partially covered structure (B) (Roodevallei, Free State Province). Shaded areas indicate the presence of *A. custodiens*.

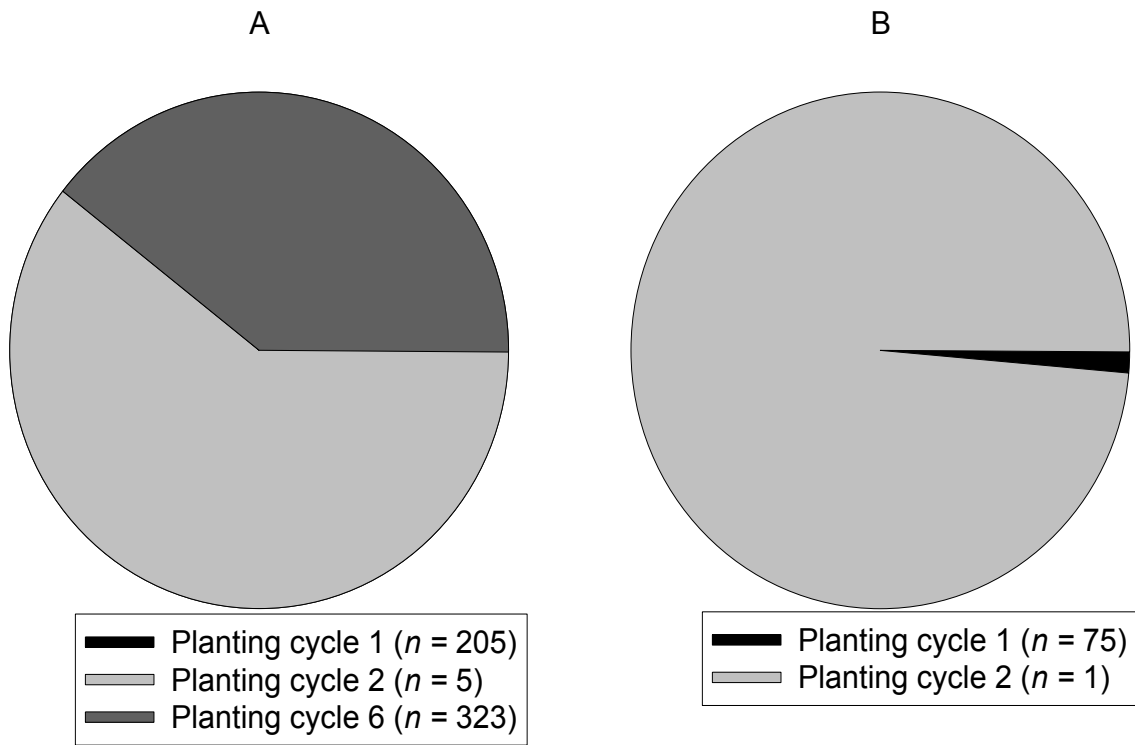


Figure 4.4: Number of predatory coccinellid larvae observed during different planting cycles in the fully covered structure (A), and partially covered structure (B) (Roodevallei, Free State Province).

Table 4.1: Different growth stages of lettuce showing corresponding aphid infestation classes (Roodevallei, Free State Province).

Growth stage	Infection level/class	Number of aphids
Seedling Stage	0	0
	1	1-10
	2	11-20
	3	21-30
	4	31-40
	5	> 41
Early Vegetative Growth Stage	0	0
	1	1-50
	2	51-100
	3	101-150
	4	151-200
	5	> 201
Late Vegetative Growth Stage	0	0
	1	1-100
	2	101-200
	3	201-300
	4	301-400
	5	> 401
Heading Stage	0	0
	1	1-100
	2	101-200
	3	201-300
	4	301-400
	5	> 401

Table 4.2: Number of aphid samples collected, and actual predator counts during each of the six planting cycles (Roodevallei, Free State Province).

Group	Planting cycle					
	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Aphids						
FCS	128	19	46	33	114	60
PCS	47	27	46	36	107	11
Predators						
FCS	228	12	3	3	6	407
PCS	118	2	27	26	33	43

FCS = Fully Covered Structure, PCS = Partially Covered Structure, PC = Planting Cycle

Table 4.3: Mean (\pm SD) maximum and minimum temperatures and recorded rainfall measured during each planting cycle (Roodevallei, Free State Province).

	Max. Temperature ($^{\circ}$ C)			Min. Temperature ($^{\circ}$ C)			Rainfall (mm)
	FCS	PCS	<i>P</i>	FCS	PCS	<i>P</i>	
PC 1	33.069 \pm 3.079 ^a	32.448 \pm 2.807 ^a	0.4258	15.621 \pm 1.791 ^a	16.534 \pm 1.695 ^a	0.0509	161
PC 2	28.509 \pm 4.671 ^a	27.228 \pm 4.119 ^a	0.1233	13.079 \pm 3.615 ^a	13.763 \pm 3.408 ^a	0.3007	310
PC 3	21.694 \pm 5.435 ^a	21.145 \pm 5.065 ^a	0.5622	3.782 \pm 5.205 ^a	4.839 \pm 4.955 ^a	0.2493	69
PC 4	19.331 \pm 3.250 ^a	18.873 \pm 3.158 ^a	0.4395	-2.144 \pm 3.251 ^a	-0.314 \pm 2.759 ^b	0.0013*	0
PC 5	22.033 \pm 5.754 ^a	21.697 \pm 5.224 ^a	0.7361	1.811 \pm 3.904 ^a	3.205 \pm 3.576 ^b	0.0420*	62
PC 6	29.762 \pm 4.065 ^a	28.779 \pm 4.174 ^a	0.1898	9.336 \pm 4.173 ^a	10.336 \pm 3.985 ^a	0.1784	160

* = Significantly different at $P < 0.05$, PC 1 = Planting Cycle 1, PC 2 = Planting Cycle 2, PC 3 = Planting Cycle 3, PC 4 = Planting Cycle 4, PC 5 = Planting Cycle 5, PC 6 = Planting Cycle 6. Means followed by the same letter in a row are not statistically different at $P < 0.05$

CHAPTER 5

THE IMPACT OF VARYING APHID POPULATIONS IN TWO DIFFERENT SHADEHOUSE STRUCTURES ON SOME PHYSICAL CHARACTERISTICS OF HEAD LETTUCE, CULTIVATED IN THE CENTRAL FREE STATE (SOUTH AFRICA)

Abstract

Introduction: Aphids are considered pests of cultivated crops, mainly because they drain phloem sap and transmit disease-causing pathogens. Direct feeding damage is caused by the removal of plant sap rather than the consumption of solid plant matter as in the case with chewing phytophages. This type of feeding damage can be either asymptomatic or symptomatic. The growth stage of the plant in relation to the time in which the aphids are present, and aphid population densities will largely determine the extent of damage that is inflicted.

Methods: Leaves of the sampled plants were counted during each of the four sampling occasions of a planting cycle, and comparisons were made between the two shadehouse structures. The number of leaves infested with one or more aphids were also counted and compared between the two structures. Correlations were then made to measure the relationship between the number of counted leaves, and the number of infested leaves in each structure. Lettuce plants used for sampling purposes were removed and weighed at the end of each individual planting cycle to compare fresh head weight between the two structures.

Results: The December 2005 – January 2006 (planting cycle 1) and October – November 2006 (planting cycle 2) planting cycles showed a significant difference in fresh head weight between the two structures, and a higher aphid infestation level in the fully covered structure. More aphid-infested leaves were also noted in this structure during these planting cycles, and there were significant correlations between the number of counted leaves and the number of leaves infested by aphids. The April – May 2006 planting cycle (planting cycle 3) also had a significant lower head weight in the fully covered structure, but aphid infestation

levels were higher in the partially covered structure during this time. During both the June – July 2006 (planting cycle 4) and August – September 2006 (planting cycle 5) planting cycles, mean number of aphid-infested leaves and aphid infestation levels, were significantly higher in the partially covered structure. August – September 2006 was also the only occasion in which the partially covered structure had a significant positive correlation between the number of counted leaves and the number of aphid-infested leaves.

Conclusions: Visible feeding damage to the lettuce crop was restricted, but asymptomatic damage in terms of a decrease in lettuce fresh head weight did occur. The microclimate in each structure also contributed to this. Therefore, aphid feeding only had any real impact under less favourable growing conditions for the lettuce crop. The physical presence of aphids on the crop is more important from a phytosanitary point of view. More aphids would imply that more leaves are infested, and under severe infestation levels, almost all of the leaves will bear aphids.

Key words: Aphid feeding; Lettuce head weight; Damage; Injury

5.1 INTRODUCTION

Aphids are considered pests of cultivated crops, mainly because they drain plant phloem sap, transmit disease-causing phytopathogenic microbes, and because they inject plant elicitors (Parker *et al.*, 2002; Ng & Perry, 2004). In addition, aphids also produce honeydew through their feeding activities, which could encourage the growth of sooty moulds (Bovi *et al.*, 2004). Their vectoring capabilities and honeydew-producing habits left aside, the direct damage they cause to plants through their feeding is due to the removal of plant sap rather than the consumption of solid plant matter as in the case of chewing phytophagous insects. Therefore, symptoms exhibited by aphid-infested hosts would differ from those of other types of phytophagous insect pests which have different feeding mechanisms.

Harm caused through the feeding of aphids has recently been reviewed by Quisenberry & Ni (2007), who mention that it is necessary to differentiate between the terms 'damage' and 'injury' as a result of aphid feeding. In short, damage (reduction in growth of the host or yield loss) can be viewed as a direct result of injury (a change in the physiological process of the host plant). According to these authors, the damage aphids cause can be either asymptomatic (no obvious feeding damage) or symptomatic. In the case of symptomatic damage, symptoms will range from desistance (stunting, chlorosis, etc.) to neoplasm (leaf curling, formation of galls, etc.). In most cases, direct feeding damage to lettuce by aphids can be attributed to the morphs which have a high rate of reproduction (Williams & Dixon, 2007). High population densities can lead to the development of symptomatic damage, with leaves becoming discolored and wilted as a result of the removal of plant sap (Harris, 1992) and shading of leaves by the aphid bodies and by their honeydew (Kaakeh *et al.*, 1992). Tjallingii (2004) has shown that even moderate aphid numbers will cause considerable damage in certain cases. The growth stage of the lettuce plant in relation to the time in which the aphids are present will also largely determine the extent of damage conducted (Irwin *et al.*, 2007). Thus, younger plants are far more likely to be damaged to such an extent that they may be unable to recover,

whilst older plants are more resistant to such attacks (University of California, 1992). Quantifying the impact that aphid feeding has on some physical characteristics of head lettuce is vital in maximizing yield and economic income.

This study aimed at determining the impact that varying aphid infestation levels has on lettuce head weight, and the number of leaves the plant will form under such conditions. It also investigated the relationship between the number of leaves head lettuce will form and the number of leaves aphids will typically infest.

5.2 MATERIALS AND METHODS (Refer to Chapter 2)

Area of research and time frame: Refer to Chapter 2 (section 2.1)

Trial design and experimental layout: Refer to Chapter 2 (section 2.2, 2.3, 2.4 & 2.7)

Aphid sampling procedure: Refer to Chapter 4 (Materials and methods)

Head weight measurements: The lettuce which were sampled, were removed and weighed (in grams) at the end of each individual planting cycle. This was achieved by carefully removing the plant from the soil, cutting off the root-mass just above soil-level, and then immediately weighing the plant on a portable electronic scale.

Leaf formation and infestation levels: Leaves of the sampled plants were counted during each of the four sampling occasions of a planting cycle. The number of leaves infested with one or more aphids were also noted and regarded as 'infested'. During the fourth sampling occasion of each planting cycle (which was conducted just after harvesting of the heads), the tightly packed, yellow-colored leaves of the heads were also counted by means of the whole plant destructive sampling method. If heads already started forming during the third sampling occasion of a planting cycle, only the wrapper loose leaves were

counted in order not to injure the plant, and also to keep disturbances to aphids to a minimum.

Statistical analysis: Differences in the mean number of counted leaves between the two structures, and differences in the mean number of leaves infested with aphids between the two structures, were both tested using the one-way ANOVA procedure (SAS, 2004). The same procedure was followed to test for differences in fresh head weight of lettuce between the two structures. Means were not separated because only two treatments were tested. Pearson's correlation was employed to compare the mean number of leaves per plant with the mean number of aphid-infested leaves per plant (SAS, 2004).

5.3 RESULTS

Head weight and aphid infestation level comparisons: Planting cycle 1 (Dec 2005 – Jan 2006) revealed an extremely significant difference in mean (\pm SD) lettuce head weight between the two structures ($F = 19.64$, $df = 1$, $P < 0.001$) (Figure 5.1A) as a result of an overall higher head mass in the partially covered structure (843.8750 ± 169.2542 vs. 642.7917 ± 144.0957). Aphid infestation levels were significantly higher ($P < 0.05$) in the fully covered structure during this planting cycle (Table 5.1). A similar situation was observed during planting cycle 3 (Apr – May 2006) ($F = 5.68$, $df = 1$, $P = 0.0213$) in which the partially covered structure again attained a higher mean (\pm SD) head mass (168.2917 ± 30.2187 vs. 141.6667 ± 45.6105) (Figure 5.1C). However, aphid infestation levels were higher in the partially covered structure during this period (Table 5.1). Planting cycle 6 (Oct – Nov 2006) also had a significant difference in head mass between the two structures ($F = 5.70$, $df = 1$, $P = 0.0211$), again as a consequence of a higher mean (\pm SD) head mass in the partially covered structure (203.3333 ± 111.7083 vs. 135.7083 ± 82.2977) (Figure 5.1F). The fully covered structure attained the highest aphid infestation levels during this time (Table 5.1).

Comparison between counted leaves and aphid-infested leaves: Despite aphid populations reaching high levels in the fully covered structure during the warmer months, and moderately higher levels in the partially covered structure during the cooler months (Table 5.1), the mean (\pm SD) number of leaves counted per plant, remained relatively similar between the two structures throughout the trial period ($P > 0.05$). The only exceptions were observed during planting cycles 5 (Aug – Sep 2006) and 6 (Table 5.2).

Differences in the number of infested leaves between the two structures were more pronounced (Table 5.3). During planting cycle 1, the last three sampling occasions all showed a significant difference ($P < 0.05$) in the mean (\pm SD) number of leaves infested with aphids between the two structures, as a result of the fully covered structure having on average more aphid-infested leaves (Table 5.3). An extremely significant correlation ($P < 0.0001$) between the number of leaves counted and the number of leaves infested by aphids was also witnessed during the last two sampling occasions of this planting cycle ($r = 0.88788$ and $r = 0.73646$, respectively) in the same structure (Figure 5.2). The leaf infestation rate during the first three sampling occasions of planting cycle 6 also differed significantly between the two structures ($P < 0.05$), again as a result of the fully covered structure on average attaining more aphid-infested leaves (Table 5.3). Sampling occasions 2 ($r = 0.95954$) and 3 ($r = 0.82919$) of this planting cycle, all showed an extremely significant correlation ($P < 0.0001$) between the number of leaves counted and the number of leaves infested with aphids in the fully covered structure (Figure 5.2). However, during both planting cycles 4 (Jun – Jul 2006: third sampling occasion) and 5 (first two sampling occasions), the mean (\pm SD) number of aphid-infested leaves were significantly higher in the partially covered structure (Table 5.3). Aphid infestation levels were also mostly higher in this structure during these two periods (Table 5.1), although not statistically different during planting cycle 5. Planting cycle 5 was the only time during which the partially covered structure had a significant positive correlation between the number of counted leaves and the number of aphid-infested leaves during the third ($r = 0.94962$, $P < 0.0001$) and fourth

($r = 0.70661$, $P = 0.0001$) sampling occasions (Figure 5.2). The fully covered structure also had a significant correlation between these two parameters during this and the third planting cycle (Figure 5.2).

5.4 DISCUSSION

Phytophagous insect feeding damage is considered to be a function of their population densities (Bale, 1991), and aphids are known to reach high population numbers under favourable conditions, as witnessed during this study. However, direct feeding damage to the host by aphids has been considered to be not very obvious (Gao *et al.*, 2008). This is due to these insects only feeding on the phloem sieve element, after intercellular probing through the epidermal and mesophyll cell layers has taken place (Gao *et al.*, 2008). In this study, symptoms of direct feeding damage on the lettuce crop was indeed insignificant, with only some degree of localized necrosis were the aphids had penetrated the plant tissue with their stylets, and a slight degree of leaf curling in some cases. However, asymptomatic damage symptoms did exist to some extent with regard to head weight reduction.

The differences observed in lettuce head weight between the two structures was actually a combination of both aphid feeding and environmental conditions. Higher aphid densities did partially contribute to the significantly lower head weights in the fully covered structure during planting cycles 1 and 6. High aphid numbers can remove substantial quantities of plant sap, interfering with the physiological processes of the plant which could inevitably lead to a decrease in fresh weight, as observed in other crops (Van Emden, 1990). However, the specific conditions (microclimate) experienced within a particular shadehouse structure, also played a role. This was evident from the fully covered structure which also reached a lower mean head mass during planting cycle 3, despite the fact that there were actually less aphids present compared to the partially covered structure. Lettuce is essentially a cool-weather crop (Harris, 1992) and higher temperature and moisture levels in the fully covered structure during the warmer months of planting cycles 1, 3 and 6 could have additionally contributed

to the lower head weights in this structure. Therefore, the differences in head weight between the two structures is also partly a function of the microclimate within a particular structure, but high aphid infestation levels could accelerate head weight reduction under the less favourable growing conditions.

Lettuce plantings from a previous planting cycle were removed prior to planting a new cycle, implicating that aphids had to newly re-infest the crop each time. Therefore, their populations were lower during the seedling stage (first sampling occasion) and had to increase over the short growth period of the crop, which was mostly the case during this study (refer to Chapter 4). Low aphid densities during the seedling stage, in turn, effectively prevented serious damage, as lettuce is vulnerable to insect attack during this time (Grafton-Cardwell *et al.*, 2005). This explains why even the high aphid infestation levels in the fully covered structure (summer months) and the moderately higher infestation levels in the partially covered structure (winter and spring months), did not have any real impact on the number of leaves formed by the plant. However, extremely high aphid infestation levels delayed leaf formation during planting cycles 5 (partially covered structure) and 6 (fully covered structure) as a result of stunting (Cerkauskas *et al.*, 1998). Due to the fact that this did not occur during the vulnerable seedling stage, the plants were able to recover in both cases. This was especially evident during planting cycle 6 when aphid numbers dwindled as the last sampling occasion was drawing closer (as a result of high coccinellid larval numbers).

It is to be expected that more leaves will be infested with aphids under such crowded conditions than those witnessed during planting cycles 1 and 6 in the fully covered structure. This led to the extremely significant differences in the number of aphid-infested leaves observed between the two structures during these planting cycles. Reasons for higher aphid densities in the fully covered structure during these periods have already been discussed (Chapter 4). In short, it entails the presence of *Anoplolepis custodiens* which could have preyed on the aphids and removed/killed coccinellid larvae and eggs in the partially covered

structure, lower abundance of adult coccinellid (and absence of *A. custodiens*) in the fully covered structure, and the fully covered structure hindering alatae from dispersing. As mentioned, the fully covered structure also reached a higher mean temperature range and humidity level which could favour the development of certain species. More infested leaves in the partially covered structure as opposed to the fully covered structure during planting cycles 4 and 5, is also a result of higher aphid populations in this structure. Several factors which are also explained in Chapter 4 contributed to this. In short, minimum temperatures were not as low as those measured in the fully covered structure which favoured aphid populations, whilst coccinellid larvae were absent, and *A. custodiens* were absent or occurred in low numbers.

Significant positive correlations between the number of counted leaves and the number of aphid-infested leaves during planting cycles 1, 5 and 6 is to be expected, since high aphid populations would eventually disperse to most of the leaves. The implication of this is that most of the leaves will be infested with aphids under such crowded conditions. Quantifying the degree to which leaves are infested with aphids is important because, despite the direct damage aphids are capable of inflicting onto lettuce through extracting phloem sap, their mere presence may also render the crop unattractive and unmarketable (Van Helden *et al.*, 1993). Lettuce heads contaminated with aphids, shed skins and honeydew, are not acceptable from a phytosanitary point of view.

5.5 CONCLUSIONS

Visible feeding damage to the lettuce crop was restricted, but asymptomatic damage in terms of a decrease in lettuce fresh head weight did occur. However, the microclimate experienced within the particular shadehouse structure also contributed to this. Therefore, aphid feeding only had any real impact under less favourable growing conditions for the lettuce crop. The physical presence of aphids on the crop is more important from a phytosanitary point of view. More aphids would imply that more leaves are infested. Under severe infestation

conditions, most of the leaves will be contaminated with the presence of aphids, which could lead to the rejection of the crop on certain markets.

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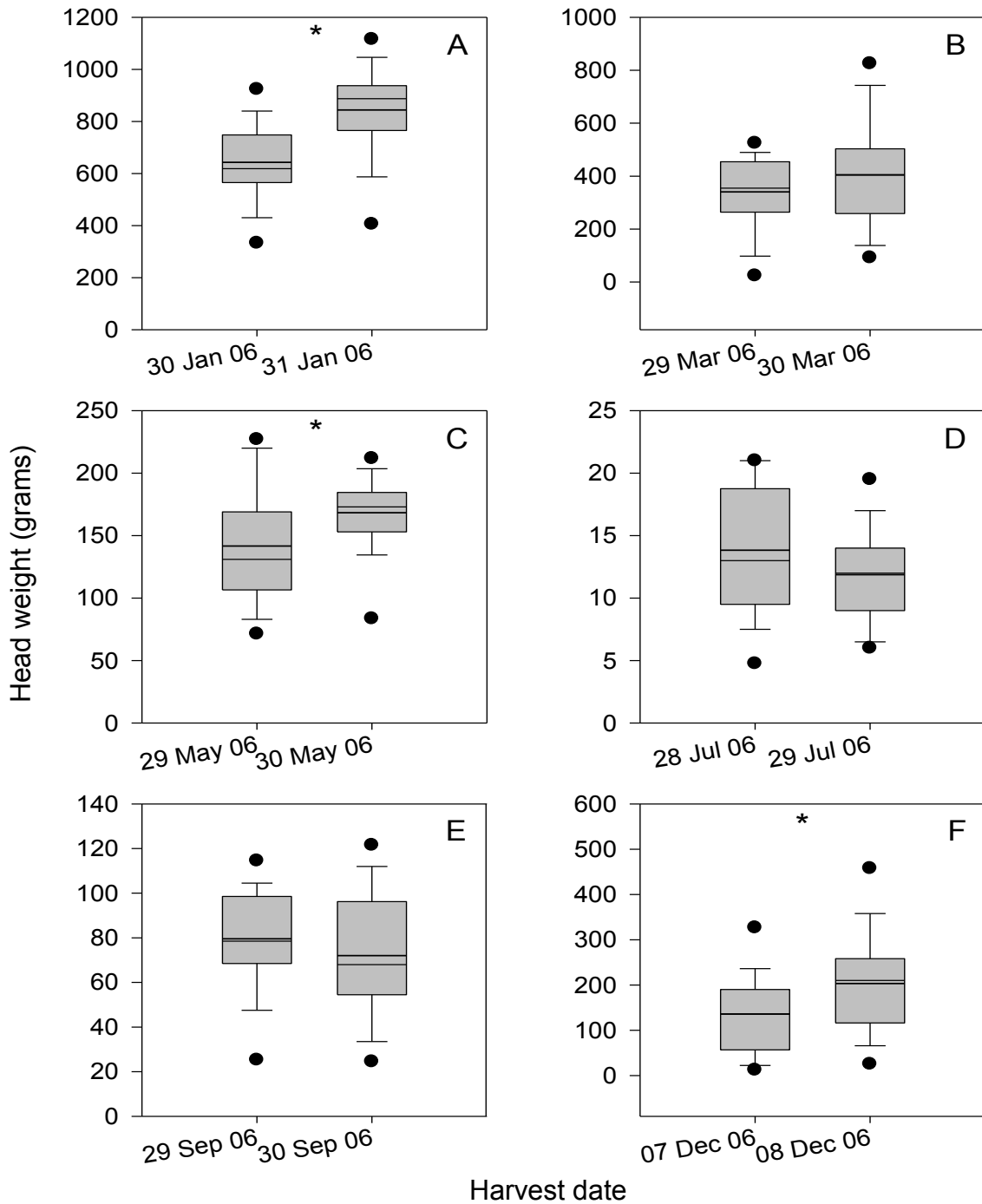
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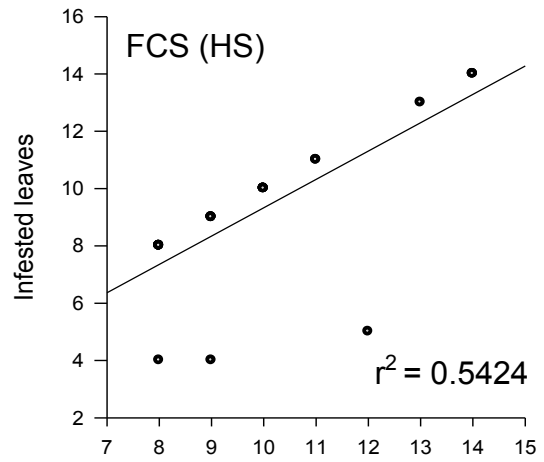
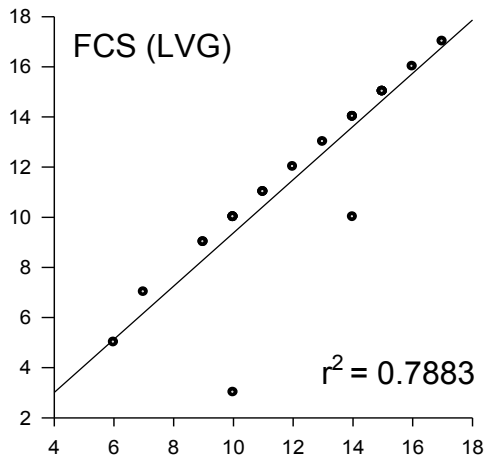
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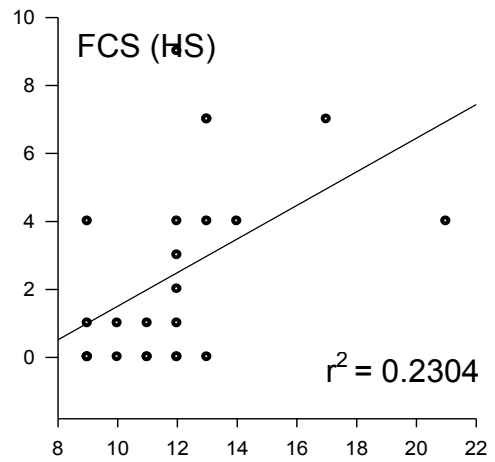
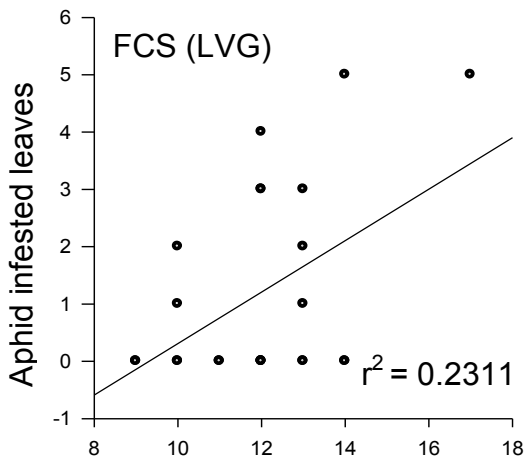
* = Significantly different at $P < 0.05$

Figure 5.1: Fresh lettuce head weight (mean \pm SD) measured in the fully covered structure (left-side box in each figure) and partially covered structure (right-side box in each figure) during each planting cycle (Roodevallei, Free State Province).

Planting cycle 1



Planting cycle 3



Planting cycle 5

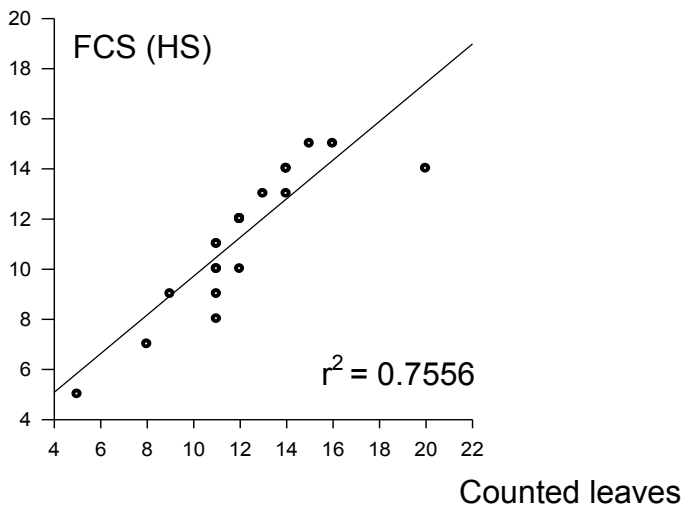
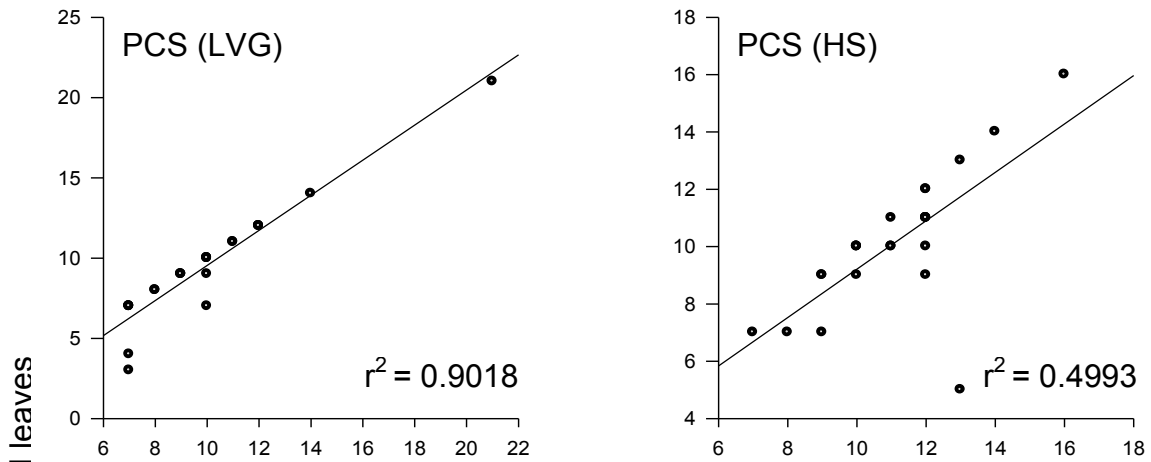
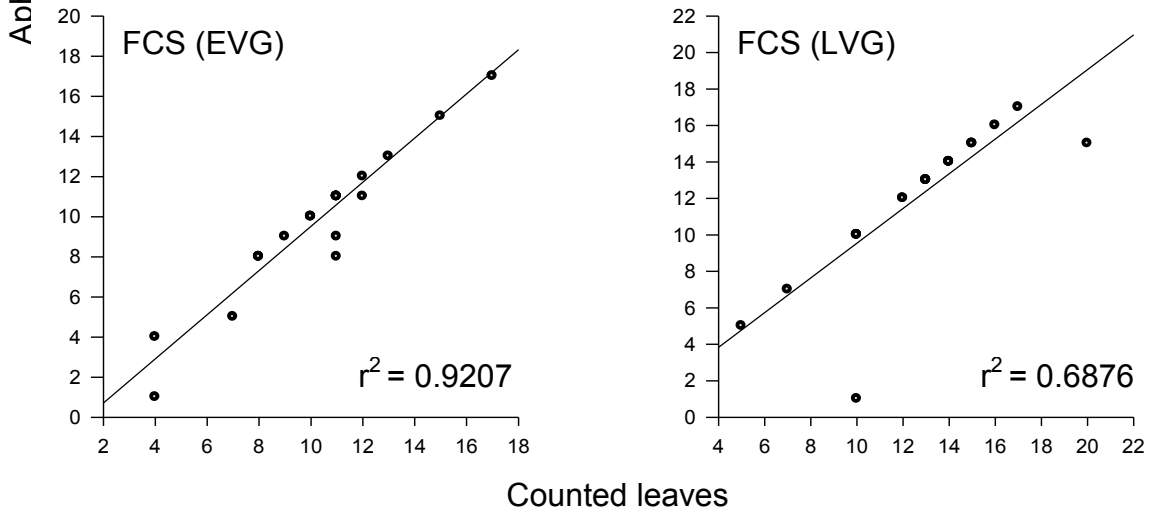


Figure 5.2: Continued on next page.

Planting cycle 5



Planting cycle 6



FCS = Fully Covered Structure, PCS = Partially Covered Structure, EVG = Early Vegetative Growth, LVG = Late Vegetative Growth, HS = Heading Stage

Figure 5.2 (continued from previous page): Relationship between the number of lettuce leaves counted and the number of leaves infested with aphids during the indicated sampling occasions and planting cycles (Roodevallei, Free State Province).

Table 5.1: Level (mean \pm SD) of aphid infestation observed in each structure per growth stage (sampling occasion) of the lettuce crop during each planting cycle (Roodevallei, Free State Province).

PC	GS	Structure type		F-value	P-value	n	SL
		FCS	PCS				
PC 1	SS	0.7083 \pm 1.3010	0.5417 \pm 0.5090	0.34	0.5619	24	ns
	VG 1	1.3333 \pm 1.5510	0.5417 \pm 0.5882	5.47	0.0238	24	*
	VG 2	3.4583 \pm 1.6150	0.5417 \pm 0.5090	71.24	<0.0001	24	***
	HS	4.4167 \pm 1.1390	0.7917 \pm 0.4149	214.66	<0.0001	24	***
PC 2	SS	0.0417 \pm 0.2041	0.1250 \pm 0.3378	1.07	0.3064	24	ns
	VG 1	0.0833 \pm 0.2823	0.1667 \pm 0.3807	0.74	0.3935	24	ns
	VG 2	0.3333 \pm 0.4815	0.3750 \pm 0.4945	0.09	0.7688	24	ns
	HS	0.5417 \pm 1.0210	0.4167 \pm 0.5036	0.29	0.5931	24	ns
PC 3	SS	0.4167 \pm 0.5036	0.2500 \pm 0.4423	1.48	0.2294	24	ns
	VG 1	0.0417 \pm 0.2041	0.2917 \pm 0.4643	5.83	0.0198	24	*
	VG 2	0.4167 \pm 0.5036	0.7917 \pm 0.4149	7.93	0.0071	24	**
	HS	0.7500 \pm 0.4423	0.9167 \pm 0.2823	2.42	0.1266	24	ns
PC 4	SS	0.0417 \pm 0.2041	0.0417 \pm 0.2041	0.00	1.0000	24	ns
	VG 1	0.1667 \pm 0.3807	0.5000 \pm 0.5108	6.57	0.0137	24	*
	VG 2	0.2500 \pm 0.4423	0.5833 \pm 0.5036	5.94	0.0188	24	*
	HS	0.7917 \pm 0.4149	0.7083 \pm 0.4643	0.43	0.5153	24	ns
PC 5	SS	0.2500 \pm 0.4423	0.5833 \pm 0.5036	5.94	0.0188	24	*
	VG 1	0.9167 \pm 0.2823	0.9583 \pm 0.2041	0.34	0.5608	24	ns
	VG 2	1.3333 \pm 0.5647	1.3333 \pm 0.4815	0.00	1.0000	24	ns
	HS	2.5000 \pm 0.8847	2.5417 \pm 1.0210	0.02	0.8805	24	ns
PC 6	SS	1.6250 \pm 1.7150	0.0417 \pm 0.2041	20.18	<0.0001	24	***
	VG 1	3.7500 \pm 1.4220	0.5833 \pm 0.5036	105.77	<0.0001	24	***
	VG 2	3.4167 \pm 1.7670	0.0833 \pm 0.2823	83.26	<0.0001	24	***
	HS	0.1250 \pm 0.3378	0.0417 \pm 0.2041	1.07	0.3064	24	ns

PC = Planting Cycle, GS = Growth Stage, FCS = Fully Covered Structure, PCS = Partially Covered Structure, SL = Significance Level, SS = Seedling Stage, EVG = Early Vegetative Growth, LVG = Late Vegetative Growth, HS = Heading Stage, * = Significant Difference, ** = Highly Significant Difference, *** = Extremely Significant Difference

Table 5.2: Number (mean \pm SD) of lettuce leaves counted in each structure per growth stage (sampling occasion) of the crop during each planting cycle (Roodevallei, Free State Province).

PC	GS	Structure type		F-value	P-value	n	SL
		FCS	PCS				
PC 1	SS	6.7500 \pm 1.7998	6.7500 \pm 2.0483	0.00	1.0000	24	ns
	EVG	11.9167 \pm 2.3015	12.6667 \pm 2.7767	1.04	0.3136	24	ns
	LVG	12.0000 \pm 2.9192	12.5417 \pm 2.4313	0.49	0.4884	24	ns
	HS	10.0000 \pm 2.0430	10.6250 \pm 2.6012	0.86	0.3594	24	ns
PC 2	SS	6.0833 \pm 1.4720	6.0833 \pm 1.3160	0.00	1.0000	24	ns
	EVG	10.1250 \pm 1.9630	11.2917 \pm 3.3555	2.16	0.1483	24	ns
	LVG	11.0833 \pm 2.3759	10.9167 \pm 2.5007	0.06	0.8139	24	ns
	HS	13.2083 \pm 2.4134	13.7500 \pm 2.4002	0.61	0.4396	24	ns
PC 3	SS	7.5833 \pm 2.1853	8.1667 \pm 2.3157	0.81	0.3741	24	ns
	EVG	8.5000 \pm 2.1669	9.7083 \pm 2.2357	3.61	0.0635	24	ns
	LVG	12.0000 \pm 1.8415	11.7917 \pm 2.8127	0.09	0.7628	24	ns
	HS	11.9167 \pm 2.7174	11.7917 \pm 3.1894	0.02	0.8844	24	ns
PC 4	SS	5.5000 \pm 1.3513	5.9583 \pm 2.0104	0.86	0.3588	24	ns
	EVG	7.2917 \pm 1.6280	7.8333 \pm 2.1602	0.96	0.3317	24	ns
	LVG	8.3333 \pm 2.5820	8.6667 \pm 2.9291	0.17	0.6777	24	ns
	HS	9.3333 \pm 2.6320	8.7083 \pm 2.4931	0.71	0.4027	24	ns
PC 5	SS	6.4167 \pm 1.4421	6.0000 \pm 2.0000	0.69	0.4120	24	ns
	EVG	9.7083 \pm 1.7315	9.0000 \pm 2.3956	1.38	0.2465	24	ns
	LVG	11.6667 \pm 2.8993	9.9167 \pm 3.0633	4.13	0.0479	24	*
	HS	12.0833 \pm 2.8117	11.1250 \pm 1.9850	1.86	0.1792	24	ns
PC 6	SS	8.8750 \pm 2.8332	9.4167 \pm 2.9476	0.42	0.5195	24	ns
	EVG	10.2083 \pm 2.8889	11.9167 \pm 2.5007	4.80	0.0336	24	*
	LVG	12.7500 \pm 3.2067	12.0000 \pm 2.4672	0.82	0.3685	24	ns
	HS	13.1667 \pm 3.5834	12.5417 \pm 3.2434	0.40	0.5295	24	ns

PC = Planting Cycle, GS = Growth Stage, FCS = Fully Covered Structure, PCS = Partially Covered Structure, SL = Significance Level, SS = Seedling Stage, EVG = Early Vegetative Growth, LVG = Late Vegetative Growth, HS = Heading Stage, * = Significant Difference

Table 5.3: Number (mean \pm SD) of aphid-infested lettuce leaves counted in each structure per growth stage (sampling occasion) of the crop during each planting cycle (Roodevallei, Free State Province).

PC	GS	Structure type		F-value	P-value	n	SL
		FCS	PCS				
PC 1	SS	0.6667 \pm 1.0901	0.6250 \pm 0.6469	0.03	0.8728	24	ns
	EVG	4.5833 \pm 4.5389	1.5833 \pm 2.1247	8.60	0.0052	24	**
	LVG	11.5000 \pm 3.4891	1.0417 \pm 1.2676	190.49	<0.0001	24	***
	HS	9.3333 \pm 2.7452	0.5417 \pm 0.7790	227.80	<0.0001	24	***
PC 2	SS	0.0417 \pm 0.2041	0.1250 \pm 0.3378	1.07	0.3064	24	ns
	EVG	0.1250 \pm 0.4484	0.4167 \pm 1.0598	1.54	0.2207	24	ns
	LVG	0.5833 \pm 1.1389	0.7917 \pm 1.4440	0.31	0.5816	24	ns
	HS	1.5833 \pm 3.5621	0.6250 \pm 0.8242	1.65	0.2055	24	ns
PC 3	SS	0.5417 \pm 0.7790	0.2500 \pm 0.4423	2.54	0.1176	24	ns
	EVG	0.0833 \pm 0.4082	0.3750 \pm 0.6469	3.49	0.0681	24	ns
	LVG	1.2083 \pm 1.7189	1.7500 \pm 1.4521	1.39	0.2444	24	ns
	HS	2.4583 \pm 2.7972	1.7500 \pm 1.1516	1.32	0.2572	24	ns
PC 4	SS	0.0417 \pm 0.2041	0.0417 \pm 0.2041	0.00	1.0000	24	ns
	EVG	0.3750 \pm 1.0959	0.7083 \pm 0.8065	1.44	0.2362	24	ns
	LVG	0.3750 \pm 0.7697	1.0417 \pm 1.2329	5.05	0.0295	24	*
	HS	1.6250 \pm 1.4982	1.0833 \pm 0.9743	2.20	0.1444	24	ns
PC 5	SS	0.2917 \pm 0.5500	0.9583 \pm 1.0826	7.23	0.0099	24	**
	EVG	2.2500 \pm 1.2938	3.4583 \pm 1.9106	6.58	0.0136	24	*
	LVG	8.7500 \pm 3.5047	9.4583 \pm 3.5260	0.49	0.4887	24	ns
	HS	11.3333 \pm 2.4964	10.1667 \pm 2.3713	2.76	0.1037	24	ns
PC 6	SS	2.1250 \pm 2.5760	0.0417 \pm 0.2041	15.6	0.0003	24	***
	EVG	9.7500 \pm 3.3133	1.3750 \pm 2.0602	110.58	<0.0001	24	***
	LVG	12.1667 \pm 3.6792	0.0833 \pm 0.2823	257.36	<0.0001	24	***
	HS	0.0000 \pm 0.0000	0.0000 \pm 0.0000	0.00	1.0000	24	ns

PC = Planting Cycle, GS = Growth Stage, FCS = Fully Covered Structure, PCS = Partially Covered Structure, SL = Significance Level, SS = Seedling Stage, EVG = Early Vegetative Growth, LVG = Late Vegetative Growth, HS = Heading Stage, * = Significant Difference, ** = Highly Significant Difference, *** = Extremely Significant Difference

CHAPTER 6

RECOMMENDATIONS FOR APHID PEST CONTROL ON LETTUCE, WITH SPECIFIC REFERENCE TO THE ROLE OF COCCINELLIDAE PREDATORS IN PLANT HEALTH MANAGEMENT

6.1 INTRODUCTION AND OBJECTIVES

As discussed in Chapter 1, integrated pest management (IPM) is receiving extensive attention worldwide, mainly due to concerns about the negative impact that the excessive use of harmful chemicals has on human health, the environment and arthropod natural enemies (Croft & Brown, 1975; Hand *et al.*, 2003; Mazlan & Mumford, 2005). The attention given to IPM is evident from the vast literature source relating to this topic, as evidenced in scientific articles in journals and books and popular articles on the web and in various magazines. Research has been conducted on a wide variety of crops in order to identify the pest species associated with them, control options which will enhance agricultural sustainability, and the impact of such control options on the society and environment. Extensive research has shown that control options might differ for the same pest species on different crops, or even for the same pest species on the same crop in different regions of the world or during different seasons of the year. Considering this, and the fact that many insect and other arthropod pest species are known from almost all crops around the world, research on pest management is a colossal field with ample work still awaiting. In addition, some crops are under production in non-autochthonous areas, establishing vacant niches which could lead to unknown pest species becoming a threat under these circumstances.

The objectives of this study is summarized in Chapter 1 (Section 1.10) and the purpose of this chapter is only to aid lettuce producers in the central Free State (and hopefully in other regions of Southern Africa with similar aphid pest species) to identify the most common aphid species and their associated predatory guilds (Coleoptera: Coccinellidae). In addition, it also aims at informing producers of

potential control practices which could lead to enhanced plant health, with some suggestions on techniques to lower aphid abundance. It is not intended to be a concise guide with step by step aphid pest management strategies, but rather a tool which can be adapted to suit the specific needs of the producer under his own unique circumstances. An overview on aphid pest status, monitoring for these insects, and identification are also discussed in order to better understand the threat these organisms hold and to aid in practical scouting procedures.

6.2 IPM AND PLANT HEALTH MANAGEMENT FOR LETTUCE

Although the number and species of aphids colonizing lettuce around the world is relatively well-known (Blackman & Eastop, 2000), little is known about which species colonizes the crop in specific regions of the world. Tatchell (2007) mentions that the aphid species complex encountered on a crop will differ in different climatic and zoogeographical regions of the world. In some cases, lettuce farmers in certain parts of the world move their production to other areas when unfavourable conditions set in, resulting in a whole new array of pest species which they have to deal with (Tatchell, 2007). It is therefore essential to determine exactly which aphid pest species are present in a particular production region, and on the basis of this, develop a plant health management system that will best be able to face the challenges of the specific area.

Lettuce is ready for consumption as soon as maturity is reached. Due to this, yield is not the ultimate indicator for success, but rather the number of heads which have an attractive appearance and which are free from insects or any traces of their presence (Tatchell, 2007). Lettuce therefore has a low economic threshold. It immediately springs to mind that pesticides will resolve the problem of insect pests, but pesticide residues on the harvested product is another concern with which producers have to deal with in this short growth-season crop. The challenges facing producers of leafy salad crops are thus clear and quite different from those of producers which cultivate other crops, e.g. grains, legumes, etc. In certain areas of the world, standards for lettuce product quality are set to which producers must adhere if they wish to sell their produce to

certain retailers. In Europe, EurepGAP (Euro Retailer Produce Working Group Good Agricultural Practice) has been founded by retailers. It is aimed at setting standards to which suppliers must adhere (Tatchell, 2007) when they wish to sell their product to certain retailers. Standards to producers in the UK have also been established (known as the Assured Produce Scheme or APS) which are aimed at addressing production issues (of which aphid control forms a part) within the framework of integrated crop management (Tatchell, 2007). South African retailers have also set standards to which lettuce must adhere, and good crop management practices are therefore required in order to ensure success.

Developing an IPM system against aphids in order to enhance plant health necessitates the use of three functional components, namely fundamentals, tactics and strategies (Irwin, 1999). Fundamentals include knowledge regarding the identity, biology, and virus transmission capabilities of the aphid pest species (Irwin *et al.*, 2007). Tactics are deployed in order to reduce threats that aphids pose to the crop, and include such practices as host plant resistance, biological control, chemical control and habitat manipulation (Irwin *et al.*, 2007). Tactics should be employed timely and at the correct target, which is only possible through using the correct strategy. More than one tactic is usually necessary to ensure that individual precautionary procedures do not become ineffective (Jones, 2004). Applying more than one control tactic will also ensure that these tactics complement each other.

6.3 MONITORING FOR APHIDS ON LETTUCE

Aphids are small and easily overlooked, especially if colonies are absent or in the initial phases of expansion. The implication of this is that a monitoring programme for aphids should be carried out with precision and with the utmost attention to detail. When scouting for aphids on lettuce, as many plants (and leaves) as possible must be visually inspected in good daylight. Some species show differences in their preferred feeding sites on a host plant (which can change with the growth season), and alates tend to move to the lower surface of leaves just after landing on the plant (Müller, 1984). This trial for instance,

showed that *Nasonovia ribisnigri* prefer to colonize the heart of the plant, but populations can spread to the outer wrapper leaves as the colony expands (Liu, 2004). Their cryptic feeding habits might therefore conceal their presence to the observer. *Acyrtosiphon lactucae* on the other hand have an exposed existence on the plant and can easily be detected in most cases (refer to Chapter 4). Aphids are easier to spot when the plants are young and have fewer leaves. Thorough monitoring a week or two after transplanting is thus strongly advised, during which time scouting would be relatively efficient and early infestations can be noted.

One of the problems facing lettuce producers and aphid pests is the mode of transport of these organisms. Most producers acquire lettuce seedlings from seedling nurseries. Under conditions in which the nursery must provide a continuous supply of plants at various time intervals, a constant availability of seedlings would be present. This is therefore a strong source from which aphids can be translocated to the production area, primarily by means of the so-called 'inadvertent' mode of transport (Irwin *et al.*, 2007). It is thus best to conduct the first scouting as early as possible to determine if aphids have been introduced into the field or structure, or to spray the seedlings with an insecticide before transplantation. As the lettuce plant matures, monitoring will become increasingly more difficult (especially if population densities are also evaluated). The challenge, however, presents itself during the heading stage when some aphids tend to feed at the heart of the plant, as discussed for *N. ribisnigri*. When scouting for aphids, it must be noted whether the aphids observed are winged (alates) or wingless (apterous). The presence of groups of wingless aphids is a direct indication that the particular species prefers the crop as a host. The presence of winged individuals could indicate that the observed aphid species is either a non-pest, potential pest, or a key pest of that crop (see discussion on aphid pest status below). Besides the physical presence of aphids, consideration should also be given to other traces of their presence, as well as plant symptoms which have developed in response to an infestation, e.g. presence of honeydew and shed skins (Figure 6.1), disturbed or abnormal leaf growth, discoloration of

the plant, and necrotic spots formed through feeding (Pettersson *et al.*, 2007). Symptoms of plant damage must be studied carefully in order to determine if their origin is indeed the result of aphid feeding damage, or some nutrient deficiency, or plant disorder which would show similar symptoms. Aphid-infested hosts will also have a higher abundance of natural enemies on them, both in the larval and adult stage (Figure 6.1).



Figure 6.1: Coccinellidae larvae amongst shed aphid skins – a clear indication of aphid presence (Roodevallei, Free State Province).

Many monitoring techniques exist which can be applied to assess aphid population dynamics, namely *in situ* counts (method used during the trial), destructive counts, vacuuming from plants, sweeping and beating (not practical on lettuce), each with its own advantages and disadvantages (Harrington *et al.*, 2007). Apart from these physical monitoring techniques, other methods can be employed, but which may prove difficult to an untrained person. These include

aerial sampling (used to measure the long distance traveling of aphids) and measuring landing rates within a particular field (provides information on the damage aphids can cause via virus transmission) (Robert, 1987; Irwin *et al.*, 2007). Suction traps and yellow sticky traps are commonly used, but their effectiveness differ (Heathcote *et al.*, 1969). For instance, *Macrosiphum euphorbiae* and *Myzus persicae*, have both been shown to be lured more efficiently to yellow sticky traps as opposed to other species (Heathcote *et al.*, 1969). Yellow sticky traps are commercially available to producers in South Africa, and once mastered and applied properly, can provide crucial information relating to aphid movement (especially of virus vectors). Suction traps have been successfully used to predict aphid outbreaks in grain crops (Howard & Dixon, 1990), but the aphid assemblage collected by these traps are governed by the location of the trap (Quinn *et al.*, 1991) and they are not commonly used in South Africa.

The study has shown that aphid numbers differed between the two types of structures. Producers are therefore advised to monitor all their structures or fields for aphid presence, as conditions may vary between them (University of California, 1992). It is of utmost importance to keep written records on all samples conducted, as well as on meteorological conditions such as minimum- and maximum temperatures, humidity levels, and daily rainfall prevailing at the time. Recording these data is an activity that must be performed daily. Environmental conditions are crucial, because it affects the development rate of both the crop and the pest (University of California, 1992). A good idea is to invest in a small weather station which will not only record temperature, rainfall, and humidity, but also day length, wind direction- and speed. The logic behind this would be to improve the capability of predicting aphid pest outbreaks, since recorded data will indicate under which environmental conditions outbreak problems were experienced. During sampling, note the aphid species present and their population densities, the predators present, the parasitoids present, growth stage of the crop, number of leaves sampled, etc. Appendix 6 provides an example of the field data form used during the study to monitor aphid

populations. Similar forms can be used for capturing data which will enable producers to compile graphs that indicate certain trends (e.g. relation between temperature and aphid abundance or differences in infestation between different structures/fields during different seasons). Also maintain a well organized reference collection of the aphid and natural enemy specimens collected for subsequent comparison between specimens collected during different times of the year (Figure 6.2).

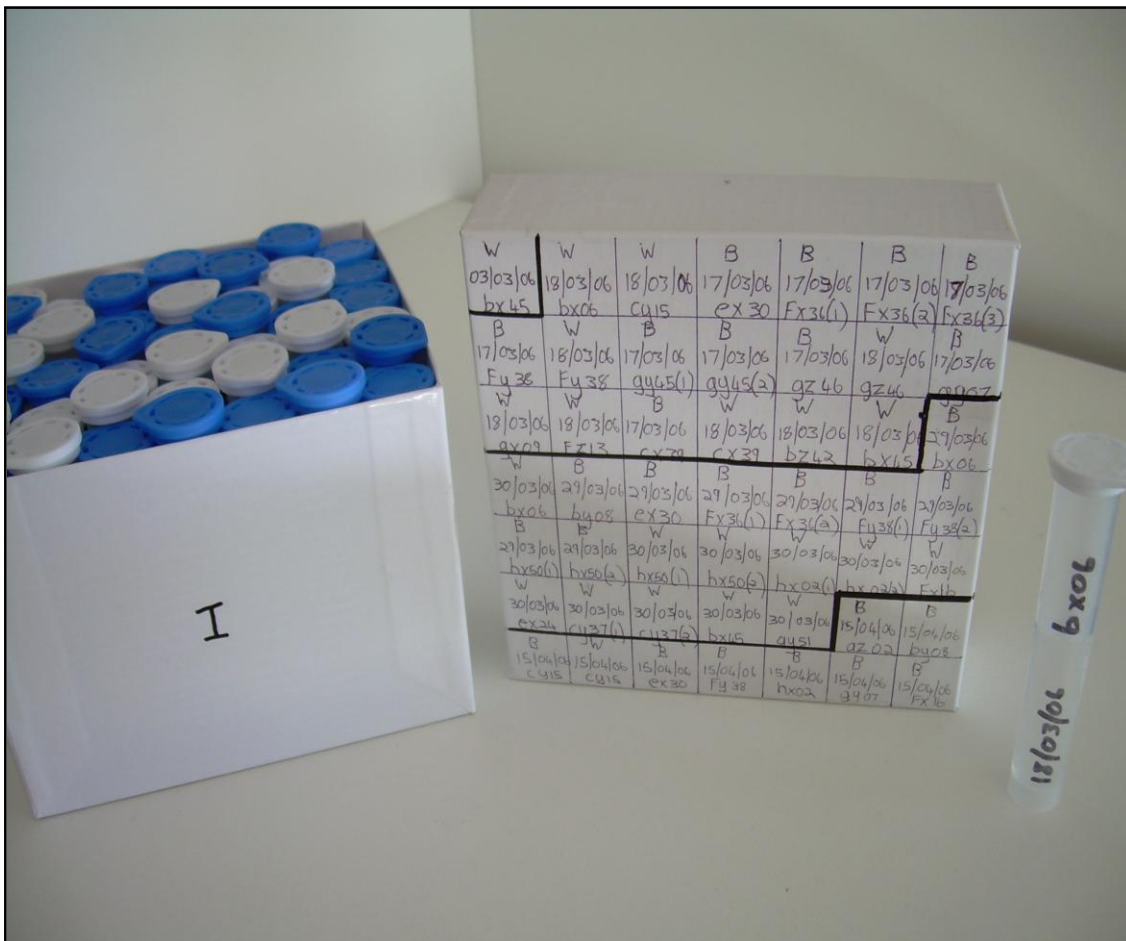


Figure 6.2: A well-organized reference collection box, containing alcohol-filled specimen vials with locality and collection dates.

6.4 APHID PEST STATUS

From the approximately 4 700 Aphididae species described, only 450 species have been recorded from cultivated crops, and of these, only 100 have fully exploited crops to become agricultural pests in the true sense of the word

(Blackman & Eastop, 2007). It can therefore be concluded that relatively few of the known aphid species can actually be considered as crop pests. Most of these pest species belong to the subfamily Aphidinae which is the largest subfamily of aphids, and they regularly feed on herbaceous plants (Blackman & Eastop, 2006). Indeed, all the pest aphid species that occur on lettuce in the central Free State also belong to this subfamily. The subfamily displays life cycles which correspond to the seasonality and phenologies of plants in the temperate northern hemisphere, where host-alteration originated. The formation of new buds and leaf fall in these regions is an indication to aphids that a new season has commenced or an existing one is nearing its end (Dixon, 1987d) and migration to or from the secondary and primary hosts will ensue in response. The generations found on the primary host are usually of no economic importance, but the parthenogenetic generations on the secondary host are the main concern to producers and it is these aphids that become pests when they form colonies on the host (Williams & Dixon, 2007). Despite this, only about 15% of the Aphidinae still make use of host alteration (Blackman & Eastop, 2007). As a matter of fact, most aphid species are monoecious and are restricted to a particular genus or family of plants (Dixon, 1987c). During this study *A. lactucae* and *M. euphorbiae* provided proof of this by being present on lettuce throughout the year. However, *M. persicae* and *N. ribisnigri* exhibited seasonality which could be attributed to heteroecy. It is important that each producer takes note of the life cycles displayed by the particular aphid species found within the particular cropping system (refer to Chapter 1 for a detailed description of aphid life cycles under Section 1.7.2). Knowledge of aphid life cycles will assist producers in developing and improving management measures (Williams & Dixon, 2007) and predict periods of higher risk.

Feeding preferences are another determining factor regarding potential pest status. Polyphagy is restricted mainly to summer morphs of heteroecious Aphidinae which feed on herbaceous hosts (such as lettuce). A possible explanation for polyphagy found in these species might be that a change in climatic conditions, which could affect one or a few hosts negatively, will not

affect other hosts. In this way, polyphagous aphids will have an improved chance of survival, compared to aphid species which are monophagous (Dixon, 1987a). Despite the obvious advantage, only 5% of all known aphid species display polyphagy, while the remaining species are either monophagous or oligophagous (Pettersson *et al.*, 2007). However, highly polyphagous aphid species (i.e. *M. persicae*) were witnessed to infest lettuce plantings during this study.

Along with host alteration, and feeding preferences, cyclical parthenogenesis is another key to the success of aphids on short-lived crops such as lettuce (Blackman & Eastop, 2000). Aphids can rapidly infest crops in new environments and establish large populations in a relatively short space of time which can complicate control (Williams & Dixon, 2007). Host quality is another major contributing factor which determines the rate at which an aphid pest population develops. High quality host plants, such as lettuce which must be healthy and attractive to the consumer, is more likely to accommodate bigger and faster-developing aphids (Dixon, 1987b). Knowledge of host alteration, non-host alteration, feeding preferences and population dynamics of aphids are all important factors for producers to be able to group them according to the threat they pose for the crop.

6.5 APHID IDENTIFICATION AND THE CLASSIFICATION OF PEST STATUS

6.5.1 Identification

The correct identification of any pest species is crucial, and is the next logical step after scouting has taken place and aphids have been found to infest the crop. Identification is the key to understanding the biology of the species concerned and provides critical information relating to its natural enemies (Blackman & Eastop, 2007). These two authors provide a good example of a scenario which occurred on soybean crops in the U.S.A. during 2000 which demonstrates the value and necessity in correctly identifying a pest species. Large numbers of an aphid resembling *Aphis gossypii* infested the soybean crop. Correct identification, however, showed that the species was *Aphis glycines*, which originates from the Far East. Accurate identification in this case provided

researchers with the required information on the biology of the species, since the species is well-studied in Eastern Asia where it is a pest of certain crops.

With aphids, the correct identification based on morphological characteristics can be problematic because phenotypical expression is influenced by environmental conditions (Blackman & Eastop, 2007). For any aphid species, there may be a variety of different forms or morphs with morphological differences. The difference between the fundatrix and later parthenogenetic females has already been described in Chapter 1 (Section 1.7.3). Other factors which could give rise to morphological differences within the same species include environmental factors, crowding, day length, etc., which could lead to winged (alate) or wingless (apterous) parthenogenetic females, with morphological differences between the two regarding different body parts. In addition, these differences in body parts may not be specific, because some wingless individuals could also display characteristics typical in winged forms. There may also exist forms that are intermediate between parthenogenetic females and oviparous females (Blackman & Eastop, 2007). Furthermore, plant nutrition could also determine the size of aphids, with poor quality hosts leading to a decrease in the physical body size in certain generations. In addition the generations found on the primary host may differ morphologically from the generations found on the secondary host (Blackman & Eastop, 2007). Differences in temperature might also influence pigmentation or the size or length of specific body parts (Blackman & Eastop, 2007). Although all these possibilities might prove discouraging to anyone attempting to identify aphids to species level, it is necessary to take note of them. Due to these differences between morphs, it is advisable to collect large series of specimens for correct identification, and to use the help of professional persons.

Identification of an aphid up to species level does not imply that everything about the aphids' life history is known. Even species that belong to the same taxospecies can have a mixture of agamospecies and biospecies (species which are incapable or capable of amphimixis) (Shaposhnikov, 1987). Knowledge about aphid population structures and organization, as well as factors leading to

speciation, would be advantageous to producers, because races could develop suddenly on cultivated crops which show different responses to certain factors. These include host specificity, pesticide resistance, capability to transmit diseases, etc. (Shaposhnikov, 1987). It is exactly this rapid adaptation which has led to some aphid species becoming pests (Blackman & Eastop, 2007). A good example encountered during this study is *A. lactucae* which showed dominance in the fully covered structure due to the absence of pugnacious ants, and certain morphological adaptations (wax covering) which protected it from the humid conditions in the structure.

Morphological characters are commonly used in order to identify the species a specific organism belongs to. Taxonomic keys are commonly used to identify insect species. However, the use of these keys can prove to be quite a challenge – even to the specialist. In their excellent work '*Aphids on the world's crops: An identification and information guide*' Blackman & Eastop (2000) provide an account on identification of pest aphid species occurring on different crops by using morphological keys. The same keys were applied (and slightly modified in certain circumstances) in order to enable producers to accurately identify the four most common aphid pest species they can expect on lettuce in the central Free State (see Appendix 7). In Appendix 8 photos of slide-mounted aphid specimens were also included, together with annotations and descriptions of some outstanding morphological characteristics which can be used to distinguish between the different species. Also depicted in Appendix 8 are the most important coccinellid predators associated with these pest aphid species.

6.5.2 Categorizing aphid pest status

The most important aphid species found to infest lettuce during this study (*A. lactucae*, *N. ribisnigri*, *M. euphorbiae* and *M. persicae*) are all well-known pests and they are also exotic to South Africa. Unfortunately, pest aphid species seem to retain their status as pests when they are translocated from temperate areas to regions with milder climates (Dixon, 1987a). It is essential to determine exactly what threat each aphid species occurring on a crop within a specific

region poses to the crop. A process for doing this is known (Irwin *et al.*, 2007) and it is aimed at classifying aphid species according to their ability to colonize crops and to transmit diseases (also refer to Chapter 1 section 1.7.4). The mentioned four categories (transient non-vectors, transient vectors, colonizing non-vectors and colonizing vectors) are aimed at predicting the threat that a specific aphid species would have to the crop, should they enter the cropping system. However, this classification system is applied to aphids landing on the crop (alates), after which they are then assigned to one of the categories based on available information regarding its damage- and colonizing abilities. This is executed regardless of the fact whether or not the specific aphid forms a colony on the crop during the specific season and within the specific region. On the basis of this pest classification system and the fact that aphid pest species do not always colonize crops in certain regions or under certain circumstances (even though they might be present in the surrounding environment), an enhanced methodology for classifying the pest status of aphids occurring on lettuce is hereby proposed. It is envisaged that the suggested pest status classification process will increase the accuracy of aphid pest categorization and is aimed to be region and season specific, while being user-friendly due to its perceived simplicity. Three main categories are therefore proposed, namely:

- Key pest species (which would include colonizing non-vectors and colonizing vectors),
- Potential pest species (which would include transient vectors, colonizing non-vectors, and colonizing vectors), and
- Non-pest species (which would include transient non-vectors)

Grouping aphids according to these three categories is a relatively simple task, which even an untrained producer will soon find to be effortless and accurate. With this system, aphids are still grouped according to their vectoring capabilities, as discussed by Irwin *et al.* (2007), but the three additional main categories render the level of distinction more accurate and, as such, simply depend on the

ability of the aphid pest to colonize the crop under certain circumstances and in certain regions of the world.

Key pest species: Once an aphid species has formed colonies on lettuce in a specific area, they will be considered a key pest of the crop in that region. Accurate records of these key pests and the environmental conditions under which they prevail must be kept, because their occurrence might differ between seasons and even between years. If the aphid species are capable of forming colonies on the crop (and are therefore considered key pests), they can then be further classified into either colonizing non-vectors, or colonizing vectors, on the basis of their viral transmitting capabilities. Both should be regarded as a serious problem, since even if a species proves to be incapable of transmitting disease, high population numbers will lead to the excretion of excess honeydew which will give rise to secondary complications, such as the formation of sooty mould. Large aphid populations will also hamper head weight increase under less optimum growing conditions, leading to an inferior product (refer to Chapter 5). Contamination with shed skins and the aphids themselves would give rise to phytosanitary complications. Once an aphid has become a key pest in a certain region, it will retain this status with regard to crop production and it will have to be monitored on a constant basis. There is apt to be no more than four or five key pest aphid species on any crop that is produced in any given region of the world (Irwin *et al.*, 2007). True to this, this study has shown that, under shadehouse conditions, only four aphid species can be classified as key pests in the central parts of the Free State.

Potential pest species: If an aphid species is observed on lettuce and the species is known to be a common pest of the crop (based on literature), but colonies are absent during a specific planting cycle, it can be regarded as potential pest species. It can therefore be said that colonizing non-vector and colonizing vector aphid species are potential pests if they are observed on lettuce without evidence of colony formation, and are present as alates. These species therefore have the capacity to become key pests (by forming colonies) once

optimum conditions prevail. Also included as potential pest species are the transient vectors which are capable of transferring viral diseases to lettuce through their search for a potential host plant. They are included as potential pest aphid species since they don't necessarily always transmit diseases, but that the probability does exist under certain conditions. Aphid species which were recorded to feed on lettuce plants during this study and for whom no colonies formed, were *Aphis craccivora* and *Aphis pseudocardui*. Potential pest species must be considered with the same level of seriousness as key pest species, since, as mentioned previously, pest populations could develop under favourable environmental conditions. It is possible that the optimum conditions for development of these aphids were lacking during the study period and that a slightly 'different' year, with regard to biotic and abiotic conditions, could lead to their outbreak.

Non-pest species: Most aphid species lack the ability to form colonies on non-hosts, signifying a serendipitous encounter with the crop. Their presence can therefore be considered a mere coincidence and no control needs to be recommended. They are the non-pest aphid species of which only transient non-vectors form part. During this study this was encountered when *Aphis* sp. 1, was recorded in relatively large numbers during the seedling stages of the crop. If not categorized correctly a phenomenon such as this could cause unnecessary pandemonium among lettuce producers. *Rhopalosiphum nymphaeae* and *Rhopalosiphum rufiabdominalis* were also examples of this category of aphids.

6.6 APHID DAMAGE TO LETTUCE

Aphid colonies exert immense strain on host plants, sometimes resulting in the formation of visible feeding damage and malformations (e.g. discoloration, galling, etc.) and asymptomatic damage (e.g. changes in the quality of the host plant) (Pettersson *et al.*, 2007; Quisenberry & Ni, 2007). Results from this study have shown that visible damage could be less obvious and restricted in lettuce, but that asymptomatic damage (reduction in head weight) will be accelerated under less favourable growing conditions for the crop.

However, the big concern, when aphids are considered from a pathological point of view, is undoubtedly the vector capability of some species whereby certain viral diseases can be transmitted (Irwin *et al.*, 2007). The aphid pest species observed during the trial all have the capability to transmit viral diseases. *A. craccivora*, for instance, is known to transmit about thirty plant viruses (Blackman & Eastop, 2000). *M. euphorbiae* is another aphid species with excellent vectoring capabilities and is reported to be able to transmit over forty non-persistent viruses and five persistent viruses (Blackman & Eastop, 2000). Perhaps the best known plant virus vector of all species is *M. persicae*, which is known to transmit in excess of a hundred plant viruses (Kennedy *et al.*, 1962). *N. ribisnigri* can also transmit viral diseases to various crops. Despite their vectoring capabilities, not all of these aphid species are efficient transmitters of viral diseases to lettuce, or would necessarily transmit viruses to the crop. It has been shown that *M. persicae* and *M. euphorbiae* transmitted Lettuce mosaic virus (LMV) very efficiently, while *N. ribisnigri* is not able to transmit the virus (Nebreda *et al.*, 2004; Moreno *et al.*, 2007). Knowledge concerning the processes involved in virus transmission, as well as the viral transmission capabilities of different aphid species, is crucial for producers in order to assess the risks that the presence of certain aphid species hold.

6.7 PEST CONTROL OPTIONS TO ASSIST IN PLANT HEALTH MANAGEMENT

6.7.1 Commonly used practices

Various control measures can be employed in a plant health management system, e.g. chemical control, cultural control, genetic manipulation of the host plant, biological control, etc. Since biological control (using natural occurring predators in specific to curb the pest) is the focus of this study, only brief reference will be made to the other control options.

6.7.2 Chemical control

Insecticides are the first line of defense most commonly employed against aphid pests. In some cases insecticides are even used in conjunction with other

substances, such as alarm pheromones (Ester *et al.*, 1993) and mineral oils (Ferreles, 2000), in order to increase control. The most commonly used chemicals against aphid infestations are carbamates, organophosphates and pyrethroids, while the use of neonicotinoids is also increasing (Van de Steene *et al.*, 2003; Dewar, 2007; Smith & Chaney, 2007). Spraying is usually conducted as soon as infestation is noted, but more sophisticated spraying programmes require populations to reach a certain threshold before implementation (Klingauf, 1987). Despite this practice, Lykouressis & Mentzos (1995) reported chemical spraying to be most successful at the onset of rapid population growth. An above-mentioned discussion which pointed out that lettuce is a low pest threshold crop (Dewar, 2007) with a short growth season, illustrates the relevance of early management implementation. Chemical spraying of aphids should therefore preferably be conducted soon after pest aphid infestation has been noted by means of the scouting methods discussed above. It is also necessary to determine aphid population size prior to the heading stage, since control after heading can be problematic and inefficient. Attention should be paid to the period after pesticide application in which the crop is not suitable for human consumption. Later pesticide applications, especially after the heading stage, can therefore prove insufficient.

The pest category to which the aphid species belongs is very important when deciding which type of chemical to use. Slow acting pesticides would prove insufficient against transient vectors which are capable of rapidly spreading through the crop stand and transmit non-persistent viruses in a short period of time (Ferreles, 2000). They would, however, be adequate for use against colonizing non-vector and colonizing vector aphid species (Irwin *et al.*, 2007). The use of chemicals could in some cases lead to increased aphid populations due to induction for vector movement, resistance, and re-colonization of the crop (Katis *et al.*, 2007). Pesticide resistance is a significant problem with aphids (Van de Steene *et al.*, 2003), especially where frequent sprayings occur when aphids are not the main pest of concern (Irwin *et al.*, 2007). An aphid species present during this study, and which is well-known for its pesticide resistance, is

M. persicae (Blackman & Eastop, 2007). The species is virtually resistant to all known aphicides in Europe, and it has been the focus of several different studies in order to better understand the underlying mechanisms of resistance (Foster *et al.*, 2000; Foster *et al.*, 2007). *M. persicae* was not the only aphid species recorded during the study which is known to be pesticide resistant, with *N. ribisnigri*, *A. craccivora* and *M. euphorbiae* also sharing the trait (Kift *et al.*, 2004; Workman *et al.*, 2004; Foster *et al.*, 2007;). Heavy insecticide spraying against *N. ribisnigri* in parts of Europe has led to resistance against certain aphicides in this species (Rufingier *et al.*, 1999), stressing the importance of avoiding over-spraying and carefully planning the outcomes prior to spraying (Foster *et al.*, 2007). The species showed resistance to primicarb and lower and varied resistance to pyrethroids and organophosphates (Barber *et al.*, 1999). It is therefore best not to rely only on one group of insecticides, but to rotate different groups of pesticides with each other in order to reduce the possibilities of resistance development.

Another drawback of pesticides is its negative impact on natural enemies of aphid pests. Both insecticides and herbicides are able to reduce the numbers of coccinellids directly or indirectly (Obrycki & Kring, 1998). These disadvantages stress the importance of not relying solely on chemical spraying to control aphid pests, but to use as many control tactics as possible in an integrated approach. Entomopathogenic fungi, insecticidal soaps and plant extracts (BioNeem) have, for instance, proved to be successful against *M. euphorbiae*, *M. persicae* and *N. ribisnigri* under greenhouse conditions (Fournier & Brodeur, 2000) and serve as examples of how other less potent control agents can be used to combat aphids. Other examples of such products include selective-insecticides, spinosad, primicarb, virus-based insecticides, etc. These options are also more user-friendly and deliver a more socially acceptable product.

6.7.3 Cultural control

The term cultural control encompasses a wide array of agronomic and phytosanitary approaches (Jones, 2004) that are deployed to manage pest

populations. This is perhaps one of the more successful ways in which aphids can be managed on lettuce. Its preventative effects on the spread of aphid-borne viruses can also be significant. However, using some of these tactics also requires knowledge on the aphid pest species complex in the region of concern, as well as the damage they are able to cause (Irwin *et al.*, 2007). Tactics which the producer can readily deploy in order to manipulate host selection include the use of barrier crops, interplanting, mixed cropping systems, reflective surfaces, and manipulation of dispersal behaviour (Klingauf, 1987).

Barrier crops (trap crops) can be effective, although limitations with regard to their degree of success does exist, e.g. height of the barrier crop and the extent of nutrient competition which might arise between the barrier and the lettuce crop (Feres, 2000; Katis *et al.*, 2007). The idea is to lure the pest species away from the host crop towards an alternative host species planted around the edge of the field (Hokkanen, 1991; Khan *et al.*, 2008). The pests can then be effectively controlled by various means (chemical, mechanical, etc.), without concern of damaging the actual crop, or disturbing natural enemies within the crop. Planting a non-host species amongst the lettuce crop (intercropping) is aimed at making the crop less obvious to the pest, and increasing the biodiversity within a field, which will in turn enhance the natural balance between pests and their natural enemies (Theunissen, 1997). Natural enemy abundance has been demonstrated to be more species-rich in more diverse cropping systems as opposed to monocultures (Tonhasca, 1993; Griffiths *et al.*, 2008). This method of cultural control also harbours certain other advantages such as an increase in soil nitrogen, improved conservation of soil water and a decrease in weeds (Jarenyama *et al.*, 2000; Wratten *et al.*, 2007). Also, productivity is increased, since water, nutrients, etc., are utilized more efficiently (Midmore, 1993). Grass planted around a lettuce field could also act as a refuge for natural enemies (Wratten *et al.*, 2007). It is always a good idea to provide some source to which natural enemies can retreat and survive in times when either the crop or aphids, or both are absent. Such refuges may include windbreaks, unsprayed plants, or grasses and trap crops as discussed above (Khan *et al.*, 2008). Regrettably,

limited research that focuses on the use of such strategies in lettuce production has been conducted.

The use of reflective surfaces (e.g. straw or tin foil) is based on the idea of using materials which reflect short-wavelengths of light which is placed around the base of the plants (Smith *et al.*, 1964). However, such an exercise would be costly on a large scale for low value crops. Shadehouse structures, on the other hand, lend themselves perfectly to this technique, pending that the value of the crop permits its use. It is also a labour intensive task which must be executed correctly in order to be effective. When using straw it is essential to monitor the crop constantly for fungal diseases, since this is an excellent inoculum for pathogen development.

Sanitation is important when one is faced with aphid pest problems, since certain weeds, as well as plant debris, could harbour viral diseases that can be transmitted to the crop, or they can act as refuge to aphids, especially with regard to polyphagous species, during times when the crop is absent (Duffus, 1971; Irwin *et al.*, 2007). However, weed management might be an expensive operation and careful planning regarding timing of control may be necessary. There is also evidence that a limited degree of weed cover could actually enhance integrated control of some aphids. This is because aphids are much more prone to land on crops surrounded by bare soil, as opposed to crops which are surrounded by other plants (Smith, 1976). The use of virus-free propagative material is also essential in ensuring crop health, especially against seed-borne, non-persistent viruses such as Lettuce Mosaic Virus (Katis *et al.*, 2007). Removal of infested plants as soon as infestation is observed may also prove effective on a small scale (Katis *et al.*, 2007).

6.7.4 Genetic manipulation (host plant resistance)

By alternating the genetic structure of plants, resistance to aphid pests and the viruses they transmit is possible (Irwin *et al.*, 2007). Van Helden *et al.* (1993), has shown how aphid-resistant lettuce lines can impede feeding, reproduction, and

development of *N. ribisnigri*. Similar results have also been attained with other vegetable crops (Cooper *et al.*, 2004). Despite the availability of aphid resistant lettuce cultivars, resistance against some aphid species (e.g. *M. persicae*) is lacking, forcing producers to still rely heavily on pesticides (Hand *et al.*, 2003). Another alternative would be to manipulate aphid host selection. This entails the breeding of plants that aphids find unattractive (size, colour, etc.), or which would deter the aphid after it has landed on the plant (interference with chemical stimuli). It is possible for genetically manipulated lettuce cultivars to play an increasingly important role in future agriculture, since the possibilities of resistance development is reduced when using such plants. However, the success of using this tactic would be governed by the availability of such cultivars.

Its success is also dependant on public acceptance. There is immense resistance against the cultivation of genetically modified organisms in certain countries and amongst groups of people, which could discourage producers from growing these products due to a lack of available markets. This is regrettable, because the use of insect resistant varieties can hold several advantages in aphid pest control.

6.8 OVERVIEW OF BIOLOGICAL CONTROL OPTIONS FOR PLANT HEALTH MANAGEMENT ON SHADEHOUSE CULTIVATED LETTUCE IN THE FREE STATE PROVINCE

Control of aphids by natural enemies is usually focused on releasing these organisms into the field where a problem persists (refer to Chapter 1 for a discussion of these practices). On the other hand, the use of natural occurring enemies (by making use of conservation biological control) has received little attention (Obrycki & Kring, 1998; Gurr & Wratten, 2000; Powell & Pell, 2007). This is regrettable, because the use of such natural enemies has several advantages, including the use of understandable/producer-friendly concepts which simplifies its implementation, the fact that all growers can adopt the practice, and the fact that it can be used to support marketing strategies due to

more environmentally-friendly pest control measures (Jonsson *et al.*, 2008). There is ample evidence to suggest that biological control can be enhanced through the conservation of natural enemies (Straub *et al.*, 2008). The cultural techniques discussed above (habitat modification through intercropping and more diverse ecosystems), lend themselves perfectly to the enrichment and deployment of natural occurring enemies against phytophagous pests (Khan *et al.*, 2008). Biological control through the use of natural occurring enemies also has the potential to be economically beneficial in intensive land use scenarios such as in the use of shadehouses, by resulting in improved yields, lower input costs (savings on pesticides), and the production of more socially acceptable products (Cullen *et al.*, 2008).

However, studies investigating the benefits and costs of this practice are scarce and the additional costs of rendering an area or production unit more natural enemy friendly, without any guarantee of additional benefits, may deter producers from adopting the strategy. Producers may also find the idea of 'no immediate control' discouraging (Cullen *et al.*, 2008). A possible solution towards this perception problem is the implementation of small trials to test the effect that the conservation of natural enemies has on pest populations. This is also an excellent way to evaluate the seasonality and species complex of both pest and natural enemies, and the interaction among them and other organisms (e.g. ants). Positive results should be encouraging to producers who may then be more likely to adopt the new innovations.

Conserving natural enemies requires knowledge on the ecology of the natural occurring enemy species, and a good understanding of the ecological community within which these organisms function (Cullen *et al.*, 2008; Jonsson *et al.*, 2008), and small trials will contribute towards establishing such a knowledge base. For example, there is bound to be a selection effect, where one (or a few) natural occurring enemies are more reliable biocontrol options than others (Straub *et al.*, 2008). This study proved this when only two of the observed coccinellid species were present in high numbers throughout the year (*Hippodamia variegata* and

Scymnus sp. 1), while others were also present in high numbers, but only during certain times of the year (*Cheilomenes lunata* and *Exochomus flavipes*), whilst one species was only observed once (*Harmonia* sp. 1). Another example is that the physical size of the predator in relation to its prey is also important when identifying reliable top performing predators, because smaller predators (e.g. *Scymnus* sp. 1) will consume less prey (Straub *et al.*, 2008). However, Diehl (1993) has found that this in itself is not necessarily disadvantageous, since the simultaneous presence of a larger predator (*H. variegata*, *C. lunata* or *E. flavipes* in this study) could serve to enhance pest control. This is based on the premise that smaller predators are less likely to become intraguild prey due to the fact that they do not drastically lower prey populations and are thereby not directly in competition with other predators. However, abundant smaller predators (as witnessed in this study) would consume more prey compared to a situation in which they are not abundant. This in turn could then lead to direct competition with larger predators. Knowledge regarding the former is essential for understanding the levels at which organisms interact with each other.

Natural coccinellid abundance can be enhanced through the reduction of harmful pesticides, use of resistant lettuce varieties, and the establishment of more diverse agro-ecosystems (Obrycki & Kring, 1998; MacLeod *et al.*, 2004; Griffiths *et al.*, 2008; Jonsson *et al.*, 2008). In the latter regard limited information on the true effects of a more diverse ecosystem on aphid feeding damage and yield reduction is available (Cullen *et al.*, 2008). This study has shown that a more insect diverse agroecosystem (such as the partially covered structure during the warmer months in this study) will ensure adequate biological control of aphids, and ensure higher yields. Producers are therefore strongly advised to make use of production practices (as mentioned above) which will have the minimum impact on natural occurring aphid predators. On the other hand, it is also known that the effect of this type of biological control may prove to have a negative impact under certain circumstances.

Natural enemies of aphids rarely occur in isolation of each other (as seen in this study) and this will have an effect on the ultimate success of aphid control (Völkl *et al.*, 2007). If natural enemies do not compete for prey, improved biological control can be the result (Chang, 1996). However, a situation in which natural enemies compete for the same aphid prey, or where they feed on each other or on the eggs of their own kind, could result in lowered efficiency in biological control (Hochberg & Lawton, 1990; Santi *et al.*, 2003). Lower aphid numbers could also result in a situation where coccinellid predators feed on each other (Agarwala & Dixon, 1992) or disperse from the field with lowered aphid abundance (Elliot *et al.*, 1996). However, coccinellids are more generalist predators than is the case with parasitic wasps, and therefore have a better chance of survival should such shortages occur (Östman, 2004; Straub *et al.*, 2008).

The pest status of the aphid species is also important when one considers biological control options and the effectiveness of such control. Transient vectors can be used as an example of this: due to the fact that these aphids move rapidly from plant to plant in search of a suitable host (whilst spreading viral diseases in the process), natural enemies will be unable to control their numbers effectively, except when released on a regular basis at the source from which the aphids spread (Irwin *et al.*, 2007). A downside to natural enemy application is that both field and laboratory studies have shown that the presence of coccinellid predators in the crop could enhance the speed with which aphids spread viruses (Roitberg & Myers, 1978; Smyrnioudis *et al.*, 2001). It is therefore obvious that natural occurring enemies of aphids will be more successful if employed against colonizing non-vectors and colonizing vectors, compared to transient vectors. This again stresses the importance of acquiring knowledge regarding the biology and vectoring capabilities of aphid pest species.

Despite the drawbacks discussed above, the benefits of this practice makes it an attractive option which can be meaningful, as was observed in this study where

almost complete control of aphid populations can be achieved without the use of expensive chemicals.

6.9 ANTS AND APHIDS: IMPLICATIONS FOR PLANT HEALTH MANAGEMENT ON SHADEHOUSE CULTIVATED LETTUCE

Ants are reported to be beneficial to aphids by cleaning their environment, protecting them against predators and parasites, and, in certain cases controlling the appearance of alates (Kawada, 1987). However, aphids are rarely attended by ants in annual crops (Williams & Dixon, 2007). This study yielded similar results with no aphid attending behaviour being observed for the pugnacious ant, *Anoplolepis custodiens* (see Appendix 9 for a representation of these ants). This species was the dominant soil-dwelling ant species observed during the study and is reported to be a pest due to their attendance behaviour to coccids and aphids (Skaife, 1979). However, it is more likely that this is the case in perennials, and they would rather exploit the aphids close to the soil surface on a short season growth crop such as lettuce as a food source. Despite this, it is again stressed (as in Chapter 4) that more controlled studies are necessary to determine the exact behaviour of this ant species towards lettuce pest aphid species under more controlled environmental conditions.

Whatever the outcome of such studies might be, one important lesson can be learnt from these interactions, namely that none of the organisms discussed in this study occur in isolation. They all operate on a multitrophic level with a multitude of other organisms directly or indirectly affecting their behaviour, development, occurrence, and population dynamics. This interaction is not only restricted to above-soil level circumstances, but is also applicable to below the soil-level where other organisms can alter the nutritional quality of the host which will in turn have an impact on above-soil level pests (De Deyn *et al.*, 2007). Bearing this in mind, when attempting to enhance plant health through the use of more biological orientated strategies, is key to the success of such management strategies. As an example, and to illustrate the complexity of these interactions on a coccinellid-aphid-ant interaction level, various authors (Bradley, 1973;

Jiggins *et al.*, 1993; Sloggett & Majerus, 2000) have demonstrated that coccinellids will be more abundant in areas from which ants have artificially been excluded. Therefore, while it might seem to be a good idea to include pugnacious ants into the system due to their reducing impact on aphid numbers, they will in turn have a negative impact on coccinellids (especially on the larvae, as seen in this study). Producers are therefore again urged to conduct preliminary trials in order to better understand the extent of interactions between these organisms, and to reach a decision on how to construct their shadehouses and which organisms to tolerate within these structures. Only then will it be possible to assess the risks and benefits associated with each insect species associated with the lettuce crop.

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CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Shadehouse structures provide a relatively cheap solution to producers seeking effective means of protecting their lettuce crop against some of the harsher environmental conditions experienced on the South African highveld (e.g. frost, hail, high winds, direct sunlight, etc.). The type of shadehouse structure used for lettuce cultivation also influences the detrimental and beneficial insects associated with lettuce produced under such conditions.

This study indicated that not all aphids observed on lettuce should be considered as pests, and also that not all pest species are present on a permanent basis throughout the year. Only four of the collected aphid species can be considered true (key) pests of lettuce in the region, namely *Acyrtosiphon lactucae*, *Macrosiphum euphorbiae*, *Myzus persicae* and *Nasonovia ribisnigri*, all of which are exotic (Chapter 3). *Aphis craccivora* which is known to utilize lettuce as a host was also collected, but observable colonies were lacking, classifying it a potential pest species which could have the capacity to infest lettuce in the area (Chapter 3 & 6). Seasonality was observed for some of the pest species, resulting in a situation where *N. ribisnigri* can be present during the warmer months of the year in both structures, whilst *M. persicae* can be present during the cooler months of the year (Chapter 3). *N. ribisnigri*, in particular, seemed better adapted to thrive in the presence of the pugnacious ant, *Anoplolepis custodiens*, during the summer months due to its cryptic feeding habits, but it was absent during the seedling stage of the crop. This could be ascribed to a lack of protection from the ants when the lettuce plants are still in the seedling stage (Chapter 3). The aphid species *A. lactucae* can be expected throughout the year in both structures and in all growth stages of the crop, showing no indication of seasonality or holocycly (Chapter 3). Its dominance, however, seemed to be restricted by the presence of *A. custodiens* in the partially covered structure, and it appeared as if this species were better adapted to the more

humid conditions experienced in the fully covered structure (Chapter 4). *M. euphorbiae* is another species showing an anholocyclic life cycle in the region, and which must be monitored continuously in both structures and in all growth stages of the crop (Chapter 3). It also appears as if the presence of *A. custodiens* could suppress their numbers during the warmer months of the year in structures to which these ants have access. Should these ants become scarcer or absent during the cooler winter months, this species would replace *N. ribisnigri* as the most abundant species (Chapter 4).

Of all the coccinellid species observed during the trial, only *Hippodamia variegata* and *Scymnus* sp. 1 (and to a lesser degree the larvae) were regularly observed in both structures (Chapter 3). It can be assumed with relative certainty that *H. variegata* is an important natural enemy of the aphid species, but the small size of *Scymnus* sp. 1 renders it an uncertain natural enemy. It is possible for this species to feed on the smaller aphid nymphs, but continuous investigation is required to determine its feeding preferences. Their smaller size will also imply that they consume less prey compared to the larger species. The remaining coccinellid species had a lower occurrence, but *Cheilomenes lunata* and *Exochomus flavipes* could also be promising natural enemies during the warmer months if access to fully covered structures is provided, as discussed below (Chapter 3). However, more controlled studies are required to accurately establish the feeding rates and preferences of these species in order to assess their value in conservation biological control.

A common misperception regarding shadehouse structures is that they can exclude insect pests. This trial has shown that a 25% shade-providing net doesn't have the capacity to exclude smaller-sized pests such as aphids, as was observed with the fully covered structure (Chapter 3). In fact, this structure harboured similar aphid pest species as those observed in the partially covered structure (Chapter 3). Furthermore, aphid infestation levels attained far greater proportions when compared to the partially covered structure during the warmer months of October – January (due to the dominance of *A. lactucae* in this

structure). It is therefore clear that their use as an insect proof barrier is most likely limited to a few bigger sized insect pest species (e.g. Orthoptera, Lepidoptera, and Coleoptera), pending adequate covering of the structure. Despite the fact that similar adult coccinellid species as those observed in a partially covered structure are also able to find their way into this type of structure (Chapter 3), the larger body will render entry cumbersome, resulting in the presence of less adult coccinellids (Chapters 3 & 4). In light of this, a fully covered shadehouse structure could hamper conservation biological control. Providing access to the natural occurring enemies into such structures is therefore recommended. This can be achieved by partially opening the structure (one or more sides left uncovered), or by making use of access flaps which can be opened or closed as required. Fully covered structures also reach a higher mean temperature range and humidity level throughout the year, which could favour aphid population growth (especially for the morphologically adapted *A. lactucae*) (Chapter 4) and the establishment of certain pathogens (especially fungi) during the warmer months of the year. Furthermore, they hamper the dispersal of alates from the structure which could increase aphid population growth rates dramatically (Chapter 4). Access flaps will therefore improve air circulation and cooling of such structures, and allow dispersing aphids to vacate these structures. In addition, these structures reach lower mean minimum temperatures during the winter months (Chapter 3). This proved advantageous, because it restricted aphid population growth during these periods (Chapter 4), concomitant to higher mean maximum temperatures during the same months that appeared to favour lettuce growth (Chapter 5).

Partially covered shadehouses harbour fewer aphids during the warmer months (when *N. ribisnigri* is dominant in this structure), mainly due to the presence of the pugnacious ant, *A. custodiens*, and the higher presence of adult coccinellids, especially the species *C. lunata*, *E. flavipes*, and *H. variegata* (Chapter 4). The presence of the pugnacious ant could therefore prove positive in as far as their predation and disturbance on aphids are concerned, but negative in the sense that they remove/kill coccinellid larvae in this structure which could lead to a

lower abundance of the larvae (Chapter 4). The decision of whether or not to allow access for these ants into shadehouse structures is complicated and it is recommended that the interaction between these ants and aphid colonies are monitored in preliminary trials (Chapter 6). While they may have preyed on the aphids during this study, the situation could just as well be reversed during other years or at different localities. More accurate studies are therefore necessary to establish the interaction between these ants and lettuce pest aphids in order to determine their impact on one another. On the other hand, the partially covered structure will harbour more aphids during the winter months (mainly due to the higher presence of *M. euphorbiae*) as a result of an absence or lower occurrence of ants and coccinellids, and also because mean minimum temperatures are more favourable for aphid development (Chapter 4). The decision concerning shadehouse structures must therefore not be centered on which type of structure to use, but rather which type of structure to use during specific times of the year (see Table 7.1 for the characteristics of each type of shadehouse). The ideal would be to use a shadehouse structure which can be opened on the sides during the warmer months, and closed during cooler months.

The direct feeding damage aphids caused to the lettuce crop were more asymptomatic rather than symptomatic, and entailed a reduction in head weight. The microclimate experienced in each structure were important in this regard, and it became evident that head weight were significantly reduced by aphid feeding if the microclimatic conditions were not optimal for lettuce production (e.g. in the fully covered structure during spring and summer months). Higher aphid populations will also entail that more leaves will become infested, while almost all the leaves of a plant can harbour aphids under severe infestation conditions. This could drastically lower the attractiveness of the crop, which could in turn, lead to market rejection. It can therefore be concluded that lettuce has a low economic threshold for aphid pests.

Scouting or monitoring for aphids must be conducted with attention to detail and with the utmost thoroughness. Written records of environmental conditions, aphid

populations, predator abundance, etc., must be kept in order to more accurately predict pest outbreaks. After aphids have been detected, ensuring plant health in lettuce shadehouse cropping systems necessitates the use of three components, namely: fundamentals, tactics, and strategies (Chapter 6). Fundamentals include information regarding the identity of the pest species, as well as information of its vectoring capabilities and pest status (key pests, potential pests, and non-pests). Identification of aphid pest species could prove problematic to the untrained eye, and identification by a specialist is therefore recommended. Correct identification is a crucial component to managing plant health and is the point of departure for the producer and allows him access to all the necessary information pertaining to the biology of the species concerned, and in determining its pest status (Chapter 6). Tactics which can be employed to combat aphid pests, include chemical control, cultural control, and biological control (Chapter 6). These tactics should be integrated to ensure increased aphid management and to undercut the possibilities of pesticide resistance and eradication of natural enemies. Strategies define how these tactics can be employed in order to ensure plant health on a sustainable level. When making use of biological control (especially conservation biological control), it must be borne in mind that the success of this strategy relies on an understanding of the multitrophic interaction between the pest, its natural enemies and other organisms in the system. A sound knowledge of the interactions between these organisms and the biology of each, are therefore necessary to evaluate the benefits of deploying such a strategy (Chapter 6).

Future research: As with most other studies, the research led to further questions that need to be investigated. Therefore, more studies are required to answer the following questions that arose during the trial:

- The intrinsic rate of population growth of each of the four important aphid species that infest shadehouse cultivated lettuce (*A. lactucae*, *N. ribisnigri*, *M. persicae* and *M. euphorbiae*).

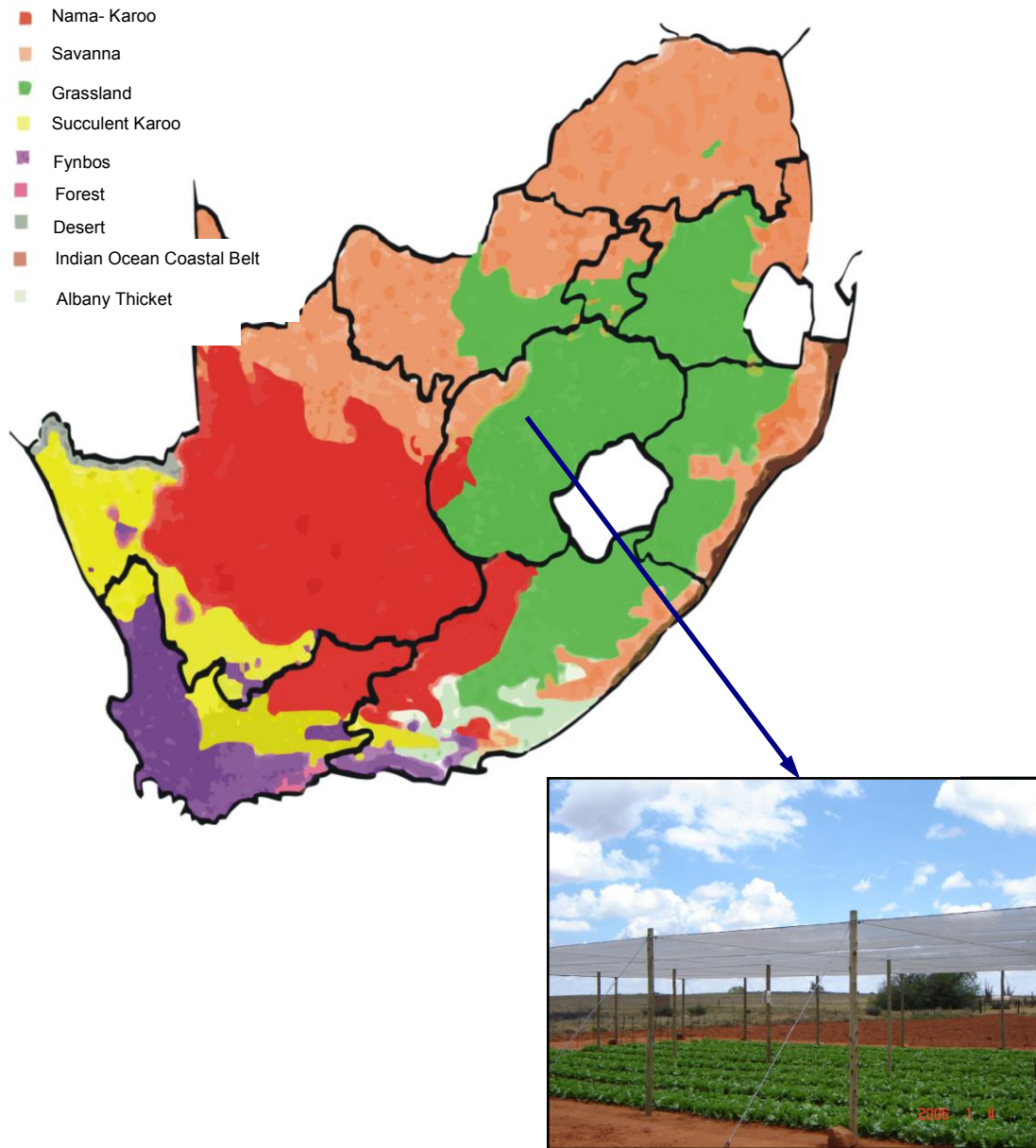
- The individual and combined performance of each of these aphid species in the presence/absence of the pugnacious ant, *A. custodiens*, under more controlled circumstances.
- The exact behaviour of *A. custodiens* towards these aphid species in the presence/absence of alternative food sources.
- Feeding preferences of the coccinellid, *Scymnus* sp. 1.
- Exact prey choice of the coccinellids, *H. variegata*, *E. flavipes* and *C. lunata*, when provided with the four aphid species.
- Prey consumption rates and generation times of these coccinellids.

Table 7.1: Different characteristics of each shadehouse structure when compared to each other (Roodevallei, Free State Province).

Parameter	Structure	
	Fully covered structure (FCS)	Partially covered structure (PCS)
Construction costs	Higher compared to PCS	Lower compared to FCS
Ability to exclude aphids	Poor	Poor
Aphid infestation levels during warmer months	Significantly higher compared to PCS	Significantly lower compared to FCS
Aphid infestation levels during cooler months	Significantly lower compared to PCS	Significantly higher compared to FCS (but not as high as FCS during warmer months)
Ability to exclude coccinellid predators	Larger species find it harder to access the structure	Poor
Presence of adult coccinellids	Lower compared to PCS	Higher compared to FCS
Presence of larval coccinellids	High during warmer months	Low throughout
Maximum temperature range	Higher compared to PCS	Lower compared to FCS
Minimum temperature range	Lower compared to PCS	Higher compared to FCS
Humidity level	Higher compared to PCS	Lower compared to FCS
<i>A. custodiens</i>	Aphid and coccinellid larvae numbers were high when this ant was excluded	Lowered aphid and coccinellid numbers during their presence in the warmer months

APPENDIX 1:

STUDY SITE



Appendix 1: Locality of the study site at Roodevallei, central Free State, South Africa (Map redrawn from: Rutherford *et al.*, 2006).

APPENDIX 2:
TRAIL LAYOUT

BLOCK 1			BLOCK 2			BLOCK 3			BLOCK 4			BLOCK 5			BLOCK 6			BLOCK 7			BLOCK 8		
ax	ay	az	bx	by	bz	cx	cy	cz	dx	dy	dz	ex	ey	ez	fx	fy	fz	gx	gy	gz	hx	hy	hz
51			51			51			51			51			51			51			51		
50			50			50			50			50			50			50			50		
49			49			49			49			49			49			49			49		
48			48			48			48			48			48			48			48		
47			47			47			47			47			47			47			47		
46			46			46			46			46			46			46			46		
45			45			45			45			45			45			45			45		
44			44			44			44			44			44			44			44		
43			43			43			43			43			43			43			43		
42			42			42			42			42			42			42			42		
41			41			41			41			41			41			41			41		
40			40			40			40			40			40			40			40		
39			39			39			39			39			39			39			39		
38			38			38			38			38			38			38			38		
37			37			37			37			37			37			37			37		
36			36			36			36			36			36			36			36		
35			35			35			35			35			35			35			35		
34			34			34			34			34			34			34			34		
33			33			33			33			33			33			33			33		
32			32			32			32			32			32			32			32		
31			31			31			31			31			31			31			31		
30			30			30			30			30			30			30			30		
29			29			29			29			29			29			29			29		
28			28			28			28			28			28			28			28		
27			27			27			27			27			27			27			27		
26			26			26			26			26			26			26			26		
25			25			25			25			25			25			25			25		
24			24			24			24			24			24			24			24		
23			23			23			23			23			23			23			23		
22			22			22			22			22			22			22			22		
21			21			21			21			21			21			21			21		
20			20			20			20			20			20			20			20		
19			19			19			19			19			19			19			19		
18			18			18			18			18			18			18			18		
17			17			17			17			17			17			17			17		
16			16			16			16			16			16			16			16		
15			15			15			15			15			15			15			15		
14			14			14			14			14			14			14			14		
13			13			13			13			13			13			13			13		
12			12			12			12			12			12			12			12		
11			11			11			11			11			11			11			11		
10			10			10			10			10			10			10			10		
9			9			9			9			9			9			9			9		
8			8			8			8			8			8			8			8		
7			7			7			7			7			7			7			7		
6			6			6			6			6			6			6			6		
5			5			5			5			5			5			5			5		
4			4			4			4			4			4			4			4		
3			3			3			3			3			3			3			3		
2			2			2			2			2			2			2			2		
1			1			1			1			1			1			1			1		
BLOCK 1	BLOCK 2		BLOCK 3			BLOCK 4			BLOCK 5			BLOCK 6			BLOCK 7			BLOCK 8					
ax	ay	az	bx	by	bz	cx	cy	cz	dx	dy	dz	ex	ey	ez	fx	fy	fz	gx	gy	gz	hx	hy	hz

Appendix 2: Trial layout demonstrating the plant numbering system used to provide each plant with an accession number (Roodevallei, Free State Province).

APPENDIX 3:
FERTIGATION DATES, pH AND EC VALUES

Appendix 3: Fertigation dates indicating amount of fertilizer used, as well as pH and EC quantified from each occasion (Roodevallei, Free State Province).

<u>Date</u>	<u>Hygroponic[®] (kg)</u>	<u>Calcium-nitrate (kg)</u>	<u>Water (l)</u>	<u>EC</u>	<u>Ph</u>
2005/12/03	1	0.8	1000	2.5	6.9
2005/12/11	1	0.8	1000	2.3	7.4
2005/12/17	1.2	1.5	1500	2.5	7.2
2005/12/24	1.4	1.7	1500	2.5	7.2
2005/12/31	2.5	2.1	1500	2.9	7.1
2006/01/07	1.7	1.5	1500	2.5	7.2
2006/01/14	1.7	1.5	1500	2.5	6.9
2006/02/04	*	*	*	*	*
2006/02/11	1	0.8	1000	2.5	7.1
2006/02/18	1	0.8	1000	2.4	7.2
2006/02/25	*	*	*	*	*
2006/03/04	1.5	1.2	1500	2.5	6.7
2006/03/11	*	*	*	*	*
2006/03/18	*	*	*	*	*
2006/04/01	1	0.8	1000	2.2	7.2
2006/04/08	1	0.8	1000	2.4	7.2
2006/04/15	*	*	*	*	*
2006/04/22	*	*	*	*	*
2006/04/29	1.5	1	1500	2.2	7.2
2006/05/06	1.7	1.5	1500	2.5	7.1
2006/05/14	2.5	2.1	1500	2.4	7.2
2006/05/21	*	*	*	*	*
2006/06/04	1	0.8	1000	2.3	7.3
2006/06/11	1	0.8	1000	2.4	7.3
2006/06/18	1	0.8	1000	2.4	7.3
2006/06/25	1	0.8	1000	2.6	7.4
2006/07/03	1.2	1	1500	2.5	6.9
2006/07/09	1.5	1.2	1500	2.5	7.2
2006/07/16	2.5	2.1	1500	2.6	7.1
2006/07/21	2.5	2.1	1500	2.4	7.2
2006/08/12	1	0.8	1000	2.5	7.3
2006/08/20	1	0.8	1000	2.4	7.6
2006/08/26	*	*	*	*	*
2006/09/01	1.5	1.2	1500	2.6	7.4

* = No data available

APPENDIX 4:
MINIMUM AND MAXIMUM TEMPERATURES

Appendix 4.1: Maximum temperatures (°C) recorded from fully covered shadehouse structure during trial period December 2005 - November 2006 (Roodevallei, Free State Province).

<u>Day</u>	<u>Dec</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>
1	*	28.5	28.5	33	28	20.5	23	16	8.5	22	32	27.5
2	*	33	33.5	30.5	29	19	22	18.5	4	24	27	19.5
3	*	28.5	30	17	29	19.5	18.5	17.5	11	22.5	25	22.5
4	*	34	32	27.5	29.5	20.5	19	18.5	12	24.5	20.5	25
5	*	36	37	26	30	22	18	20.5	12.5	23	22	22.5
6	*	34	21.5	27	30	18	21	22.5	18	25	24	28
7	*	33	26.5	30	29.5	16.5	22	15.5	21.5	25	24	29
8	*	28	26	33	26.5	19	21	18.5	22	24	23	33.5
9	*	27.5	28	35	28	19	20.5	19	21	27	24	31.5
10	*	29	33.5	33	21	19	20	19.5	22	28.5	28	32
11	*	31	33	32	21	22	22	19	23	30	31	31.5
12	*	34	23	22	25	23	22.5	21	16.5	28.5	31	30
13	*	35	32.5	26.5	28	23	19	19	23	27.5	30	31
14	*	35	34	26	28.5	23	14	19	25	30	31.5	31.5
15	*	36	36	25	28	24	16	21	15.5	24	33	36
16	*	35.5	30	27	29	22	18	21	16	27	30	34.5
17	*	37	34.5	25	22	19	20	21	20.5	17	25	30
18	*	33.5	34.5	27	24	18	21	22	20	18	31	28
19	*	35	32	27	21.5	14	20	23	23.5	22	28	27
20	*	36.5	33	30	26.5	9	20	25	24	22	30.5	28.5
21	*	29	34	21	23	11	20.5	19	22	25	32	33
22	*	31	35	26	22	12	14	11	19.5	26	31.5	32.5
23	*	30.5	28	25	9	12	15	13	19.5	29	36	34
24	*	32.5	20	28	18	12	16	20	16	28	35	33.5
25	*	35	20.5	26	20	14	17	24	18	31	35.5	32
26	34.5	37	23	30	22	18	16	25	20	20	29.5	31.5
27	37	25.5	30	22.5	23	20	13	25.5	22.5	25	34.5	31.5
28	37	30	31.5	25	24.5	17	15.5	24.5	24	29.5	30.5	33
29	36.5	30	*	23	23.5	18.5	16	24.5	21.5	31.5	29	36.5
30	33.5	33	*	26.5	23.5	22	16.5	23.5	12	33	29	34
31	30	34	*	29.5	*	23	*	20.5	20	*	32	*
Avg:	34.8	32.5	30	27.2	24.8	18.4	18.6	20.2	18.5	25.7	29.2	30.4

Appendix 4.2: Minimum temperatures (°C) recorded from fully covered shadehouse structure during trial period December 2005 - November 2006 (Roodevallei, Free State Province).

<u>Day</u>	<u>Dec</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>
1	*	15.5	14.5	10.5	9	8	-1	1	-0.5	-2	3	14.5
2	*	16.5	14.5	8.5	12	8	0	-3.5	0	1	3	6
3	*	15	13	6	12	2	-1.5	-3.5	-4	6	-0.5	2
4	*	15.5	13	6	7.5	1.5	-2	-5	-4	2	-1.5	5
5	*	18	15	4.5	8.5	4	-4	-2.5	-4	3	5	2
6	*	16	16	7	8	-3	-5	-2.5	-4.5	6	7	5.5
7	*	17	15	8	8.5	-3	-3	-4	-2.5	5	13	6.5
8	*	15	15	11.5	8	1.5	-5	-4.5	-2	4	6	11.5
9	*	16	14	14	9.5	3	-4	-5	0	3	4	10.5
10	*	14	16	12	11	-1	-3	-6	2	3.5	7	7
11	*	15	17	12	9	-1	5	-5	0.5	4.5	7	9
12	*	17	15	5	5.5	-1	-3.5	-1.5	-2	9	6.5	12
13	*	15	15.5	6	7	0	-3	2.5	-1.5	7	12	8.5
14	*	15	15.5	15	5	2	-4	-2	5	11	14	10
15	*	17.5	17.5	16	6.5	2	-2	5	-2	3.5	11	5.5
16	*	18	17	15	12	5	-3	5	-2.5	6	4	16
17	*	20	16.5	16	10	1	1	0	-1.5	7	10	14
18	*	15.5	17.5	16	12	2	-0.5	0	-0.5	-3.5	12	11.5
19	*	15	16	13.5	11	4	-3	3.5	-1	-1	11	14.5
20	*	16	16.5	15	9	-2	-3	7	6	-2	11	15
21	*	15.5	17	12	11	-4	-4	-0.5	6.5	0	6	8
22	*	17.5	17	11.5	6	-3	-6	-9.5	8	2.5	8	11
23	*	16	16	12	1	-5	-5	-9.5	8.5	2.5	7.5	14.5
24	*	17	17	12	2	-5	-5	-3.5	2.5	3	9	9
25	*	18	15.5	10	1	-6	-5	-1.5	2	1	12	12
26	12	16.5	14	12	1.5	-3	0	2	3	0	12	14
27	11	16	16	9	2	-3	-2	1	4	0	14.5	12
28	14	14	15	8.5	3	-4	-2	0	5.5	3	13	14
29	13.5	14	*	8.5	4.5	-3	-4.5	-3	-4.5	6	13	14
30	15	16	*	9.5	7.5	-1	-3	-1.5	-4	8	9	15
31	16	13.5	*	8.5	*	-2.5	*	7.5	-1	*	11	*
Avg:	13.6	16	15.6	10.7	7.35	-0.2	-2.7	-1.3	0.37	3.3	8.37	10.3

Appendix 4.3: Maximum temperatures (°C) recorded from partially covered shadehouse structure during trial period December 2005 - November 2006 (Roodevallei, Free State Province).

<u>Day</u>	<u>Dec</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>
1	*	28	28.5	31	27	21	22	16	15	21	32	28
2	*	33	32	29	30	20	21	18	5	24	26	19.5
3	35.5	28	28	15.5	28	19	18	17	11.5	21.5	24	22
4	35	32.5	31	28	28	20	18	18	11.5	24	19	19
5	33	34.5	30	26	28	21.5	18	20	12	23	20	22
6	32	33	22	26.5	28	18	20	22	17.5	25	22.5	27
7	34	33	21.5	28.5	28	16	21	15	21	25	23.5	28
8	31	28	27	31.5	26	17	20	18	21.5	24	22	31.5
9	33	27	27.5	32.5	27.5	19	19.5	18	20	25	23	30
10	32	30	31.5	31.5	21	19	20	19	22	27.5	28	31
11	29.5	32	31	30	20	21	21	19	23	28	29	31
12	28	33	22.5	22	24.5	22	24	21	16.5	28	29	28.5
13	33	34	30	24.5	27	22	19	19	23	27	28	30
14	30	34.5	32	21	28	22	14	19	25	28.5	30.5	31
15	30	35	34	25	27	24	15	21	15.5	24	31.5	35
16	34	35	30	27	28	22	17	21	16	26	29	34
17	33	35.5	32.5	25	22	19	19.5	21	20.5	16.5	24.5	29
18	34	33	33	26	24	18	20.5	21.5	19	18	30	27.5
19	34.5	33	30	25	21	14	19.5	23	22	21.5	27.5	26.5
20	32	35	32	28	25.5	9	19	24.5	24	22	29	28
21	35	29	31.5	21	23	11	20	19	22	28	31	32
22	34	30	34	25	21	12	14.5	13	19	25	30.5	31.5
23	35.5	30.5	27	25	10	12	15	13	19.5	28	33	34
24	36	31.5	20	26.5	17	13	11	20	15.5	27	34.5	34
25	35	33.5	21	25	19	14	16	23	18	30	34	31
26	34.5	36	23	28	21	16	16	24	20	20	28.5	31.5
27	36	24.5	29	22	23	20	14	25	21.5	24	34	31.5
28	37	30.5	30	24.5	23.5	17	15	23.5	23	28	30	32.5
29	36	29.5	*	23.5	22.5	18.5	15.5	23	22	30	28.5	34
30	32	32	*	25	22	21	16	22	12	31	28.5	33.5
31	29	33	*	27.5	*	22	*	20	19	*	31	*
Avg:	33.2	31.8	28.6	26	24	18.1	18	19.9	18.5	25	28.1	29.5

Appendix 4.4: Minimum temperatures (°C) recorded from partially covered shadehouse structure during trial period December 2005 - November 2006 (Roodevallei, Free State Province).

<u>Day</u>	<u>Dec</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>
1	*	16	14.5	12	10.5	9	0	2.5	1	0.5	4.5	15
2	*	16.5	15	10	12	4	1.5	-1	1.5	4	4	8
3	15	16.5	13.5	7	13	3.5	0	-1	-2.5	8.5	0.5	4
4	13	16	14	7	9	3	0	-3	-2	3	0	7.5
5	15	19	20	6	10	5	-2	-2	-1.5	4	4	3.5
6	14	17	16	8.5	9	-1	-2	-1	-3	7.5	6	6.5
7	11	18	15.5	9	10	-1	-1	-2	-1.5	6.5	14	8
8	13	16	16	12	9	0	-3	-2.5	0	5.5	5	12
9	16	17	15.5	14	10.5	-1	-2.5	-3	2	5	5	12
10	10.5	16	16.5	12	11.5	1	-1.5	-4	3	5	8	9
11	10.5	18	17	14	9.5	-1	5	-3.5	1	6	8	10
12	8	17	16	6.5	7	1	-1	0	0	9	7	12.5
13	11.5	16	16	7	8	2	-0.5	4	0.5	8	12.5	10
14	15	16	16.5	15	6.5	4	-2	0	6	12	14.5	11
15	16	18	18	17	8	3	-2	5	0	5	12	11
16	14	19	17	15.5	12	7	-1	6	0	7	4.5	17
17	14	20.5	17	16	11	3	3	1	0	0	11	15
18	10.5	16.5	17.5	16	12	3	1.5	2	1	-0.5	12.5	12.5
19	13	16	16.5	14	12	5	-1	5	0.5	1.5	12	15
20	12	17	17	15.5	10	0	-1	7.5	7	0	11.5	15.5
21	12	16	17	12.5	12	-2	-2	1	7	3	7.5	9.5
22	12	18	16.5	12	7	-2	-4	2	9	3	9	12
23	11	16.5	16.5	12	1	-3	-4	-6.5	9.5	4	10	16
24	15.5	17	17	13.5	4	-3	-3	-2	4.5	5	10.5	11
25	13	18.5	15.5	10.5	2.5	-4	-3	0.5	3.5	2	13	12.5
26	13.5	17	14	13	3	-3	0.5	3	4	1	13	14
27	12	17	16	10	3.5	-1	0	2.5	5.5	2	14	13
28	14	14	15.5	9	4	-3	-1	2	6.5	4.5	14	15
29	14.5	13	*	9.5	5.5	0.5	-3	-1	-3	7	14	14
30	16	16	*	10	8.5	0	-1	0	-2	9	10.5	14.5
31	17	14	*	9.5	*	0	*	9	0	*	12	*
Avg:	13.2	16.7	16.2	11.5	8.38	0.94	-1	0.66	1.85	4.6	9.16	11.6

APPENDIX 5:
RAINFALL MEASURED IN THE RESEARCH
AREA

Appendix 5: Daily rainfall (mm) in study area from December 2005 - November 2006 (Roodevallei, Free State Province).

<u>Day</u>	<u>Dec</u>	<u>Jan</u>	<u>Feb</u>	<u>Mar</u>	<u>Apr</u>	<u>May</u>	<u>Jun</u>	<u>Jul</u>	<u>Aug</u>	<u>Sep</u>	<u>Oct</u>	<u>Nov</u>
1		7							15			53
2		3.5				13			9			22
3			5									
4			1.5									
5			14.5									
6			6									
7		11	22									
8			50									
9												
10			1	18	7							
11			50.5	1.5	3							
12			4.5								20	
13		4										
14	4	9.5										
15	0.5										2.5	
16	10				5	5					1	1
17			2	3	1.5	3					13	
18				10	2						3	15
19			7	16		8					10	
20	7	48		28	2	3					2.5	
21		8		30	8.5							
22		8							16			
23					8				12			
24			10	4.5					10			
25			19									
26			1									
27		4		5							6	
28		22										
29											8	
30	1											
31	1.5	12									3	
Tot:	24	137	194	116	37	32	0	0	62	0	69	91

APPENDIX 6:
SAMPLING SHEET

Appendix 6: Example of an aphid and coccinellid sampling sheet used during the study (Roodevallei, Free State Province).

	FIELD NR / STRUCTURE NR				
Cycle:	Predominant aphid species:				
Observation dates:	1	_____	1	_____	
	2	_____	2	_____	
	3	_____	3	_____	
	4	_____	4	_____	
Observation times:	Predominant predator species:				
	1	_____	1	_____	
	2	_____	2	_____	
	3	_____	3	_____	
Plant weight:	4	_____	4	_____	
Observations					
Description:	1	2	3	4	
Leaves observed:					
Leaves infested:					
Aphids observed:					
Class:					
Predators observed:					
Larvae observed:					
<i>Anoplolepis</i> sp. (Y/N):					
Samples					
Counting:	Sample:	Species:	Sample:	Species:	
1					
2					
3					
4					

APPENDIX 7:

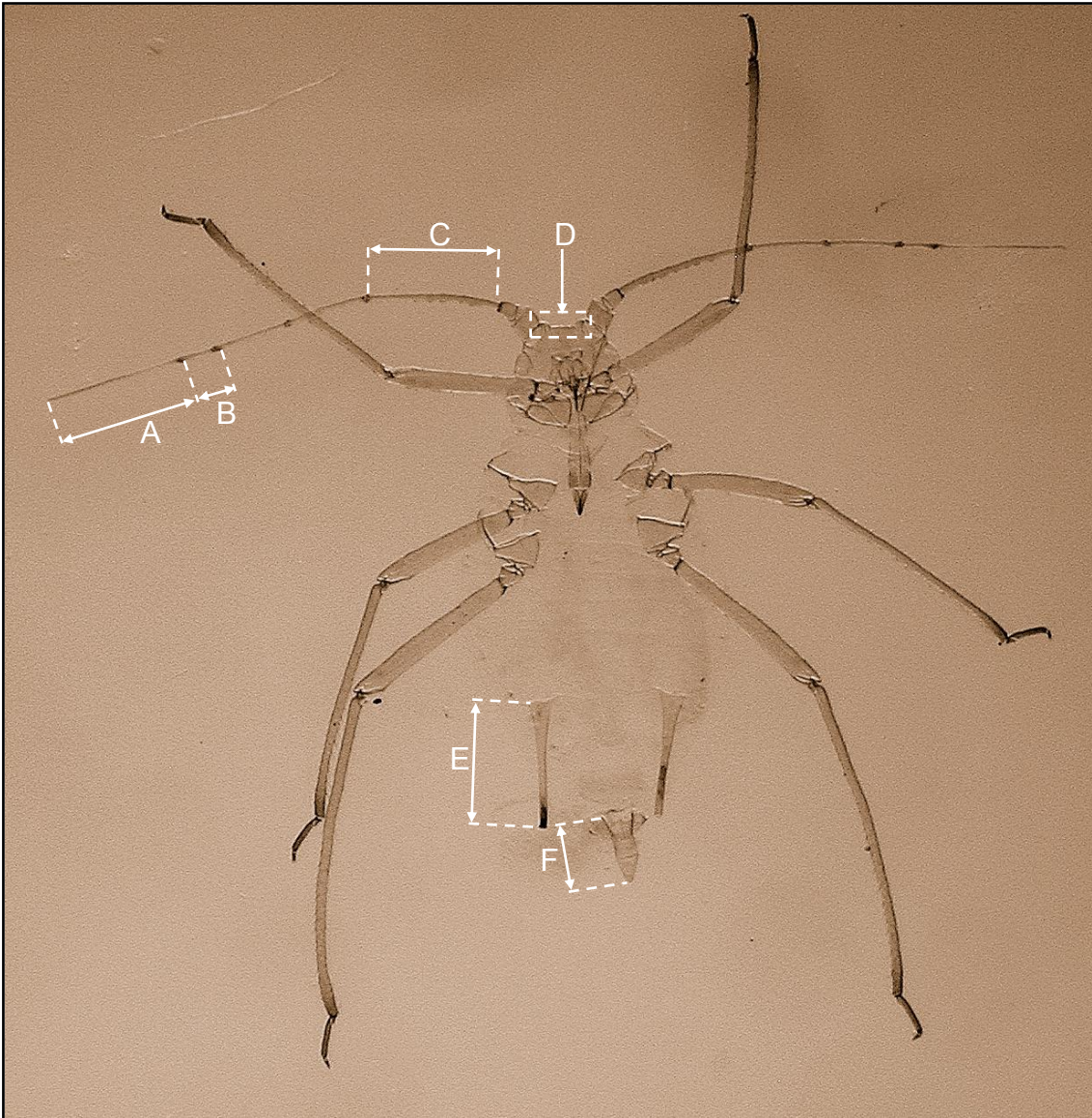
**KEY MORPHOLOGICAL CHARACTERISTICS
OF THE ECONOMICALLY IMPORTANT APHID
PEST SPECIES OF LETTUCE**

Appendix 7 illustrates the diagnostic aphid morphological characteristics used in the key provided below. Distinctive features of all four aphid pest species found on lettuce in the central Free State of South Africa, are i) the terminal process of the antennae is longer than the base of the last antennal segment (Appendix 7A & B), ii) the antennal tubercles are well developed (Appendix 7D); iii) siphunculi are pale with a darker distal portion (Appendix 7E) (Blackman & Eastop, 2000). From this, and additional morphological information, a key to these species can be compiled (adapted from Blackman & Eastop, 2000).

1. Dorsal surface of abdomen contains paired dark intersegmental markings, and the terminal antennal process is 6-9x longer than base of last antennal segment *Nasonovia ribisnigri* (Mosley)
 - Not as above 2

2. In dorsal view, the inner faces of the antennal tubercles are convergent. Siphunculi appear slightly clavate and the same length (or longer) than third antennal segment *Myzus persicae* (Sulzer)
 - In dorsal view, the inner faces of the antennal tubercles are divergent. Siphunculi appear either cylindrical or tapering and are less than twice as long as cauda 3

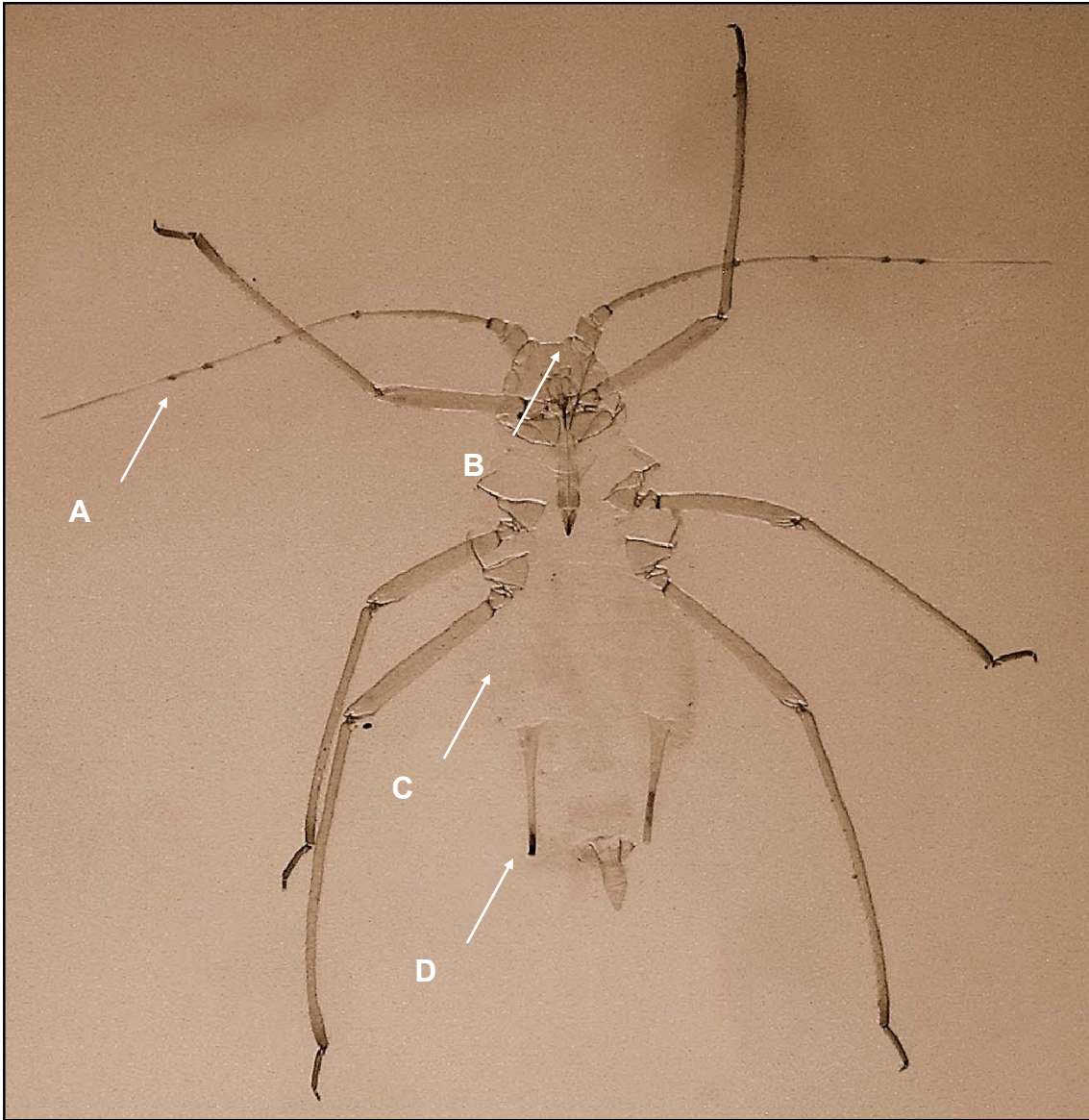
3. Siphunculi a little shorter than the distance between their bases, and without subapical reticulation *Acyrtosiphon lactucae* (Passerini)
 - Siphunculi longer than the distance between their bases, and with subapical reticulation *Macrosiphum euphorbiae* (Thomas)



Appendix 7: Slide-mounted aphid specimen, showing A) terminal process of last antennal segment, B) base of last antennal segment, C) third antennal segment, D) inner faces of antennal tubercles, E) siphunculi, and F) cauda.

APPENDIX 8:

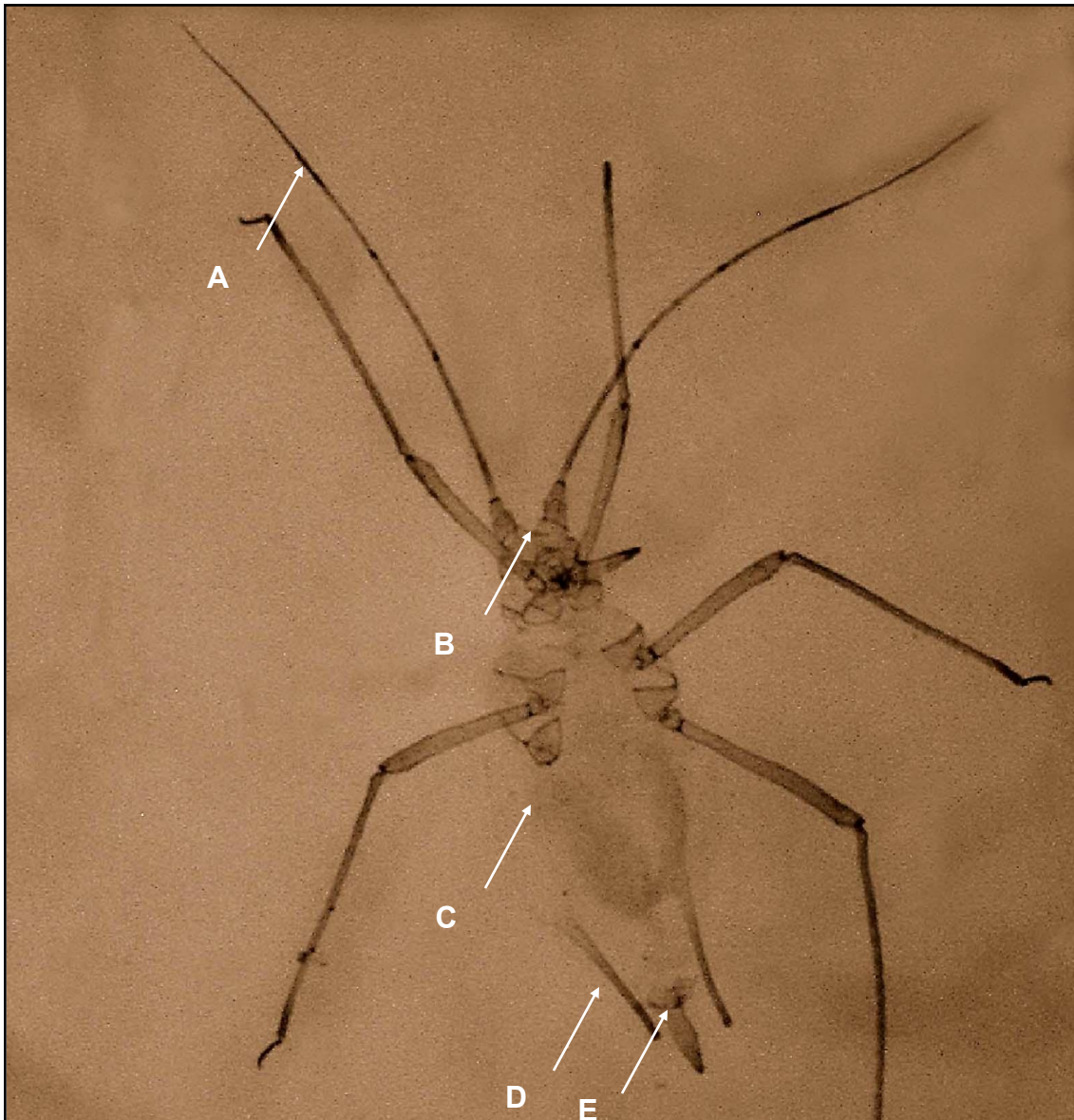
**ADDITIONAL MORPHOLOGICAL
CHARACTERISTICS OF LETTUCE APHID
PEST SPECIES AND THEIR PREDATORY
COCCINELLIDAE GUILD**



Appendix 8.1: *Acyrthosiphon lactucae* Passerini showing A) terminal process of antennae, B) inner faces of antennal tubercles, C) abdomen, and D) siphunculi.

This aphid has a distinct lighter area between the base of the last antennal segment and the base of the terminal process of the last antennal segment, as seen in this slide (A). The inner faces of the antennal tubercles are divergent when studied under a stereo microscope (B). The body of this species also has a waxy coating, giving it a powdery appearance (C) and different color forms (dark green, light green, and pink) may occur in a single colony. The eyes are bright

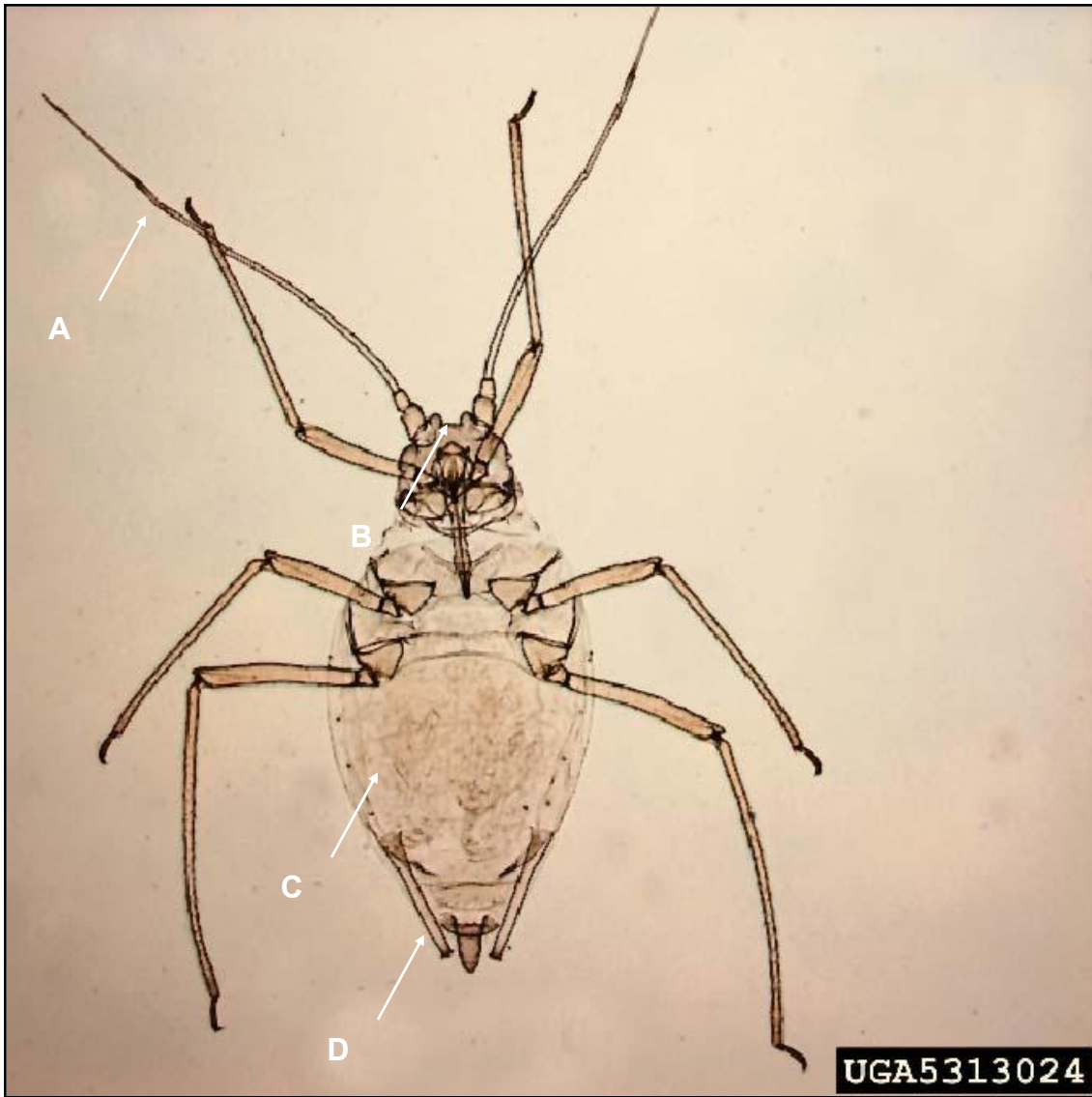
red in live and recently preserved specimens. The dark-tipped siphunculi (D) are visibly shorter than those of *Macrosiphum euphorbiae* (Appendix 8.2).



Appendix 8.2: *Macrosiphum euphorbiae* Thomas showing A) base of last antennal segment, B) inner faces of antennal tubercles, C) abdomen, D) siphunculi, and E) cauda.

This aphid is relatively larger than the other aphid species encountered on lettuce and it doesn't have a distinct lighter area between the base of the last antennal segment and the base of the terminal process of the last antennal segment. Instead, the area has a dark appearance (A). The inner faces of the antennal tubercles are also divergent (B) as in the case with *A. lactucae* (Appendix 8.1). The body of this species has a shiny appearance (C). Dark-tipped siphunculi (D)

are visibly longer than those of the other aphid pest species, and appear as if they converge at their near-distal ends. The cauda is also longer than those of the other three aphid pest species (E). Additionally, the nymphs of these aphids tend to cluster around the adults.



Appendix 8.3: *Myzus persicae* Sulzer showing A) base of last antennal segment, B) inner faces of antennal tubercles, C) abdomen, and D) siphunculi (Image taken from: Pest and Disease Library, Bugwood.org).

A distinctly shaped aphid species. The area between the base of the last antennal segment and the base of the antennal process is not clearly defined as in *A. lactucae* (A). Unlike the two species discussed previously, the inner faces of the antennal tubercles are convergent (B), in other words, they grow towards each other. As is the case with *M. euphorbiae*, the body also has a shiny appearance with a coloration ranging from pale green to yellow, or dark (C). Another distinctive characteristic of this aphid which sets it apart from the other

three species, is the clavate siphunculi which have a slightly bulging appearance towards the middle (D). Additionally, the nymphs of these aphids spread out and are rarely clustered, except under extremely crowded conditions.



Appendix 8.4: *Nasanovia ribisnigri* Mosley showing A) terminal process of last antennal segment, B) inner faces of antennal tubercles, C) abdomen, and D) siphunculi.

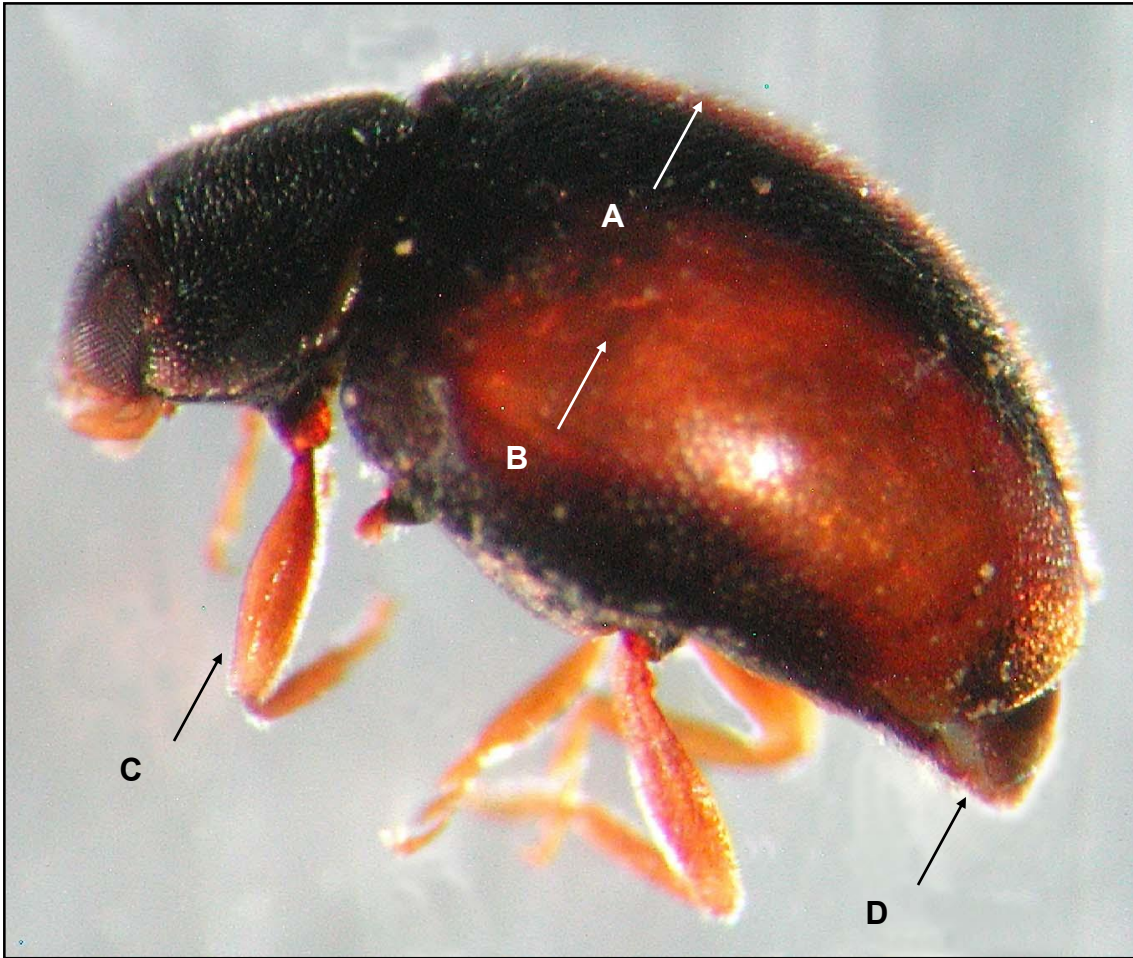
This species has a very long terminal process of the antennae (A), and the inner faces of the antennal tubercles are also divergent (B) as in *A. lactucae* (Appendix 8.1) and *M. euphorbiae* (Appendix 8.2). However, this species is readily distinguishable from the other aphid species by bearing dark bilateral-symmetric

markings on the dorsal surface of the abdomen which can be seen with the naked eye (C). The siphunculi are also dark-tipped as in the other species (D).



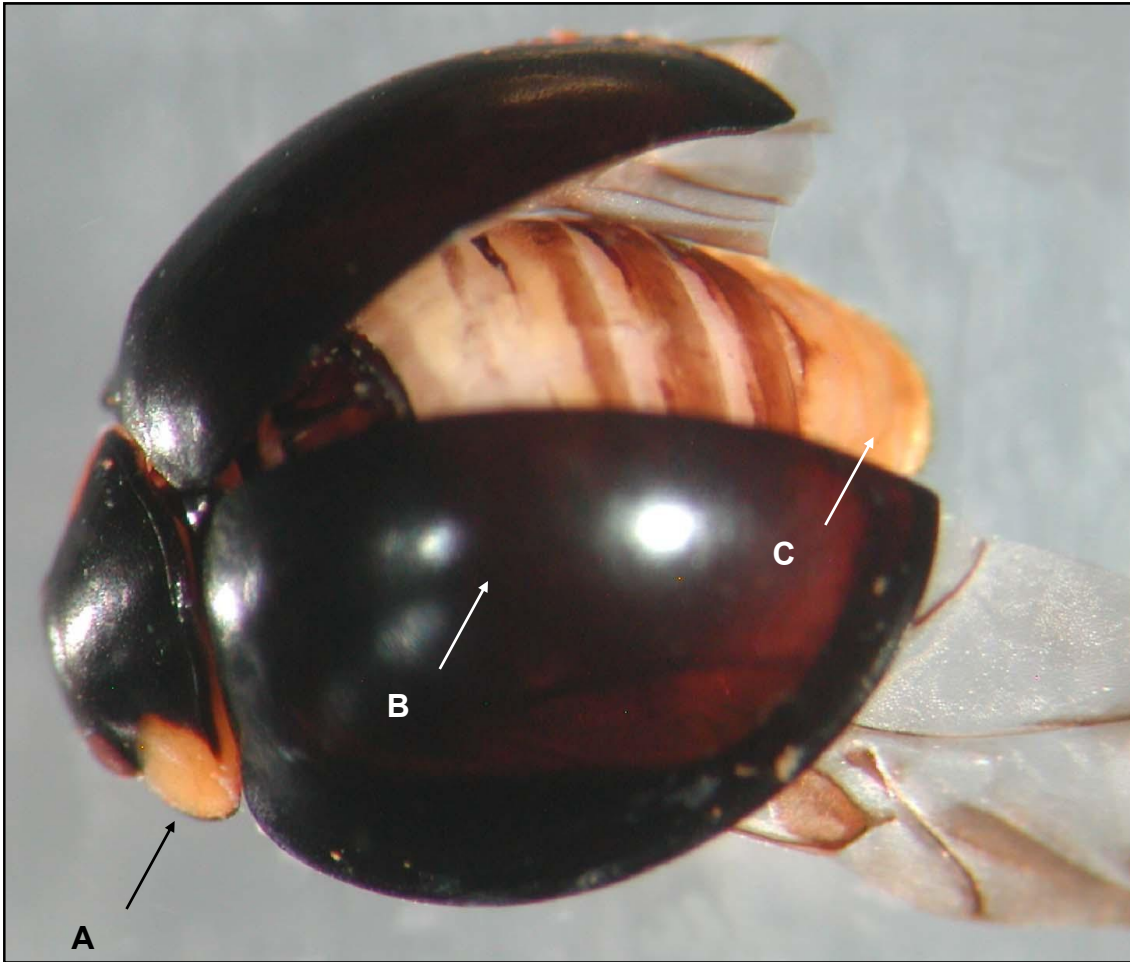
Appendix 8.5: *Hippodamia variegata* Goeze showing A) outer margin of thorax, B) elytra, and C) dark spots on elytra.

This species has a light margin around the edges of the black thorax, which also bears two pale spots (A). The elytra have a distinctive orange coloration (B) with a variable number of black spots (C), of which the four big spots (sometimes fused to form two large spots) in the centre are usually a key characteristic.



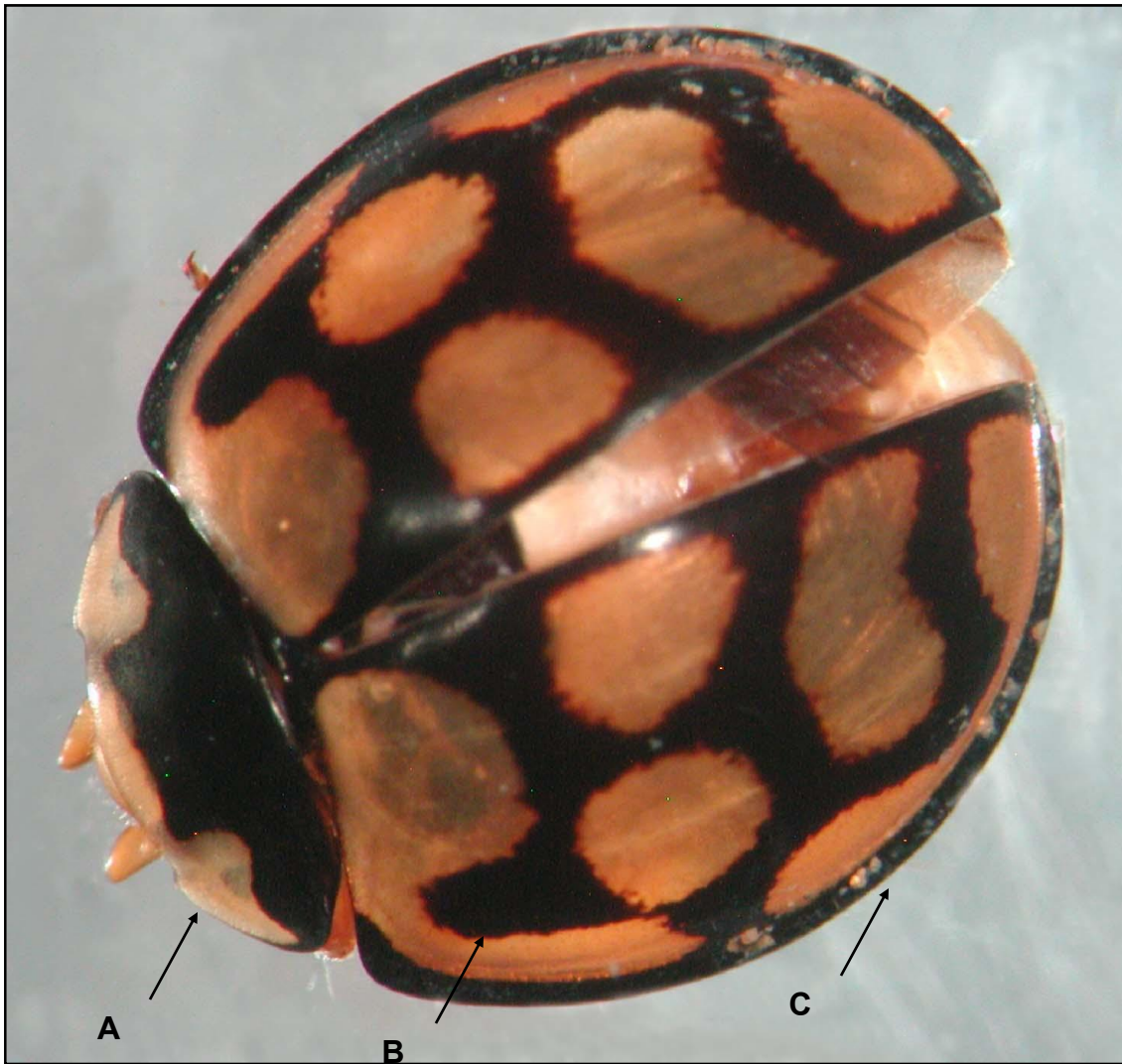
Appendix 8.6: *Scymnus* sp. 1 showing A) setae on elytra, B) elytra, C) legs, and D) abdominal tip (pygidium).

The smallest of the important coccinellid species observed during the study, with a total body length of a few millimeters. Under a stereo microscope, the body has a dull appearance due to the presence of setae (pubescence) (A). Each elytron also bears a distinctive orange patch surrounded by a darker area (B). The tip of the abdomen (pygidium) is exposed beyond the elytral apex (D).



Appendix 8.7: *Exochomus flavipes* Thunberg showing A) outer margin of thorax, B) elytra, and C) abdomen.

An easy distinguishable species with two orange-cream 'cheeks' on the thorax (A) and dark wing covers (B). When the elytra are parted, or in a ventral view, the abdomen has peach-cream color (C).



Appendix 8.8: *Cheilomenes lunata* Fabricius showing A) outer margin of thorax, B) elytra, and C) outer margin of elytra.

This is was the biggest coccinellid species collected during the study and it also bears a thorax with lighter colored areas as in *H. variegata* (Appendix 8.5), but without the two light spots (A). The spots on the elytra are also not clearly defined as that found on *H. variegata*, and appears as orange or red spots surrounded by black lines (B). The whole outer margin of the wing covers is also darker colored, rendering it easy to distinguish this species from *H. variegata*.

APPENDIX 9:

ANOPOLEPIS CUSTODIENS



Appendix 9: The pugnacious ant, *Anoplolepis custodiens*.