

**An assessment of the potential use of
pheromone traps to monitor the
green mirid, *Creontiades dilutus*
(Stål) in Australian cotton.**

By

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Declaration

I, Suzette Argent, declare that this is my own work and it has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published and unpublished work of others has been acknowledged in the text and a list of references is given.

Signature

(Suzette Argent)

30th October 2008

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Abstract

The potential use of pheromone traps as a monitoring tool for the cotton pest green mirid (*Creontiades dilutus*) was studied at three sites. The trials compared pheromone catch numbers to the number of adult and nymph green mirids, the sex ratio, and the percentage of mated females in the field population that were sampled using visual, suction, beat sheets and sweep nets. At the Narrabri site results showed no association between pheromone trap catches with the number of green mirids in the field, the sex ratio, or the mated status of the female in the field population. The Goondiwindi site had a significant association between the pheromone trap catch numbers and the absolute number of adult and nymphs in the field. At the Boggabri site there was a correlation between pheromone trap catches and the number of green mirid nymphs in the field and the percentage of mated females in the field population, with high trap numbers being correlated to high percentages of females in the population being mated. A possible explanation for the difference in the Goondiwindi and Boggabri sites is the variation in the percentage of females mated at each site and immigration. At Goondiwindi there was little variation in the percentage of females mated while at Boggabri it was highly variable. At Goondiwindi the green mirid might have a short pre-reproductive period and little migration, whereas the opposite may have occurred at Boggabri. Overall the results from this trial suggest that pheromone traps on their own may not be reliable monitoring tools for green mirid populations in the field. However they may be more valuable for studying the population dynamics of mirids, hence achieving a better understanding of green mirid ecology.

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Chapter One:

Review of literature

1.1 Introduction

This dissertation focuses on an insect pest of Australian cotton, the green mirid *Creontiades dilutus* Stål (Hemiptera: Miridae), hereafter referred to as GM. The GM has emerged as a new insect pest through changes in pesticide use in the industry. Its high mobility, feeding habits and potential to cause rapid damage is why the GM is a pest of concern. As mentioned by Andrew Watson (Cotton grower) last year (06/07) experienced levels of GM above the economic threshold suggested in the industry, yet he did not spray and did not end up losing any crop yield. This dissertation has a strong emphasis on improving the management of GMs through analysing current and potential sampling and monitoring techniques. This review argues to continue research into the effectiveness of current sampling methods. This review also supports further research into the use of the GM pheromones identified by Lowor (2006) as a monitoring technique to help understand GM chemical ecology and behaviour. The pheromone might also form the basis of a control technique.

There is relatively limited information on the green mirid (GM), *Creontiades dilutes* (Stål), particularly as a pest of cotton (*Gossypium* spp.). Where appropriate, reference will be made to species closely related to the green mirid such as the *Lygus lineolaris*. The genus *Lygus* occurs in America and is of importance as it is similar to the GM in relation to biology, ecology and pest management on cotton (Gregg 2007, Threlfall *et al.* 2005/06). There is more literature on the American *Lygus* species and given its similarity to the GM is used as a reference point. Where reference cannot be made to the GM or *Lygus lineolaris* the next closest related species will be used.

1.2 What is a Pest?

A pest is an organism that has characteristics that are regarded by humans as unwanted or damaging (Foster & Harris 1997, Moore 2004). An organism is normally considered a pest by humans when it is competing for a resource, especially when large numbers of pests are present. In an agricultural system, weeds, diseases and insects are considered the main pest organisms (Metcalf & Luckmann 1994). Wallner (1987) and Metcalf & Luckmann (1994) emphasise two main theories and concepts in regards to pests. Firstly, that not all pests cause intolerable damage to resources and secondly, that some insects are only considered as pests at certain times of the year. Furthermore, it is assumed by Metcalf & Luckmann (1994) that it is impossible to eliminate pest problems through the removal of the activities which promote them. It is impossible to eliminate pest problems, which develops the concept of controlling a pest so it does not cause economic damage instead of eliminating it. The aim is therefore pest management rather than pest removal.

1.3 General Principles of Pest Management

Pest management involves controlling a pest by some means to prevent or reduce damage to a resource (Foster & Harris 1997). Pest management can be implemented through the use of chemical, biological, cultural, physical, genetic and/or regulatory methods of control (Fitt 1994, Metcalf & Luckmann 1994). Chemical methods of control include the use of growth inhibitors, sterilants, pesticides, attractants and repellents. Chemical control can also involve the use of behavioural manipulation. Foster & Harris (1997) use the term behavioural manipulation to mean changing a pest's behaviour in some manner which is beneficial towards its' control.

Chemical means of control are the most widely implemented methods for controlling insect pests (Metcalf & Luckmann 1994, Fitt 1994, Foster & Harris 1997, Fitt 2000). Within chemical control the most widely and successful tool is the use of insecticides. However, the over reliance on insecticides has caused problems. Metcalf & Luckmann (1994) and Christiansen (2002) state that overreliance on specific

insecticides has resulted in pests developing resistance to the active constituents of those insecticides. This has reduced their efficiency in controlling pests, resulting in higher rates of chemicals being applied and an associated increased cost of chemicals for producers. Furthermore, significant environmental harm can be caused by pesticides. A move towards more environmentally friendly chemistry and approaches is occurring (Fitt *et al.* 2004, CRC 2008).

1.3.1 Integrated Pest Management

The Australian cotton industry is encouraging a shift towards the use of Integrated Pest Management (IPM) with the aim of reducing the use of environmentally damaging chemicals (Fitt *et al.* 2004, CRC 2008). Dent (1991) defines IPM as the use of a combination of suitable techniques that are less environmentally damaging, which also reduces and/or maintains pest populations at a level which is not economically damaging to resources. Fitt (1994 & 2000) states that IPM techniques achieve the goal of being more environmentally friendly by using control techniques that are more host specific. Some of these techniques include the use of resistant plant varieties, the use of natural predators of the pest, selective and biological chemicals (soft chemicals), behavioural manipulation and cultural practices. Dent (1991) also emphasises that IPM is based on the concept of management not eradication, and is often a cheaper and more sustainable approach.

Insect pest management is a complex problem due to many complicated interactions (Dent 1991, Fitt 2000). For example, an understanding of the relationships between weather, natural enemies and crop stage on the population dynamics of a pest. Other complications include knowing how many insects will cause economic damage and how to determine this point to implement control (Foster & Harris 1997, Suckling 2000). A broad understanding of a pest's ecology along with how it interacts with biotic and abiotic factors is needed to achieve successful management.

The success of IPM relies on an extensive evaluation of the type of problem at hand to ensure an appropriate and successful management strategy can be implemented. Although pests might be causing visible damage to a crop this does not always lead to

a reduction in yield. Resource damage can be dependent on the number of pests present, the stage of the pest's life cycle, the growth stage of the plant and the time of year (Wallner 1987; Metcalf & Luckmann 1994, Frisbie *et al* 1989a and Dent 1991). It is vital to identify when a pest is causing or is going to cause economic damage to a resource, therefore monitoring is required.

Sampling techniques and damage/cost assessments can be combined to give economic thresholds (When the cost of control outweighs the cost of production loss). Regular sampling can identify whether a population is below, above or reaching this economic threshold throughout the season (Frisbie *et al.* 1989a, Metcalf & Luckmann 1994, Dent 1991). IPM should be based on sampling systems and population thresholds to allow better timing of pesticide application and only when needed. (Fitt 2000). Thus, sampling of pests plays a very important role in IPM.

1.4 General principles of sampling

Sampling obtains information about the population of interest (Bodnaruk 1987). Field sampling is used to collect data on an insect's population dynamics to assist decision making (Dent 1991, Frisbie *et al.* 1989b). Accurate sampling provides a useful representation of the range of insect abundance in the area of interest sampled, which can lead to better management decisions (Dent 1991, Frisbie *et al.* 1989b). Inaccurate and insufficient sampling typically results from an individual's personal bias or an inappropriate sampling method for the type of pest or stage of crop growth (Dent 1991). This can be overcome by using random and stratified sampling techniques, and choosing sampling methods appropriate to the pest and crop.

A variety of sampling methods are available to monitor insect populations. Among the many sampling methods available for use outlined by Southwood (1968) there are four which were used and compared in this dissertation. These were beat sheets, sweep nets, visual searches and suction sampling (D-vacs). Sampling methods can be classified on the basis of whether or not they collect all the individuals in the sampled space (absolute) or collect a proportion of the individuals (relative).

1.4.1 Absolute estimate sampling methods

Absolute sampling methods count the number of insects within a predefined unit of habitat (Southwood 1968; Dent 1991). Southwood (1968) explains that an absolute estimate of the number of insects in a particular unit area allows for the estimation of the insects density in that unit of area. Some examples of habitat units mentioned by Dent (1991) include numbers of individuals per volume, per metre, per dwelling, per plant or plant part (such as a leaf). Absolute sampling methods provide insect densities which are analytically comparable over time and space (Dent 1991).

Despite the usefulness of absolute sampling methods they have numerous limitations. For example, to obtain useful and accurate information from absolute sampling methods intensive labour and repetitive tedious sampling is needed (Southwood 1968). Furthermore, sampling error has the potential to occur from two primary sources, sampling inefficiency and inaccurate assessment of insect numbers. Conversely, Dent (1991) demonstrates that a regression analysis between the relationship of counted numbers in a field and laboratory counts can result in some elements of sampling error being removed.

1.4.2 Relative estimate sampling methods

Relative sampling methods count the insects but do not relate this to a definite unit of area and are therefore regarded as only of representative value (Dent 1991, Southwood 1968). Relative sampling methods are typically conducted without establishing factors such as the unit of area, volume, plant or plant part. In addition relative methods vary according to immediate environmental effects such as climatic and seasonal changes. Traps, such as pheromone traps, are relative sampling methods due to the fact that the specific location of the insect population data obtained is unknown (Southwood 1968). A requirement for the comparison of relative estimates is that conditions are known to be of similar states (Dent 1991). Despite relative

estimates providing only a representative value, they have the potential to reflect actual pest population densities.

Southwood (1968) and Dent (1991) suggest that relative methods are useful as they permit large amounts of data to be collected easily. Southwood (1968) suggests that relative sampling methods can collect large amounts of data as they physically collect insects, a feature absolute methods generally lack. Dent (1991) explains that entomologists often use traps to collect data on mobile aerial pest species like moths, which have less mobile immature stages damaging to crops. Because relative sampling is fast, relative methods also have the advantage of giving an early warning of potential infestations occurring. This allows for earlier organization of pest management that would not have been possible with absolute methods.

Both relative and absolute sampling methods have strengths and limitations. Both relative and absolute methods also share errors of measurement that occur from two main sources. These are poorly calibrated instruments and various forms of human error. Dent (1991) states to remove operator error (individual differences) the same person should conduct all samples (collections). If this is not possible an attempt to quantify individual differences should be undertaken in order to remove this effect from the data.

1.5 The cotton industry and developments in pest management

The cotton industry faces many complex problems specific to pest management. Insect pest control in cotton has previously been focused on the use of insecticides. However, insecticide resistances, ever increasing costs and environmental concerns have resulted in insect control changes with a move towards integrated pest management (Fitt 1994, Christiansen 2002). These changes have led to changes in the major pest species present in the cotton industry. The main change in cotton pest management over the past decade has come from the introduction of transgenic cotton (Bollgard II®). Transgenic cotton has led to a reduction of broad spectrum insecticide use (Wu *et al.* 2002, Finlay 2006, CRC 2007a). The reduction in use of, and reliance

on, pesticides has improved the implementation of integrated pest management. However, Fitt (2000) and Fitt *et al.* (2004) emphasises that the introduction of transgenic Bt cotton should not be considered as a “magic bullet” but as an opportunity to promote change in pest control towards a sustainable and environmentally friendly future for the cotton industry as a whole.

Changes in insect pest control have lead to alterations in the key pests present within the cotton industry. These changes have resulted in the GM becoming a significant pest (Khan *et al.* 2004a, Khan 2003, Miles *et al.* 1992). Literature suggests that the GM was originally controlled unintentionally with intensive spray program against *Helicoverpa* species (Goolsby *et al.* 2005, CRC 2007a, b). Fitt *et al.* (2004), Grundy (2004), Goolsby *et al.* (2005) and CRC (2007a) suggest that the GM has become a serious pest due to the reduction of broad spectrum insecticide use as a result of the introduction of transgenic Bt cotton.

1.5.1 Current Insect pest management focus – Sucking pests

Research and literature regarding new control methods for Australian cotton pests in recent years has been focused on a new emerging group of sap sucking insects commonly regarded as sucking pests by cotton growers (Miles *et al* 1992, Miles *et al* 1994, Goolsby *et al* 2005, Knight *et al* 2007). This group also includes white fly (*Bemissia tabaci*). GM is a sucking insect belonging to the Family Miridae of the Order Hemiptera, and is native to Australia. Fitt (1994) stated that GM is more abundant in Queensland than New South Wales, but concluded its pest status was unclear. Literature from two decades ago originally suggested the GM was considered a beneficial as it had been observed consuming *Helicoverpa* eggs and larvae (Sterling *et al.* 1989). However, in more recent times GM has been viewed as an increasingly serious pest due to its feeding habits on the cotton plant and the associated economic damage (Khan *et al.* 2004a, CRC 2007b).

1.6 What is known about the GM?

To develop an understanding of how the GM functions and why it is a pest, knowledge of its biology and ecology is required. The GM has two main ecological characteristics which make it a significant pest to cotton (Khan *et al.* 2004a, CRC 2007b). The first is its ability to cause rapid damage to a crop via its feeding habits and second is its high mobility both within a crop and over large spatial areas make it a difficult pest to monitor and therefore control. These characteristics will now be examined to assist in understanding the biology and ecology of the GM.

The adult GM is winged and therefore able to fly away when disturbed. The GM is typically active when either flying to food sources or scurrying around on the host plant. The GM is also an opportunist of favourable conditions such as optimal weather and food sources. Like other mirids, GM's can be highly invasive (migrate in large numbers) and reproduce rapidly as they prioritise energy towards reproduction. These characteristics assist mirids to colonise and utilise favourable environmental conditions (Campbell *et al.* 1974, Chapman *et al.* 1986).

1.6.1 Life Cycle and ecology

In order for the control of the GM to be successful and also continue to support the cotton industries move towards a sustainable future, an understanding of the green mirid's ecology is needed. The lifecycle of the GM consists of five nymphal stages and an adult stage (As shown in figure 1.1). A female adult lays eggs singly by inserting them into the plant tissue, cutting slits and leaving the oval cap of the egg protruding (Khan *et al.* 2004a). Nymphs emerge after approximately seven to ten days. Each of the five nymphal stages takes about two to three days, with the whole life cycle completed in approximately three weeks. Upon reaching the adult stage of development GM's live for approximately three to four weeks however this is very dependent on weather conditions.

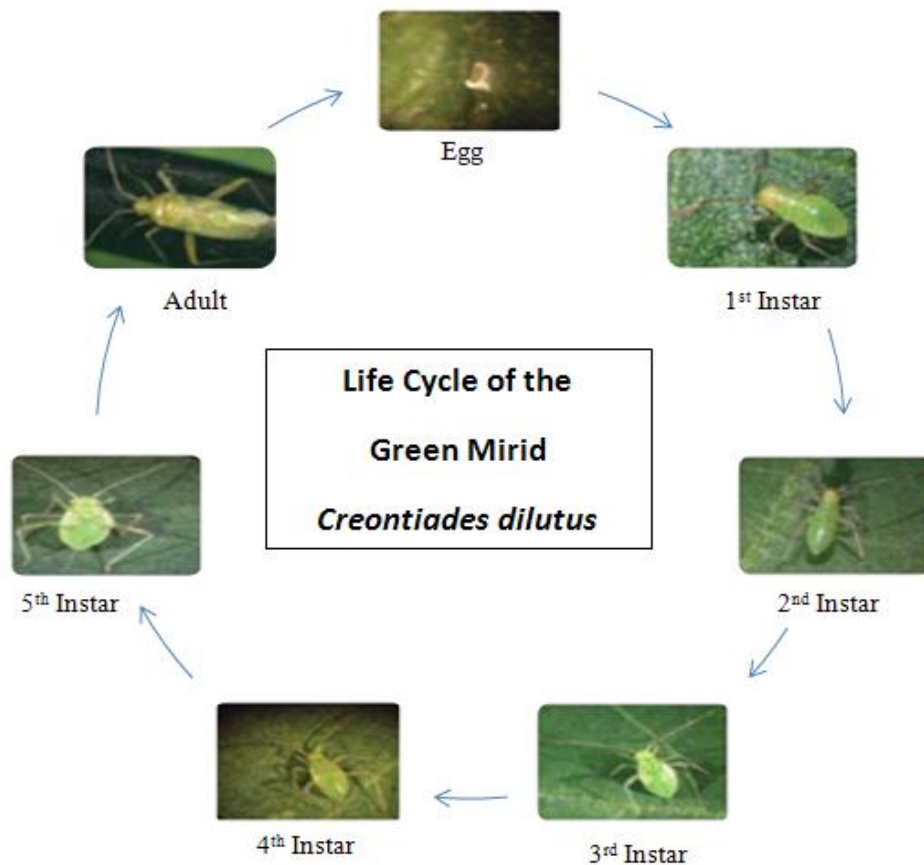


Figure 1.1 Life cycle of the Green Mirid. Photos Author: Dr Moazzem Khan of QDPI&F

1.6.2 Climatic effects on the GM

Temperature and weather conditions play an important role in a mirid's life cycle (Ridgway & Gyrisco 1960). Foley & Pyke (1985) demonstrated the GM has a rapid rate of development, requiring an estimated 280 day-degrees (Function of time that varies with temperature). This is lower than other major pests such as the *Helicoverpa armigera* which has a developmental requirement of 475 day-degrees. Foley & Pyke (1985) believe this rapid development can enable populations to build up rapidly making GM a great risk to cotton production due to potential sudden outbreaks. Khan & Quade (2008) identify temperatures around 30-32 °C as optimal for development from egg to adult. The same results have been found for other mirids, such as *Lygus lineolaris* which does not occur in Australia (Ridgway & Gyrisco 1960).

Excessive temperatures and other climatic conditions can adversely affect the GM. Khan (1999) identified that dry periods, heavy rainfall and high winds lower GM numbers. Khan (1999) also provided evidence that temperature, humidity and other physical habitat conditions affect behavioural patterns of the GM. As a result favourable conditions result in high metabolic activity causing an increased rate in development. Khan (1999) found that temperatures below 11°C and above 38°C have detrimental effects on egg laying and adult survival. It was also identified that the highest fecundity rates were observed between 20- 30°C, with the optimum at 30°C. It was also identified that nymphal survival and development was highest in the temperature range 20-30°C, with once again highest development occurring around 30°C. Thus literature suggests temperature is a crucial variable in the lifecycle of the GM.

1.6.3 Feeding habits

The GM feeds by piercing plant tissue and releasing a chemical (pectinase) which destroys the cells in the feeding zone (Ellington *et al.* 2007, Khan *et.al* 2004a, Pyke & Brown 1996). The cell destruction associated with their feeding habits results in the loss of terminals, shedding of small squares and sometimes large squares and damage to bolls. The cell destruction results in lower yields, thus GM is now considered a major pest in cotton despite past literature comments of the GM being beneficial (CRC 2007a, CRC 2007b, Ellington *et al.* 2007, Foley & Pyke 1985, Khan *et.al* 2004a, Miles *et al.* 1992).

The GM has a large host range including crop species such as cotton, lucerne, mung beans, sunflowers, safflower and many legume crops (Hori & Miles 1993). In addition the GM have host weed species which harbour this pest including wild turnips, common joy weed, verbena and thistles (Khan *et al.* 2004a). These ecological characteristics of a large host range along with its high mobility makes the GM such a successful pest as it can move from one crop or weed species to another.

The GM favour lucerne for oviposition in comparison to cotton if a choice is available amongst alternatives even after lucerne is cut it is still more desirable than cotton (Sevacherian & Stern 1973, Miles *et al.* 1992, Mensah & Khan 1997, Pearce & Zalucki 2005). Furthermore evidence suggests the pest numbers in the lucerne were

reduced with no more being found in adjacent cotton. Miles *et al.* (1992), Mensah & Khan (1997) and Pearce & Zalucki (2005) emphasise that the only time cotton is found more desirable is when lucerne starts drying out.

1.6.4 Migratory habits

There is evidence to suggest that repeated influxes of mirids occur from long distance migration through the aid of weather fronts, but this is not well understood. It is possible that from October to December GM are introduced into cotton growing areas by weather fronts from the same outback hosts that support *Helicoverpa punctigera* (Foley & Pyke 1985, Khan 1999). Gregg (1995) indicated that there is limited information available regarding the migration and movement of hemiptera cotton pests. However Gregg (1995) also proposed that some hemipterans are capable of very long distance travelling both during the day and at night. Specifically Gregg (1995) identifies the GM as a probable migrant, but the timing and extent of migration is unknown due to limited information.

Consequently it is necessary to identify migrants, local concentrations of populations, intercrop movement and to achieve a better understanding of the GM pre-reproduction phenomena. Gregg (1995), Rankin (1976) and Johnston (1969) explain that long-distance migration is often pre-reproductive. The energy necessary for flight takes energy away from reproduction and maturity and therefore insects which migrate tend to sexually mature later and consequently breed later (Murlis & Jones 1981, Stein 1986, Taylor 1986). Therefore an insect can either stay in one location, develop sexually and as a result breed earlier (most female adults will be mated), or migrate and develop migratory mechanisms like wing muscles and breed later. Once an insect has migrated they can become sexually active (Gregg 1995). The majority of information available in this area of research is based on lepidopteron cotton pests such as the *Helicoverpa* species which therefore will form the basis for discussion within this dissertation.

The migratory nature of the GM and its effect on its pest status in different regions of Australia is relatively unknown (Hereward 2007). Currently there is research being undertaken by Hereward (2007) utilising molecular techniques such as microsatellites

and sequence data to examine migratory traits. This research will determine and develop a theory of whether the GM present in cotton occurs due to local dispersal and/or from long distance migration (Hereward 2007).

1.6.5 Sexual Behaviour of mirids

1.6.5.1 Pre-mating period

The mating behaviour of mirids is quite complex and thus has consequences for the cotton industry. One characteristic of most mirid species includes a pre-mating period (Leston 1961, Wheeler 2001). Wheeler (2001) suggests that the exact time frame for many species varies from just a couple of hours for some species and up to seven days for others. Johnston (1969) and Gregg (1995) indicated that longer pre-mating periods are associated with long distance migration, as mentioned earlier. Khan (1999) has extensively studied the pre-mating period in the GM and found it is temperature dependent and is usually quite long, approximately a week. In addition Khan (1999) also indicates that there is limited information available specifically examining the mating behaviour of the GM. Therefore this information could suggest the GM is a long distance migrant but further research this area is needed.

1.6.5.2 Use of pheromones

To help male insects successfully locate females for the purpose of breeding pheromones are used. Pheromones are chemicals secreted externally by an organism to communicate with members of the same species (Suckling 2000, Hamilton 2001, Welter *et al.* 2005, Rumbo 1981). Pheromones are important as they help in survival and reproduction success of the species by acting as a signal from the insect which secretes the pheromone to the insect of the same species which receives the signal and both mutually benefit (Gut *et al.* 2004). Murlis (1986) explains that the female gypsy moth calls from a tree trunk by releasing sex pheromones which aid the male in finding her with an accuracy of approximately a couple of centimetres. As a result of this precision and accuracy the female is able to be fertilised (Murlis & Jones 1981). There is limited information available on the pheromones of hemipteran insects (McBrien & Millar 1999, Zhang & Aldrich 2003). Miles (1995) suggested that the

female GM releases a sex attractant because males were attracted to virgin females held in traps. However, Miles did not identify the chemicals that constitute the pheromone. While Lowor (2006) recently identified the chemical compounds of the GM sex pheromone, little is still known about how the GM behaves in relation to the pheromone.

Further, there is very limited information available on whether female mirids continue to produce pheromones and attract males after they have mated, or if males mask the females plume to prevent future mating (Zhang & Aldrich 2003). Groot & Smid (2000) studied the mirid bug *Lygocoris pabulinus* (the American equivalent to the Australian GM). They observed that males were attracted to mated females at both long and close ranges only hours after previous copulation. They also observed that males that had already mated were less attracted to virgin females. It was also observed that long time periods transpire between male matings with only 23 % of males mating 24hr after they had previously copulated. It would be valuable to know if these sexual behavioural trends are the same for GM, however no literature is available.

1.6.5.3 Mating frequency- Fertilisation requirements

An additional variable of interest for GM sexual behaviour includes the number of matings required to fertilise females. Wheeler (2001) states it is common for some species of mirids to mate several times. However, it is unknown if multiple matings were needed to effectively fertilise eggs throughout the female's egg laying life and if this is the case for the GM (Groot *et al.* 1999). Groot & Smid (2000) found there was no positive effect of multiple matings on fecundity and longevity on the *Lygocoris pabulinus*. However information for the GM is not available. Wheeler (2001) states, there is limited research and a poor overall understanding of mirid sexual behaviour in general.

1.7 Sampling for mirids!

The monitoring of mirids is undertaken through the use of sampling. Accurate sampling is essential but is hard to achieve as the GM are easily disturbed and highly mobile (Threlfall *et al.* 2005/06, Finlay 2006). This argument is supported by Miles *et al.* (1992) who states that it is hard to sample GM when they are disturbed as they are highly mobile. Furthermore, constant monitoring of approximately two to three times per week is necessary because the GM is an irregular pest (patchy dispersal within a field) with a fast life cycle, (Khan *et al.* 2004b). In addition multiple samples are also necessary to accommodate and reflect patchy invasions within the cropping system (Wu *et al.* 2002, Khan *et al.* 2004b). Therefore particular and constant attention needs to be taken in sampling the GM pest to obtain accurate results.

There are several sampling methods used for monitoring mirids. These include sweep netting, beat sheeting, visual inspection, and suction sampling (Bodnaruk 1987, Simpson & Lloyd 2007). All methods have both positive and negative characteristics and there is debate on which method is better (Morris 1960, Pyke *et al.* 1980, Bodnaruk 1987).

1.7.1 Sampling methods for mirids

1.7.1.1. Sweep netting

Literature suggests that sweep netting is a relatively easy and inexpensive method for sampling (Strickland 1961, Bodnaruk 1987, Miles *et al.* 1992, Threlfall *et al.* 2005/06). Sweep netting utilises a net similar to a butterfly net with a standard diameter of 38cm. The netting used is of a finer quality resulting in smaller insects such as the mirid being caught. The generally accepted principal used in sweep net sampling is to make 20 sweeps in front of the operator with the sweep being about 25-30cm from the top of the cotton canopy. As a result it is important that the net is moved fast enough to prevent the insects from escaping (Threlfall *et al.* 2005/06).

Sweep net samples have been found to be less accurate than visual sampling. Strickland (1961) also states that another limitation to sweep netting is the possibility

of bias caused from operator variations. De-long (1932) supports this statement as it was found that sweep net sampling is not accurate in sampling active insects like the GM. Ellington (2007) found that sweep nets only catch eight percent of the total insect population present in the sampled area of cotton. Unfortunately, another limitation to sweep netting is that a reliable population index for mirids is not achievable (Strickland 1961, Byelry *et al.* 1978, Bodnaruk 1987). In addition sweep netting cannot be carried out on young cotton plants due to potential plant damage and in wet weather and therefore cannot be used throughout a season as a standard technique (Miles *et al.* 1992).

Despite the limitations of the sweep net sampling method there are advantages. Smith *et al.* (1976) and Threlfall *et al.* (2005/066) state that sweep net samples can be converted and adjusted for quantitative use and therefore determine population density fluctuations despite error. The effectiveness of sweep net sampling was analysed by Threlfall *et al.* (2005/06) who compared the effectiveness of sweep net sampling to beat sheeting and visual sampling. Evidence suggested sweep netting and beat sheeting were the fastest methods, only taking approximately three to five minutes per sample. Further the sweep net is light, relatively easy to use and transport within a field due to being light.

1.7.1.2. Visual assessment

The visual sampling method has both weaknesses and strengths. Young & Tugwell (1975) and Miles *et al.* (1992) argue that visual sampling results in the most accurate population density for the insect of interest compared to other methods used for sampling mirids. Deutscher *et al.* (2003) showed that visual sampling is the most effective at monitoring a number of cotton pests, including both *Helicoverpa* species, mites, aphids, thrips and whitefly, but not as effective at sampling mirids. This argument is supported by evidence from Young & Tugwell (1975), Wilson & Gutierrez (1980) and Wilson & Room (1982). Literature suggests the reason the visual technique is inefficient at sampling mirids is they are very mobile and active, so they are inclined to move away or hide when scouts are coming and during the counting process (Byerly *et al.* 1978, Roach *et al.* 1979). This same argument also stands and is relevant for other sampling methods, however the argument is stronger

for the visual sampling technique due to lack of evidence against other sampling methods.

Visual sampling has another limitation. It is time consuming, taking 15 to 20 minutes per metre-row sampled compared to beat sheeting and sweep nets which take three to five minutes per sample (Threlfall *et al.* 2006). However, Bodnaruk (1987) states that visual sampling is useful as it can be effectively implemented on young cotton plants whereas beat sheeting and sweep net techniques cannot. This theory is attributed to the fact that visual sampling does not damage the cotton plants when they are young while the other techniques do. In addition Khan *et al.* (2004b) also proposes that visual sampling is just as effective as beat sheeting when cotton is below nine nodes. However Byerly *et al.* (1978) showed that the visual technique lost effectiveness as the cotton season progressed. As a result concern exists regarding the effectiveness of this method in the later part of the cotton season.

1.7.1.3. Suction sampling (D-vac)

The use of suction sampling (D-vac) is another method used to sample mirids. This method involves the use of suction whereby insects are literally sucked from the plant and the surrounding environment by the device like a vacuum cleaner. The sampler is a petrol engine driven leaf blower, used in reverse, with a collecting bag attached over the air intake tube so insects can be collected for counting (Smith *et al.* 1975). Samples are taken over 20 metres by continuously moving the instrument through the canopy of the cotton plants and repeated approximately six times.

Byerly *et al.* (1978) demonstrated that suction sampling gave a good estimate of the adults present, however was poor at sampling nymphs throughout the cotton season. An additional limitation, outlined by Simpson & Lloyd (2007), was the use of the suction method is expensive, cannot be used when the crop canopy is wet, and not convenient especially if the equipment fails during field work. Stanley (1997) states sampling via the use of a large suction machine he called the Bigvac gave efficiency in sampling mirids of 44 – 54% which is believed not to be efficient enough, yet it is probably the most efficient out of all the methods available. Byerly *et al.* (1978)

showed that suction sampling can be corrected by using a conversion factor to reflect the actual (absolute method) population.

1.7.4. Beat sheeting

Beat sheeting has numerous strengths as a sampling method. Firstly, Simpson *et al.* (1999), Deutscher *et al.* (2003) and Simpson & Lloyd (2007) demonstrated that beat sheeting is a reliable and cost effective method that helps to indicate whether mirid numbers are high enough to instigate control, while only taking three to five minutes to conduct each sample. Secondly, Khan *et al.* (2004b) also states that beat sheets are fast and consistent throughout the cotton season. Thirdly, Deutscher *et al.* (2003) showed that beat sheets were more effective at counting mobile insects like GM than visual and suction techniques. Finally, Deutscher *et al.* (2003) suggests beat sheeting is less subject to operator bias as the amount and variability of the skill needed to use this method is less than other methods, particularly visual sampling.

The beat sheet technique has been identified by Miles *et al.* (1992) as being accurate in measuring the actual population densities, but in order to achieve the results need to be converted and adjusted for quantitative use to determine population density fluctuations. Miles *et al.* (1994) also states that it is possible to devise correlations for any sampling method which will help provide accurate estimates of true mirid populations as long as a good understanding of the correlation between the real population density and the count number is known. This is only achievable by developing a sound understanding of the GM population dynamics and ecology, and how this relates to the sample numbers. Further investigation into this is mentioned as being needed by Miles *et al.* (1994).

In light of examining the strengths and limitations of each sampling method there appears to be no one perfect method for sampling mirids. Byerly *et al.* (1978) conducted an experiment which revealed that sweep net, suction and visual sampling methods are all relatively efficient but as the canopy of cotton closes the sweep net and suction are no longer adequate as they only sample the top portion of the plant. However, Byerly *et al.* (1978) also considered that visual sampling was not effective in sampling large cotton. As a result no method has been determined as efficient in

sampling large cotton. Deutscher *et al.* (2003) suggests that to achieve an accurate overall assessment of an insect population, a combination of methods should be used as no one perfect method exists.

1.7.2 GM sampling requirements

The number of samples taken per sampling method is another factor to consider when sampling. When the GMs patchy distribution within a crop is considered, more samples are required to obtain an accurate representation of the field population. Simpson *et al.* (1999) discussed the number of samples required in order to achieve an accurate representation of an insect population. Simpson *et al.* (1999) recommended six beat sheet samples as a standard number of replicates that achieves appropriate results as further increasing sample sizes often does not give extra information considering the time and resources necessary to achieve the extra accuracy. However, Khan *et al.* (2004b) indicated that research into the number of beat sheet samples that are required per field for the most accurate results that justify costs is continuing.

1.7.3 Operator variation

One variable affecting sampling accuracy which could be further investigated is operator use. In all experiments there is some form of human error and sampling for mirids is no exception. Deutscher *et al.* (2003) found no significant differences between operators using beat sheets. However, this appears to be the only study investigating operator differences in sampling for GM, and it considered only one method, beat sheeting. Therefore it can be proposed that further research is necessary into human error in sampling method accuracy.

1.7.4 Novel innovative sampling methods

Bodnaruk (1987) indicates that research into new methods such as the use of traps may be useful and revolutionary. Bodnaruk (1987) experimented with the use of 'sticky traps' as a method for sampling GM. The results showed some potential for the use of sticky traps as a monitoring tool; however this has not been adopted in commercial practice. Additional research has shown trap catches act as an indicator of the influx of mirids into cotton and were found to be correlated with the other methods available (Khan *et al.* 2004b). Other trapping methods which may also be useful include the use of sex pheromones.

1.8 Potential for sex pheromones as monitoring tool for GM

The sex pheromone of the GM was identified by Lowor (2006) as mentioned earlier. The two principle components of the female produced pheromone are hexyl hexanoate and (*E*)-2 hexenyl hexanoate. Hexyl hexanoate is the main constituent, produced by both sexes, whereas (*E*)-2 hexenyl hexanoate was produced only by females. Field trials were conducted on various crops to test the attractiveness of different blends of these two components. Lowor (2006) discovered that a blend of 5:1 of hexyl hexanoate and (*E*)-2-hexenyl hexanoate was most attractive. The pheromone traps caught only males making it clear the blend was acting as a sex attractant. Furthermore, Lowor (2006) conducted preliminary tests on the pheromone application for mating disruption and attract-and-kill styles of pest control. These trials suggested there was potential for the use of the pheromone blend, however further investigation is needed. Since this research concluded the pheromone blend is species and gender specific, there is a genuine possibility of using pheromone traps as a monitoring tool or as an attract-and-kill tool for GM.

1.8.1 Strengths of pheromones as a monitoring tool

There are numerous benefits to using pheromones as a monitoring tool for insects. Wall (1990) identifies five main strengths for using pheromones as a monitoring tool. Firstly, pheromones are often the most sensitive sampling technique due to pheromones attracting insects from very long distances away (Miller & Roelofs 1978). Secondly, pheromone traps are cost effective, relatively easy to set up, require little maintenance and are not labour intensive. Thirdly, pheromone traps are species-specific, which means they only catch the species of interest so the catch often does not require sorting. Finally the method does not necessarily require sophisticated education to use appropriately.

1.8.2 Weaknesses of pheromones as a monitoring tool

1.8.2.1 Complications in interpreting pheromone trap catches

Despite the advantages of pheromone traps as a sampling method there are limitations. The limitations of pheromones are based mainly on the complications of interpreting the catches, especially in relation to the insect's population dynamics. Wall (1990) states there is limited information available on how the sampling range is affected by various factors like climate variations, especially over time. Reidl (1980) argues that the effects of factors like rainfall and temperature on catch numbers are often unknown. As a result it is difficult to interpret exactly what pheromone trap catch numbers represent, especially with a poor understanding of the pests ecology.

1.8.2.2 Female competition phenomena

Other potential inaccuracies in the interpretation of pheromone trap catches are possibly due to the emergence of the general phenomena of female competition which presents another limitation (Carde 1979, Hartstack & Witz 1981, Miller & McDougall 1973, Riedl & Croft 1974). Female competition can occur when the male insect follows the naturally occurring pheromone source of the wild female instead of following the synthetic pheromone source (Campion *et al.* 1989). Female competition may imply that males are disproportionately attracted to pheromone traps when the wild mature female presence is low. This could happen, for example, when most

females in the population have already been mated and may be relatively unattractive. This could be a result of the female releasing less or a different pheromone blend. This would be reflected in high male catch numbers in the traps. Conversely, when unmated female numbers are high, trap numbers would presumably be low because the males would be attracted more to the real female pheromone signal. These effects may be exaggerated if the synthetic pheromone is not a perfect mimic of the natural one produced by the females (Campion *et al.* 1989).

In addition, Champion *et al.* (1989) identifies the possibility that unmated females actively search for males which might reduce the accuracy of pheromone traps. Further, Carde & Minks (1995) and Betts *et al.* (1993) explain the efficiency of the technology is also related to the motility of mated females into the area being managed. Both affect the population dynamics in the area which could result in an inaccurate assumption being made of the GM population in a field from the pheromone trap results. This is why a good understanding of the insects' ecology and behavioural trends are needed and unfortunately these behaviours are not yet known for the GM.

The result of these influences might be that instead of reflecting the population density of an insect in the field, pheromone traps reflect aspects of mating behaviour. Such information might still be useful for pest management, but the simple correlation of high catches with high numbers (and thus potential damage) would not be present. Thus, to successfully implement and use pheromone traps as a monitoring tool an accurate understanding of the pests ecology, population trends and behavioural characteristics and how these relate to trap catches is needed (Wall 1990, Suckling 2000). These complications have resulted in the failure of pheromone traps as monitoring devices in some cases (for example, Fitt 1994 and Champion *et al.* 1989).

There have been a number of cases where the relationship between trap catches and population density is well understood, and hence pheromone trapping is routinely used for monitoring. Some of these examples include the oriental fruit moth (*Cydia molesta*), the light brown apple moth (*Epiphyas postvittana*) and pink bollworm (*Pectinophora gossypiella*) (Campion *et al.* 1989, Carde & Minks 1995, Kehat *et al.* 1999, Suckling 2000). Most successful uses of pheromones in mating disruption and

as a monitoring tool have been obtained mostly with moths (Lepidoptera) (Carde & Minks 1995, Perry *et al.* 1988). However, currently there appear to be no cases where pheromone traps have been used in commercial practice to monitor a mirid pest despite the identification of females produced pheromones in several mirid species (Groot *et al.* 1999).

1.9 Conclusion and Aims of dissertation

In conclusion the GM is a significant pest of cotton and further investigation into this pest is needed to gain an understanding of its ecology, sexual and migratory behaviours and ways of control. The use of the GM pheromone identified by Lowor (2006) may be able to help gain a better understanding of the GM ecological and biological characteristics and possibly be a new successful monitoring tool. The dissertation which accompanies this literature review will investigate whether GM might prove to be the first successful example, among mirids, as a useful tool for monitoring and/or shed light on some ecological and biological characteristics of the GM. This dissertation will also compare and address problems associated with the current sampling methods available to sample the GM.

The overall aim of this dissertation is to determine the relationships between pheromone trap catches and various parameters of a population of GM in cotton to see if pheromone traps will be an appropriate monitoring method. This study also aims to provide some basic information on mirid population dynamics which are currently lacking. The population parameters the pheromone trap catches will be compared to include:

1. The population densities of GM adults and nymphs in the field
2. The sex ratio of the field population
3. The percentage of mated females in the population

These population parameters will be addressed as individual studies (1, 2, 3, respectively) that attend to the following questions and hypotheses.

1. Do pheromone traps reflect mirid population densities for either adult or nymphs in the cotton field?

Ho: Pheromone traps are not associated with mirid population densities in the cotton field

2. Are pheromone trap catches associated with the sex ratio of the field population?

Ho: Pheromone traps are not associated with the sex ratio of the field population of mirids.

3. Are pheromone trap catches associated with the percentage of mated females in the population of GM in the cotton field?

Ho: Pheromone traps are not associated with the percentage of mated females in the population of GM in the cotton field.

A preliminary study will also be conducted early in the experiment determine if variation occurs between:

- a) The ability of the four current methods available (Visual assessment (1), Suction (2), Beat sheet (3), Sweep net (4)) at sampling GM adults and nymphs.
- b) The three operators and sites at sampling adult and nymph GMs using each of the four sampling methods.

Chapter Two:

Materials and Methods

2.1 Trial sites

This honours project forms part of the green mirid (GM) pheromone trapping trials done in eight locations in Queensland and New South Wales for the Cotton Catchment Communities CRC (Project 1.5.02; *Chemical ecology of insects in Australian cotton fields*). These trials were conducted between October 2007 and February 2008 in collaboration with the Cotton CRC's extension officers and researchers. Of these eight sites, three were chosen to be used for this dissertation: Auscott in Narrabri NSW, Brigadoon in Boggabri NSW and Korolea in Goondiwindi. The author was responsible for data collection from the Boggabri site while data collections from the other two trials were conducted by Cotton CRC staff: Peter Gregg (PG) and Alice Del Socorro (ADS) at Narrabri and Rod Gordon (RG) at Goondiwindi. The locations of these sites and other Australian cotton growing areas are shown in Figure 2.1. All trials were conducted in irrigated Bollgard II® cotton. The author was responsible for the laboratory work and statistical analyses for all three sites.

Fields were chosen on the basis of accessibility during all weather conditions, surrounding crops/vegetation and infrastructure. The accessibility of the field during wet weather was considered so that trap clearing and field sampling for mirids could be done at regular intervals under all weather conditions. Surrounding vegetation such as crops and weeds and infrastructure such as bright lights from sheds or posts during the night, were also considered to minimise their effects on mirid behaviour or mirid populations in the experimental areas.

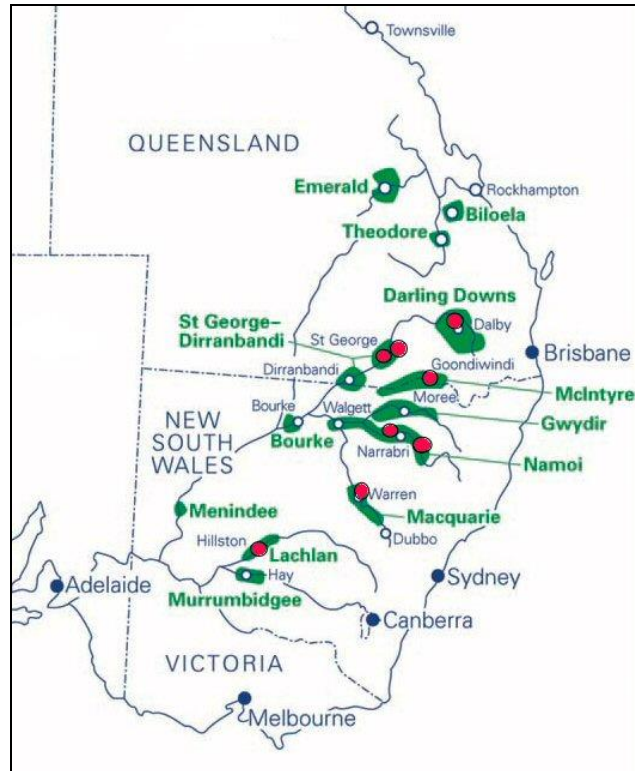


Figure 2.1. Locations of pheromone trapping trials (red dots) in the cotton growing regions (green areas) of Queensland and New South Wales. In this dissertation, only the Namoi (Narrabri and Boggabri) and the Goondiwindi sites will be considered

2.2 Pheromone Traps and Lures

The pheromone traps used were AgriSense® funnel traps (Entosol Australia Pty Ltd, Roselands, NSW, Australia) (Figure 2.2). They are also known in the literature as Universal traps (Howse *et al.* 1998). The GM pheromone lure was attached with a paper clip to the red plug on the top of the trap so it hung inside the trap as shown in Figures 2.3 and 2.4.

A 2 x 3 cm block of pest strip containing 186g/kg Dichlorvos ('Killmaster Zero', Barmac Industris Pty. Ltd, Swanbank, Qld) held in a plastic container with aeration holes, was placed inside the trap to kill any insects caught (Figure 2.5). Both the pheromone attractant lures and pest strips were replaced every six weeks throughout the experiment. The method used for preparing the pheromone lures was the same as that used by Lowor (2006). Pheromone lures consisted of rubber septa impregnated with 100 mg of a 5:1 blend of hexyl hexanoate and (*E*)-2 hexenyl hexanoate. These components are highly volatile, hence easily lost. To improve the longevity of

pheromone lures the rubber septa was coated in araldite glue except for a small exposed area at the tip. Coating the lures allowed the slow release of the pheromone for much longer time compared with uncoated ones (A. Del Socorro & P. Gregg, unpublished data, 2007).



Figure 2.2. AgriSense® Trap

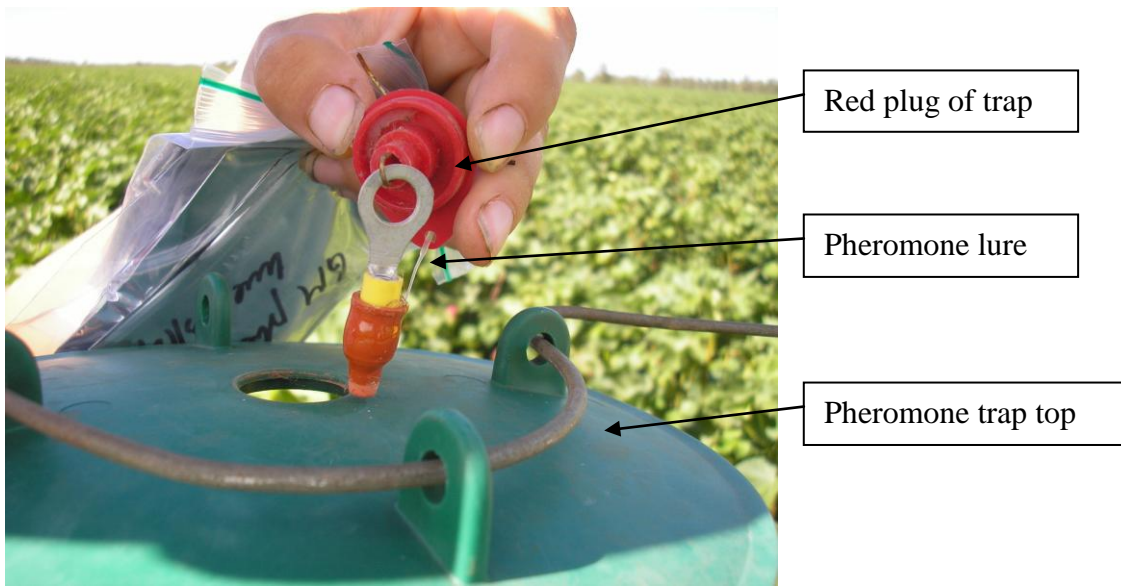


Figure 2.3: Top of Agrisense® pheromone trap with pheromone lure attached, about to be hung inside the trap.



Figure 2.4. (a) side view of pheromone trap with pheromone lure hanging inside, (b) inside view of pheromone trap with pheromone lure hanging inside.



Figure 2.5. Pest strip inside the pheromone trap.

Traps were mounted to flexible PVC electrical conduit posts with thick wire so the traps hung above the canopy of the cotton crop. Traps were adjusted as the cotton plants grew to keep them approximately 30cm above the plant canopy (Figure 2.6). Pheromone traps were cleared at least twice a week. The number of mirids in each trap was recorded but the trap catches were not kept. Lowor (2006) found that all GM caught in such traps were male (as would be expected with a sex pheromone), and that control traps lacking pheromone lures did not catch any GM of either sex.



Figure 2.6. Pheromone trap placed above the cotton crop canopy by using flexible PVC electrical conduit pipe and strong wire.

2.3 Field Layout of Traps

Four pheromone traps (2 traps x 2 rows) were set up at each site. Depending on the size of the field, traps were placed between 50-100 m in from the edges of the field and 50-200 m between rows. In order to locate the traps in the field throughout the season as the cotton crop grew, flag posts were placed at both ends of the rows. Flexible PVC electrical conduit was used for both the pheromone trap posts and the flag posts to reduce the interference of the experiment with common cropping practices and to prevent damage to the traps while machinery went over them. An example of the layout of traps (represented by o) and flag posts (represented by flags) in the field is shown in Figure 2.7.

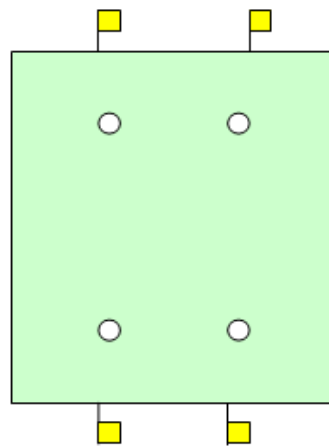


Figure 2.7. Field layout of traps in the field. The white circles represent the pheromone traps and the yellow flags represent the flags to help identify where the traps were in the field.

2.4 Identifying sampling locations within the field

Insect densities can vary both across, up and down a field. To address this irregularity of population densities throughout the field, the experiment was set up using the concept of Latin square designs to identify where each sampling method would occur on a particular sampling date. The Latin square design allowed for random assignment of each sampling method to each site throughout the field. There were four Latin square designs constructed (as shown in Figure 2.8) and each was used on separate sampling dates. For example:

- Date 1 = Latin square 1
- Date 2 = Latin square 2
- Date 3 = Latin square 3
- Date 4 = Latin square 4
- Date 5 = Latin square 1

By designing and using four separate Latin square designs, each of the four sampling methods could be used in every plot site throughout the field (Ie a plot site is one small square with a number allocated which represents the sampling method to be used). Perry *et al.* (1980) reviewed the uses and advantages of using a Latin square design.

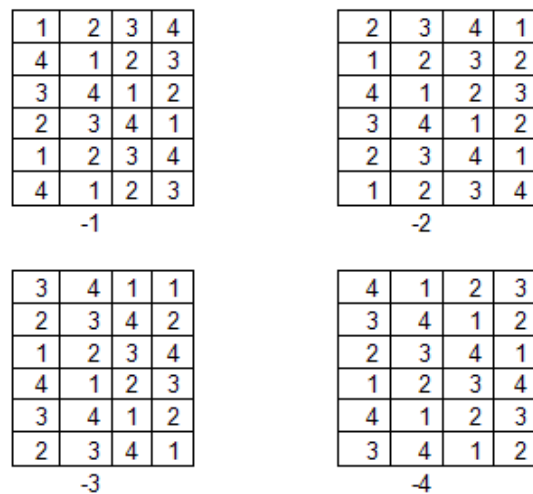


Figure 2.8: Four Latin square designs used for allocation of site within the field for using each sampling method on a particular date.

*Note: Each small square (

1

) is considered a plot but plots were not mapped. Further not all sampling methods were used on each sampling occasion, so the full Latin square design was not required.*

2.5 Clearing pheromone traps

The pheromone traps were cleared at least twice a week at all sites, and usually every second day at Boggabri. On the same days sampling occurred in the field (Section 2.6). The number of mirids in each trap was recorded but the trap catches were not kept for reasons discussed above.

2.6 Sampling for mirids in the field

Field sampling for mirids was done when traps were cleared. Four methods were used to sample the field population of mirids and each method was assigned a number. The sampling methods included visual counts (1), suction or D-vac (2), sweep net (3) and beat sheet (4).

Each sampling method was replicated six times on each sampling occasion. All GM adults and nymphs from each sample were kept in vials and labelled according to site, date and sampling method. All samples were kept in an insulated container with an ice box inside to keep them cool while in the field. Upon return to the laboratory, samples were sorted, sexed and kept in vials containing 70% alcohol until dissection.

Not all of the four sampling methods were employed on each sampling date. For example, very early in the season, only visual counts on cotton plants at the seedling stage were done so as not to damage the plants (which would have resulted from sweep netting or beat sheeting), or contaminate the sample with dirt (as would have occurred with the suction sampling). As the plants matured suction, sweep net and beat sheet methods or a combination of two or more methods were used.

2.6.1 Method 1 - Visual counts (whole plant check)

Visual counts were conducted by randomly selecting a one meter plot of cotton plants where each plant was carefully checked for adults and nymphs (see section 2.4). Adult mirids are highly mobile when disturbed so plants were checked as quietly as possible. Checks were first made for adults, starting at the top of the plant and progressing downwards while very gently touching the individual plants. This was followed by a more rigorous check and close inspection of terminals, squares, flowers and bolls of the plants for adults and nymphs (as shown in figure 2.9). Any adult mirids that escaped from the plants within the 1 m plot were also included in the counts.



Figure 2.9: Visual assessment of cotton plants for green mirids. Photo: Peter Gregg

2.6.2 Method 2 –Suction/vacuum sampler (D-Vac samples)

The suction sampler (Ryobi 31 cc) was a small portable vacuum machine, with a 120 mm diameter cone and a nozzle speed of approximately 10 m per second, powered by a two cycle engine.

Suction sampling was done over a 20 m length of plants in a row. The 20 m length was first marked with a PVC post and sampling was done two rows away parallel from this post where plants were not disturbed by the operator walking along the row. Insects were collected in a cone-shaped nylon cloth bag approximately 25 cm long, inserted into the suction tube and secured by rubber bands. The suction tube end where the nylon bag was attached was then passed over the cotton plants for 20 m for each replicate (As shown in Figure 2.10). Once a 20 m suction was completed, the nylon bag was removed and secured while the engine was still running to prevent any mirids escaping. Upon return to the laboratory, collecting bags were kept in the freezer to kill the insects before sorting for mirids was done. Suction sampling was usually

done later in the morning when plants were not wet to prevent samples from becoming soggy and damaging insects.

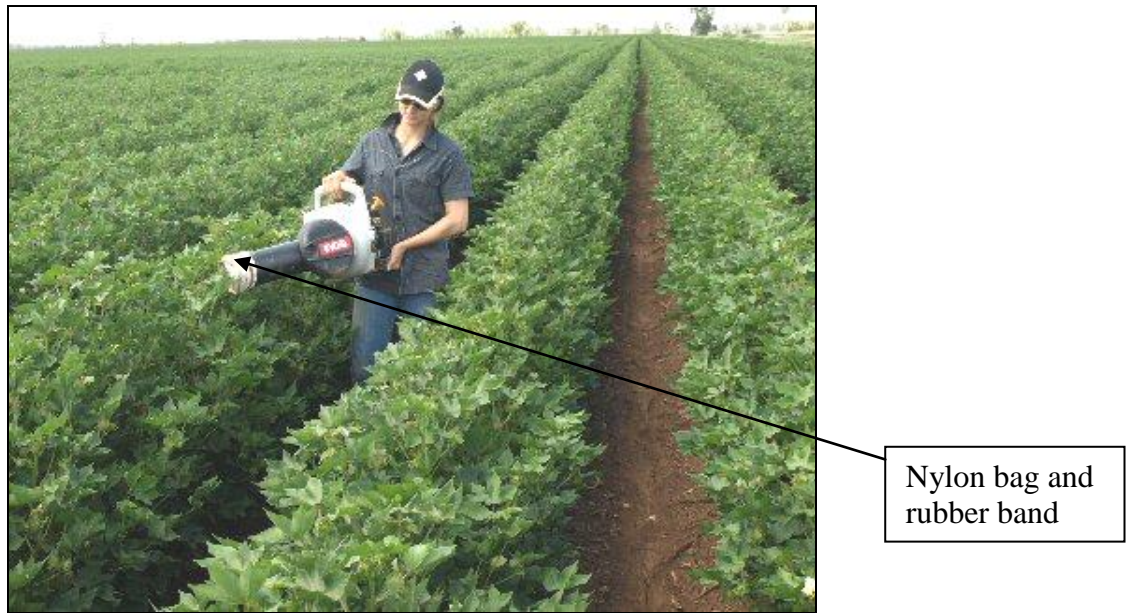


Figure 2.10: Suction sampling of cotton plants for green mirids. Photo by: Peter Gregg

2.6.3 Method 3 – Sweep net

Sweep netting was done using a 38 cm diameter net made of very fine netting material that prevented small insects like the GM from escaping. Each sweep net sampling consisted of 20 sweeps (as shown in Figure 2.11). The GM adults and nymphs for each sample were placed into separate vials, labelled accordingly and the numbers recorded.



Figure 2.11: Sweep net sample being conducted on cotton for green mirids.

Photo: Peter Gregg

2.6.4 Method 4 – Beat sheets

The beat sheet method was done by rigorously beating the plants with a 1m stick onto a yellow plastic sheet measuring approximately 1.5m x 1.5m. This plastic sheet was laid down under the cotton plants and opened up over the plants in the opposite row (shown in figure 2.12). Plants were beaten onto the sheet rigorously 10 times. All mirid adults and nymphs found on the sheet after beating were counted and collected into vials and labelled accordingly.



Figure 2.12: Beat sheet sample being conducted on cotton for green mirids.

One problem encountered with the beat sheet method of sampling was that it was not possible when the ground was very wet, or when the field was being flood irrigated.

2.7 Time of sampling

The time when sampling was conducted was in the morning. When this was not achievable sampling was conducted in the afternoon. These times were used to avoid the heat of the day. They were selected because Bodnaruk (1987) found mirids are most active in the morning and at dusk.

2.8 Control

An ideal control for this study might have been to sample another field at each site, where no pheromone traps were present, to determine if the traps affected the number and population dynamics of the GM. However, such controls are rarely used in pheromone research because the spatial scale of the treatments, which exploit the behaviour of mobile insects, makes it difficult. It would be necessary to site the

control so far away from the treated area that differences in other factors (eg surrounding vegetation or crop factors) would invalidate it. At Boggabri some degree of control was provided by reference to the agronomist's weekly checks from other fields and it was found that trends were broadly consistent with those in the experimental field.

2.9 Recording observations.

Field observations and sexing information was recorded on a summary sheet similar to the template shown in Figure 2.13 and then placed into Excel spread sheets where further analysis could be conducted.

Note: To make all data directly comparable between the four sampling methods all collections were made on per meter basis.

Date:	
Day:	
Location:	
Notes:	

Trap	# mirids
1	
2	
3	
4	
Total	

	# females	# males	# nymphs
1			
2			
3			
4			
5			
6			
Total			

	# females	# males	# nymphs
1			
2			
3			
4			
5			
6			
Total			

	# females	# males	# nymphs
1			
2			
3			
4			
5			
6			
Total			

	# females	# males	# nymphs
1			
2			
3			
4			
5			
6			
Total			

Figure 2.13: Record sheet for field observations

2.10 Dissection of adult mirids

Female mated status was determined according to the description by Strong *et al.* (1970) for the American mirid, *Lygus hesperus*, which scored a female as mated when the seminal depository appeared enlarged and the genital pouch inflated and whitish in colour. In this study, reproductive development in female mirids was categorised into the following:

1 = No eggs and No white seminal depository (SD)

2 = No eggs and white SD

3 = Eggs and No white SD

4 = Eggs and white SD

A female green mirid was scored as mated under categories 2 and 4, when white seminal depository usually appeared enlarged and full.

2.11 Statistical analysis

Normality of data was tested using MINITAB v14 (Ryan *et al.* 1992). If normality was not present in the raw data, square-root transformations were applied and normality re-tested. If normality still had not been achieved by square-root transformations, log (x+1) transformations were applied and used. All data sets were either normal or were rendered normal by one of these transformations. All data were analysed using analysis of variance, and where the analysis proved significant, means were separated by Fisher's Least Significant Difference test at 5 % levels.

2.12 Studies 1, 2 and 3 respective to aims.

Study 1 only used the field data and not the dissection data. The raw data and statistical analysis and further methodology for study 1 are in Appendix 3. Study 2 and 3 used both the field and dissection data. The raw data, statistical analysis and further methodology used for studies 2 and 3 are in Appendices 4 and 5 respectively.

Chapter Three

Results

3.1 Comparison of sampling methods

A preliminary analysis of trapping and sampling data was conducted early in the trials to determine if any variation occurred between:

- the effectiveness of the four sampling methods (1,2,3,4) at sampling adults and nymphs
- the effectiveness of sampling methods for both adult and nymphs in the hands of different operators

This preliminary analysis was needed to identify any variation between operators and sampling methods so conversion factors could be made for further analysis, so the data were consistent between sites and dates.

To minimise the effect of crop growth stage from this preliminary analysis three days were chosen for each operator, on which all four sampling methods had been used. The dates were selected as close as possible to each other relative to the stage of crop growth (days after planting - DAP) (as shown in Table 3.1).

Table 3.1: The dates used for the preliminary study, relative to the stage of crop growth for each operator

Operator/Location,	Date 1	Date 2	Date 3
Operators initials	DAP	DAP	DAP
1 –Narrabri (PG-ADS)	99	113	120
2 –Boggabri (SA)	103	114	117
3 –Goondiwindi (RG)	98	117	125

Once the dates were chosen, normality tests were conducted to establish if the data were normally distributed before statistical analyses were done. Both the methodology for the normality tests and other preliminary statistical analyses conducted are further explained in detail in Appendix 1.

Figures 3.1 to 3.3 show the mean number of nymphs and adult mirids caught using the four different sampling methods by three different operators on the three separate dates. The results showed there was no single best method for sampling nymphs or adult mirids. In general, on each sampling date, the efficiency of sampling methods differed between locations/operators. It was also found that differences in the effectiveness of sampling methods were affected by date. For all three dates there was a significant interaction between the sampling method used and the operator, affecting the numbers of adult mirids caught. A summary of the findings based on the preliminary analyses done for the three sites on the three sampling dates is given in Table 3.2.

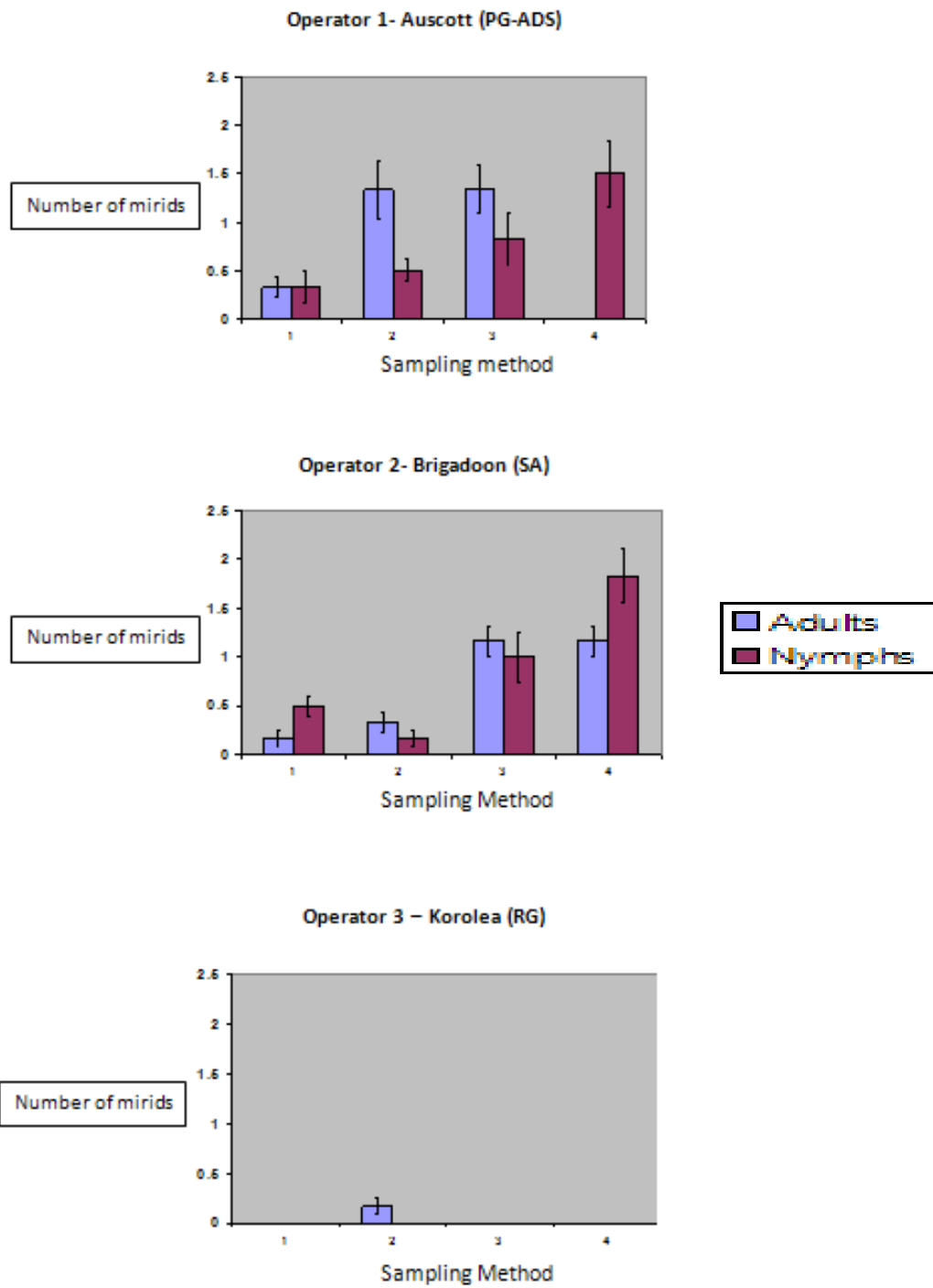


Figure 3.1. Mean number of GMs caught per sample using visual (1), sweep net (2), suction (3) and beat sheet (4) methods at Auscott, Brigadoon and Korolea on **Date 1**. Note where no mirids are shown for a particular sampling method at a particular site it means that no mirids were caught in that sample, not that the sampling method was not used. Error bars indicate standard error of means.

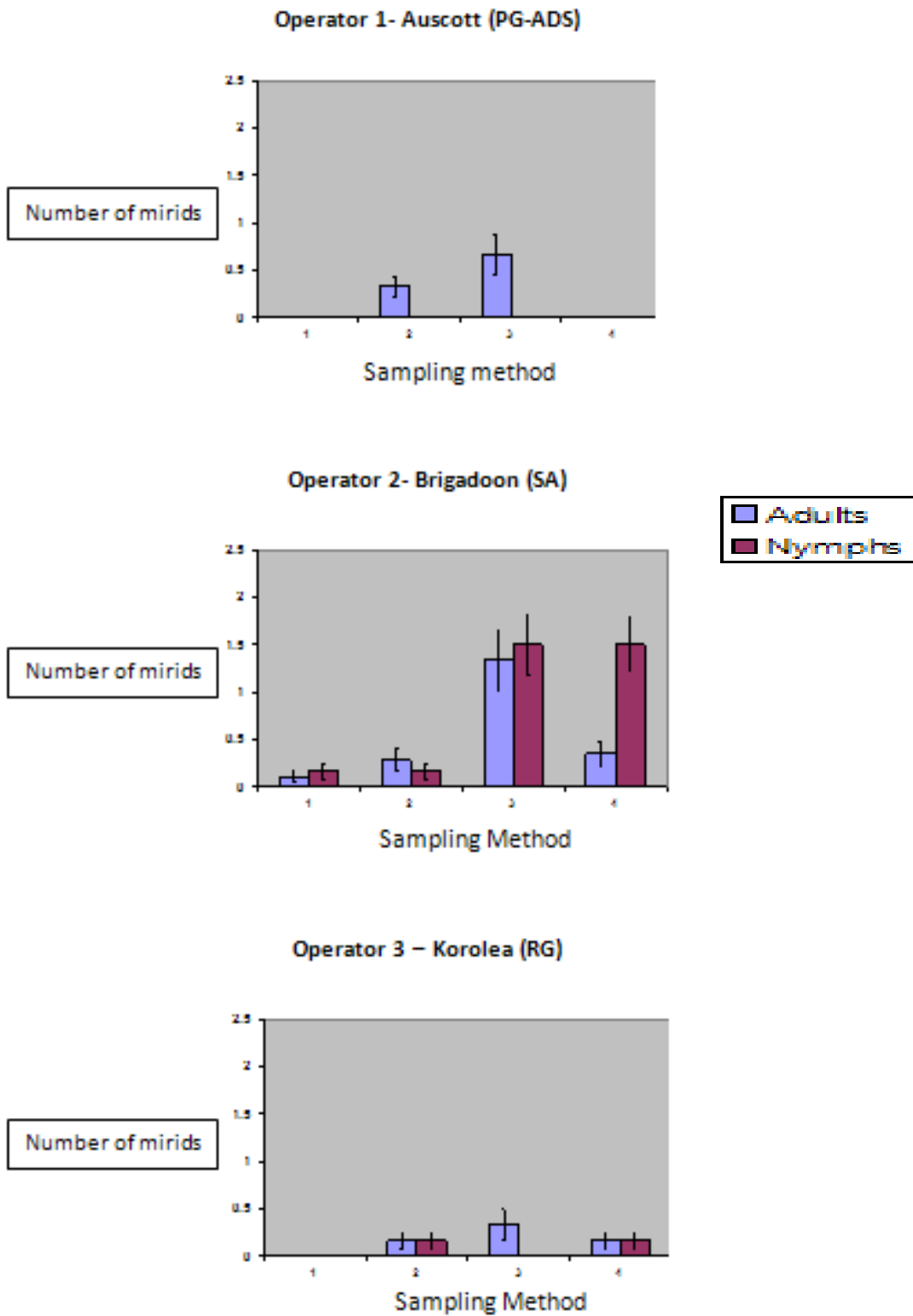


Figure 3.2. Mean number of GMs caught per sample using visual (1), sweep net (2), suction (3) and beat sheet (4) methods at Auscott, Brigadoon and Korolea on **Date 2**. Note where no mirids are shown for a particular sampling method at a particular site it means that no mirids were caught in that sample, not that the sampling method was not used. Error bars indicate standard error of means.

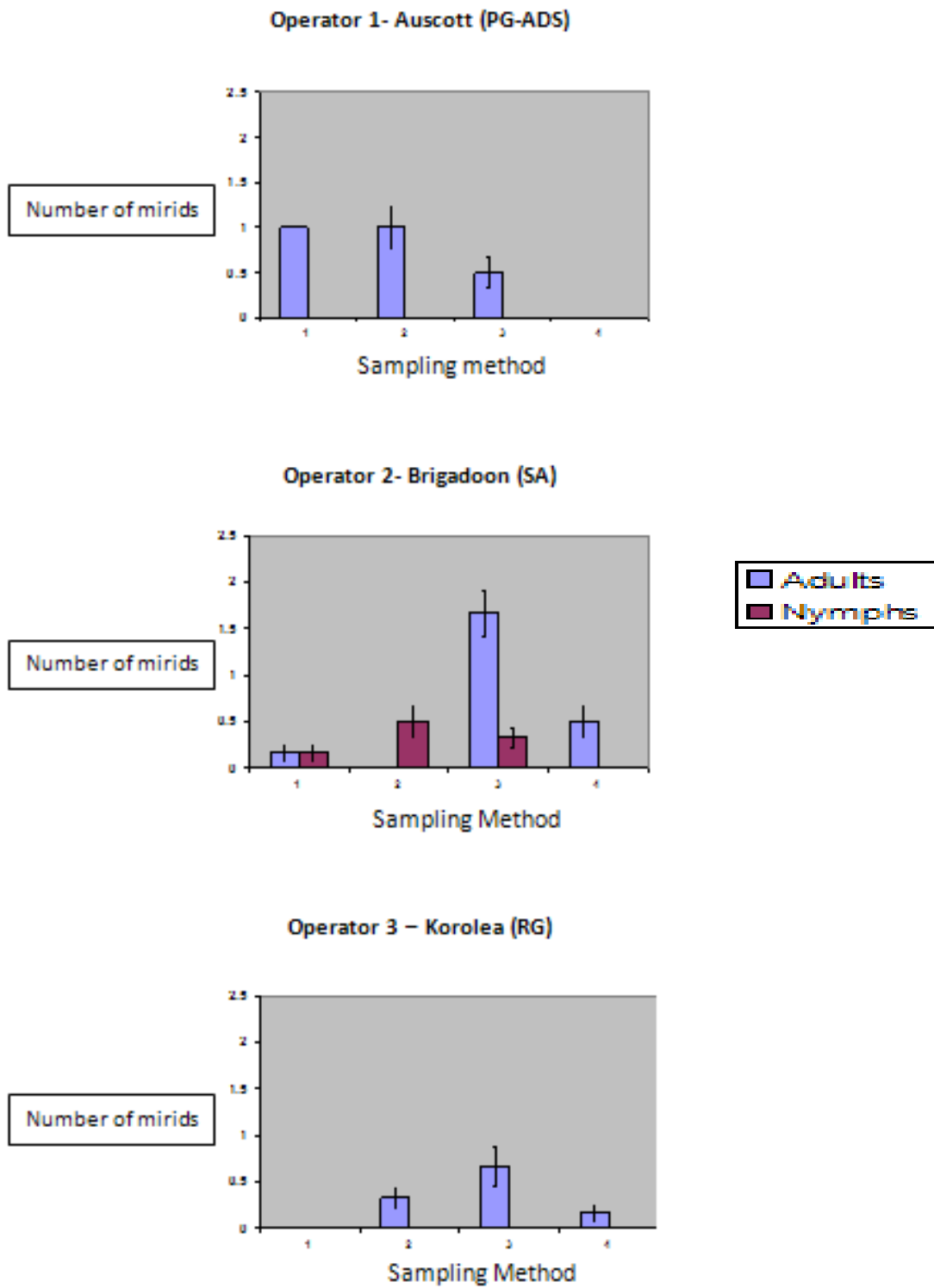


Figure 3.3. Mean number of GMs caught per sample using visual (1), sweep net (2), suction (3) and beat sheet (4) methods at Auscott, Brigadoon and Korolea on **Date 3**. Note where no mirids are shown for a particular sampling method at a particular site it means that no mirids were caught in that sample, not that the sampling method was not used. Error bars indicate standard error of means.

Table 3.2 summarises the results of statistical analyses conducted on the data illustrated in Figures 3.1 to 3.3. Details can be found in Appendix 1.

Table 3.2. Summary of statistical findings for Figures 3.1 to 3.3 (see Appendix 1)

Date	Adults	Nymphs
1	<ul style="list-style-type: none"> *A significant interaction between method and operator ($P=0.007$). * No significant difference in method effectiveness for both operators 1 ($P=0.067$) and 3 ($P=0.413$). Methods (1 = 2 = 3 = 4) * Significant difference between methods for operator 2 ($P=0.014$). Methods 3 and 4 significantly different to method 1. * For operator 2 sampling methods 3 and 4 are significantly more effective than methods 1 and 2 but no significant difference between them is evident. (1 = 2 < 3 = 4) 	<ul style="list-style-type: none"> * No significant interaction between operator and method ($P=0.339$). * Significant difference between operators ($P=0.001$). *Operator 3 significantly different to operators 1 and 2 (but 1 and 2 are not significantly different). ($3 \neq 1 = 2$) * Significant difference among sampling methods exists ($P=0.048$). Method 4 is significantly different from Method 1. * No significant difference in method effectiveness between operators.
2	<ul style="list-style-type: none"> * A significant interaction between method and operator ($P=0.002$). * No significant difference between methods for either operator 1 ($P=0.190$) or 3 ($P=0.766$). * Method 3 is the most effective for operator 2. 	<ul style="list-style-type: none"> * A significant interaction between method and operator ($P=0.014$). * No significant difference between methods for either operator 1 ($P=***$) or 3 ($P=0.582$). * Significant difference between methods for operator 2 ($P=0.042$). Methods 1 and 2 are significantly different to Methods 3 and 4. * For operator 2 sampling methods 3 and 4 are significantly more effective than methods 1 and 2 but no significant difference between them is evident. (1 = 2 < 3 = 4)
3	<ul style="list-style-type: none"> *A significant interaction between method and operator ($P=0.001$). * Significant differences between methods for operators 1 ($P=0.013$) and 2 ($P=0.004$) *For operator 1 sampling methods 1 and 2 are significantly more effective than methods 3 and 4 but no significant difference between them is evident. (1 = 2 > 3 = 4) * Method 3 was significantly different from other methods for operator 2. ($3 \neq 1 = 2 = 4$) 	<ul style="list-style-type: none"> * No significant interaction between operator and method exists ($P=0.470$). * There is a Significant difference in operators ($P=0.005$). *Operator 3 is statically different to operators 1 and 2 (but 1 and 2 are not statistical different from each other). * There is no significant difference between the effectiveness of methods for any of the operators. (1 = 2 = 3 = 4)

On the basis of these results, conversion factors were calculated to come up with the corrected mean numbers of adults and nymphs across means of up to four sampling methods for each sampling date on each site. Calculations for these conversion factors for each site are detailed in Appendix 2.

Even though there was no significant best method at sampling either adults or nymphs or for each individual operator, from Figures 3.1, 3.2 and 3.3 there are some observable trends of the most effective method at sampling adult and nymph green mirids for each operator. Table 3.3 below identifies these observable trends. From Figures 3.1, 3.2 and 3.3 it seems for adult mirids method 3 followed by method 2 might be more effective at sampling adults for each operator, while methods 3 and 4 seem to show a higher effectiveness at sampling nymphs.

Table 3.3: A summary of the observable differences in the order of effectiveness of each method at sampling green mirid adults and nymphs for each operator. Note (>) identifies which methods are more effective and (=) shows methods where their effectiveness seems to be the same. For example with operator 1 the effectiveness of methods 2 and 3 are equal (2 =3) in their effectiveness at sampling adults and are more effective than method 1 (>) and even greater (>) in effectiveness than method 4.

Operator location	Adults	Nymphs
Operator 1 – Auscott (PG-ADS)	(2 = 3) > 1 > 4	4 > 3 > 2 > 1
Operator 2 – Brigadoon (SA)	3 > 4 > (1 = 2)	3 > 4 > (1 = 2)
Operator 3 – Korolea (RG)	3 > 2 > 4 > 1	(2 = 4) > (1 = 3)

3.2 Pheromone trap catches versus mirid numbers in the field

The relationship between pheromone trap catches and the estimated numbers of mirids in the field (calculated as described in Appendix 2) varied at the three locations. Significant correlations between pheromone trap catches and both mirid adults and nymphs were shown at Goondiwindi, and between trap catches and mirid nymphs at Brigadoon. On the other hand, there was no significant correlation with either adults or nymphs at Narrabri. Statistical analyses of data for each site are given in Appendix 3.

3.2.1 Goondiwindi, Korolea

Pheromone trap catches were significantly correlated with both mirid adults and nymphs at Goondiwindi (Figs. 3.4, 3.5). Peak trap catches appeared to coincide with peak numbers of mirids in the field.

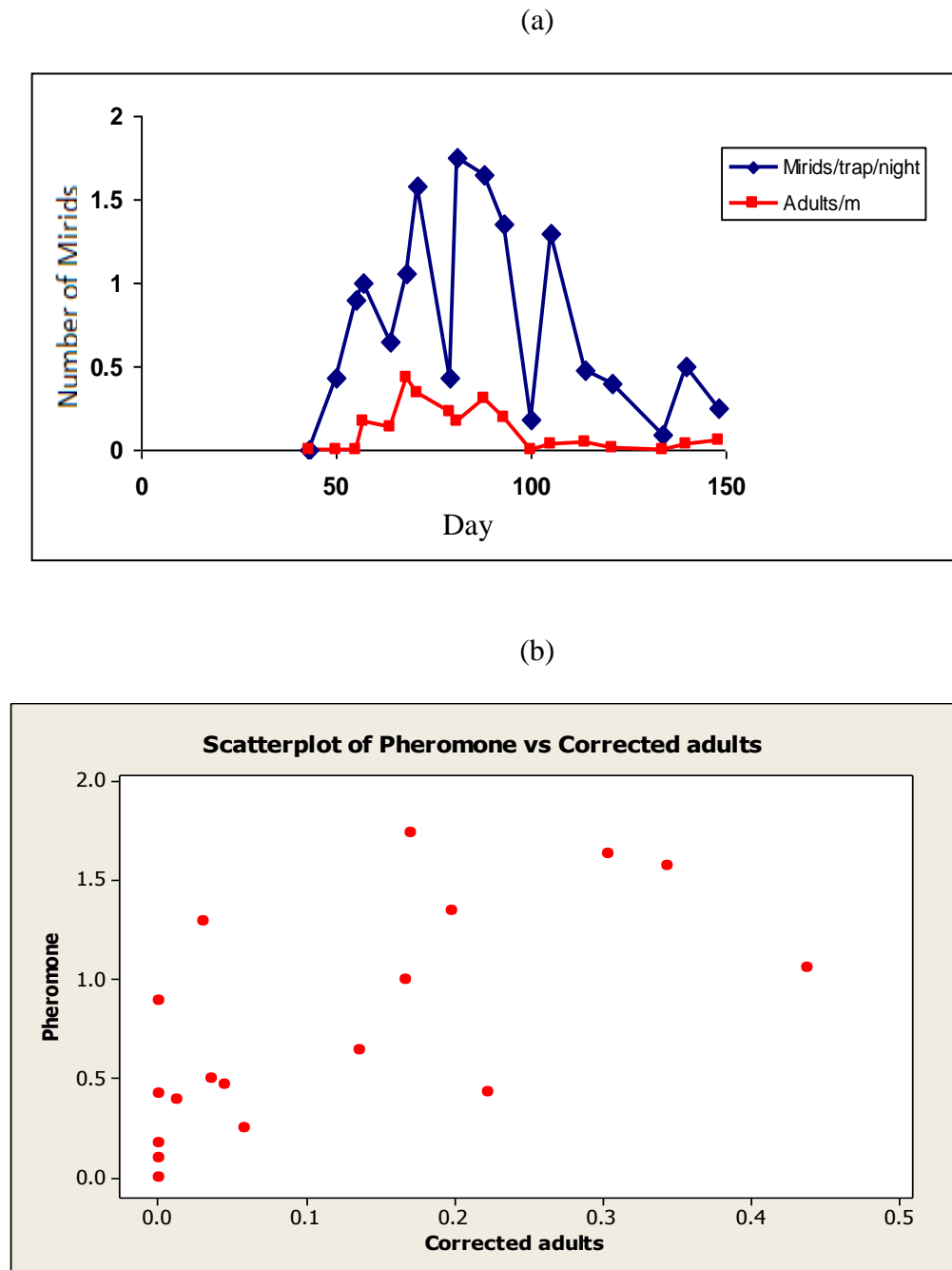
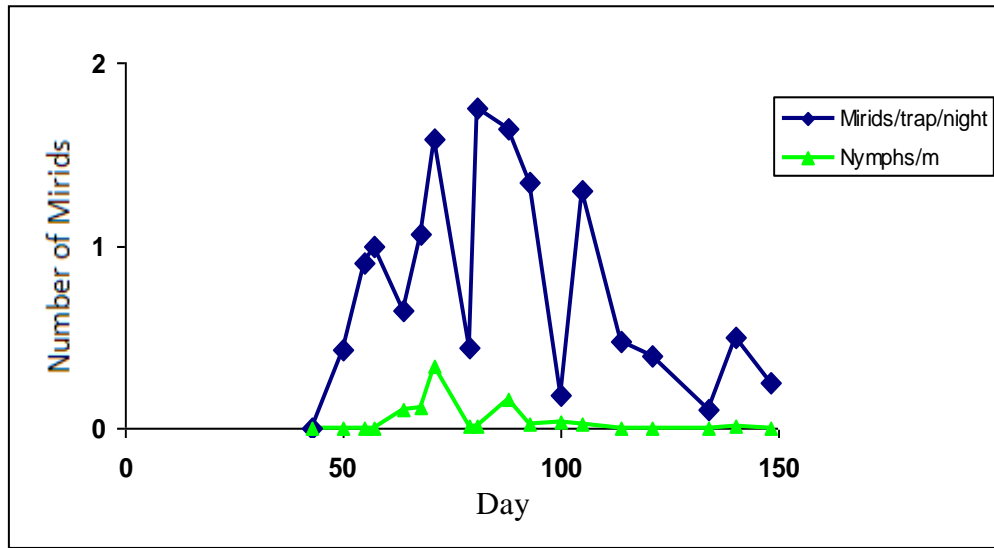


Fig. 3.4. Pheromone trap catches and corrected numbers of mirid adults plotted against time (a) and scatter plot of pheromone trap catches and corrected numbers of adult mirids (b) at **Goondiwindi**. Regression analysis indicated a significant positive correlation between trap catches and corrected numbers of adults ($p < 0.01$, $R^2 = 50.9$).

(a)



(b)

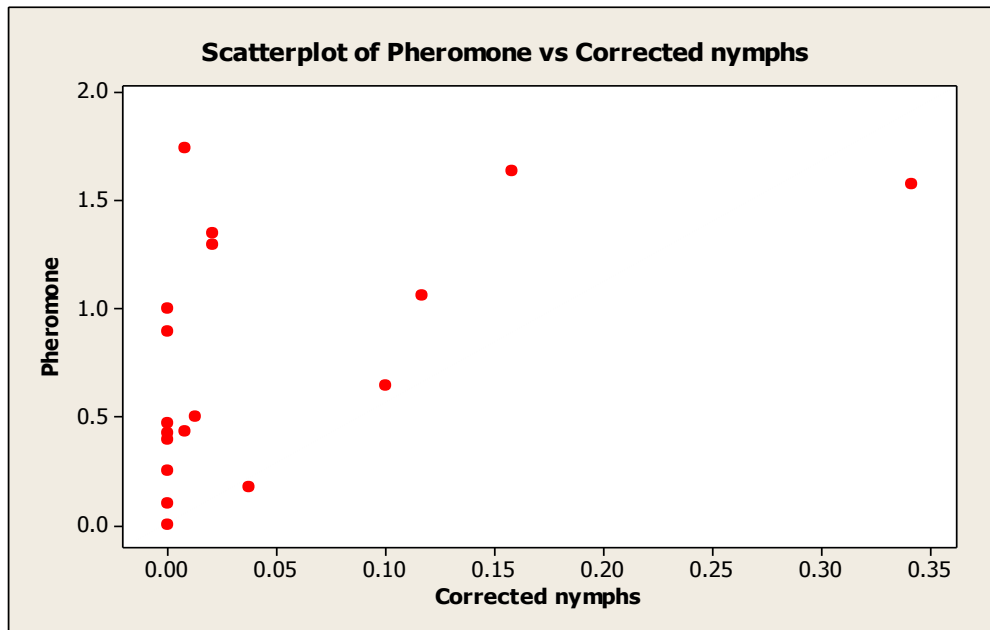
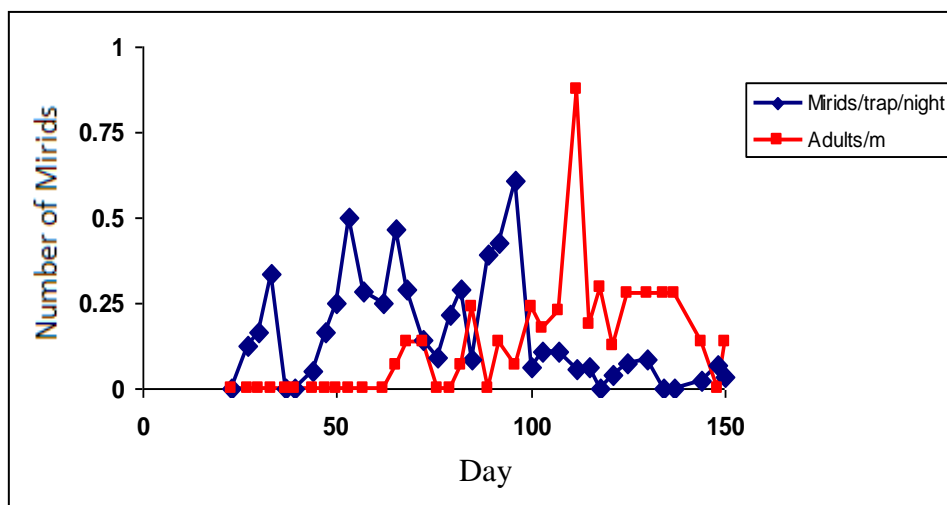


Fig. 3.5. Pheromone trap catches and corrected numbers of mirid nymphs plotted against time (a) and scatter plot of pheromone trap catches and corrected numbers of mirid nymphs (b) at **Goondiwindi**. Regression analysis indicated a significant positive correlation between trap catches and corrected numbers of nymphs ($p < 0.01$, $R^2 = 35.1$).

3.2.2. Boggabri, Brigadoon

At Boggabri, pheromone trap catches were not significantly correlated with adult mirids, but there was a significant correlation with mirid nymphs (Figs. 3.6, 3.7). Earlier in the season, pheromone traps caught mirids when there were no adults in the field as indicated by visual sampling. Later in the season, there was a decline in trap catches when mirid adults and nymphs were still found in the field.

(a)



(b)

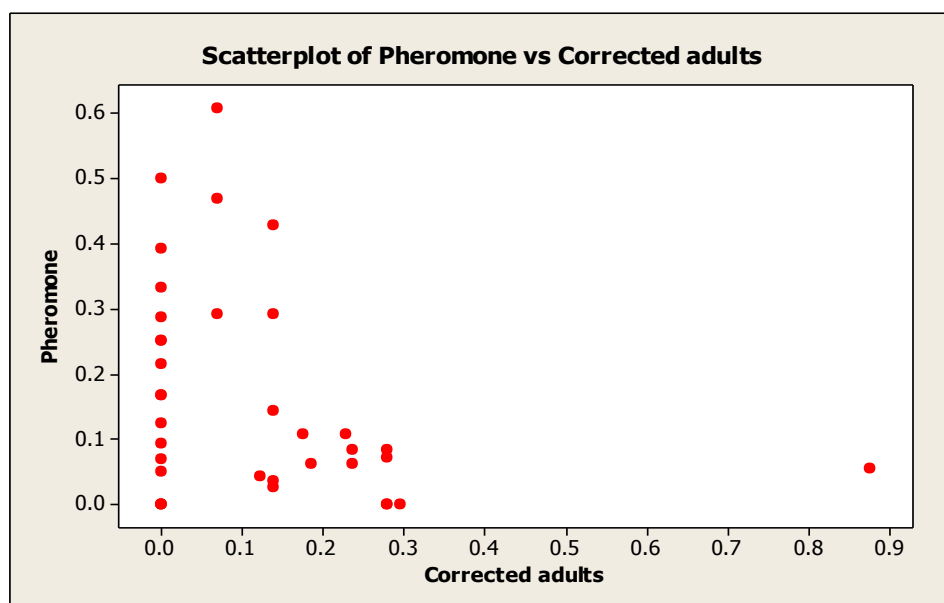
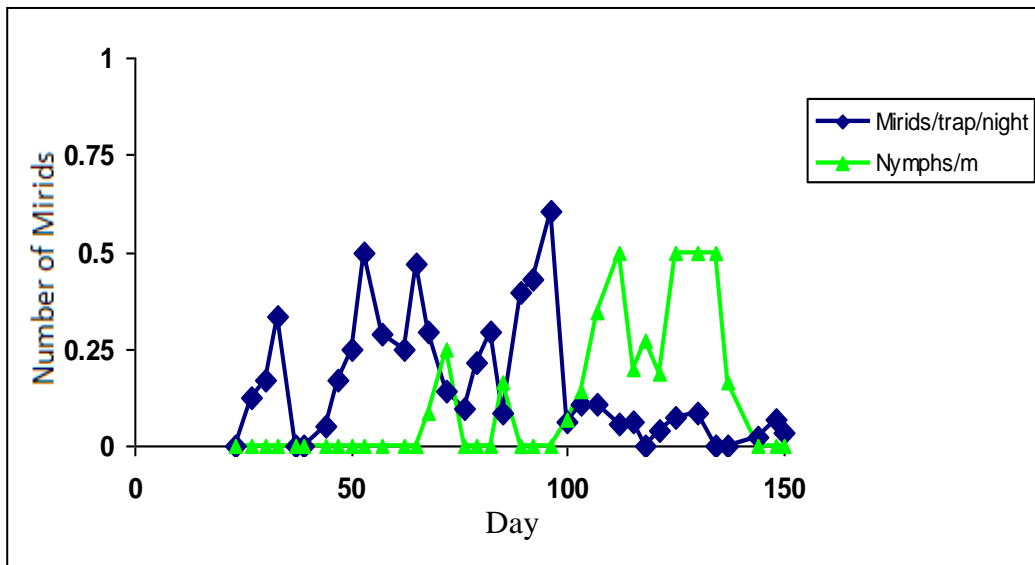


Fig. 3.6. Pheromone trap catches and corrected numbers of mirid adults plotted against time (a) and scatter plot of pheromone trap catches and corrected numbers of adult mirids (b) at **Boggabri**. Regression analysis indicated no significant correlation between trap catches and corrected numbers of adults ($p > 0.05$).

(a)



(b)

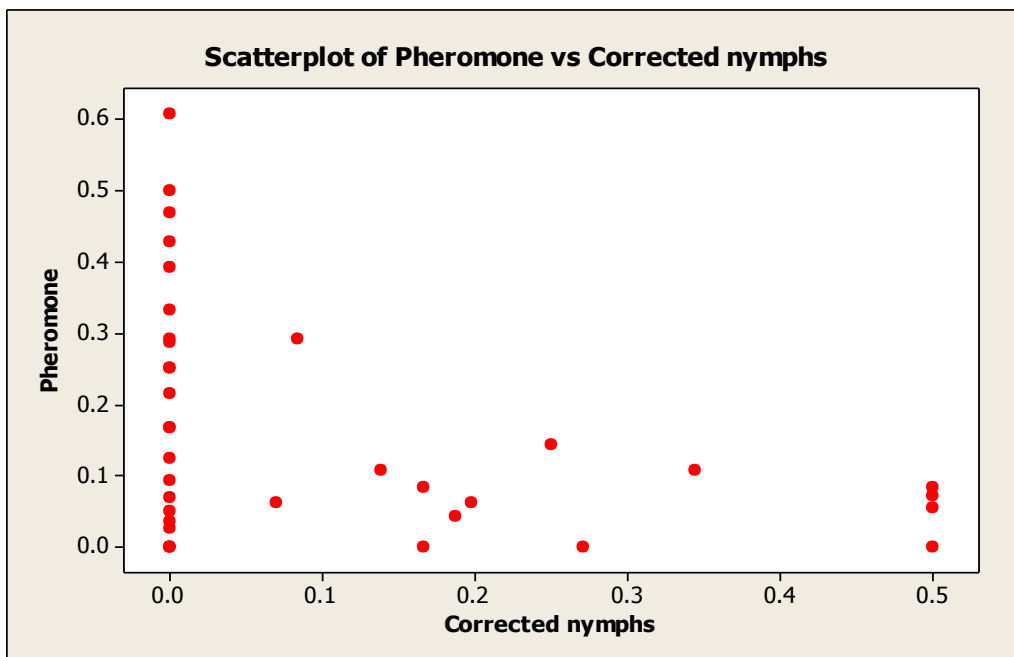
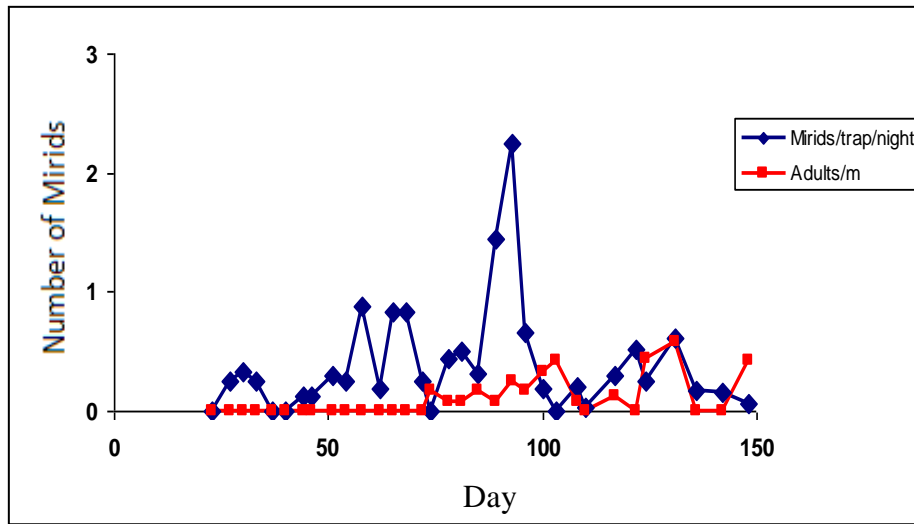


Fig. 3.7. Pheromone trap catches and corrected numbers of mirid nymphs plotted against time (a) and scatter plot of pheromone trap catches and corrected numbers of mirid nymphs (b) at **Boggabri**. Regression analysis indicated a significant negative correlation between trap catches and corrected numbers of nymphs ($p < 0.05$, $R^2 = 14.9$).

3.2.3 Narrabri, Auscott

Pheromone catches were not significantly correlated with either the corrected numbers of mirid adults or nymphs at Narrabri (Figs. 3.8, 3.9). Pheromone traps caught mirids earlier in the season when there were no mirids seen in the field by visual sampling. This observation was also identified at Boggabri.

(a)



(b)

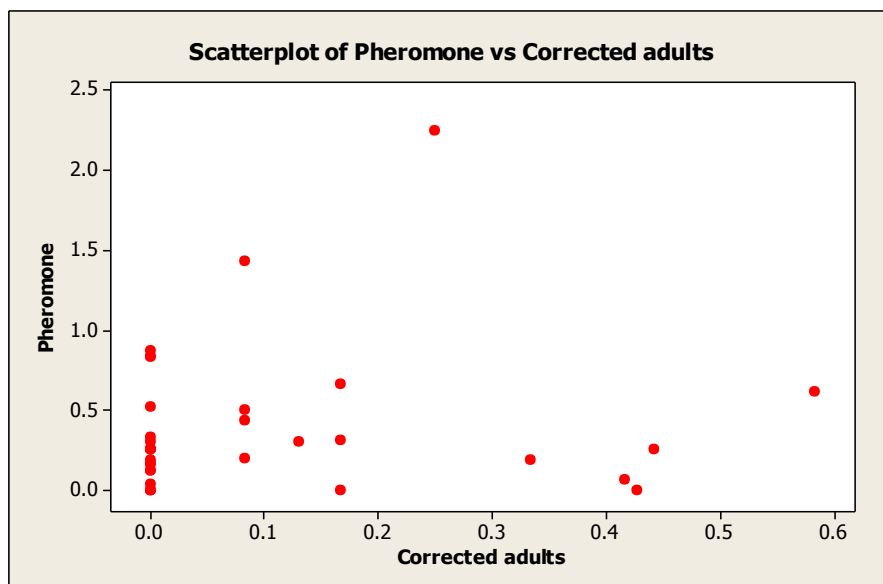
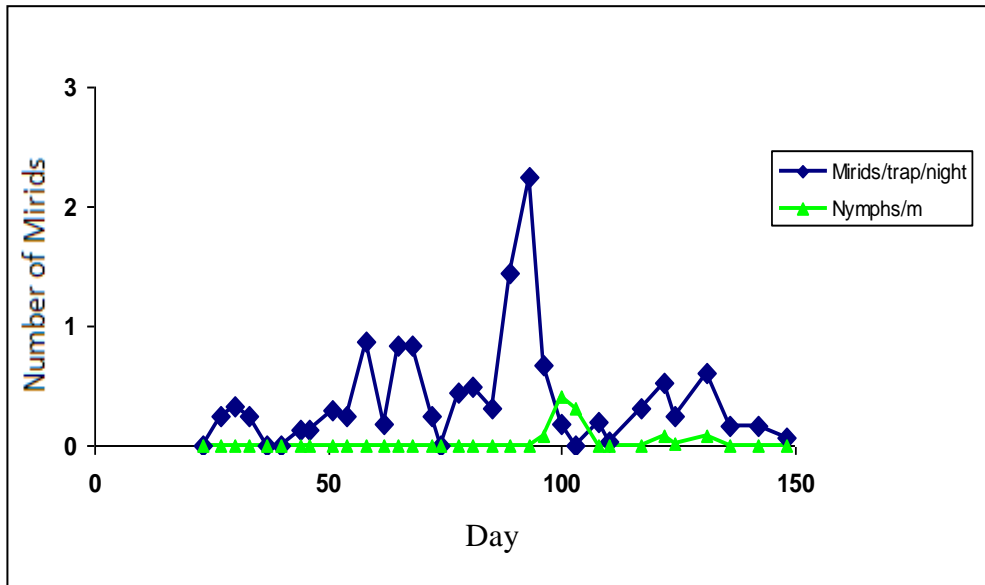


Fig. 3.8. Pheromone trap catches and corrected numbers of mirid adults plotted against time (a) and scatter plot of pheromone trap catches and corrected numbers of adult mirids (b) at **Narrabri**. Regression analysis indicated no significant correlation between trap catches and corrected numbers of adults ($p = 0.532$).

(a)



(b)

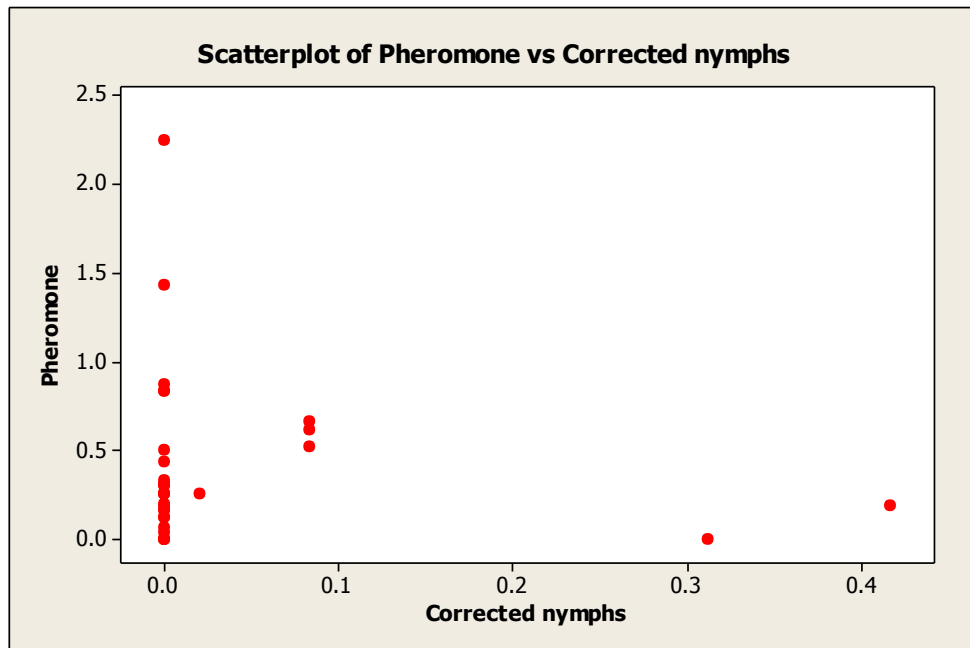


Fig. 3.9. Pheromone trap catches and corrected numbers of mirid nymphs plotted against time (a) and scatter plot of pheromone trap catches and corrected numbers of mirid nymphs (b) at **Narrabri**. Regression analysis indicated no significant correlation between trap catches and corrected numbers of nymphs ($p > 0.05$).

3.3. Pheromone trap catches and percentage of females in population

Mirid samples were dissected to determine sex and mated status of females. Figures 3.10, 3.11, 3.12 show percentages of females at each location on dates when there were mirid adults sampled from the field. At Boggabri and Narrabri, the patterns of the percentage of females in the population appeared to be very variable compared with that at Goondiwindi.

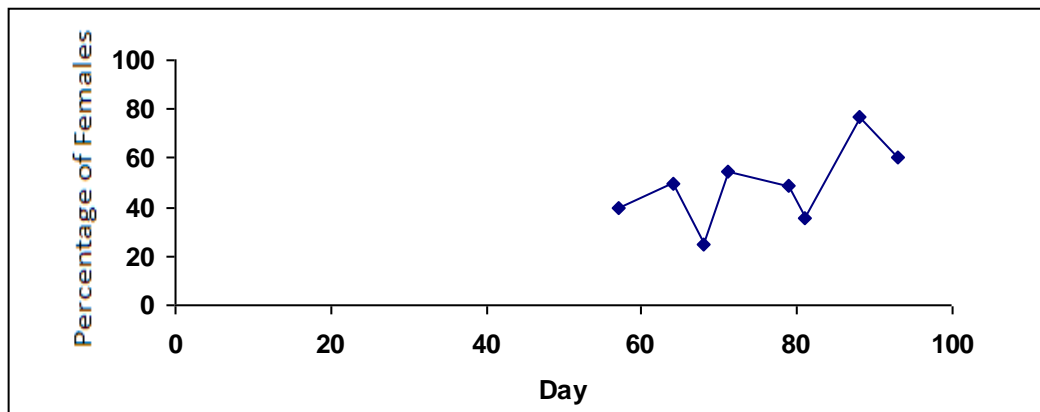


Fig. 3.10. The percentage of females in the field at **Goondiwindi** over time.

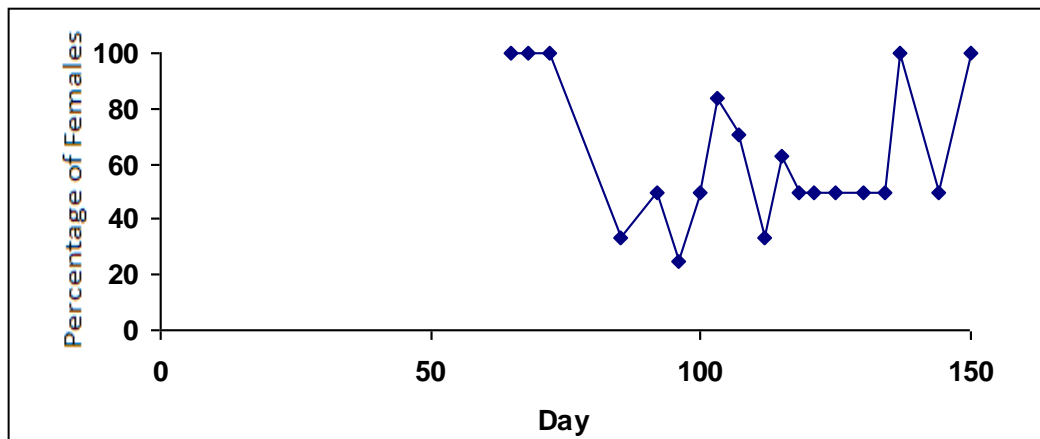


Fig. 3.11. The percentage of females in the field at **Boggabri** over time.

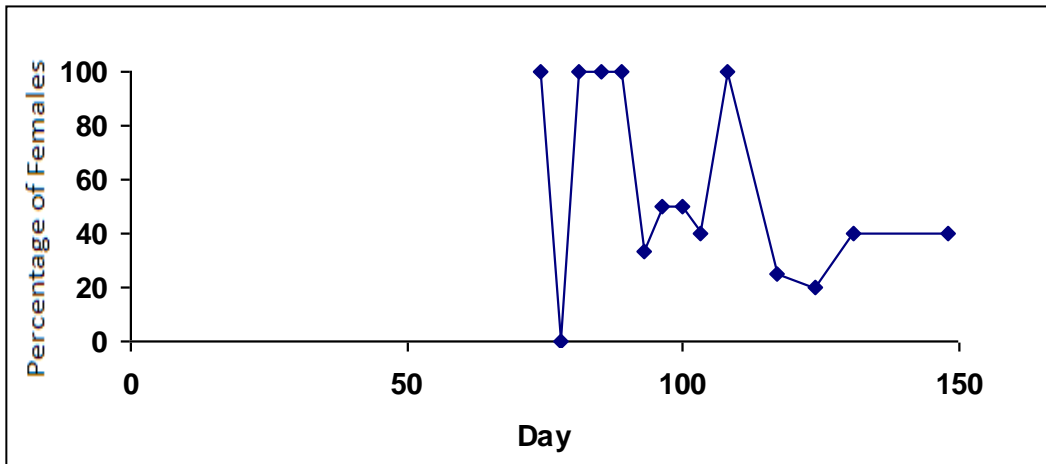


Fig. 3.12. The percentage of females at **Narrabri** over time.

Regression analyses yielded no significant correlations between pheromone catches and the percentage of females in all three locations (shown in Appendix 4). Scatter plots of trap catches against percentage of females are shown in Figs. 3.13, 3.14, 3.15.

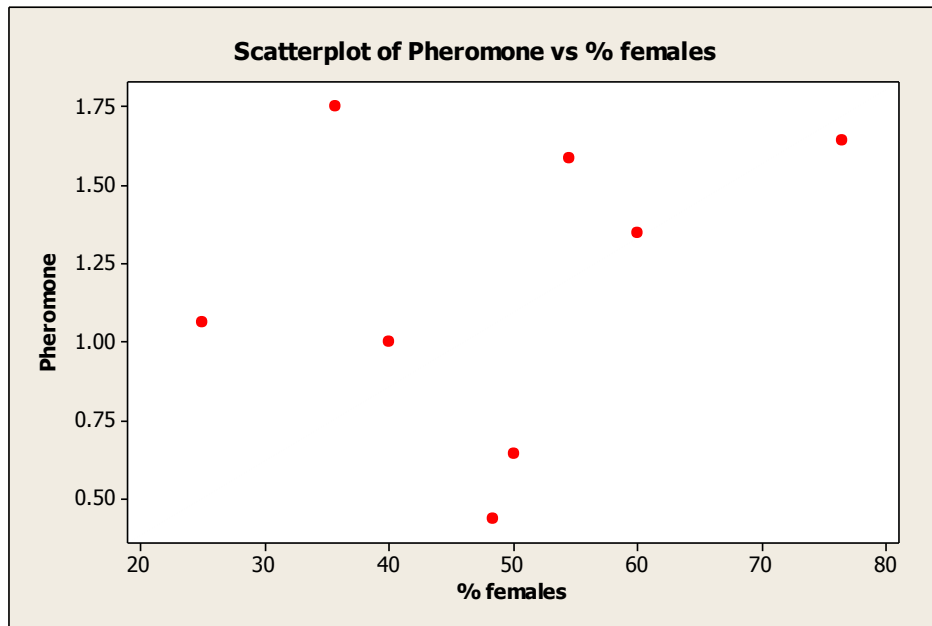


Fig. 3.13. Scatter plot of pheromone trap catches and % females at **Goondiwindi** ($p=0.540$).

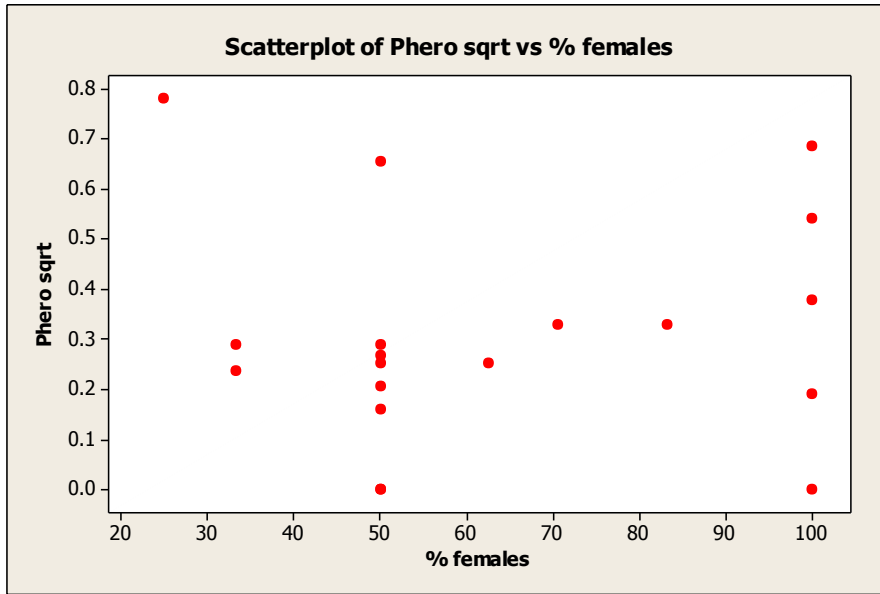


Fig. 3.14. Scatter plot of pheromone trap catches and the percentage of females at **Boggabri** ($p=0.904$).

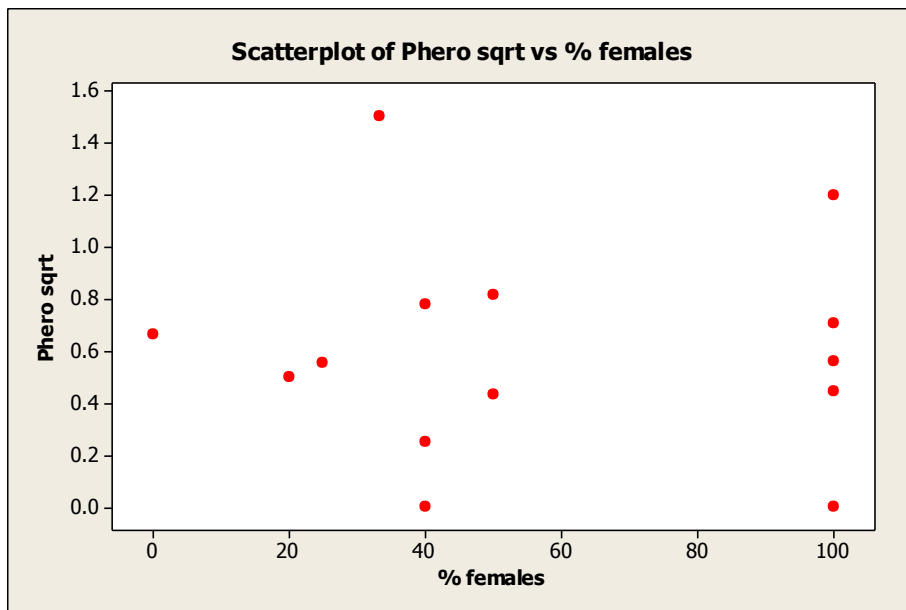
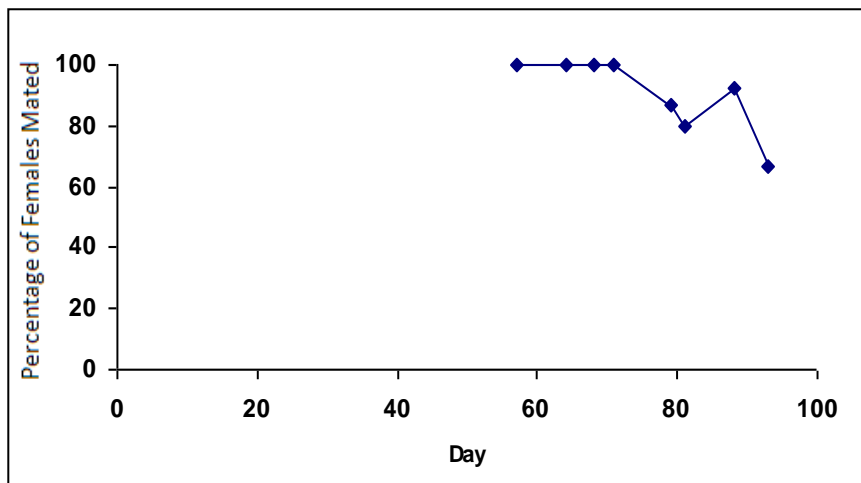


Fig. 3.15. Scatter plot of pheromone trap catches and the percentage of females at **Narrabri** ($p=0.848$).

3.4 Pheromone trap catches and the percentage of females mated

Percentages of mated females and the relationship between trap catches and the percentage of mated females at the three locations are shown in Figs. 3.16, 3.17, 3.18. Regression analyses were done on pheromone catches versus per cent mated females for each location. A significant correlation between trap catches and the percentage mated was observed at Boggabri, but not at Narrabri and Goondiwindi.

(a)



(b)

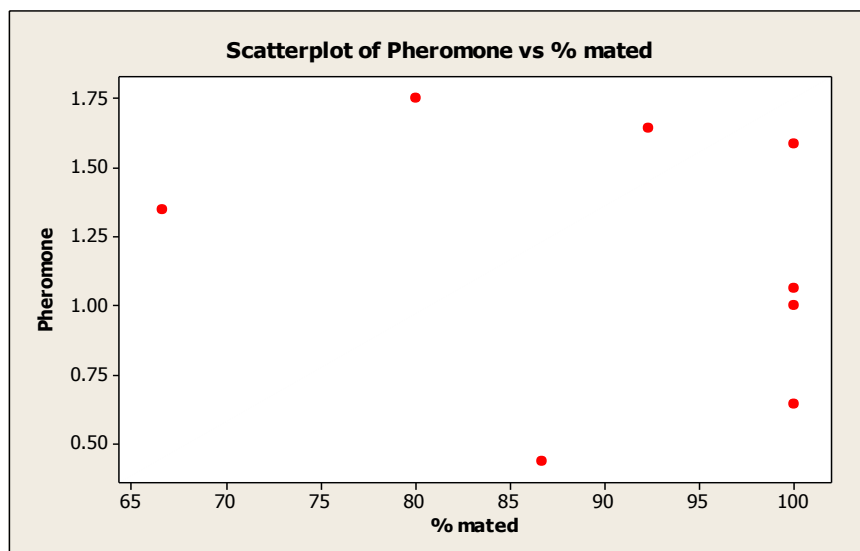
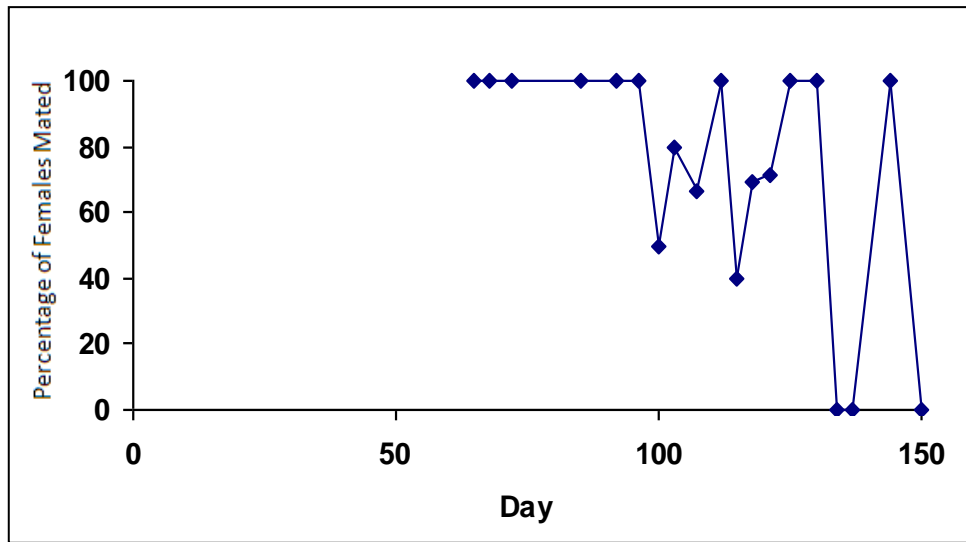


Fig. 3.16. Percentage of mated females plotted against time (a) and scatter plot of pheromone trap catches and the percentage of females mated at (b) at **Goondiwindi**. Regression analysis indicated no significant correlation between trap catches and the percentage of females mated ($p > 0.05$).

(a)



(b)

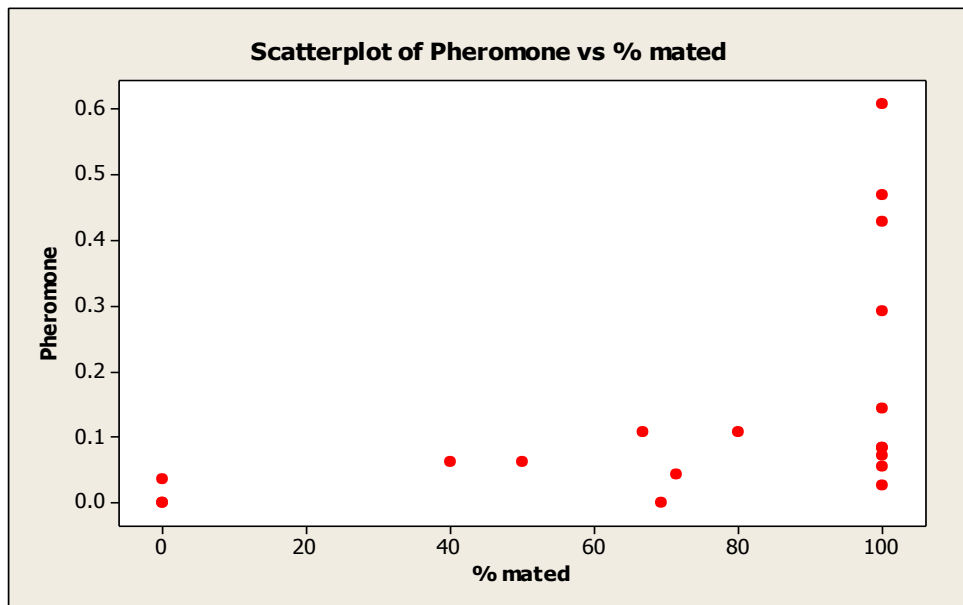
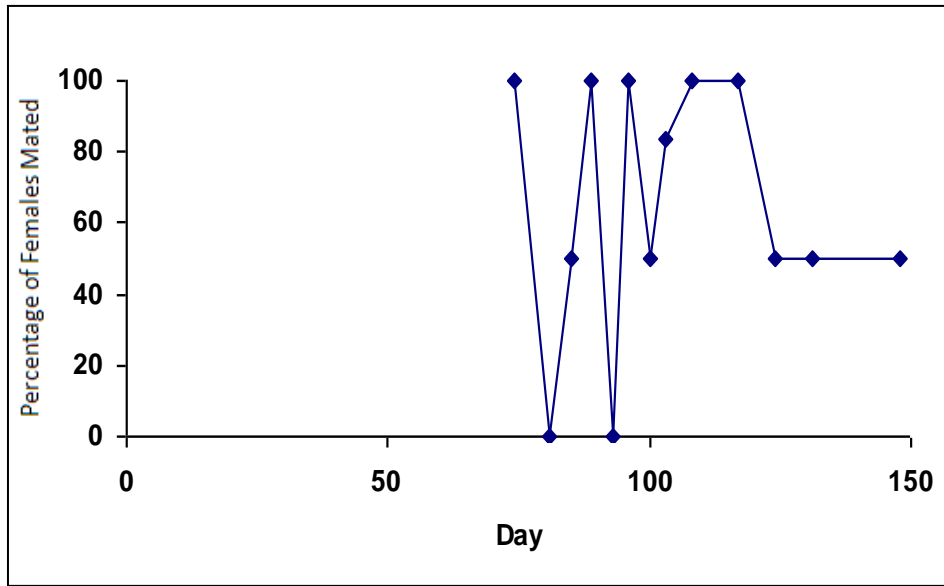


Fig. 3.17. Percentage of mated females plotted against time (a) and scatter plot of pheromone trap catches and the percentage of females mated at **Boggabri**. Regression analysis indicated a significant positive correlation between trap catches and the percentage of females mated ($p < 0.05$, $R^2 = 36.8$).

(a)



(b)

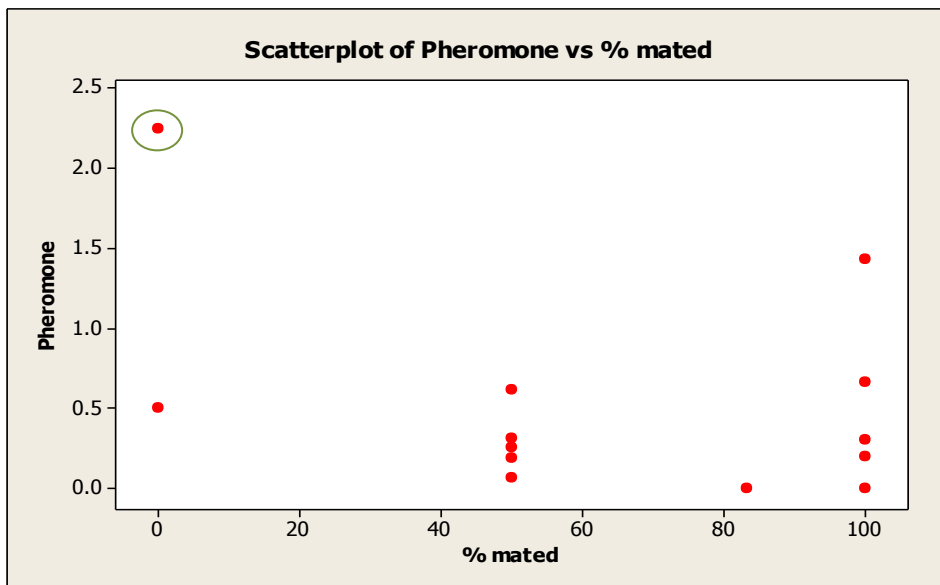


Fig. 3.18. Percentage of mated females plotted against time (a) and scatter plot of pheromone trap catches and the percentage of females mated at (b) at **Narrabri**. Regression analysis indicated no significant correlation between trap catches and the percentage of females mated ($p>0.05$).

One of the deficiencies of the trials was that at Goondiwindi, clearing of traps and mirid sampling was not done at regular intervals, hence there were big gaps between some data points. In addition, some of the sampled mirids were not collected and thus, data on the percentage of females and mated status of females were not available on some sampling dates. Nevertheless, the results suggest that at Boggabri, pheromone traps appeared to catch large numbers of mirids, only at times when most of the females were mated. At Goondiwindi, this appeared not to be so, though in the latter case it is difficult to draw any conclusions because most females were mated on most sampling occasions. At Narrabri, there appeared to be a trend towards a pattern similar to that at Boggabri, especially if one outlying data point (circled in Figure 3.18) is excluded, but the regression analysis did not indicate a significant correlation.

Chapter 4:

Discussion & Conclusion

4.1 Findings

4.1.1 Effectiveness of sampling methods at collecting either GM adults or nymphs

There was a significant difference in sampling method effectiveness at sampling GM adults and nymphs. The effectiveness of each sampling method at catching GM adults and/or nymphs was also affected by both date and operator. The effectiveness of each sampling method varied over operators at catching both adults and nymphs. Therefore from the preliminary study it is clear that there is no overall more effective method at sampling either GM adults or nymphs.

The findings in the preliminary study both support and challenge previous literature and research. The results contradict Deutsher *et al.* (2003) who found no significant difference occurred between operators at using beat sheets. In most experiments there is always some form of human error and human variation (Strickland 1961). It was found in this study that human variation (operator) was a significant factor affecting the effectiveness of sampling methods at sampling either GM adults or nymphs. The variation from operator could be due to inexperience at sampling and their ability to identify GM adults and nymphs.

The operator * method interaction found indicates that some operators can use certain methods more effectively than other operators. In some cases there were clear reasons for this. For example, the suction sampling method was very ineffective in the hands of Operator 2, who was a relatively small woman. Effective use of the suction sampler requires that it be pushed firmly downwards into the crop canopy

from above, and in the high crop in which these comparisons were done. Operator 2 had more difficulty with this than Operators 1 and 3, who were both tall men. In other cases the reasons were not clear. For example, beat sheeting was a very poor method for sampling adults in the hands of Operator 1 compared to the other two operators. Presumably this relates to some difference in sampling methods which occurred in spite of attempts to standardise techniques at the start of the experiment.

From literature there are both advantages and disadvantages for every sampling method available even in relation to the ease of their use (Morris 1960, Pyke *et al.* 1980, Bodnaruk 1987). For example Deutscher *et al.* (2003) stated that beat sheeting was a relatively easy sampling method to learn to use, while other methods like visual checks were not. It was also discussed that operators bias can affect sweep net effectiveness (Strickland 1961). Further the suction method is challenging to use due to the weight, long distances and time it is carried for. These issues could affect the operators' ability to effectively use this sampling method as physical strength, height and fitness would account for variability in the operators' ability to use this method appropriately and these issues are most likely why a difference in method effectiveness was found between operators in the preliminary study.

Another factor which was observed to affect sampling method effectiveness and use was the stage of the cotton season. These findings are supported by Byerly *et al.* (1978), Bodnaruk (1987), Dent (1991) and Miles *et al.* (1992) who all explain that some sampling methods cannot be used throughout a season due to the potential risk of damage to the plant and/or varying effectiveness. Further the GM population may change within the crop over time. These are all plausible reasons why 'Days After Planting (DAP)' showed up as a contributing factor to the effectiveness of each method at collecting GMs. Therefore there is no one perfect method at sampling either GM adults or nymphs as it can change with operator and the stage of cotton growth irrespective of the advantages and disadvantages of each method mentioned in chapter 1.

4.1.2 Pheromone trap catches association with population densities, the sex ratio and mated status of GMs in the field.

The pattern of pheromone trap data was not consistent in the three trial sites. At Narrabri and Boggabri in the Namoi Valley, pheromone traps caught mirids early in the season when visual or sweep net sampling yielded no mirid adults in the field. Conversely, at Goondiwindi, the traps caught mirids when sampling indicated they were present in the field. These results suggest that pheromone traps can detect the presence of mirids in the field before field sampling can (Wall 1990). It is also possible that when plants are small, mirids can easily fly away when distracted before visual or sweep net sampling can be done (Miles 1992, Threlfall *et al.* 2005/06, Finlay 2006). During visual sampling the operator usually approached the plants quietly and slowly in an attempt to not disturb any insects in them, but this may have been unsuccessful.

The differences between sites suggest that the population dynamics of mirids between locations varied. For example, at Goondiwindi, there was a significant correlation between trap catches and mirid numbers in the field, whilst at Narrabri, no such correlation was observed. A possible explanation for the difference between these two sites is the variation in the pattern of the percentage of mated females at each site. At Goondiwindi, this pattern was more or less consistent whilst at Narrabri and Boggabri, it was very variable.

The high variability in the female mated status observed at Boggabri and Narrabri could be a result of periodic immigration of unmated sexually immature females into the area. When insects migrate they are generally not sexually mature as they put energy from their food resources into flight rather than egg maturation, a phenomenon known as the oogenesis-flight syndrome (Gregg 1995, Rankin 1976, Johnston 1969). This could explain why such variability in the mated status of females is apparent at Boggabri and Narrabri.

At Boggabri, the results indicated that the only time pheromone traps caught large numbers of mirids was when a high proportion of the females in the field were mated, and this was not the case at Goondiwindi. Despite the lack of significance at the

Narrabri site, the trends in the data resembled those found at Boggabri. This relationship between pheromone trap catches and the mated status of females could be a result of female competition.

Female competition is a phenomenon in which males are attracted to the real female pheromone plume over the synthetic pheromone in the traps. In many insects, unmated females are more attractive to males than either mated females or synthetic pheromones. This could be because the pheromones females produce are qualitatively or quantitatively different to those produced by mated females or by the synthetic lures, or perhaps because they have other methods of communication (visual or auditory) which supplement the effects of the pheromone. This means when unmated female numbers in the field are high, pheromone catches are low, as males can distinguish real females and therefore do not respond to the synthetic lures in the traps (Campion *et al.* 1989).

The results suggest that ultimately the pheromone trap catches are a function of two population parameters: the numbers of mirids present and of the relative abundance of mated and unmated females. At Goondiwindi pheromone traps might more closely reflect the field population numbers of GMs due to the lack of immigration of unmated females into the field. However at Narrabri and Boggabri frequent immigration of unmated females from nearby sources might be disrupting the relationship between mirid numbers and population density, due to the female competition effect.

A working hypothesis is thus proposed from these findings. At sites like Goondiwindi, mirids might have short pre-reproductive times and little immigration of unmated females into the field, and so the pheromone traps might be effective monitoring tools. However at sites like Brigadoon, mirids might have longer pre-reproductive times and/or there may be immigration of unmated females into the area, and so the pheromone traps would not be effective monitoring tools.

There is potential to further analyse the current data through the removal of early data, especially at Narrabri and Boggabri, where no adults or nymphs were sampled in the field, yet pheromone trap catches were occurring. This phenomenon suggests that

pheromone traps could serve as an early warning mechanism, providing evidence of the presence or absence of GM earlier than is possible with current sampling methods. However, the inclusion of data from these periods may have been masking correlations which otherwise might have been found in the data. Unfortunately time did not permit more extensive analysis of the data in this dissertation. Further analysis of the data could also be attempted using time series regression analysis, in which correlations could be sought between changes in pheromone trap catches and changes in field populations which occurred sometime previously or after the changes in pheromone catches.

Additional trials might be useful to get some more data on the relationship between trap catches and mirid population dynamics in the field that might validate or disprove the proposed working hypothesis above. Pheromone traps may be valuable for studying the population dynamics of mirids. For example, more intensive pheromone trapping and field sampling in a site where a potential source of mirids (eg lucerne) was located close to cotton might help clarify the relationship between movement and mating in GM.

4. 2 Problems experienced

4.2.1 Experimental design

Two challenges are associated with experimental designs. These include the difficulty in detecting and estimating the effects of interference (other factors), and the challenge in determining an experimental design that minimises biased estimates of treatment differences (Dyke *et al.* 2002). All of these factors were considered in the experimental design process but still presented problems for this study. Some reasons for this include the procedure is cumbersome, costly and often impracticable, and this is why the same operator was not used at each site and the most desirable control for this experiment was not implemented. As explained in Chapter 2 the distance needed between the trial site and a control to remove the effect of the synthetic pheromone as a result of GMs being able to identify this pheromone kilometres away makes the

control no longer an appropriate control as differences in other factors (e.g. surrounding vegetation or crop factors) would be invalidated.

Another limitation to the experimental design was the lack of accuracy as to where each sample was to be undertaken, despite the construction of the Latin squares design. Even though this was considered in the design process it was not possible due to costs, impracticability and difficulty. A suggestion to overcome this problem in the future is to use a GPS (Global Positioning System) to specifically identify the sampling site location within the field. However sampling in the same spot could result in effects from the previous sampling event occurring, for example damage to the crop and the removal of insects from previous sampling events.

4.2.2 Environmental effects

Interference for this study mainly consisted of environmental effects especially in relation to weather conditions. In the preliminary study it was hard to obtain all four samples from each of the three sites on similar days. This was due to weather conditions, particularly rainfall, and irrigation. Sweep netting and suction sampling cannot be conducted when the canopy of the cotton is wet (Miles *et al.* 1992, Simpson & Lloyd 2007). Further wet ground and/or flooded ground due to irrigation can prevent beat sheeting. These factors are unavoidable which is one reason why the data is somewhat patchy, and why it was hard to obtain more than 3 dates where all four methods could be used at all three sites while the cotton crop was at similar growth stages.

4.2.3 Low numbers of Green mirid adults and nymphs

Another problem faced by the study was the low numbers of GMs. At all sites it was a particularly quiet year for the GM as a pest. For example at Brigadoon the GM was observed within the field but no control measures were implemented throughout the season because the GM never exceeded the economic threshold (did not cause intolerable damage) and thus was not considered a pest.

4.2.4 Weather conditions

Several factors could have contributed to the low numbers of GM at all three sites. These include unfavourable weather, migratory patterns and the presence of more desirable host crops, particularly lucerne. Boggabri normally experiences average high temperatures of 32-38°C during the summer months (Reading 2006), but from October 2007 to February 2008 the mean monthly average temperature was approximately 2-3°C below the average, with some hot, dry spells and periods of short heavy rainfall (Doggett 2008). In Chapter 1 the effect of weather, particularly temperature on GM population dynamics was explained. A temperature range of 20-30°C was identified as most desirable for GM growth, development, fecundity and survival (Khan 1999). With the lower than average temperatures it would be assumed that GM numbers would be high as temperatures experienced in Boggabri were only marginally higher than the most desirable temperature range for the GM. This suggests that other weather conditions experienced in the season like heavy rainfall events may have prevented the GM population from increasing.

4.2.5 Presence of other host crops

At both Narrabri and Boggabri other host crops were grown nearby. At the time of site selection at Boggabri it was known that one neighbour grew lucerne so a field far away was selected. There was not a lot of vegetation around due to the lack of rainfall at the time so the site selected seemed to be ideal in relation to surrounding crops and vegetation like lucerne. However in December frequent rainfall was experienced and as a result the grazing property next door had lucerne appear in the grazing system.

Lucerne is a more desirable host crop than cotton for oviposition and as a food source, even after lucerne has been cut (Miles *et al.* 1992, Mensah & Khan 1997, Pearce & Zalucki 2005). This occurrence could explain the influxes of unmated females into the field and the lack of nymphs observed, especially at the start of the cotton season at Boggabri and Narrabri as the females might have been ovipositing on other more desirable host crops. Therefore the presence of the lucerne in the vicinity of the fields of which the experiment was conducted could have affected the number of GM's present in the fields.

This introduces the idea of landscape management (Parajulee *et al.* 2007) as a result of the movement (migration) of the pest being not just within a field but from surrounding fields, crops and environments. In the future it would be ideal to survey the surrounding properties and ask if they grow crops, like lucerne, or have weed species present that are hosts for the GM.

4.2.6 Lack of information – Migratory habits

We have limited information about patterns of both short and long range movements in GMs. The immigration of GMs into the field population could be one reason why at Boggabri the pheromone trap numbers were not correlated with the absolute adult numbers in the field but were correlated with nymph numbers. Nymphs are not winged and therefore cannot migrate.

4.2.7 Operator effect - Human error

The preliminary study found that variation in effectiveness of each sampling method for both GM adults and nymphs was affected by operator. There are three main issues associated with the operator variation: bias, ability and experience in conducting each sampling method. The identification of variation assigned to ‘operator’ through the preliminary study allowed for the formation of a population index by using the available sampling methods with conversion factors for each operator (Refer to appendix 2). The conversion factors helped to remove operator effects and sampling method effects from the data set. Nevertheless, the need for this procedure illustrates a common problem in entomological research in which substantial sampling efforts need to be made across time and space, requiring more than one operator.

4.3 Conclusions and the future

There is limited research into the ecology of the GM, a major cotton pest. The ecology of the GM makes it a successful pest. It is hard to monitor, and current monitoring methods available for this pest are inadequate.

The problems faced with the field experiment and the variable results over the different sites indicate that it is difficult to find an association between trap catches and the insect's population dynamics that can be used for monitoring purposes (Reidl 1980). To add to the challenge there is very little known about the sexual behaviours or migratory habits of the GM. These factors could be affecting the relationship between the pheromone trap catch numbers and the field population, but precisely how is unknown. Further research should persist.

The results suggest at some sites, mirids might have short pre-reproductive times and little immigration of unmated females into the field, whereas at others they might have longer pre-reproductive times and/or immigration of unmated females. This dissertation suggests that pheromone traps on their own may not be reliable at monitoring the GM populations in the field at the moment due to lack of an understanding of the pest's ecology and how this relates to trap catches. Therefore currently the pheromone traps may be more valuable for studying the population dynamics of mirids to gain a better understanding of GM ecology.

References

1. Betts, M., Gregg, P., Fitt, G. & MacQuillan, J. 1993, 'A field trial of mating disruption for *Helicoverpa* spp. in cotton', In *Pest Control and Sustainable Agriculture*, eds Corey, S., Dall, D. and Milne, W., CSIRO Press, Melbourne, pp. 174–177.
2. Bodnaruk, P. 1987, Monitoring of mirids in cotton in south east Queensland, New South Wales, PhD thesis, University of New England.
3. Byerly, K., Gutierrez, A., Jones, R. & Luck, R. 1978, 'A comparison of sampling methods for some arthropod populations in cotton', *Hilgardia*, vol.46, no.8, pp.257-282.
4. Campbell, A, Frazer, B., Gilbert, N., Guitierrez, A. & Mackauer, M. 1974, 'Temperature requirements of some aphids and their parasitoids', *Journal of applied ecology*, Vol.11, No.2. pp. 431-437.
5. Campion, G., Critchley, R. & McVeigh, J. 1989. 'Mating disruption' in *Insect pheromones in plant protection*, eds Jutsum, R. & Gordon, S., John Wiley & Sons Ltd, Brisbane.
6. Carde, T., 1979. 'Behavioural responses of moths to female-produced pheromone and the utilization of attractant-baited traps for population monitoring', in *Movement of highly mobile insects: Concepts and methodology in research*, eds Rabb, L. & Kennedy, G., North Carolina State university, pp.286-315.

7. Carde, T. & Minks, K. 1995 'Control of moth pests by mating disruption: successes and constraints', *American review of entomology*, Vol. 40, pp. 559-585.

8. Chapman, B., Perman, D. & Hicks, P. 1986. *National pest control, An Australian guide for commercial growers for orchardists and farmers*, Reed Methuen publishers Ltd, New Zealand.

9. Christiansen, I. 2002. 'Extension and profitability-implementation of profitable sustainable approaches', *Proceedings 11th Australian cotton conference*, pp.65-74.

10. CRC. 2008 'Strategic plan 2006-2012', Cotton Catchment communities CRC, CRC, Retrieved 20th October 2008 from
www.cottoncrc.org.au/files/9228d248-7eb0-4696-9f05-9a8100be1b4a/Cotton%20CRC%202008%20Plan1

11. CRC 2007a. 'Bugs in cotton', Cotton Catchment communities CRC, CRC, Retrieved 5th August 2007 from
<http://web.cotton.crc.org.au/content/Industry/Publications/PestsandBeneficials/CottonInsectPestandBeneficialGuide/Pestsbycommonname/Bugsincotton.aspx>

12. CRC 2007b, 'Green mirid', Cotton Catchment communities CRC, CRC, Retrieved 5th August 2007 from
<http://web.cotton.crc.org.au/content/Industry/Publications/PeatandBeneficials/CottonInsectPestandBeneficialGuide/Pestsbycommonname/Greenmirids.aspx>

13. De-Long, M. 1932. 'Some problems encountered in the estimation of insect populations by the sweeping method ', Annals of entomological society of America, Vol. 25, pp. 13-17.
14. Dent, D. 1991. *Insect Pest Management*, CAB International, UK.
15. Deutscher, S., Dillon, M., Mansfield, S., Staines, T. & Lawrence, L. 2003. 'Giving insects a good beating', The Australian cotton grower, vol.24, no.3, pp.24-27.
16. Doggett, S. 2008. 'Temperature Maps', rainfall and temperature maps, NSW Arbovirus surveillance and vector monitoring program, Retrieved 29th September 2008 from www.arbovirus.health.nsw.gov.au/areas/arbovirus/climate/rainfall_maps.htm#temp
17. Dyke, G., Todd, A. & Jenkyn, J. 2002. 'Some problems in the design and analysis of field experiments subject to inter-plot interference', Journal of agricultural science, Vol.139, pp.295-305.
18. Ellington, J., Carrillo, T., McCauley, J., McWilliams, D., Lillywhite, J., Pierce, J. & Drake, J. 2007. 'Precision cotton production', cooperative extension service, New Mexico state university. Retrieved 7th May 2008 from <http://cahe.nmsu.edu/pubs/-a/>
19. Finlay, J. 2006. 'Bio-pesticide for emerging cotton pest', department of primary industries for NSW, Retrieved 5th August 2007 from http://www.dpi.nsw.gov.au/archive/agriculture-todaystories/agriculture_today_april_2006/2006-004/biopesticide_for_emerging_cotton_pest

20. Fitt, G. 1994. 'Cotton pest management: Part 3. An Australian perspective', Annual review of entomology, vol. 39, pp. 543-62.
21. Fitt, G. 2000. 'An Australian approach to IPM in cotton: integrating new technologies to minimize insecticide dependence', Crop Protection, vol.19, pp.793-800.
22. Fitt, G., Wilson, L., Mensah, R. & Daly, J. 2004 'Advances with Integrated Pest Management as a component of sustainable agriculture: the case of the Australian cotton industry', Crop Science, Vol.43, pp. 2125-2134.
23. Foley, D. & Pyke, B. 1985. 'Developmental time of *Creontiades dilutus* (Stål) (Hemiptera: Miridae) in relation to temperature', Australian Journal of entomology, Vol.24, Is.2, pp.125-127.
24. Foster, S. & Harris, M. 1997. 'Behavioural manipulation methods for insect pest-management', Annual review of entomology, vol.42, pp. 123-46.
25. Frisbie, E., Crawford, L., Bonner, M. & Zalom, G. 1989b. 'Implementing IPM in cotton' in *Integrated Pest management systems and cotton production*, eds Frisbie, E., El-Zik, M. & Wilson, T., Wiley-Interscience publication, USA.
26. Frisbie, E., El-Zik, M. & Wilson, T. 1989a. 'Perspective on cotton production and integrated pest management' in *Integrated Pest management systems and cotton production*, Wiley-Interscience publication, USA.
27. Goolsby, J., Patt, J., Pfannenstiel, R. & Adamczyk, J. 2005. 'Biologically based pest management for field and greenhouse crops', beneficial insect's research unit, Weslaco, Texas.

28. Gregg, P. 2007. 'Biology, ecology and management of the green mirid, *Creontiades* (Stal) in Australia', 2nd International Lygus Bug Symposium, 2007, Asilomar conference grounds Pacific Grove, California.
29. Gregg, P. 1995. 'Migration of cotton pests: Patterns and implications for management', in *Challenging the future proceedings of the world cotton research conference*, eds Constable, A & Forrester, W., CSIRO, Canberra.
30. Groot, A. & Smid, M. 2000. 'Polyandry in the mirid bug *Lygocoris pabulins* (L.)- effects on sexual communication and fecundity', *Invertebrate reproduction and development*, vol.38, Iss.2, pp.143-155.
31. Groot, A., Timmer, R., Gort, G., Lelyveld, G., Drijfhout, F., Van Beek, T. & Visser, H. 1999. 'Sex-related perception of insect and plant volatiles in *Lygocoris pabulinus*', *Journal of chemical ecology*, vol. 25, No. 10, pp. 2357-2371
32. Grundy. P. 2004. 'Impact of low release rates of assassin bug *Pristhesancus plagipennis* on Helicoverps spp. And *Creontiades* spp. In cotton', *Australian journal of entomology*, Vol.43, no.1, pp.77-82.
33. Gut, J., Stelinski, L., Thomson, R., Miller, R. 2004. 'Behaviour modifying chemicals: Prospects and constraints in IPM', in *Integrated Pest Management*, eds Koul, O., Dhaliwal, S. & Cuperus, W., CABI publishing, Wallingford, UK.
34. Hamilton, R. 2001, ' Chemical communication in insects' Lecture 5, State university of New Jersey, retrieved 12th of March 2008 from <http://aesop.rutgers.edu/~hamilton/lecture5.htm>.

35. Hartstack, W. & Witz, A. 1981. 'Estimating field populations of tobacco budworm moths from pheromone trap catches', *Environmental entomology*, Vol.10, pp.908-914.
36. Hereward, J. 2007. 'Is the source of mirids in cotton derived from local dispersion or long distance migration? Project 1_01_04', Cotton Catchment Communities, Australian research centres Programme, Retrieved on 7th May 2008 from http://www.cottoncrc.org.au/content/General/Research/CurrentProjects/1_01_04.aspx
37. Hori, K., & Miles, M. 1993, 'The etiology of damage to lucerne by the green mired, *Creontiades dilutus*', *Australian Journal of experimental agriculture*, Vol.33, No.1, pp. 327-32.
38. Howse, P., Stevens, I. & Jones, O., 1998, *Insect Pheromones and their use in pest management*, Chapman and Hall, Melbourne, Australia.
39. Johnston, G. 1969. *Migration and dispersal of insects by flight*, Methuen, London.
40. Kehat, M., Ansheleuich, L., Gordon, D., Harel, M., Zilberg, L. & Dunkelblum, E. 1999. 'Effects of density of pheromone sources, pheromone dosage and populaiotn pressure on mating of the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae)', *Bulletin of entomological research*, Vol. 89, pp. 339-345.
41. Khan, M. 1999. Aspects of the biology, ecology and management of the green mirid, *Creontiades dilutus* (Stål), in Australian cotton, New South Wales, PhD thesis, University of New England

42. Khan, M. & Quade, A. 2008. 'Mirids: Pictorial identification of mirids life cycle', Cotton Catchment communities, Department of Primary Industries and Fisheries, QLD.
43. Khan, M, 2003, 'Salt mixtures for mired management', The Australian cotton grower, Vol.24, No.3, pp.10.
44. Khan, M., Hichman, M., Mensah, R., Brier, H. & Wilson, L. 2004a. 'Research review, Mirid Ecology in Australian cotton', Australian cotton CRC, No.14, pp.1-4.
45. Khan, M., Hichman, M., Mensah, R., Brier,H. & Wilson.L. 2004b. 'Research review, Mirid management in Australian cotton', Australian cotton CRC, No.15, pp.1-4.
46. Knight, K., Brier, H., Lucy, M. & Kopittke, R. 2007. 'Impact of mirid (*Creontiades spp.*) pest management on *Helicoverpa spp.* The case for conserving natural enemies', Journal of pest management science, Vol.63, No.1, pp. 447-452.
47. Leston, D. 1961. 'Observations on the mirids (Hem) hosts of *Braconidae* (Hym) in Britain', Entomologist Monthly Magazine, vol. 97, pp.65-71.
48. Lowor, S. 2006. Isolation, identification and potential uses of sex pheromones for three pests of cotton in Australia, New South Wales, PhD thesis, University of New England.
49. McBrien, L. & Millar, G. 1999. 'Phytophagous bugs', in *Pheromones of non-Lepidopteran insects associated with agricultural plants*, eds Hardie, J. & Minks, K., CAB international, Wallingford.

50. Mensah, R. & Khan, M. 1997. 'Use of *Medicago sativa* (L.) inter-plantings trap crops in the management of the green mirid, *Creontiades dilutus* (Stål) in commercial cotton in Australia', International journal of pest management, vol.43, Is.3, pp.197-202.
51. Metcalf, R. & Luckmann, W.(eds) 1994, *Introduction to insect pest management*, 3rd edn, John Wiley and Sons Inc, Brisbane.
52. Miles, M., Pyke, B. & Walter, G. 1992. 'Sampling and control of Mirids in cotton', Organised by the Australian Cotton Growers' Research Association, 1992 Australian cotton conference proceedings, Broadbeach, QLD.
53. Miles, M., Pyke, B., Walter, G. Malipatil, M. 1994. 'The mirid problem and options for management', conference proceeding of the 7th Australian cotton conference, August 1994, Broad beach, Gold coast, Queensland.
54. Miles, M. 1995. Identification, pest status, ecology and management of the green mirid, a pest of cotton in Australia. PhD thesis, University of Queensland, St Lucia, Australia.
55. Miller, A. & McDougall, A. 1973. 'Spruce budworm moth trapping using virgin females', Canadian journal of zoology, Vol.51, pp.853-858.
56. Miller, R. & Roelofs, L. 1978. 'Gypsy moth responses to pheromone enantiomers as evaluated in sustained-flight wind tunnel', Environmental entomology, vol.7, pp.742-744.

57. Moore, B. (ed) 2004, *The Australian Oxford dictionary*, 2nd edn, Oxford University Press, Oxford.
58. Morris, F. 1960. 'Sampling insect populations', *Annual review of entomology*, Vol.5, pp.243-264.
59. Murlis, J. 1986. 'The structure of odour plumes', in *Mechanisms in insect olfaction*, eds Payne, T., Birch, C. & Kennedy, J, Clarendon Press, Oxford.
60. Murlis, J., & Jones. 1981. 'Fine-scale structure of odour plumes in relation to insect orientation to distant pheromone and other attractant sources', *Journal of physical entomology*, vol.6, pp.71-86.
61. Parajulee, M., Shrestha, R. & Stanley, C., 2007, 'Intercrop movement of *Lygus hesperus* in the Texas high plains: Potential for landscape management', 2nd international Lygus Bug Symposium, Asilomar Conference grounds Pacific Grove, California.
62. Pearce, S. & Zalucki, M., 2005. 'Does the cutting of Lucerne (*Medicago sativa*) encourage the movement of arthropod pests and predators into the adjacent crop?', *Australian journal of entomology*, vol.44, Is.3, pp.219-225.
63. Perry, N., Wall, C. & Clark, J., 1988. 'Close-range behaviour of male pea moths, *Cydia nigricana*, responding to sex pheromones re-released via the substrate', *Entomologica Experimentalis et Applicata*, Vol. 49, pp.37-42.
64. Perry, N., Wall, C. & Greenway, R., 1980. 'Latin square designs in field experiments involving insect sex attractants' *Ecological Entomology*, Vol.55, pp.385-396.

65. Pyke, A. & Brown, E. 1996. *The Cotton Pest and Beneficial Guide*, Pp.51, CRDC, Narrabri and CTPM, Brisbane.
66. Pyke, B., Sterling, W. & Hartstack, A. 1980. 'Beat and shake bucket sampling of cotton terminals for cotton leafhoppers, other pests and predators', *Environmental entomology*, Vol. 9, pp. 572- 576.
67. Rankin, M. 1976. 'Hormonal control of insect migratory behaviour', in *Proceeding in life sciences: Evolution of insect migration and diapauses*, ed Dingle, H., Springer-Verlag, New York.
68. Reading, A., 2006, 'Boggabri- Our community and our home town', Boggabri community, Retrieved 29th September 2008 from www.boggabri.com.au/ourtown.htm
69. Ridgway, L. & Gyrisco, G. 1960. 'Effect of temperature on the rate of development of *Lygus lineolaris* (Hemipters: Miridae)' *Annals of the entomological society of America*, Vol. 53, pp. 691-694.
70. Riedl, H. 1980. 'The importance of pheromone trap density and trap maintenance for the development of standardized monitoring procedures for the codling moth (Lepidoptera: Tortricidae)' *The Canadian entomologist*, Vol 112, pp. 655-663.
71. Riedl, M. & Croft, A. 1974. 'A study of pheromone trap catches in relation to codling moth (Lepidoptera: Olethreutidae) damage' *Canadian entomologist*, Vol. 106, pp. 525-537.

72. Roach, H., Smith, W., Vinson, B., Graham, M. & Harding, A. 1979. 'Sampling predators and parasites of *Heliothis* species on crops and native host plants', in *Economic thresholds and sampling Heliothis species on cotton, corn, soybeans and other host plants*, ed Sterling, L, South. Coop. Ser. Bull, vol. 231, pp. 133-145.
73. Rumbo, R. 1981. 'Study of single sensillum response to pheromone in the light-brown apple moth, *Epiphyas postvittana*, using an average technique', *Journal of Physical entomology*, vol.6, pp.87-98.
74. Ryan, F., Jopiner, L. & Ryan, A., 1992, *MINITAB Handbook*. 2nd ed. PWS-Kent, Boston.
75. Sevacherian, V. & Stern, M. 1973. 'Host plant preferences of *Lygus* bugs in alfalfa inter-planted cotton fields', vol.3, pp.761-766.
76. Simpson, G. & Lloyd, R. 2007. 'Sampling for green mirids in cotton', *Cotton Catchment and Communities*. Retrieved 31st June 2008 from <http://tools.cotton.crc.org/Publicat/Pest/mirid.htm>
77. Simpson, G., Murray, D. & Lloyd, R. 1999. 'New ideas on sampling for green mirids in cotton', *The Australian cotton grower*, vol.20, no.5, pp.22-24.
78. Smith, M., Stadelbacher, A. & Gantt, W. 1976. 'A comparison of techniques for sampling beneficial arthropod populations associated with cotton', *Environmental entomology*, Vol. 5, pp. 435-444.
79. Southwood, E. 1968. *Ecological Methods: with particular reference to the study of insect populations*, Chapman and Hall, London.

80. Stanley, N. 1997. The seasonal abundance and impact of predatory arthropods on *Helicoverpa* species in Australian cotton fields. New South Wales, PhD thesis, University of New England, Armidale, Australia.
81. Stein, W. 1986. 'Dispersal of insects of public health importance', in *Insect flight: Dispersal and migration*, ed Danthanarayana, W., Springer-Verlag, Berlin.
82. Sterling, W., El-Zik, K. & Wilson, L. 1989. 'Biological controls o pest populations', in *Integrated Pest Management systems and cotton production*, eds R. Frisbie., K. El-Zik. L. Wilson, Wiley-Interscience publication, USA.
83. Strickland, A. 1961. 'Sampling crop pests and their hosts', *Annual review of Entomology*, vl.6, pp.201-220.
84. Strong, E., Sheldahl, J., Hughes, P. & Hussein, K. 1970. 'Reproductive biology of *Lygus hesperus* Knight', *Hilgardia*, Vol.40, pp.105-147.
85. Suckling, D. 2000. 'Issues affecting the use of pheromones and other semio-chemicals in orchards', *Crop protection*, vol.19, pp.677-683.
86. Taylor, R. 1986, 'The four kinds of migration', in *Insect flight: Dispersal and migration*, ed Danthanarayana, W., Springer-Verlag, Berlin.
87. Threfall, C., Deutscher, S., Wilson, L. & Staines, T., 2005-06. 'Sweeping up mirids gives a net improvement', *The Australian cotton grower*, vol.26 , no.7 , pp. 55-57

88. Wall, C. 1990. 'Principles of monitoring', in behaviour-modifying chemicals for insect management, eds, Ridgway, R., Silverstein, R. & Inscoc, M., Marcel Dekker, INC, USA.
89. Wallner, W. 1987. 'Factors affecting insect population Dynamics: Differences between Outbreak and Non-Outbreak species', Annual review of entomology, vol.23, pp.317-340
90. Welter, S., Pickel, C., Millar, J., Cave, F., Van Steenwyk, R. & Dunley, J. 2005. 'Pheromone mating disruption offers selective management options for key pests', online journal of California agriculture, vol.59, no.1. Retrieved 3rd June 2008 from
<http://CaliforniaAgriculture.ucop.edu>
91. Wheeler, A. 2001. *Biology of the plant bug*, Cornell University Press, New York.
92. Wilson, T. & Gutierrez, P. 1980. 'Within-plant distribution of immatures of *Heliothis zea* (Boddie) on cotton', Hilgardia, Vol. 48, pp.24-36.
93. Wilson, L. & Room, P. 1982. 'The relative efficiency and reliability of three methods for sampling arthropods in Australian cotton fields', Journal of Australian entomology society, vol.21, pp.175-181.
94. Wu, K., Li, W., Fen, H. & Guo, Y. 2002. 'Seasonal abundance of mirids on Bt cotton in north china', Crop protection, Vol.21, No.1, pp.997-1002.

- 95.** Young, C., & Tugwell, P. 1975. 'Different methods of sampling for clouded and tarnished plant bugs in Arkansas cotton fields', Agricultural experiment station, Vol. 219, pp.1-12.
- 96.** Zhang, H. & Aldrich, R. 2003. 'Male-produced anti-sex pheromone in a plant bug', *Naturwissenschaften*, vol.90, Issue.11, pp. 505-508.

Appendix 1:

Preliminary study

Normality tests, methodology and further statistical analyses.

Preliminary Questions:

- a) Is there any difference in effectiveness of the four methods at sampling:
 - a. Adult mirids
 - b. Nymphs

- b) Is there any difference between operators in sampling:
 - a. Adult mirids
 - b. Nymphs

A. Data Display

A.1 Auscott, Narrabri Data

Day	Per metre VisualA	VisualN	DvacA	DvacN	SweepA	SweepN	BeatA	BeatN
0								
23	0	0	*	*	*	*	*	*
27	0	0	*	*	*	*	*	*
30	0	0	*	*	*	*	*	*
33	0	0	*	*	*	*	*	*
37	0	0	*	*	*	*	*	*
40	0	0	*	*	*	*	*	*
44	0	0	*	*	*	*	*	*
46	0	0	*	*	*	*	*	*
51	0	0	*	*	*	*	*	*
54	0	0	*	*	*	*	*	*
58	0	0	*	*	*	*	*	*
62	0	0	*	*	*	*	*	*
65	0	0	*	*	*	*	*	*
68	0	0		0	0	*	*	*
72	*	*		0	0	*	*	*
74	*	*	0.016667	0	*	*	*	*
78	*	*	0.008333	0	*	*	*	*
81	*	*	0.008333	0	*	*	*	*
85	*	*	0.016667	0	*	*	*	*
89	*	*	0.008333	0	*	*	*	*
93	*	*	0.025	0	*	*	*	*
96	*	*	0.016667	0.008333	*	*	*	*
100	*	*	0.033333	0.041667	*	*	*	*
103	0.333333	0.333333	0.066667	0.025	0.066667	0.041667	0	1.5
108	*	*	0.008333	0	*	*	*	*
110	*	*	0	0	*	*	*	*
117	0	0	0.016667	0	0.033333	0	0	0
122	*	*	0	0.008333	*	*	*	*
124	1	0	0.05	0.008333	0.025	0	0	0
131	*	*	0.058333	0.008333	*	*	*	*
136	*	*	0	0	*	*	*	*
142	*	*	0	0	*	*	*	*
148	*	*	0.041667	0	*	*	*	*

A.2 Brigadoon, Boggabri data

Day	Per metre VisualA	VisualN	DvacA	DvacN	SweepA	SweepN	BeatA	BeatN
0	*	*	*	*	*	*	*	*
23	0	0	*	*	*	*	*	*
27	0	0	*	*	*	*	*	*
30	0	0	*	*	*	*	*	*
33	0	0	*	*	*	*	*	*
37	0	0	*	*	*	*	*	*
39	0	0	*	*	*	*	*	*
44	0	0	*	*	*	*	*	*
47	0	0	*	*	*	*	*	*
50	0	0	*	*	*	*	*	*
53	0	0	*	*	*	*	*	*
57	0	0	*	*	*	*	*	*
62	0	0	*	*	*	*	*	*
65	0	0	0.008333	0	*	*	*	*
68	0	0.166667	0.016667	0	*	*	*	*
72	0	0.166667	0.016667	0.01666667	*	*	*	*
76	0	0	0	0	*	*	*	*
79	0	0	0	0	*	*	*	*
82	0	0	0.008333	0	*	*	*	*
85	0.333333	0.166667	0.008333	0.00833333	*	*	*	*
89	0	0	0	0	*	*	*	*
92	0	0	0.016667	0	*	*	*	*
96	0	0	0.008333	0	*	*	*	*
100	0.5	0.166667	0.008333	0	0.033333	0.008333	*	*
103	0.333333	0.166667	0.008333	0.00833333	0.025	0.016667	*	*
107	0.166667	0.5	0.016667	0.00833333	0.058333	0.05	1.166667	1.833333
112	1.333333	0.666667	0.025	0.025	*	*	*	*
115	0.166667	0.166667	0.016667	0.00833333	0.091667	0.058333	0.333333	0.666667
118	0.333333	0.166667	0.016667	0.00833333	0.15	0.075	0.833333	1.5
121	0.166667	0.166667	0	0.025	0.083333	0.016667	0.5	0
125	*	*	0.016667	0.025	*	*	*	*
130	*	*	0.016667	0.025	*	*	*	*
134	*	*	0.016667	0.025	*	*	*	*
137	*	*	0.016667	0.00833333	*	*	*	*
144	*	*	0.008333	0	*	*	*	*
148	*	*	0	0	*	*	*	*
151	*	*	0.008333	0	*	*	*	*

A.3 Korolea, Goondiwindi Data

Day	Per metre		SweepA	SweepN	DvacA	DvacN	BeatA	BeatN
	VisualA	VisualN						
0	0	0	*	*	*	*	*	*
43	0	0	*	*	*	*	*	*
50	0	0	*	*	*	*	*	*
55	0	0	*	*	*	*	*	*
57	0.166667	0	*	*	*	*	*	*
64	0.166667	0.166667	0.041667	0.016667	*	*	*	*
68	0.5	0.166667	0.15	0.033333	*	*	*	*
71	0.5	0.5	0.075	0.091667	*	*	*	*
79	0	0	0.016667	0.016667	0.141667	0	0	0
81	0.166667	0	0.025	0.016667	0.066667	0	0.166667	0
88	0.166667	0.166667	0.058333	0.033333	0.125	0.066667	0.5	0.833333
93	0.166667	0	0.05	0.016667	0.05	0.008333	0.666667	0.333333
100	*	*	0	0	0	0.008333	0	0.166667
105	*	*	0.016667	0	0.041667	0	0	0.166667
114	*	*	0.033333	0	0.041667	0	0	0
121	0	0	0	0	0.008333	0	0	0
134	0	0	0	0	*	*	0	0
140	0	0	0.016667	0	0.008333	0.008333	0.166667	0.166667
148	0	0	0.033333	0	0.016667	0	0.166667	0

B. Normality tests

The test used is the Ryan-Joiner or Shapiro wilks test in MINITAB (Ryan *et al.* 1992). The results show (shown in Figures B1 and B2) that neither the adult or nymph data set had normally distributed residuals as the P-values were both less than 0.1 which in this analysis is used as the significant value therefore providing evidence against the null hypothesis.

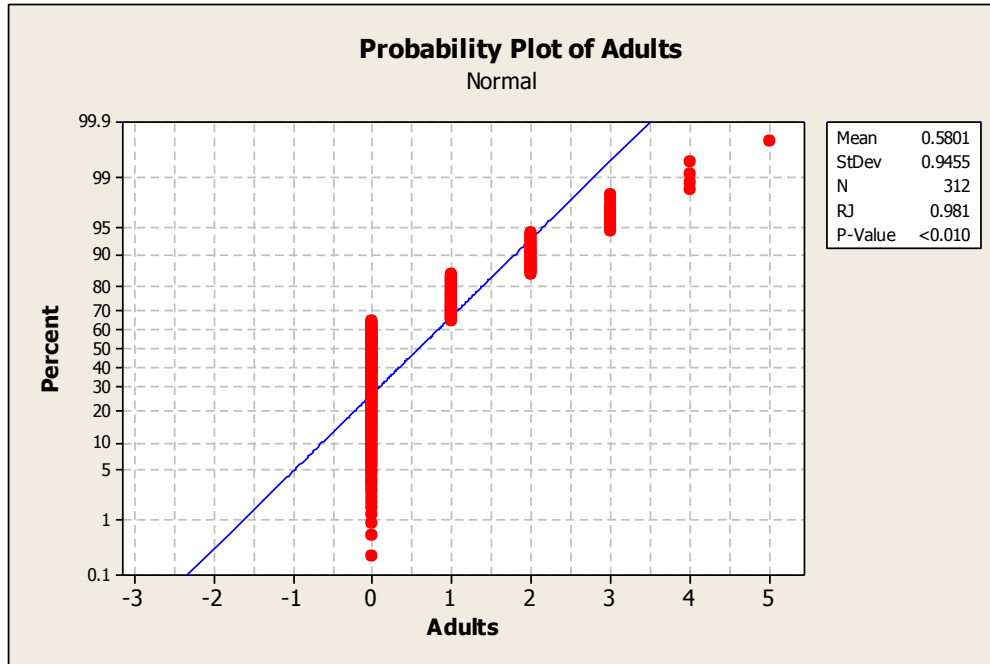


Figure B1: Probability plot and test for normality in the adult data set.

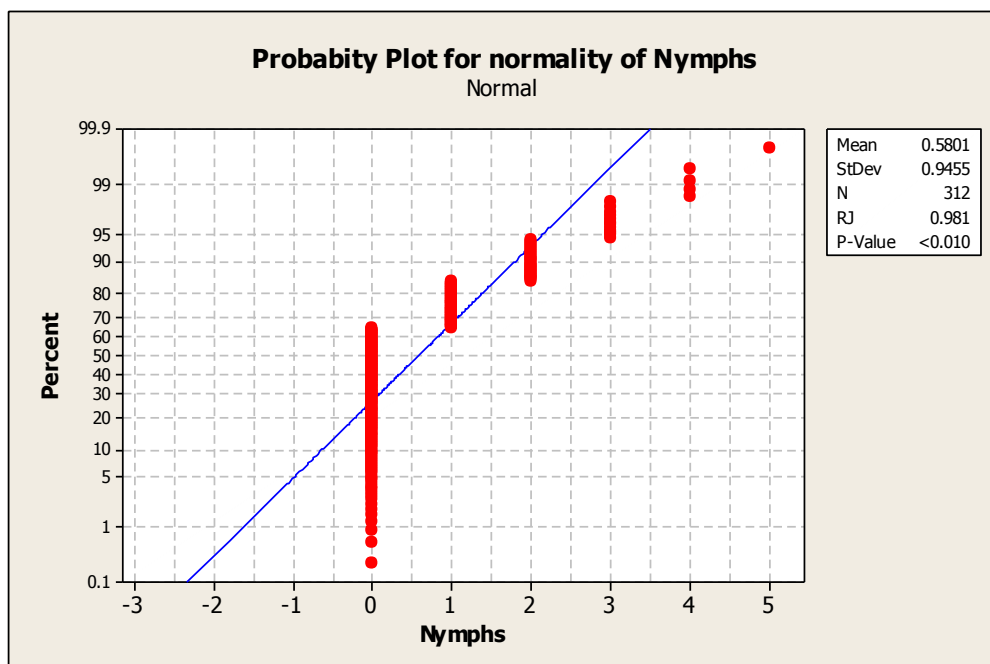


Figure B2: Probability plot and test for normality in the nymph data set.

To achieve normality Log transformations were tested and used. This transformation rectified the problem of non normality as the P-Value for both Log(adults) and Log(nymphs) (P-value >0.1) therefore giving no evidence to reject the null hypothesis of normally distributed residuals.

The probability plots and P-values for the transformed adult and nymph data is represented in Figures B3 and B4. The residual plots for the Log transformed data appears to have solved the non-normality problem despite the large frequency of zeros in the data. Therefore further analysis will be conducted with the data Log transformed data so a normal distribution of residuals is evident.

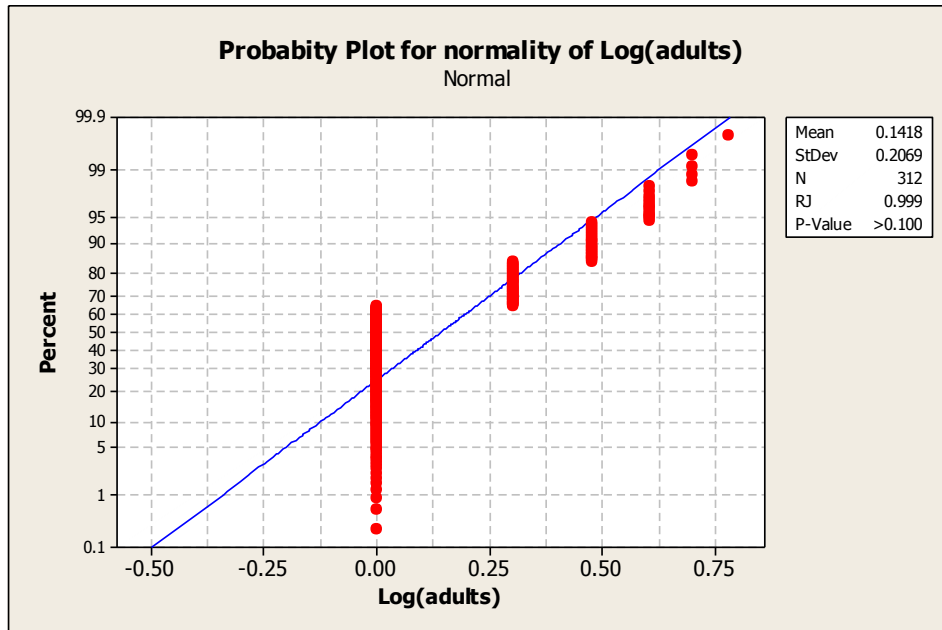


Figure B3: Probability plot and test for normality in the adult data set after Log transformations.

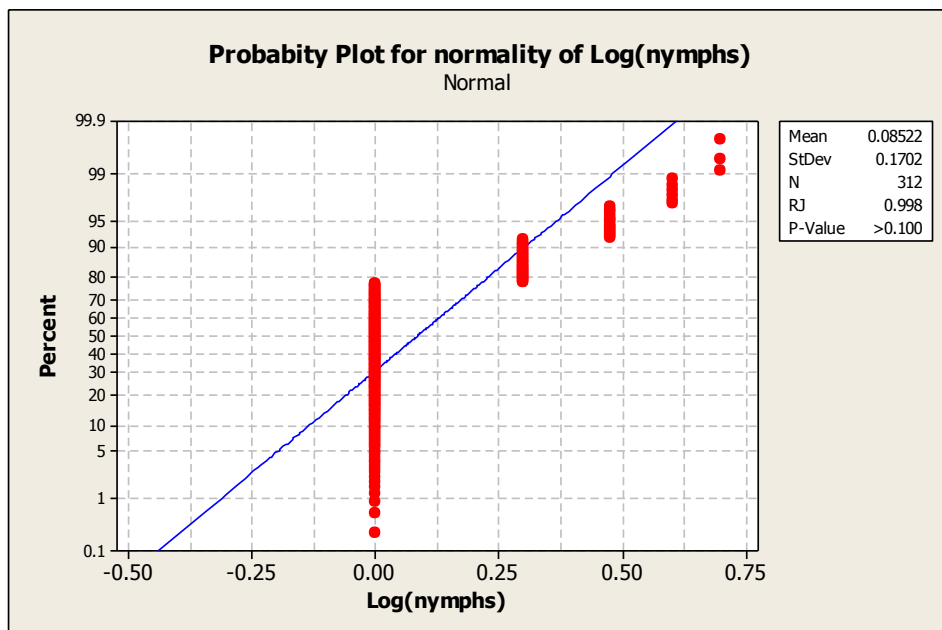


Figure B4: Probability plot and test for normality in the nymph data set after Log transformations.

C. Analysis on Date

Note: In order to determine if data could be pooled over the variable 'date' before conducting the preliminary study an Analysis to determine if date explained any of the variability in the number of adults and nymphs was needed first.

C.1 Analysis of covariance for transformed data on Adult mirid numbers versus DATE and Method

Analysis of Variance for LogAdult, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DATE	2	0.16069	0.16069	0.08035	2.34	0.105
Method	3	1.00576	1.00576	0.33525	9.77	0.000
DATE*Method	6	0.23169	0.23169	0.03862	1.12	<u>0.359</u>
Error	60	2.05975	2.05975	0.03433		
Total	71	3.45789				

S = 0.185281 R-Sq = 40.43% R-Sq(adj) = 29.51%

C.2 Analysis of covariance for transformed data on Adult mirid numbers versus DATE and Operator

Analysis of Variance for LogAdult, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DATE	2	0.00324	0.00324	0.00162	0.05	0.954
Op_loc	2	0.81398	0.81398	0.40699	11.77	0.000
DATE*Op_loc	4	0.37395	0.37395	0.09349	2.70	<u>0.032</u>
Error	207	7.16048	7.16048	0.03459		
Total	215	8.35166				

S = 0.185988 R-Sq = 14.26% R-Sq(adj) = 10.95%

C.3 Analysis of covariance for transformed data on Nymph numbers versus DATE and Method

Analysis of Variance for LogNymph, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DATE	2	0.43198	0.43198	0.21599	13.62	0.000
Method	3	0.16339	0.16339	0.05446	3.43	0.022
DAP*Method	6	0.21848	0.21848	0.03641	2.30	<u>0.046</u>
Error	60	0.95163	0.95163	0.01586		
Total	71	1.76548				

S = 0.125939 R-Sq = 46.10% R-Sq(adj) = 36.22%

From the tests there are several significant interactions between predictors and DATE on the number of both response variables, adult and nymph numbers. For adults the variable DATE does not have a significant interaction with Method (P-value 0.359) but does a significant interaction with operator (P-value 0.032) meaning there is an effect between DATE and Operator on adult numbers. The adult data therefore can not be pooled over dates due to this significant interaction, so each date has to be considered separately

For nymphs, the DATE has a significant interaction with Method (P-value 0.046). The significant interaction means there is an effect between DATE and Methods on nymph numbers sampled. The nymph data therefore cannot be pooled over the three DATES as some of the variability in nymph numbers is due to the interaction between DATE and Methods. In conclusion separate analyses for each DATE for both adult and nymphs are necessary.

D. Analysis on Preliminary Study

D.1- DATE 1

D.1a – Adult

Analysis of covariance for Log transformed data on Adult versus Operator and Method

Analysis of Variance for LogAdult, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Op_loc	2	0.46673	0.46673	0.23336	9.26	0.000
Method	3	0.23469	0.23469	0.07823	3.10	0.033
Op_loc*Method	6	0.50564	0.50564	0.08427	3.34	<u>0.007</u>
Error	60	1.51202	1.51202	0.02520		
Total	71	2.71909				

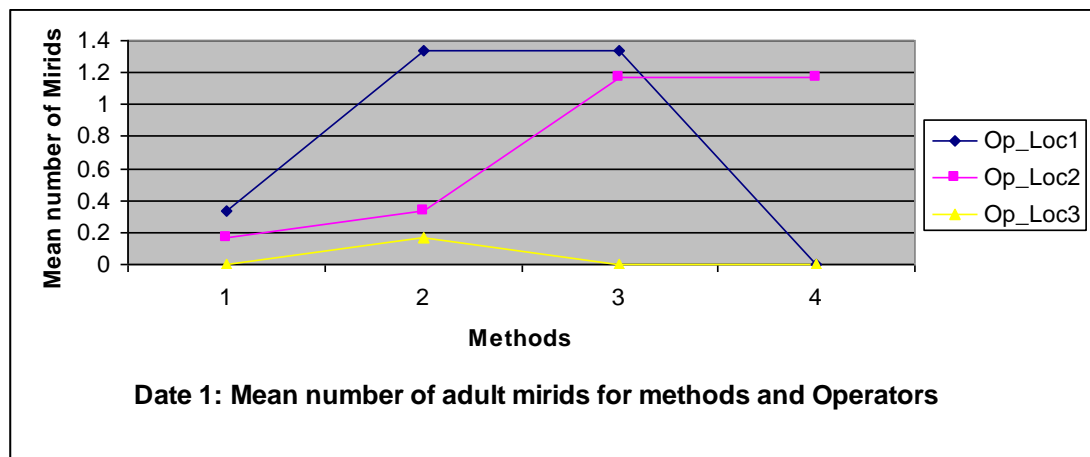
S = 0.158746 R-Sq = 44.39% R-Sq(adj) = 34.20%

Ho: The interaction between Operator and Method is equal to zero

The F-test has a P-value of 0.007 for the interaction of the two predictor variables. This P-value if is less then 0.05 (<0.05) making the interaction between Operator and Method significant. Therefore it can be concluded that there is significant evidence against the null hypothesis indicating there is an interaction effect between Operator and Method on the number of Adult mirids sampled. This means that significant

variability in the data is explained through the interaction of the two predictor variables Operator and Method.

There is evidence of a difference among the Operator in terms of the most effective sampling method for adult mirids. On comparison of the three graphs for Date 1 it is evident that the effectiveness of method 4 is very different between Operator 2 and the others.



The effectiveness of methods in sampling adult mirids changes over the varying levels of operators. From this graph it can be noted that methods 2 and 3 are most effective at sampling adult mirids for Operator 1, while Op_Loc2 shows methods 3 and 4 seem most effective at sampling adult mirids and method 2 seems the most effective method for Operator 3.

Further analysis was conducted to identify if the trends observed were significant

D.1a1- Operator 1

One-way ANOVA: LogAdult versus Method

Source	DF	SS	MS	F	P
Method	3	0.3921	0.1307	2.79	0.067
Error	20	0.9358	0.0468		
Total	23	1.3279			

S = 0.2163 R-Sq = 29.53% R-Sq(adj) = 18.96%

P-value = 0.067 (>0.05)

From the 1-Way ANOVA test the P-value is larger than 0.05. This shows there is no evidence against the null hypothesis indicating there is no significant difference between methods and the mean number of adult mirids caught. Therefore even though

there is a trend that could suggest that methods 2 and 3 are more effective sampling methods there is no statistical evidence to support this observation in the data.

Fisher 95% Individual confidence intervals: pair wise comparison.

1a	Note: <i>a,b</i> represent pair wise comparisons among levels of method. Even though the confidence intervals and the fisher comparison shows trends of similarity, there is not a significant difference between any of the methods.
2ab	
3ab	
4a	

D.1a2- Operator 2

One-way ANOVA: LogAdult versus Method

Source	DF	SS	MS	F	P
Method	3	0.3369	0.1123	4.49	0.015
Error	20	0.5007	0.0250		
Total	23	0.8377			

S = 0.1582 R-Sq = 40.22% R-Sq(adj) = 31.26%

P value = 0.015 (<0.05)

From the 1-Way ANOVA test the P-value is less than 0.05. This shows there is evidence against the null hypothesis indicating there is significant difference between methods and the mean number of adult mirids caught for operator two. Therefore there is a trend that could suggest that methods 2 and 3 are more effective sampling methods for operator 2. To confirm if the observed trend is the same trend showing significance a Fisher 95% Individual confidence intervals pair wise comparison among levels of method was done.

Operator 2: Fisher 95% Individual confidence intervals: pair wise comparison.

1a	Note: <i>a,b,c</i> represent pair wise comparisons among levels of method.
2ab	
3 bc	
4 bc	

The confidence intervals and the fisher comparison shows trends of similarity and where significant differences between methods is evident. This supports the observations in Figure 5 that 3 and 4 are more effective at catching adults then method 1 but are not statistically different form each other.

D.1a3- Operator 3

One-way ANOVA: LogAdult versus Method

Source	DF	SS	MS	F	P
Method	3	0.01133	0.00378	1.00	0.413
Error	20	0.07552	0.00378		
Total	23	0.08684			

S = 0.06145 R-Sq = 13.04% R-Sq(adj) = 0.00%

P-value = 0.413 (>0.05)

From the 1-Way ANOVA test the P-value is larger than 0.05. This shows there is no evidence against the null hypothesis indicating there is no significant difference between methods and the mean number of adult mirids caught. Therefore even though there is a trend that could suggest that method 2 is the most effective sampling method for operator 3 there is no statistical evidence to support this observation in the data.

Fisher 95% Individual confidence intervals: pair wise comparison.

1a 2ab 3abc 4abc	Note: <i>a,b,c</i> represent pair wise comparisons among levels of method.
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Further evidence of no significant trend comes from the Fisher pair wise comparison as there is not a significant difference between any of the methods.

D.1- DATE 1

D.1b – Nymph

General Linear Model: LogNymph versus Op_loc, Method						
Analysis of Variance for LogNymph, using Adjusted SS for Tests						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Op_loc	2	0.60041	0.60041	0.30021	8.65	0.001
Method	3	0.35867	0.35867	0.11956	3.44	0.022
Op_loc*Method	6	0.24209	0.24209	0.04035	1.16	<u>0.339</u>
Error	60	2.08326	2.08326	0.03472		
Total	71	3.28443				

S = 0.186336 R-Sq = 36.57% R-Sq(adj) = 24.94%

Ho: The interaction between Operator and Method is equal to zero

The F-test has a P-value of 0.339 for the interaction of the two predictor variables. This P-value is larger than 0.05 (>0.05) making the interaction between Operator and Method not significant. Therefore it can be concluded that there is significant evidence supporting the null hypothesis indicating there is no interaction effect between Operator and Method on number of Nymphs caught on date 1. As there is no interaction between the predictor variables the main effects will be used to analyse the variability in the data.

From the GLM model the p-values for both main effects of the predictor variables are <0.05 indicating that variability in the data is due to significant linear relationships between at least operator and the number of Nymphs sampled and a linear relationship between at least on sampling methods and the number of Nymphs sampled. From the GLM it is also known there is a significant difference between at least one operator and one method but are unsure of where the difference is. To observe where the significant relationship is coming from further analysis is needed.

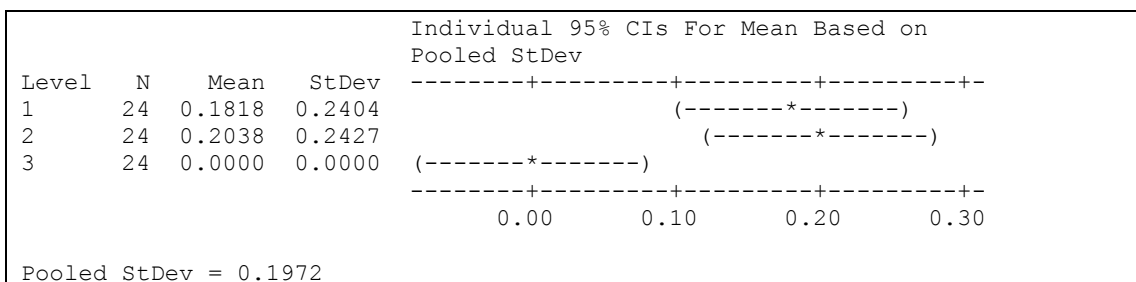
One-Way ANOVA are needed to find where the significant difference in operators and methods is evident.

D.1b1- Nymph vs Operator

One-way ANOVA: LogNymph versus Op_loc

Source	DF	SS	MS	F	P
Op_loc	2	0.6004	0.3002	7.72	0.001
Error	69	2.6840	0.0389		
Total	71	3.2844			

S = 0.1972 R-Sq = 18.28% R-Sq(adj) = 15.91%



From the One-Way ANOVA the P-value is less than 0.05 concluding there is a relationship between number of nymphs and at least one operator. From the confidence intervals it is evident that operators 1 and 2 are both significantly different from 3. The same result was also achieved when a Fisher pair wise comparison was conducted.

D.1b2- Nymph vs Method

One-way ANOVA: LogNymph versus Method

Source	DF	SS	MS	F	P
Method	3	0.3587	0.1196	2.78	0.048
Error	68	2.9258	0.0430		
Total	71	3.2844			

S = 0.2074 R-Sq = 10.92% R-Sq(adj) = 6.99%

Fisher 95% Individual confidence intervals: pair wise comparison.

1a 2ab 3abc 4 c	Note: <i>a,b,c</i> represent pair wise comparisons among levels of method.
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The F-test produced a P-value <0.05 indicating there is a relationship between number of nymphs and at least one of the methods. The Fisher pair wise test shows that method 4 is statically different from methods 1 and 2 but not 3. From Figure 3.1 it can be seen that for Date 1 method 3 and 4 have the higher numbers of nymphs caught for both operators 1 and 2.

Fisher pair wise tests and One-Way ANOVAs were run for individual operators to see if method was significantly better than another and none of the methods.

Fisher 95% Individual confidence intervals: pair wise comparison.

Operator	P-value from ANOVA	Fisher pairwise comparison
Operator 1	0.398 (Not significant)	1a 2ab 3abc 4abc
Operator 2	0.054 (Not Significant)	1a 2ab 3abc 4 c
Operator 3	*****	*****No nymphs were sampled

From the One-way ANOVA and Fisher pair wise test it is evident that the observable trend in methods is not significant for either operator. This means that there is no difference in the effectiveness of methods at catching nymphs for either operator even though there is an observable difference in sampling method catch numbers. Therefore the significant difference in the effectiveness of methods comes from the pooling of data from all three operators. It could be concluded on date 1, methods 3 and 4 were more effective at catching mirid nymphs.

D.2- DATE 2

D.2a – Adult

General Linear Model: LogAdult versus Op_loc, Method

Analysis of Variance for LogAdult, using Adjusted SS for Tests						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Op_loc	2	0.61320	0.61320	0.30660	13.95	0.000
Method	3	0.62001	0.62001	0.20667	9.40	0.000
Op_loc*Method	6	0.51465	0.51465	0.08578	3.90	<u>0.002</u>
Error	60	1.31894	1.31894	0.02198		
Total	71	3.06680				

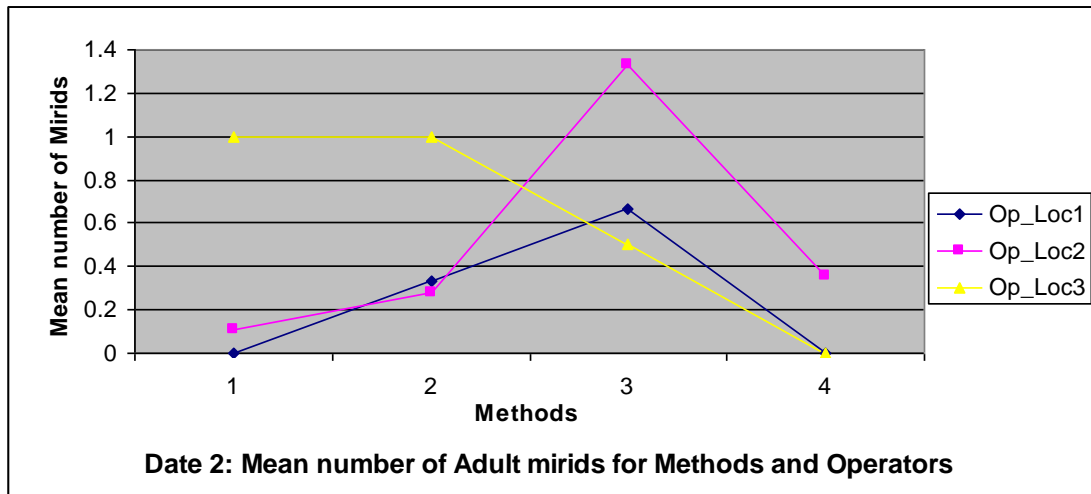
S = 0.148264 R-Sq = 56.99% R-Sq(adj) = 49.11%

Ho: The interaction Operator and Method is equal to zero

The F-test has a P-value of 0.002 for the interaction of the two predictor variables. This P-value is less than 0.05 (<0.05) making the interaction between operators and Method significant. Therefore it can be concluded that there is significant evidence against the null hypothesis indicating there is an interaction effect between operator and Method on number of Adult mirids. This means that variability in the data is explained by the interaction of the two predictor variables, operator and Method.

There is evidence of a difference among the operator in terms of the most effective sampling method for adult mirids. On comparison of figure 3.2 for date 2 it seems there is a difference between operators and between the effectiveness of sampling methods.

To get a better understanding of the interaction a plot of mean adult numbers for operators and methods can be used.



The effectiveness of methods in sampling adult mirids changes over the varying levels of operators. As an interaction is apparent between operators and levels of method, the effectiveness of each method for operators needs to be considered separately. From this graph it can be noted that methods 1 and 2 are most effective at sampling adult mirids for Operator 3, while method three is most effective at sampling adult mirids for both operators 1 and 2. This shows that the effectiveness of methods is different between operators.

Further analysis is needed to identify if the observed trends are significant

D.2a1- Operator 1

One-way ANOVA: LogAdult versus Methods

Source	DF	SS	MS	F	P
MethodN_1	3	0.1113	0.0371	1.75	0.190
Error	20	0.4244	0.0212		
Total	23	0.5356			

S = 0.1457 R-Sq = 20.77% R-Sq(adj) = 8.89%

P-value = 0.190 (>0.05)

From the One-Way ANOVA test the P-value is larger than 0.05 showing there is no evidence against the null hypothesis indicating there is no difference in mean number of adult mirids caught with each method for operator 1. Therefore even though there is a trend in figure 3.2 that could suggest methods 2 and 3 are more effective for

operator 1, this is not supported statistically or by the Fisher 95% pair wise comparisons.

Fisher 95% Individual confidence intervals: pair wise comparison.

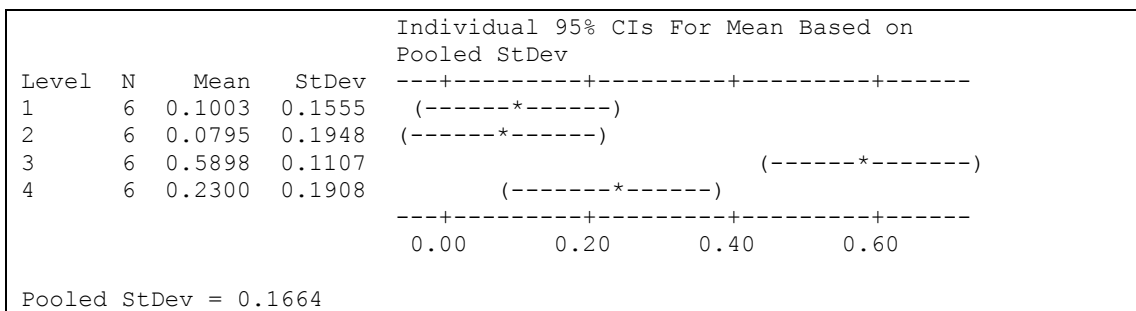
1a	Note: <i>a,b,c</i> represent pair wise comparisons among levels of method.
2ab	
3abc	
4ab c	

D.2a2. Operator 2

One-way ANOVA: LogAdult versus Methods

Source	DF	SS	MS	F	P
Method	3	1.0038	0.3346	12.08	0.000
Error	20	0.5539	0.0277		
Total	23	1.5576			

S = 0.1664 R-Sq = 64.44% R-Sq(adj) = 59.11%



P-value = 0.000 (<0.05)

From the One-Way ANOVA test the P-value is less than 0.05. This shows there is evidence against the null hypothesis indicating there is a significant difference between methods and the mean number of adult mirids caught for operator2.

Fisher 95% Individual confidence intervals: pair wise comparison.

1a	Note: <i>a,b</i> , represent pair wise comparisons among levels of method.
2ab	
3	
4ab	

The confidence Intervals and Fisher pair wise comparison test show where the significant difference between methods is evident. From these analyses that a significant difference between method four and the other three methods is evident.

Therefore with the observed trend in graph 3.2 for date 2 operator 2 and the statistical analysis it is evident that method three is the most effective method at sampling adult mirids for operator 2 on date 2.

D.2a3. Operator 3

One-way ANOVA: LogAdult versus Methods

Source	DF	SS	MS	F	P
Method	3	0.0196	0.0065	0.38	0.766
Error	20	0.3407	0.0170		
Total	23	0.3604			

S = 0.1305 R-Sq = 5.44% R-Sq(adj) = 0.00%

P-value = 0.766 (>0.05)

From the One-Way ANOVA the P-value is larger than 0.05. This shows there is no evidence against the null hypothesis ($H_0: \mu = 0$) indicating there is no significant difference between methods and the mean number of adult mirids caught. Therefore even though an observable trend may occur in the mean number of adult mirids caught by operator 3 with the varying methods it is not a significant difference to support if a method is more effective than another. This is further supported through the CI's and the Fisher pair wise comparison below as they identify similarity in all methods effectiveness in sampling adult mirids

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	6	0.0000	0.0000	-----+-----+-----+-----+----- (-----*-----)
2	6	0.0502	0.1229	(-----*-----)
3	6	0.0795	0.1948	(-----*-----)
4	6	0.0502	0.1229	(-----*-----) -----+-----+-----+-----+----- -0.080 0.000 0.080 0.160

Fisher 95% Individual confidence intervals: pair wise comparison.

1a 2ab 3abc 4abc	Note: <i>a,b,c</i> represent pair wise comparisons among levels of method.
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D.2 - DATE 2

D.2b – Nymph

General Linear Model: LogNymph versus Op_loc, Method

Analysis of Variance for LogNymph, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Op_loc	2	0.50635	0.50635	0.25318	13.96	0.000
Method	3	0.15995	0.15995	0.05332	2.94	0.040
Op_loc*Method	6	0.31849	0.31849	0.05308	2.93	<u>0.014</u>
Error	60	1.08834	1.08834	0.01814		
Total	71	2.07313				

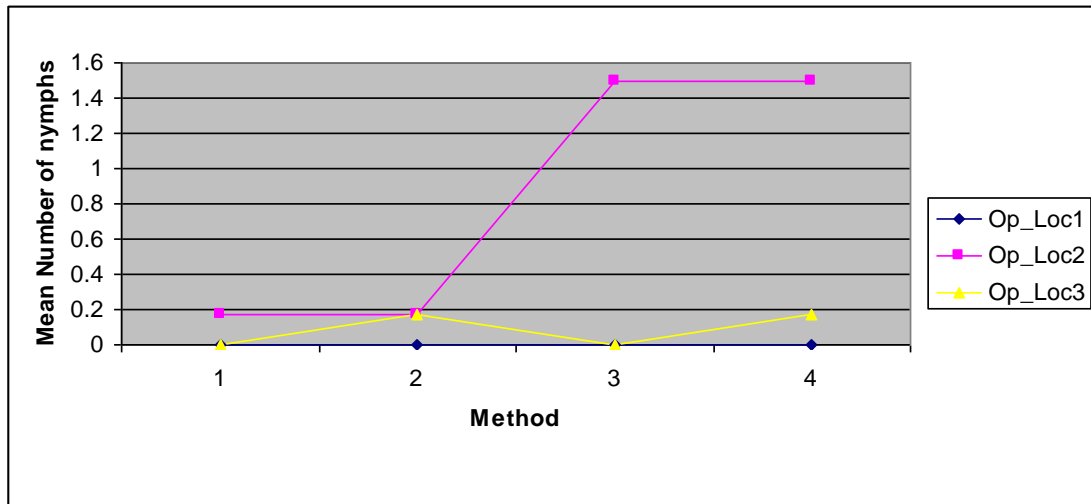
S = 0.134681 R-Sq = 47.50% R-Sq(adj) = 37.88%

Ho: The interaction between Op_Loc and Method is equal to zero

The F-test has a P-value of 0.014 for the interaction of the two predictor variables. This P-value is less than 0.05 (<0.05) making the interaction between Operator and Method significant. This means variability in the data is explained through the interaction of the two predictor variables Operator and Method. Therefore It can be concluded the effect of operator on the number of nymphs caught is not the same for all sampling methods.

On comparison of DATE 2 in figure 3.2 it is evident the effectiveness of method 3 is very different between Operator 2 and the other operators. As there is evidence of a difference among Operators in terms of the most effective sampling method for mirid nymphs. However to identify where the significant trends lay further analysis is needed.

To gain a better understanding of the interaction an interaction plot is needed



Graph :Date 2- Interaction Plot of operator and Method on mean number of Nymphs.

The effectiveness of methods in sampling nymphs changes over the varying levels of operators. From this graph it can be noted that methods 3 and 4 are most effective at nymphs for Operator 2, while methods 2 and 4 seem to be the most effective methods for operator 3. To identify if these trends are significant further individual analysis is needed.

D.2b1- Operator 1

One-way ANOVA: LogNymph versus Methods

Source	DF	SS	MS	F	P
Method	3	0.000000	0.000000	*	*
Error	20	0.000000	0.000000		
Total	23	0.000000			

S = 0 R-Sq = *% R-Sq(adj) = *%

Due to no mirid nymphs present there is no data to draw any conclusions from.

D.2b2- Operator 2

One-way ANOVA: LogNymph versus Methods

Source	DF	SS	MS	F	P
Method	3	0.4633	0.1544	3.30	0.042
Error	20	0.9373	0.0469		
Total	23	1.4006			

S = 0.2165 R-Sq = 33.08% R-Sq(adj) = 23.04%

P-value = 0.042 (<0.05)

From the One-Way ANOVA test the P-Value is less than 0.05. This shows there is evidence against the null hypothesis indicating there is a significant difference between at least one sampling method for operator 2. From the interaction plot and figure 3.2 it is observable that methods 3 and 4 seem to be more effective at sampling nymphs than methods 1 and 2. To determine if this observable trend is significant Fisher pair wise comparison is needed.

Fisher 95% Individual confidence intervals: pair wise comparison.

1a 2a 3 b 4 b	Note: <i>a,b</i> represent pair wise comparisons among levels of method.
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The Fisher pair wise comparison shows the observable trends of similarity are in fact significant. Methods 1 and 2 are not significantly different from each other but they are statically different from methods 3 and 4. However methods 3 and 4 are not significantly different from each other. Therefore it can be concluded that methods 3 and 4 are most effective at sampling mirid nymphs when used by operator 2.

D.2b3- Operator 3

One-way ANOVA: LogNymph versus Methods

Source	DF	SS	MS	F	P
Method	3	0.01510	0.00503	0.67	0.582
Error	20	0.15103	0.00755		
Total	23	0.16613			

S = 0.08690 R-Sq = 9.09% R-Sq(adj) = 0.00%

From the One-Way ANOVA test the P-value is larger than 0.05. This shows there is no evidence against the null hypothesis indicating there is no significant difference between methods and the mean number of nymphs caught. In the interaction plot and figure 3.2 it was observable that methods 2 and 4 may have been more effective, however the there is no statistical evidence for this observation. This is further supported by the Fisher pair wise comparison test below.

Fisher 95% Individual confidence intervals: pair wise comparison.

1a 2ab 3abc 4abc	Note: <i>a,b,c</i> represent pair wise comparisons among levels of method.
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D.3- DATE 3

D.3a – Adult

General Linear Model: LogAdult versus Op_loc, Method

Analysis of Variance for LogAdult, using Adjusted SS for Tests						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Op_loc	2	0.10801	0.10801	0.05400	2.12	0.129
Method	3	0.25030	0.25030	0.08343	3.27	0.027
Op_loc*Method	6	0.67247	0.67247	0.11208	4.39	<u>0.001</u>
Error	60	1.53175	1.53175	0.02553		
Total	71	2.56252				

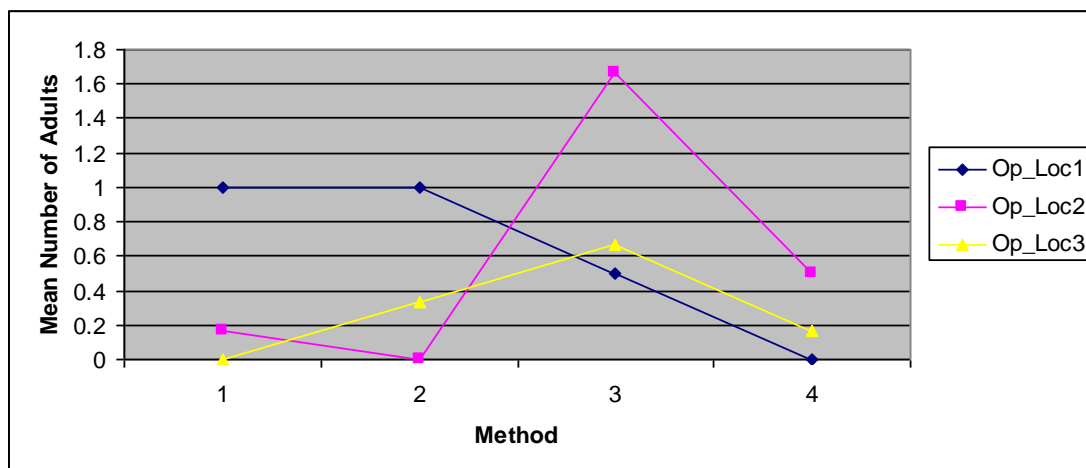
S = 0.159778 R-Sq = 40.22% R-Sq(adj) = 29.27%

Ho: The interaction between Op_Loc and Method is equal to zero.

The F-test has a P-value of 0.001 for the interaction of the two predictor variables. This P-value is less than 0.05 (<0.05) making the interaction between Operator and Method significant. This means variability in the data is explained through the interaction of the two predictor variables Operator and Method. Therefore it can be concluded the effect of operator on the number of adults caught is not the same for all sampling methods.

On comparison of DATE 3 on the figure 3.3 it is evident the effectiveness of method 3 is very different between Operator 2 and the other operators. There is evidence of a difference among Operators in terms of the most effective sampling method for adult mirids. However to identify where the significant trends lie further analysis is needed.

To gain a better understanding of the interaction an interaction plot is needed



The effectiveness of methods in sampling adult mirids changes over the varying levels of operators. From this graph it can be noted that methods 1 and 2 are most effective at sampling adults for operator 1, while method 3 seems to be most effective at sampling adults for both operators 2 and 3. To identify if these trends are significant further individual analysis is needed.

D.3a1- Operator 1

One-way ANOVA: LogAdults versus Methods

Source	DF	SS	MS	F	P
Methods	3	0.3254	0.1085	4.58	0.013
Error	20	0.4741	0.0237		
Total	23	0.7995			

S = 0.1540 R-Sq = 40.70% R-Sq(adj) = 31.80%

P-value = 0.013 (<0.05)

From the One-Way ANOVA test the P-Value is less than 0.05. This shows there is evidence against the null hypothesis indicating there is a significant difference between at least one sampling method for operator 1. To determine if the observed trend is significant Fisher pair wise comparison is needed.

Fisher 95% Individual confidence intervals: pair wise comparison.

1a	Note: <i>a,b,c</i> represent pair wise comparisons among levels of method.
2ab	
3abc	
4 c	

The Fisher pair wise comparison test shows trends of similarity and where significant differences between methods are evident. The fisher shows there is no statistical difference between method 1 and 2 but both are significantly different from 4. Method 4 however is not significantly different from 3. As there were no adults sampled with method 4 it can be considered that method 4 is the least effective method at sampling adult mirids and methods 1 and 2 are the effective methods for sampling adults on date 3 for operator 1.

D.3a2- Operator 2

One-way ANOVA: LogAdults versus Methods

Source	DF	SS	MS	F	P
Methods	3	0.5138	0.1713	6.14	0.004
Error	20	0.5578	0.0279		
Total	23	1.0716			

S = 0.1670 R-Sq = 47.95% R-Sq(adj) = 40.14%

P-value = 0.004 (<0.05)

From the One-Way ANOVA test the P-Value is less than 0.05. This shows there is evidence against the null hypothesis indicating there is a difference between at least one sampling method for operator 2. To determine if the observed trend is significant Fisher pair wise comparison is needed.

Fisher 95% Individual confidence intervals: pair wise comparison.

1a	Note: <i>a,b,c</i> represent pair wise comparisons among levels of method.
2ab	
3	
4ab	

The Fisher pair wise comparison test shows trends of similarity and where significant differences between methods are evident. The fisher shows there is no statistical difference between methods 1,2 and 4 but all of these are statistically different from 3. It is observable from the interaction plot and figure 3.3 that method 3 has the highest number of adults sampled. It can be concluded that method three is most effective for operator 2 on Date 3 for sampling adult mirids.

D.3a3- Operator 3

One-way ANOVA: LogAdults versus Methods

Source	DF	SS	MS	F	P
Methods	3	0.0835	0.0278	1.11	0.367
Error	20	0.4999	0.0250		
Total	23	0.5834			

S = 0.1581 R-Sq = 14.32% R-Sq(adj) = 1.47%

From the One-Way ANOVA test the P-value is larger than 0.05. This shows there is no evidence against the null hypothesis indicating there is no significant difference between methods and the mean number of adults caught. In the interaction plot and figure 3.3 it was observable that method 3 is more effective, however there is no statistical evidence for this observation. This is further supported by the Fisher pair wise comparison test below.

Fisher 95% Individual confidence intervals: pair wise comparison.

1a	Note: <i>a,b,c</i> represent pair wise comparisons among levels of method.
2ab	Note: This trend shows that all methods are not significantly different from each other.
3abc	
4abc	

D.3 - DATE 3

D.3b – Nymph

General Linear Model: LogNymph versus Op_loc, Method

Analysis of Variance for LogNymph, using Adjusted SS for Tests						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Op_loc	2	0.078516	0.078516	0.039258	5.69	0.005
Method	3	0.019554	0.019554	0.006518	0.95	0.425
Op_loc*Method	6	0.039108	0.039108	0.006518	0.95	<u>0.470</u>
Error	60	0.413685	0.413685	0.006895		
Total	71	0.550863				

S = 0.0830346 R-Sq = 24.90% R-Sq(adj) = 11.13%

Ho: The interaction between Operator and Method is equal to zero.

The F-test has a P-value of 0.470 for the interaction of the two predictor variables. This P-value is larger than 0.05 (the critical value) therefore making the interaction between Operator and Method is not significant. It can be concluded there is significant evidence supporting the null hypothesis indicating there is no interaction

effect between Operator and Method on the number of nymphs caught on DATE 3. AS there is no interaction between the predictor variables, the main effects will be used to analysis the variability in the data.

From the GLM model the P-value for the main effect of the predictor variable Method is not significant (>0.05). This indicates there is no significant difference in the effectiveness of methods across all operators on DATE 3.

From the GLM model the P-value for the main effect of the predictor variable Op-Loc is significant (<0.05). This indicates there is a significant difference in operator's effectiveness for sampling nymphs on DATE 3. To identify where this difference is individual operator analysis is needed.

Fisher 95% Individual confidence intervals: pair wise comparison.

1a	Note: <i>a</i> , represents a pair wise comparisons among levels of method.
2	
3a	

From the Fisher pair wise comparison it is evident that operator two is significantly different to operators 1 and 3 at sampling nymphs on DATE 3. Also it is evident there is no statistical difference between operator 1 and 2 at sampling nymphs on DATE 3. From figure 3.3 it is evident that operator 2 is the only operator that collected any nymphs. Therefore it could be concluded that operator 2 is the most effective operator at collecting nymphs on DATE 3.

Appendix 2:

Conversion factors

Methodology and values.

Note: Using conversion factors allows for the removal of the effects of both Operators and methods. This is achievable as the conversion factor removes the variation in the data (adult and nymph numbers) that are of interest.

A. Auscott, Narrabri

A.1 Data Display

Comparison of 4 methods (3 dates)

ROW	Day	VisualA	VisualN	DvacA	DvacN	SweepA
1	103	0.33333	0.333333	0.0666667	0.0250000	0.0666667
2	117	0.00000	0.000000	0.0166667	0.0000000	0.0333333
3	124	1.00000	0.000000	0.0500000	0.0083333	0.0250000

ROW	SweepN	BeatA	BeatN
1	0.0416667	0	1.5
2	0.0000000	0	0.0
3	0.0000000	0	0.0

	N	MEAN	MEDIAN	TRMEAN	STDEV	SEMEAN
VisualA	3	0.444	0.333	0.444	0.509	0.294
VisualN	3	0.111	0.000	0.111	0.192	0.111
DvacA	3	0.0444	0.0500	0.0444	0.0255	0.0147
DvacN	3	0.01111	0.00833	0.01111	0.01273	0.00735
SweepA	3	0.0417	0.0333	0.0417	0.0220	0.0127
SweepN	3	0.0139	0.0000	0.0139	0.0241	0.0139
BeatA	3	0.00000	0.00000	0.00000	0.00000	0.00000
BeatN	3	0.500	0.000	0.500	0.866	0.500

	MIN	MAX	Q1	Q3
VisualA	0.000	1.000	0.000	1.000
VisualN	0.000	0.333	0.000	0.333
DvacA	0.0167	0.0667	0.0167	0.0667
DvacN	0.00000	0.02500	0.00000	0.02500
SweepA	0.0250	0.0667	0.0250	0.0667
SweepN	0.0000	0.0417	0.0000	0.0417
BeatA	0.00000	0.00000	0.00000	0.00000
BeatN	0.000	1.500	0.000	1.500

A.2-Correction factors:

Dvac Adults: $0.444/0.0444 = 10$

Dvac Nymphs: $0.111/0.01111 = 10$

Sweep net Adults: $0.444/0.0417 = 10.65$

Sweep net Nymphs: $0.111/0.0139 = 8.0$

Beat sheet Adults: $0.444/0 = \text{inf}$

Beat sheet Nymphs: $0.111/0.5 = 0.22$

B. Brigadoon, Boggabri

B.1 Data Display

Comparison of 4 methods (4 dates)

ROW	Day	VisualA	VisualN	DvacA	DvacN	SweepA
1	107	0.166667	0.500000	0.0166667	0.00833333	0.0583333
2	115	0.166667	0.166667	0.0166667	0.00833333	0.091667
3	118	0.333333	0.166667	0.0166667	0.00833333	0.150000
4	121	0.166667	0.166667	0.0000000	0.0250000	0.0833333

ROW	SweepN	BeatA	BeatN
1	0.0500000	1.16667	1.83333
2	0.0583333	0.33333	0.66667
3	0.0750000	0.83333	1.50000
4	0.0166667	0.50000	0.00000

	N	MEAN	MEDIAN	TRMEAN	STDEV	SEMEAN
VisualA	4	0.2083	0.1667	0.2083	0.0833	0.0417
VisualN	4	0.2500	0.1667	0.2500	0.1667	0.0833
DvacA	4	0.01250	0.01667	0.01250	0.00833	0.00417
DvacN	4	0.01250	0.00833	0.01250	0.00833	0.00417
SweepA	4	0.0958	0.0875	0.0958	0.0388	0.0194
SweepN	4	0.0500	0.0542	0.0500	0.0245	0.0123
BeatA	4	0.708	0.667	0.708	0.370	0.185
BeatN	4	1.000	1.083	1.000	0.828	0.414

	MIN	MAX	Q1	Q3
VisualA	0.1667	0.3333	0.1667	0.2917
VisualN	0.1667	0.5000	0.1667	0.4167
DvacA	0.00000	0.01667	0.00417	0.01667
DvacN	0.00833	0.02500	0.00833	0.02083
SweepA	0.0583	0.1500	0.0646	0.1354
SweepN	0.0167	0.0750	0.0250	0.0708
BeatA	0.333	1.167	0.375	1.083
BeatN	0.000	1.833	0.167	1.750

B.2 Correction factors:

Dvac Adults: $0.2083/0.01250 = 16.7$
 Dvac Nymphs: $0.25/0.01250 = 20$
 Sweep net Adults: $0.2083/0.0958 = 2.17$
 Sweep net Nymphs: $0.25/0.05 = 5$
 Beat sheet adults: $0.2083/0.708 = 0.29$
 Beat sheet nymphs: $0.25/1 = 0.25$

C. Korolea, Goondiwindi

C.1 Data Display

Comparison of 4 sampling methods (7 dates)

ROW	Day	VisualA	VisualN	SweepA	SweepN	DvacA
1	79	0.000000	0.000000	0.0166667	0.0166667	0.141667
2	81	0.166667	0.000000	0.0250000	0.0166667	0.066667
3	88	0.166667	0.166667	0.0583333	0.0333333	0.125000
4	93	0.166667	0.000000	0.0500000	0.0166667	0.050000
5	121	0.000000	0.000000	0.0000000	0.0000000	0.008333
6	140	0.000000	0.000000	0.0166667	0.0000000	0.008333
7	148	0.000000	0.000000	0.0333333	0.0000000	0.016667

ROW	DvacN	BeatA	BeatN
1	0.0000000	0.000000	0.000000
2	0.0000000	0.166667	0.000000
3	0.0666667	0.500000	0.833333
4	0.0083333	0.666667	0.333333
5	0.0000000	0.000000	0.000000
6	0.0083333	0.166667	0.166667
7	0.0000000	0.166667	0.000000

	N	MEAN	MEDIAN	TRMEAN	STDEV	SEMEAN
VisualA	7	0.0714	0.0000	0.0714	0.0891	0.0337
VisualN	7	0.0238	0.0000	0.0238	0.0630	0.0238
SweepA	7	0.02857	0.02500	0.02857	0.02033	0.00768
SweepN	7	0.01190	0.01667	0.01190	0.01260	0.00476
DvacA	7	0.0595	0.0500	0.0595	0.0552	0.0208
DvacN	7	0.01190	0.00000	0.01190	0.02447	0.00925
BeatA	7	0.2381	0.1667	0.2381	0.2520	0.0952
BeatN	7	0.190	0.000	0.190	0.311	0.117

	MIN	MAX	Q1	Q3
VisualA	0.0000	0.1667	0.0000	0.1667
VisualN	0.0000	0.1667	0.0000	0.0000
SweepA	0.00000	0.05833	0.01667	0.05000
SweepN	0.00000	0.03333	0.00000	0.01667
DvacA	0.0083	0.1417	0.0083	0.1250
DvacN	0.00000	0.06667	0.00000	0.00833
BeatA	0.0000	0.6667	0.0000	0.5000
BeatN	0.000	0.833	0.000	0.333

C.2 Correction factors:

Sweep net Adults: $0.0714/0.02857 = 2.5$

Sweep net Nymphs: $0.0238/0.01190 = 2$

Dvac Adults: $0.0714/0.0595 = 1.2$

Dvac Nymphs: $0.0714/0.01190 = 6$

Beat sheet Adults: $0.0714/0.2381 = 0.3$

Beat sheet Nymphs: $0.0714/0.190 = 0.38$

Table 1: Summary table of correction factors

Method/Adult or Nymph	Auscott	Brigadoon	Korolea
Sweep net Adults	10.65	2.17	2.5
Sweep net Nymphs	8	5	2
Dvac Adults	10	16.7	1.2
Dvac Nymphs	10	20	6
Beat sheet Adults	**	.29	.3
Beat sheet Nymphs	0.22	.25	.38

Appendix 3:

Statistical analyses and methodology for Aim 1, Study 1

Question 1: Do pheromone traps reflect mirid population densities in the field?

A. Auscott, Narrabri: Regression Analysis Trap catches

A.1 - Data Display

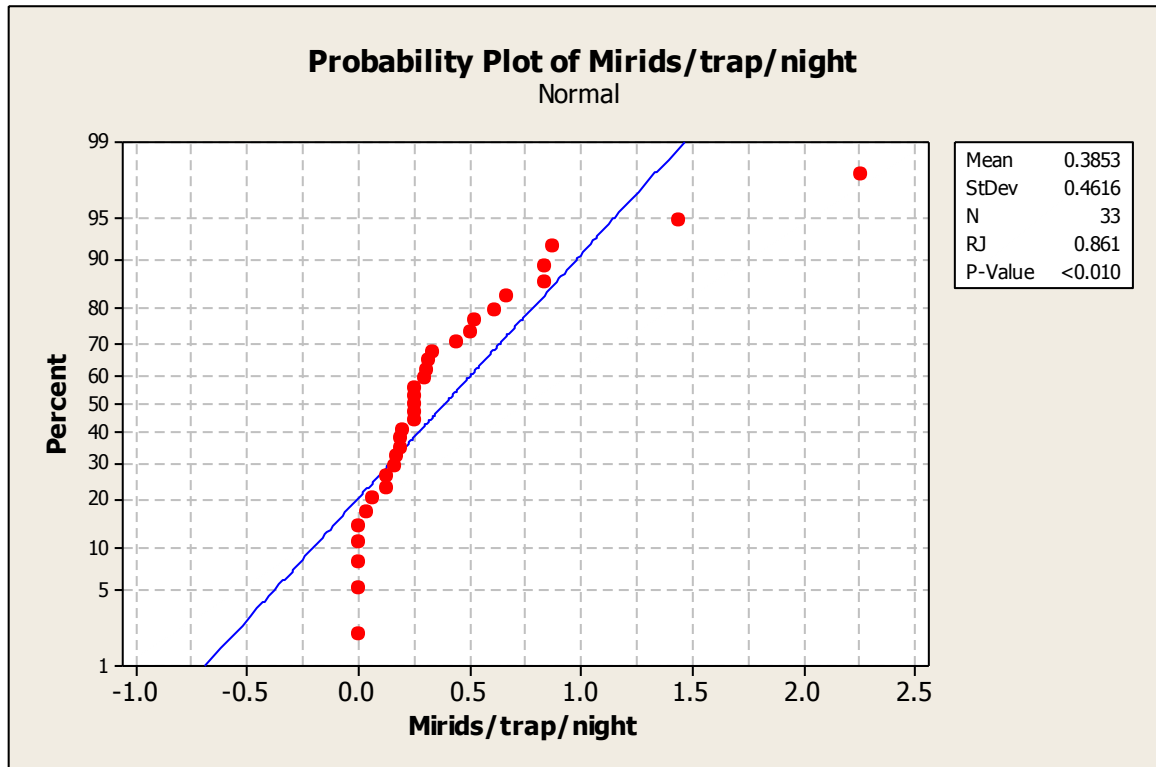
Day	Mirids/trap/night SQRtnymph	Adults/m	Nymphs/m	SQRTrap	SQRAdult
0	0.00000 0.000000	0.000000	0.000000	0.00000	0.000000
43	0.00000 0.000000	0.000000	0.000000	0.00000	0.000000
50	0.42857 0.000000	0.000000	0.000000	0.65465	0.000000
55	0.90000 0.000000	0.000000	0.000000	0.94868	0.000000
57	1.00000 0.000000	0.166667	0.000000	1.00000	0.408248
64	0.64286 0.316228	0.135417	0.100000	0.80178	0.367990
68	1.06250 0.341565	0.437500	0.116667	1.03078	0.661438
71	1.58333 0.584523	0.343750	0.341667	1.25830	0.586302
79	0.43750 0.091287	0.222917	0.008333	0.66144	0.472141
81	1.75000 0.091287	0.169792	0.008333	1.32288	0.412058
88	1.64286 0.397911	0.303125	0.158333	1.28174	0.550568
93	1.35000 0.144338	0.197917	0.020833	1.16190	0.444878
100	0.17857 0.194365	0.000000	0.037778	0.42258	0.000000
105	1.30000 0.145297	0.030556	0.021111	1.14018	0.174801
114	0.47222 0.000000	0.044444	0.000000	0.68718	0.210819
121	0.39286 0.000000	0.012500	0.000000	0.62678	0.111803
134	0.09615 0.000000	0.000000	0.000000	0.31009	0.000000
140	0.50000 0.111803	0.035417	0.012500	0.70711	0.188193
148	0.25000 0.000000	0.058333	0.000000	0.50000	0.241523

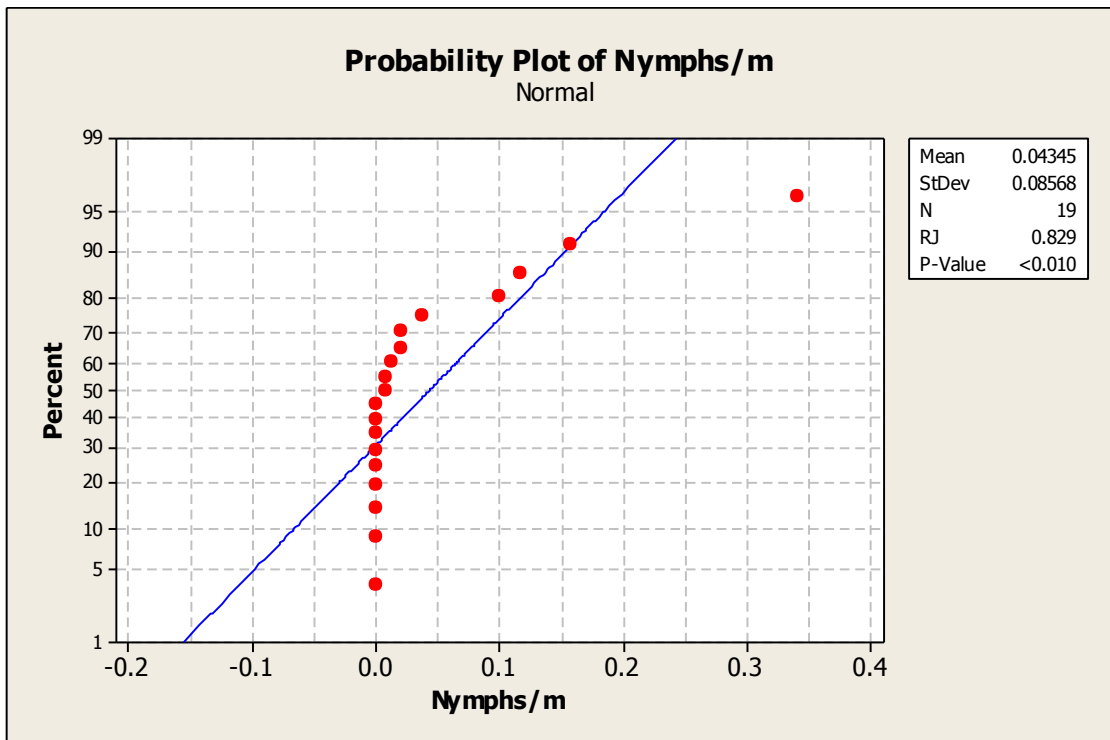
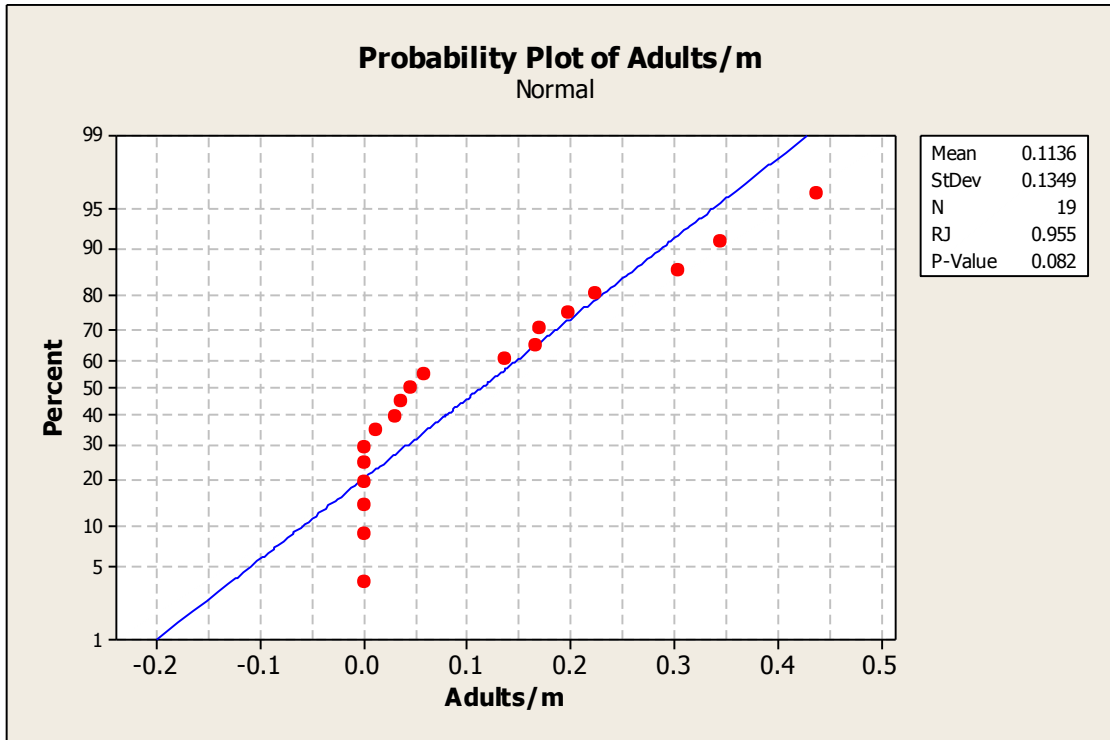
A.2 - Testing normality in the data

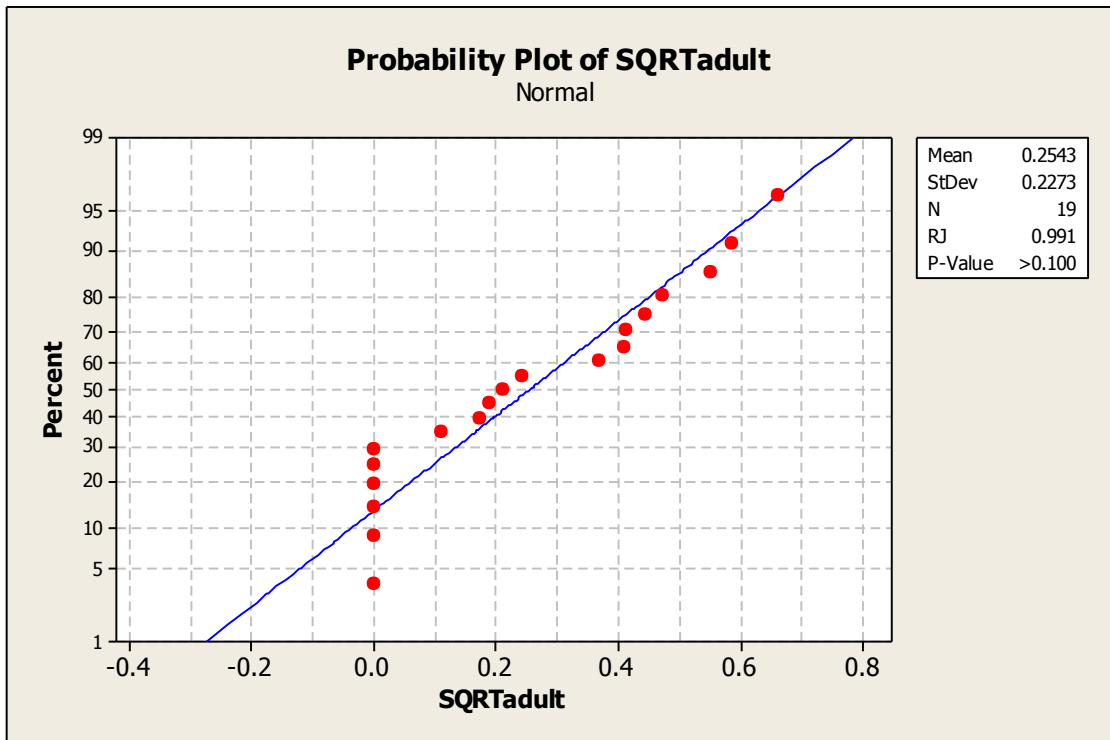
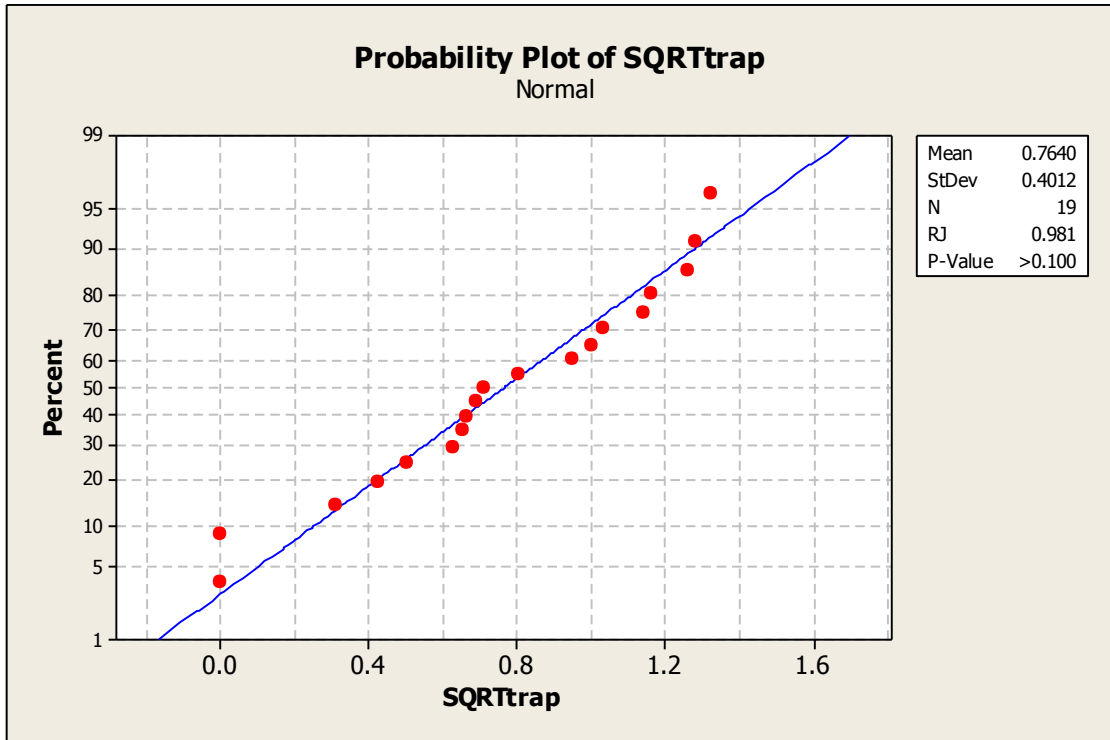
The test used is the Ryan-Joiner or Shapiro wilks test in MINITAB (Ryan *et al.* 1992).

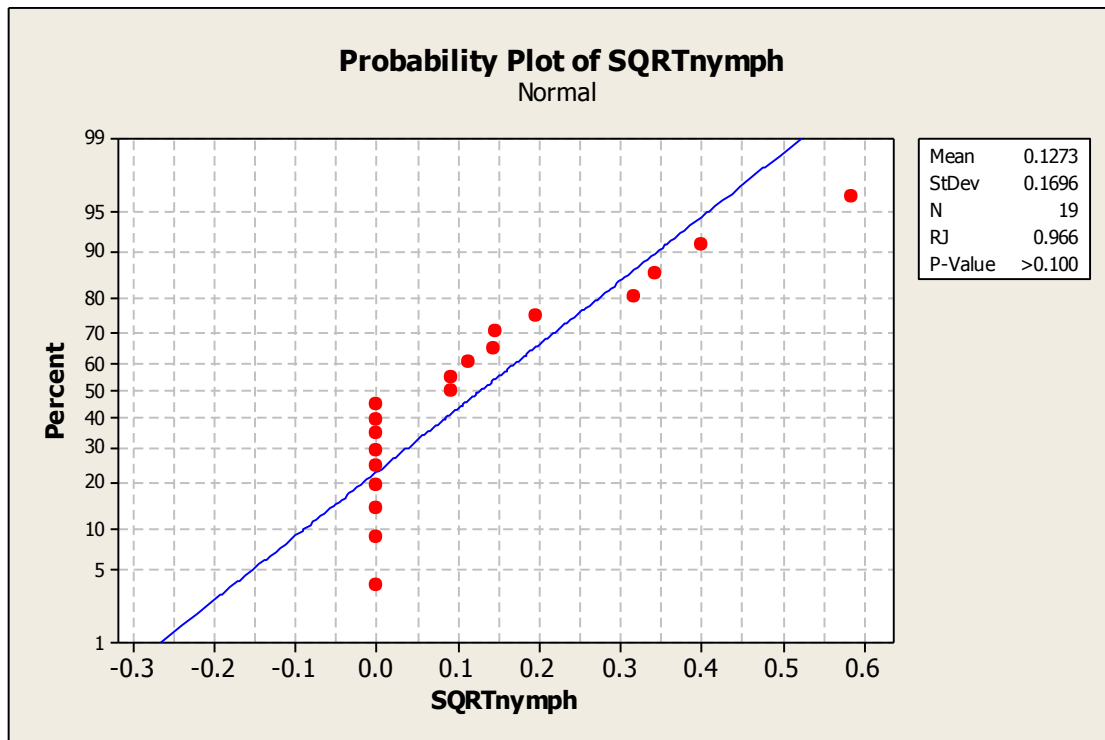
Normality was not founding the raw data so Square root transformations were used and tested. From the graphs it is evident the Square root transformation achieved normality allowing for statistical analyses to be conducted.

Note: Normality = $P > 0.100$









A.3 – Analysis of variance for Square root transformed data on Pheromone trap catch numbers versus adult numbers in the field

Regression Analysis: SQRTadults versus SQRTtrap

The regression equation is
 $SQRTadults = 0.155 + 0.084 SQRTtrap$

Predictor	Coef	SE Coef	T	P
Constant	0.15457	0.08269	1.87	0.071
SQRTtrap	0.0842	0.1332	0.63	0.532

S = 0.259707 R-Sq = 1.3% R-Sq(adj) = 0.0%

Analysis of Variance					
Source	DF	SS	MS	F	P
Regression	1	0.02696	0.02696	0.40	0.532
Residual Error	31	2.09089	0.06745		
Total	32	2.11785			

Unusual Observations

Obs	SQRTtrap	SQRTadults	Fit	SE Fit	Residual	St Resid
21	1.50	0.5000	0.2809	0.1382	0.2191	1.00 X
24	0.00	0.6538	0.1546	0.0827	0.4993	2.03R
30	0.78	0.7638	0.2204	0.0571	0.5433	2.14R

R denotes an observation with a large standardized residual.
 X denotes an observation whose X value gives it large influence

A.4 – Analysis of variance for Square root transformed data on Pheromone trap catch numbers due to nymph numbers in the field

Regression Analysis: SQRTnymphs versus SQRTtrap

The regression equation is
 $SQRTnymphs = 0.0848 - 0.0340 SQRTtrap$

Predictor	Coef	SE Coef	T	P
Constant	0.08479	0.05260	1.61	0.117
SQRTtrap	-0.03404	0.08474	-0.40	0.691

S = 0.165218 R-Sq = 0.5% R-Sq(adj) = 0.0%

Analysis of Variance					
Source	DF	SS	MS	F	P
Regression	1	0.00440	0.00440	0.16	0.691
Residual Error	31	0.84621	0.02730		
Total	32	0.85061			

Unusual Observations

Obs	SQRTtrap	SQRTnymphs	Fit	SE Fit	Residual	St Resid
21	1.50	0.0000	0.0337	0.0879	-0.0337	-0.24 X
23	0.43	0.6455	0.0700	0.0297	0.5754	3.54R
24	0.00	0.5583	0.0848	0.0526	0.4735	3.02R

R denotes an observation with a large standardized residual.
 X denotes an observation whose X value gives it large influence

B. Brigadoon, Boggabri: Regression Analysis Trap catches

B.1 - Data Display

Day	Mirids/trap/night SQRtnymph	Adults/m	Nymphs/m	SQRTrap	SQRAdult
23	0.000000 0.000000	0.000000	0.000000	0.000000	0.000000
27	0.125000 0.000000	0.000000	0.000000	0.353553	0.000000
30	0.166670 0.000000	0.000000	0.000000	0.408252	0.000000
33	0.333300 0.000000	0.000000	0.000000	0.577321	0.000000
37	0.000000 0.000000	0.000000	0.000000	0.000000	0.000000
39	0.000000 0.000000	0.000000	0.000000	0.000000	0.000000
44	0.050000 0.000000	0.000000	0.000000	0.223607	0.000000
47	0.166670 0.000000	0.000000	0.000000	0.408252	0.000000
50	0.250000 0.000000	0.000000	0.000000	0.500000	0.000000
53	0.500000 0.000000	0.000000	0.000000	0.707107	0.000000
57	0.285700 0.000000	0.000000	0.000000	0.534509	0.000000
62	0.250000 0.000000	0.000000	0.000000	0.500000	0.000000
65	0.468750 0.000000	0.069583	0.000000	0.684653	0.263787
68	0.291670 0.288675	0.139167	0.083333	0.540065	0.373050
72	0.142857 0.500000	0.139167	0.250000	0.377964	0.373050
76	0.093750 0.000000	0.000000	0.000000	0.306186	0.000000
79	0.214286 0.000000	0.000000	0.000000	0.462910	0.000000
82	0.291670 0.000000	0.069583	0.000000	0.540065	0.263787
85	0.083330 0.408248	0.236250	0.166667	0.288669	0.486056
89	0.392857 0.000000	0.000000	0.000000	0.626783	0.000000
92	0.428571 0.000000	0.139167	0.000000	0.654653	0.373050
96	0.607143 0.000000	0.069583	0.000000	0.779194	0.263787
100	0.062500 0.263523	0.237167	0.069444	0.250000	0.486998
103	0.107143 0.372678	0.175583	0.138889	0.327327	0.419027
107	0.107143 0.586302	0.227479	0.343750	0.327327	0.476948
112	0.055560 0.707107	0.875417	0.500000	0.235712	0.935637
115	0.062500 0.444878	0.185146	0.197917	0.250000	0.430286
118	0.000000 0.520416	0.294708	0.270833	0.000000	0.542870
121	0.041667 0.433013	0.123125	0.187500	0.204125	0.350892

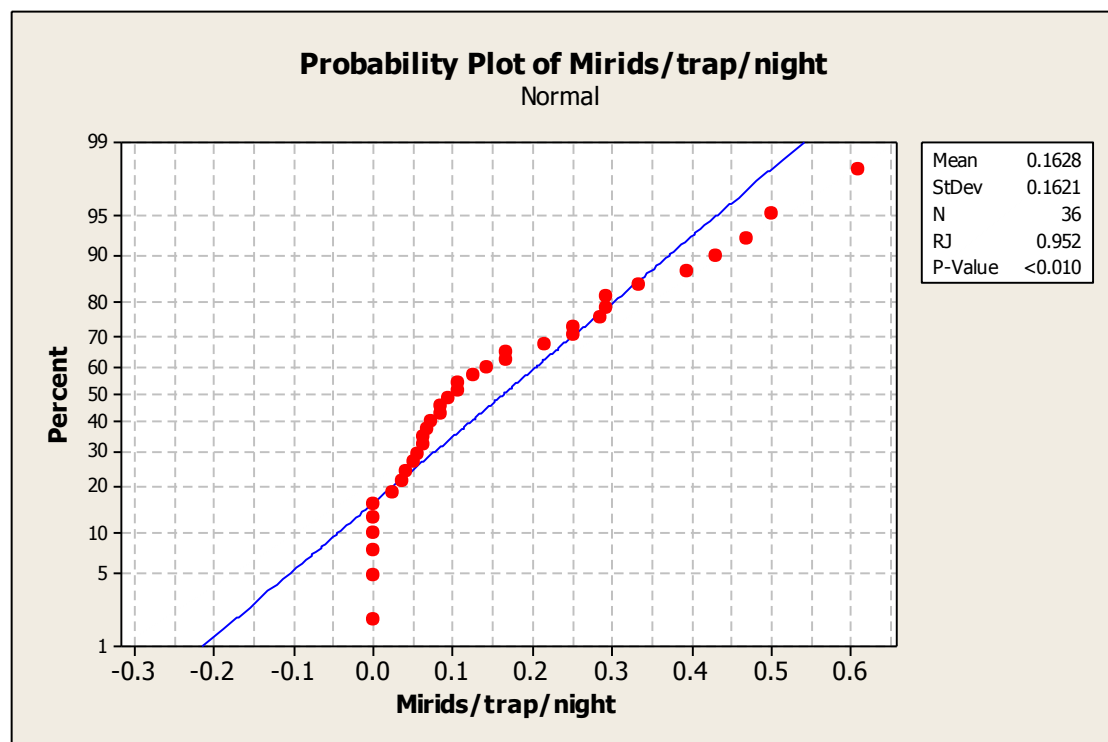
125	0.071429 0.707107	0.278333	0.500000	0.267262	0.527573
130	0.083330 0.707107	0.278333	0.500000	0.288669	0.527573
134	0.000000 0.707107	0.278333	0.500000	0.000000	0.527573
137	0.000000 0.408248	0.278333	0.166667	0.000000	0.527573
144	0.025000 0.000000	0.139167	0.000000	0.158114	0.373050
148	0.068182 0.000000	0.000000	0.000000	0.261117	0.000000
150	0.035714 0.000000	0.139167	0.000000	0.188981	0.373050

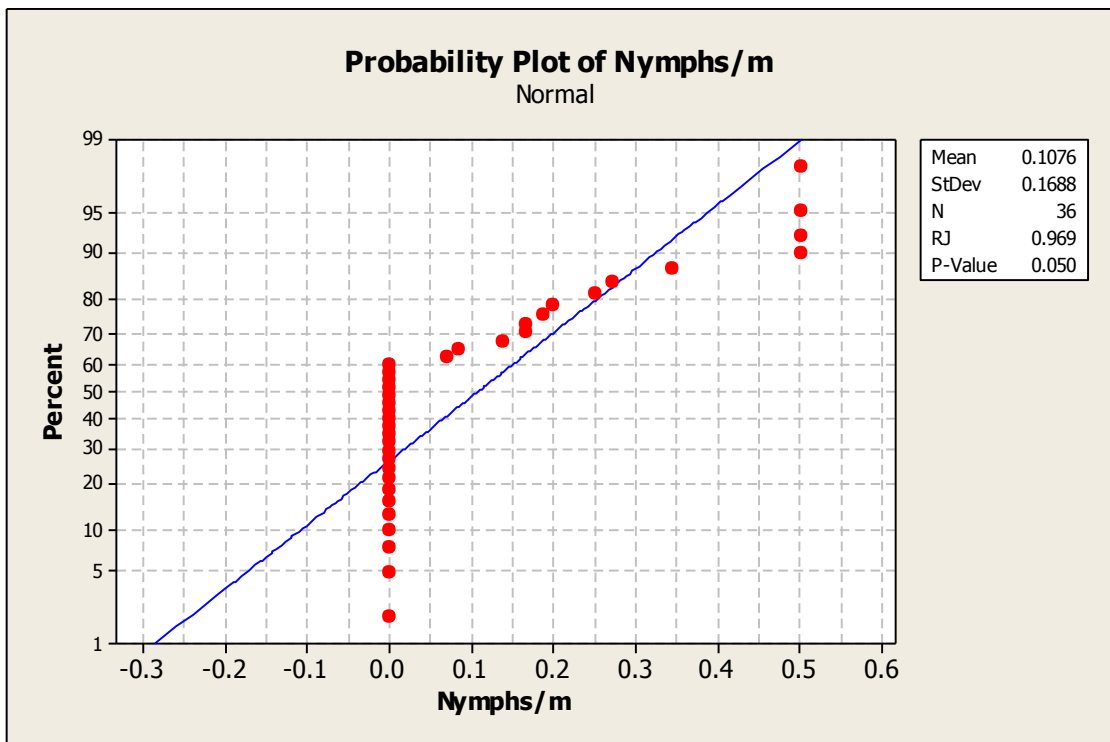
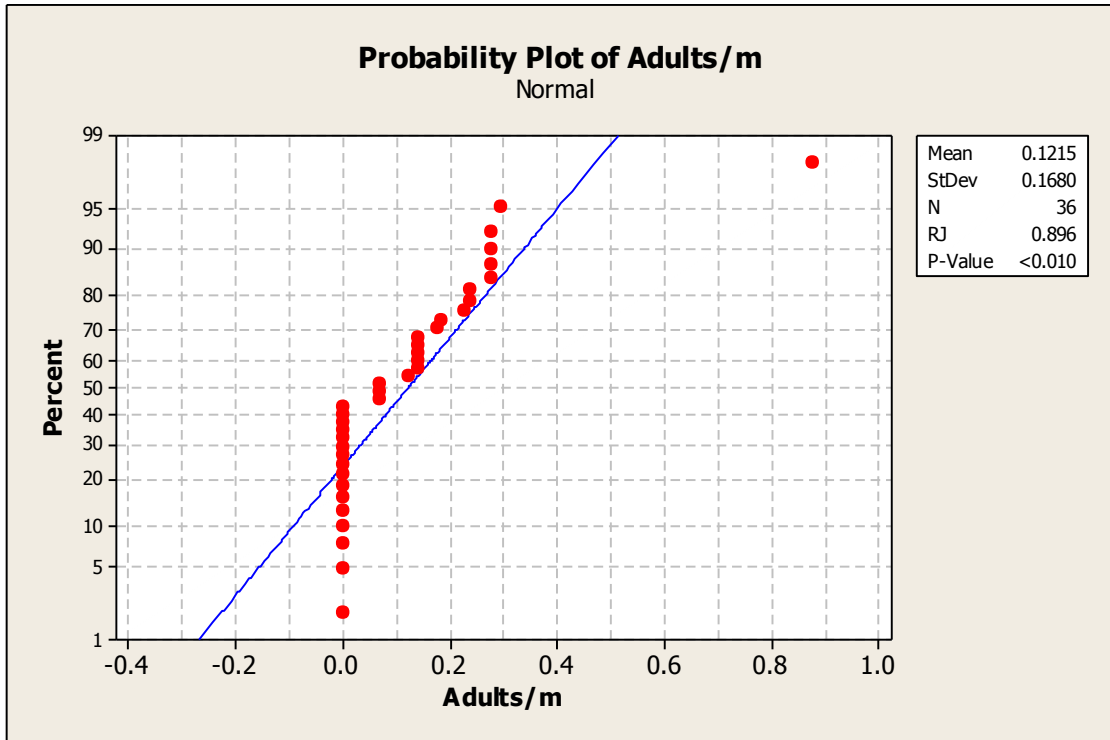
B.2 - Testing normality in the data

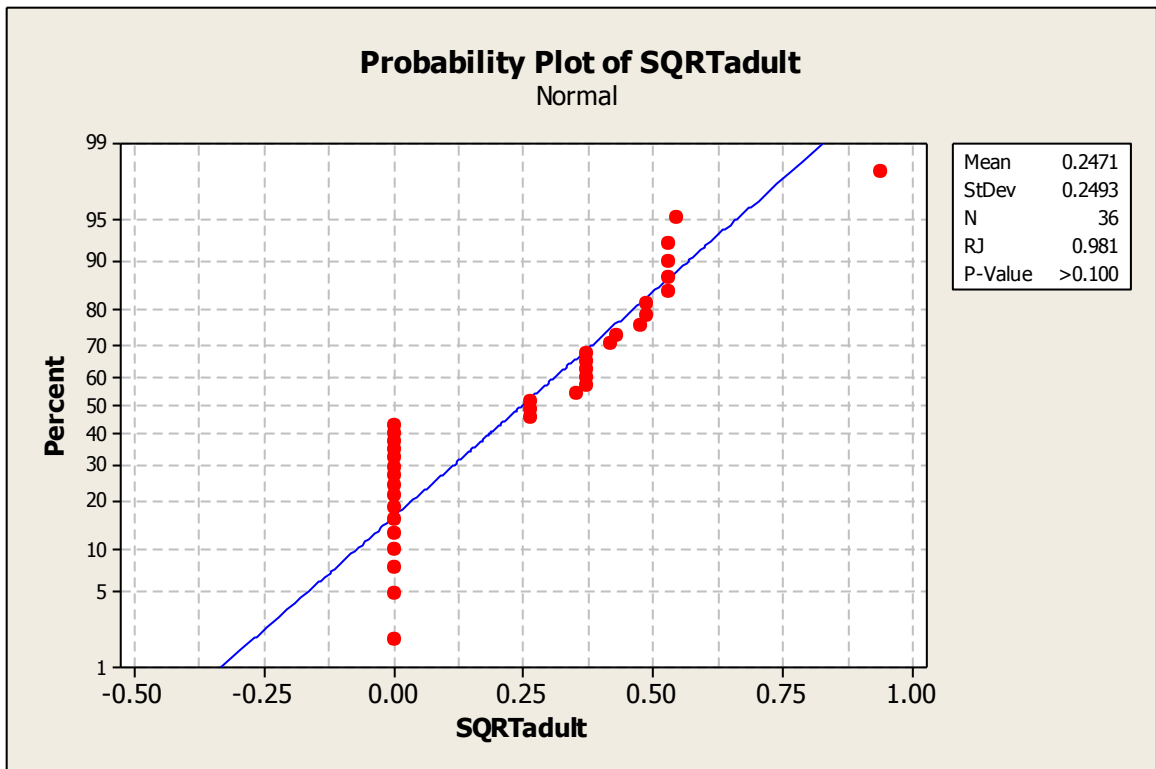
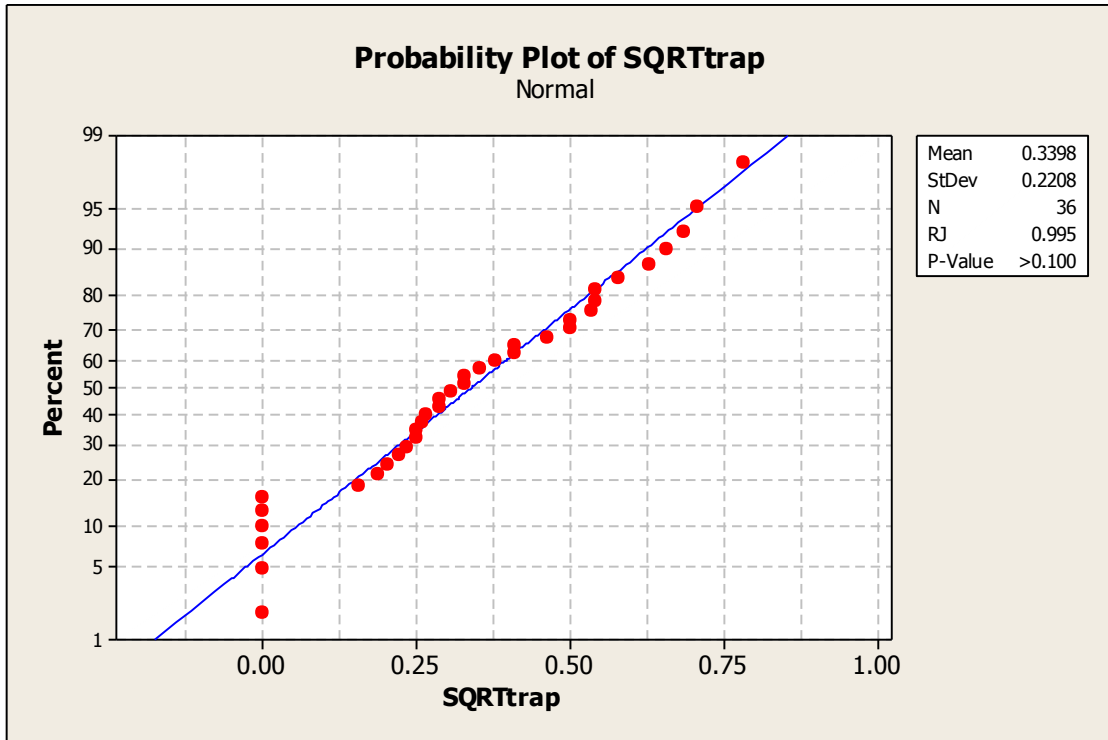
The test used is the Ryan-Joiner or Shapiro Wilks test in MINITAB (Ryan *et al.* 1992).

Normality was not found using the raw data so Square root transformations were tested. From the graphs it is evident the transformation achieved normality allowing for statistical analyses to be conducted.

Note: normality = $P > 0.100$

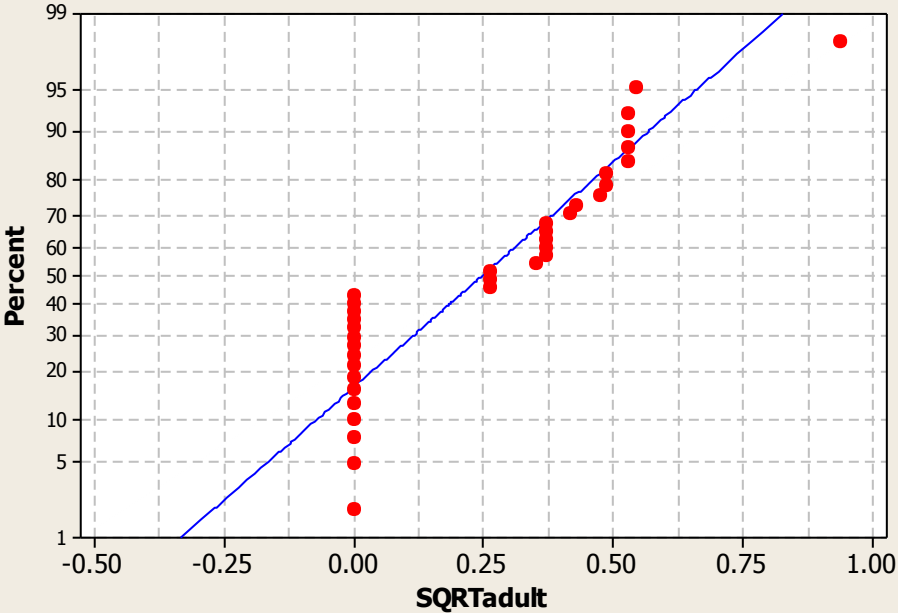






Probability Plot of SQRTadult

Normal



B.3 – Analysis of variance for transformed data on Pheromone trap catch numbers versus adult numbers in the field

Regression Analysis: SQRTtrap versus SQRTadult

The regression equation is
 $SQRT_{trap} = 0.400 - 0.243 SQRT_{adult}$

Predictor	Coef	SE Coef	T	P
Constant	0.39995	0.05090	7.86	0.000
SQRTadult	-0.2435	0.1460	-1.67	0.105

S = 0.215370 R-Sq = 7.6% R-Sq(adj) = 4.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.12892	0.12892	2.78	0.105
Residual Error	34	1.57706	0.04638		
Total	35	1.70597			

Unusual Observations

Obs	SQRTadult	SQRTtrap	Fit	SE Fit	Residual	St Resid
22	0.264	0.7792	0.3357	0.0360	0.4435	2.09R
26	0.936	0.2357	0.1721	0.1068	0.0636	0.34 X

R denotes an observation with a large standardized residual.
 X denotes an observation whose X value gives it large influence.

B.4 – Analysis of variance for transformed data on Pheromone trap catch numbers versus nymph numbers in the field

Regression Analysis: SQRTtrap versus SQRTnymph

The regression equation is
 $SQRT_{trap} = 0.402 - 0.319 SQRT_{nymph}$

Predictor	Coef	SE Coef	T	P
Constant	0.40229	0.04295	9.37	0.000
SQRTnymph	-0.3190	0.1309	-2.44	0.020

S = 0.206679 R-Sq = 14.9% R-Sq(adj) = 12.4%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.25362	0.25362	5.94	0.020
Residual Error	34	1.45235	0.04272		
Total	35	1.70597			

C. KOROLEA, GOONDIWINDI : Regression Analysis Trap catches

C.1 Data Display

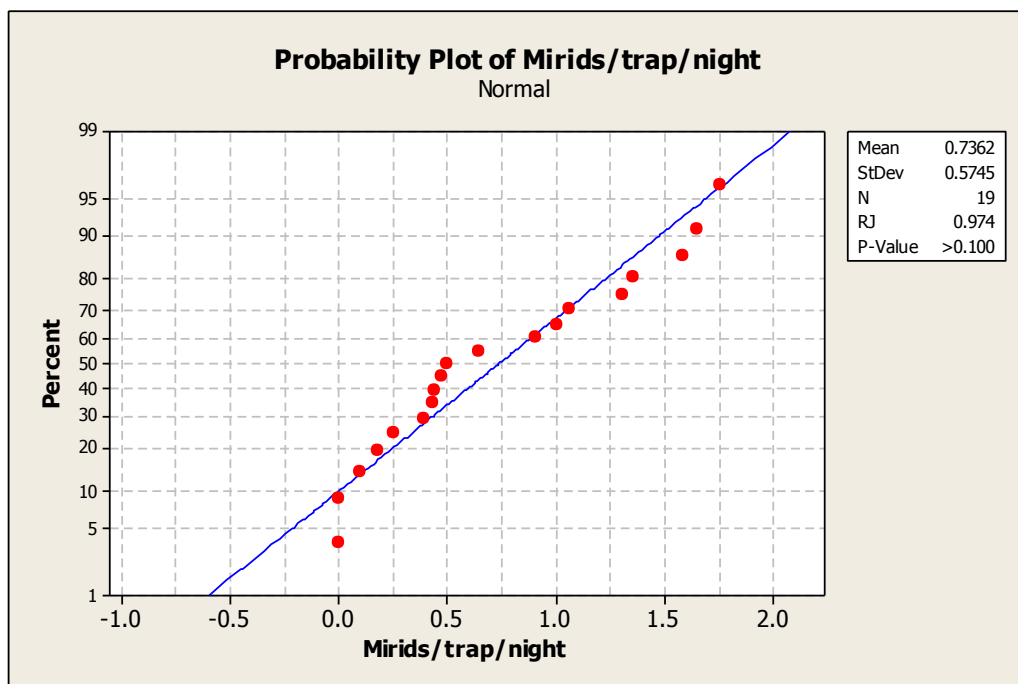
Day	Mirids/trap/night SQRTnymph	Adults/m	Nymphs/m	SQRTAdult	
0	0.00000	0.000000	0.000000	0.000000	0.000000
43	0.00000	0.000000	0.000000	0.000000	0.000000
50	0.42857	0.000000	0.000000	0.000000	0.000000
55	0.90000	0.000000	0.000000	0.000000	0.000000
57	1.00000	0.166667	0.000000	0.408248	0.000000
64	0.64286	0.135417	0.100000	0.367990	0.316228
68	1.06250	0.437500	0.116667	0.661438	0.341565
71	1.58333	0.343750	0.341667	0.586302	0.584523
79	0.43750	0.222917	0.008333	0.472141	0.091287
81	1.75000	0.169792	0.008333	0.412058	0.091287
88	1.64286	0.303125	0.158333	0.550568	0.397911
93	1.35000	0.000000	0.037778	0.000000	0.194365
105	1.30000	0.030556	0.021111	0.174801	0.145297
114	0.47222	0.044444	0.000000	0.210819	0.000000
121	0.39286	0.012500	0.000000	0.111803	0.000000
134	0.09615	0.000000	0.000000	0.000000	0.000000
140	0.50000	0.035417	0.012500	0.188193	0.111803
148	0.25000	0.058333	0.000000	0.241523	0.000000

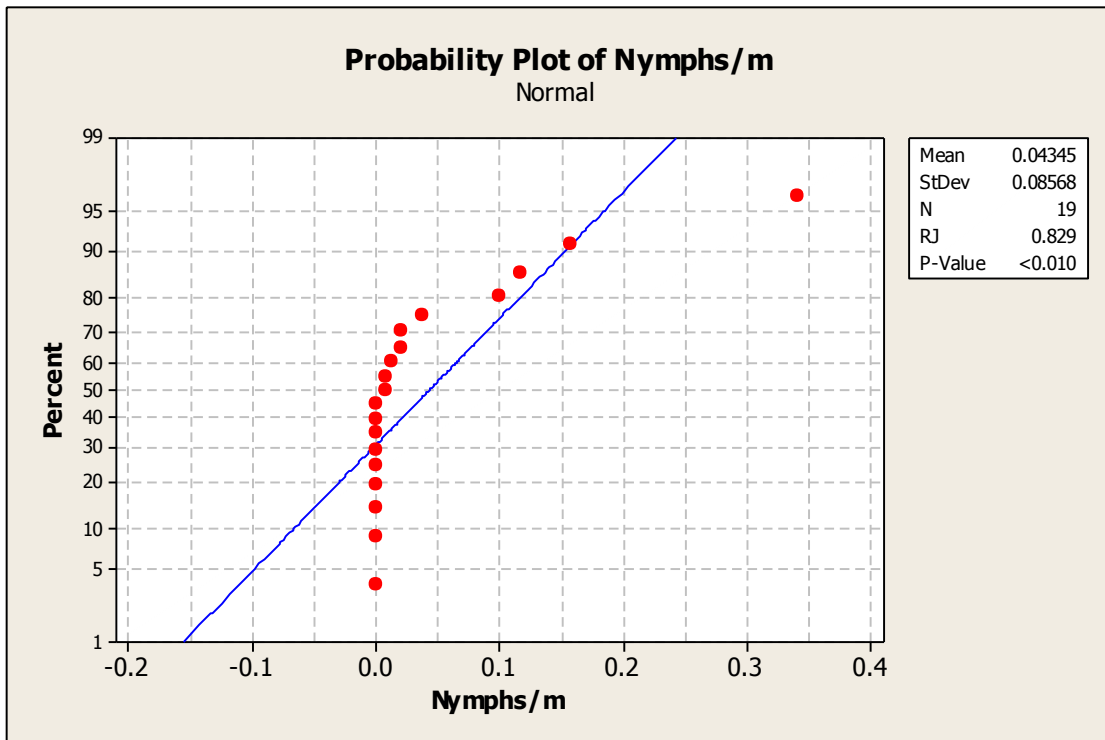
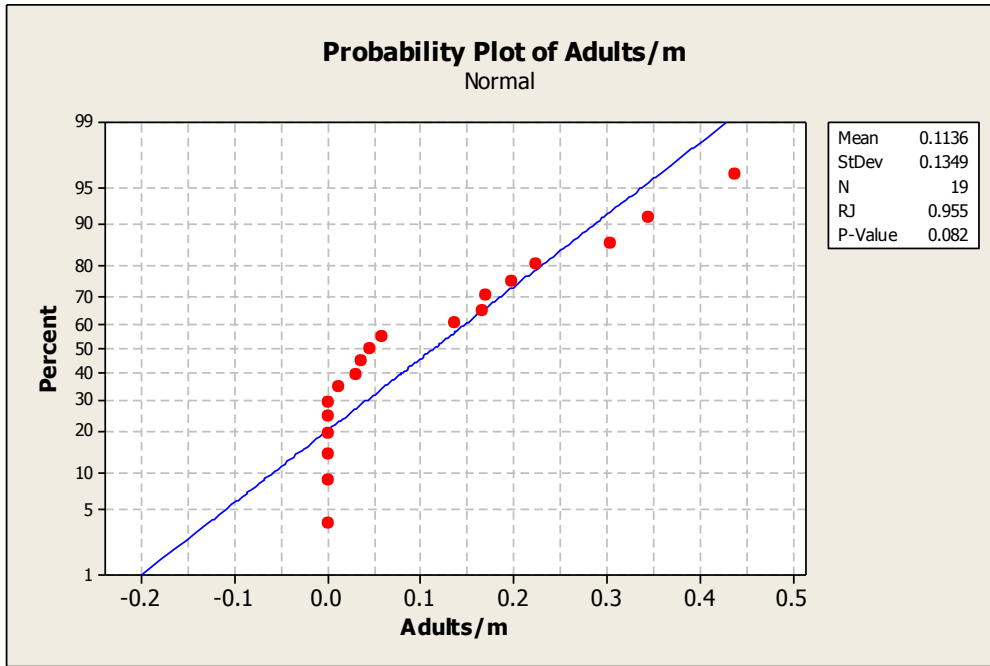
C.2 - Testing normality in the data

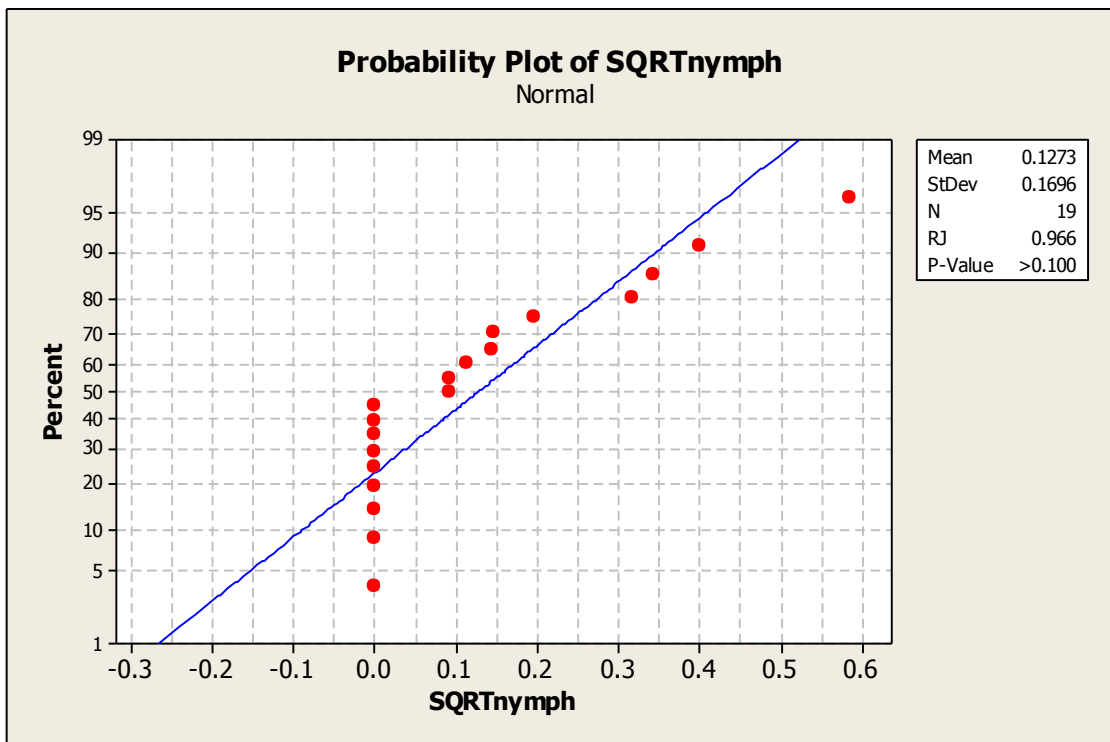
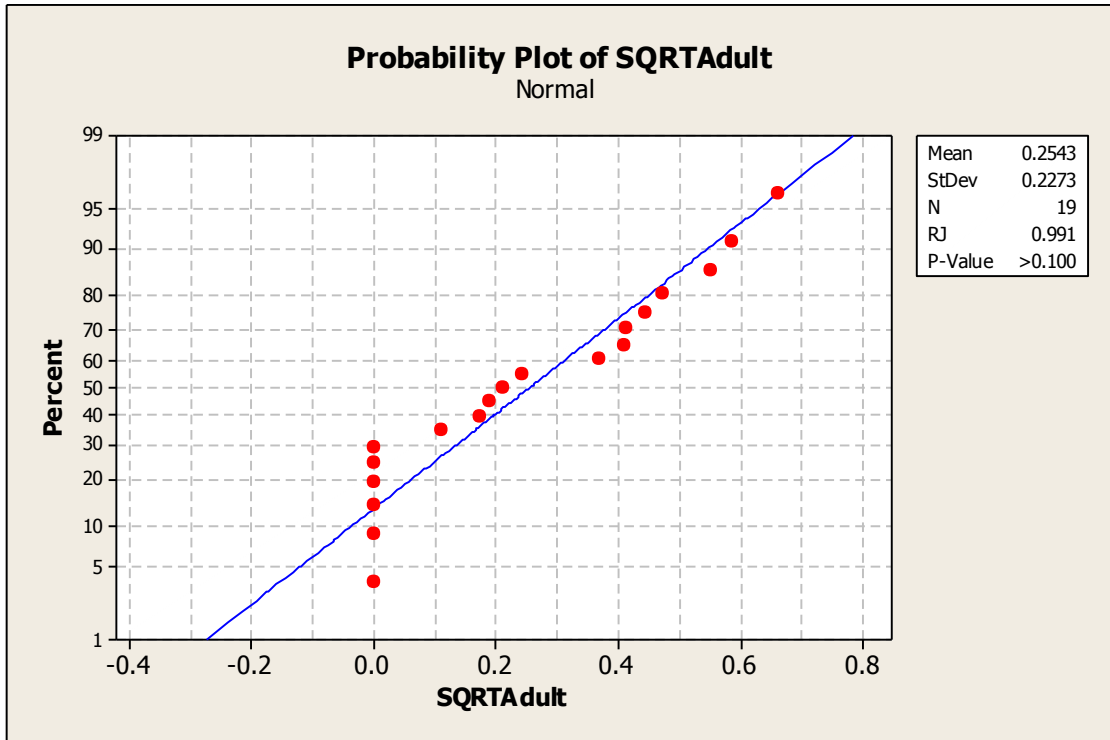
The test used is the Ryan-Joiner or Shapiro Wilks test in MINITAB (Ryan *et al.* 1992).

Normality was only found using the raw data for Trap data so Square root transformations were tested until normality on the number of adults and nymphs sampled . From the graphs it is evident the square root transformation achieved normality for adults and nymphs. Achieving normality allows for statistical analyses to be conducted.

Note: Normality = $P > 0.100$







C.3 – Analysis of variance for Log transformed data on Pheromone trap catch numbers versus adult numbers in the field

Regression Analysis: Mirids/trap/night versus SQRTAdult

The regression equation is
 Mirids/trap/night = 0.278 + 1.80 SQRTAdult

Predictor	Coef	SE Coef	T	P
Constant	0.2776	0.1447	1.92	0.072
SQRTAdult	1.8036	0.4295	4.20	0.001

S = 0.414181 R-Sq = 50.9% R-Sq(adj) = 48.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	3.0256	3.0256	17.64	0.001
Residual Error	17	2.9163	0.1715		
Total	18	5.9419			

C.4 – Analysis of variance for transformed data on Pheromone trap catch numbers versus nymph numbers in the field

Regression Analysis: Mirids/trap/night versus SQRTnymph

The regression equation is
 Mirids/trap/night = 0.481 + 2.01 SQRTnymph

Predictor	Coef	SE Coef	T	P
Constant	0.4807	0.1380	3.48	0.003
SQRTnymph	2.0069	0.6620	3.03	0.008

S = 0.476315 R-Sq = 35.1% R-Sq(adj) = 31.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	2.0850	2.0850	9.19	0.008
Residual Error	17	3.8569	0.2269		
Total	18	5.9419			

Unusual Observations

Obs	SQRTnymph	Mirids/trap/night	Fit	SE Fit	Residual	St Resid
8	0.585	1.583	1.654	0.322	-0.070	-0.20 X
10	0.091	1.750	0.664	0.112	1.086	2.35R

D. Summary of analyses

SUMMARY (Pheromone catches vs field mirids)

<u>Location</u>	<u>Adults</u>	<u>Nymphs</u>
Auscott	ns (0.532)	ns (0.691)
Brigadoon	p=0.105	p=0.02
Korolea	p=0.001	p=0.008

Appendix 4:

Statistical analyses and methodology for Aim 2, Study 2

Pheromone trap catches vs % females (sex ratio)

Question 2: Are pheromone trap catches associated with the percentage of females in the field population (sex ratio of mirids)?

A. Auscott, Narrabri: Analysis

A.1 - Data Display

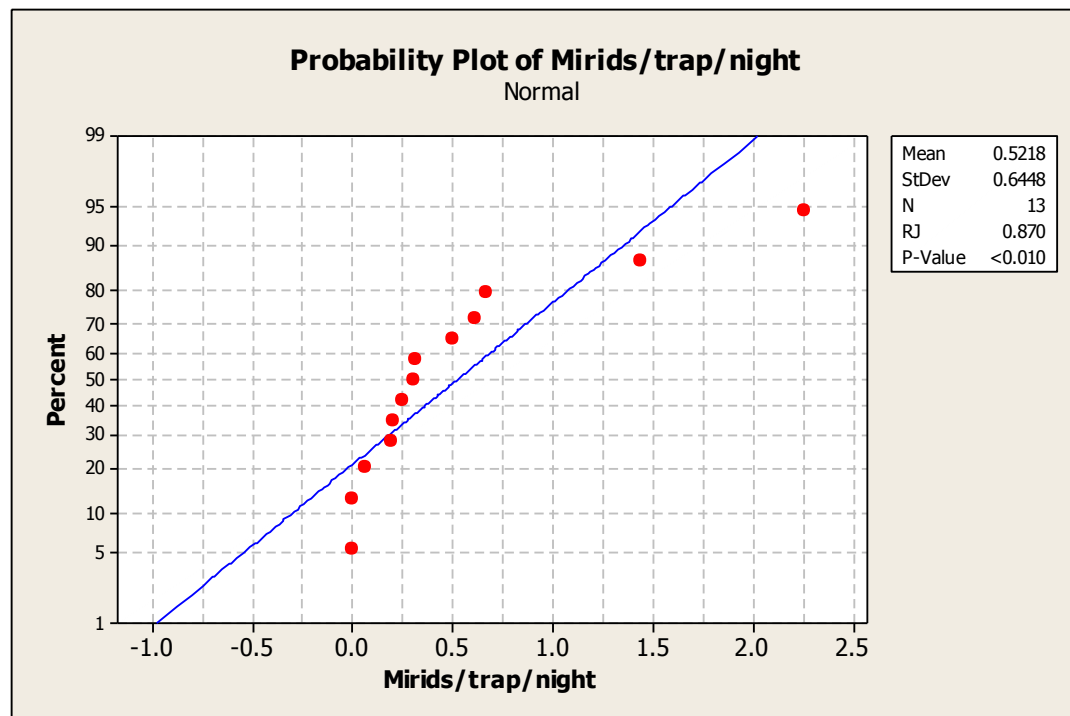
Day	Mirids/trap/night	%females	SQRTPhero
74	0.00000	1.00	0.00000
81	0.50000	1.00	0.70711
85	0.31250	1.00	0.55902
89	1.43750	1.00	1.19896
93	2.25000	0.33	1.50000
96	0.66700	0.50	0.81670
100	0.18750	0.50	0.43301
103	0.00000	0.50	0.00000
108	0.20000	1.00	0.44721
117	0.30556	0.25	0.55277
124	0.25000	0.20	0.50000
131	0.61110	0.40	0.78173
148	0.06250	0.40	0.25000

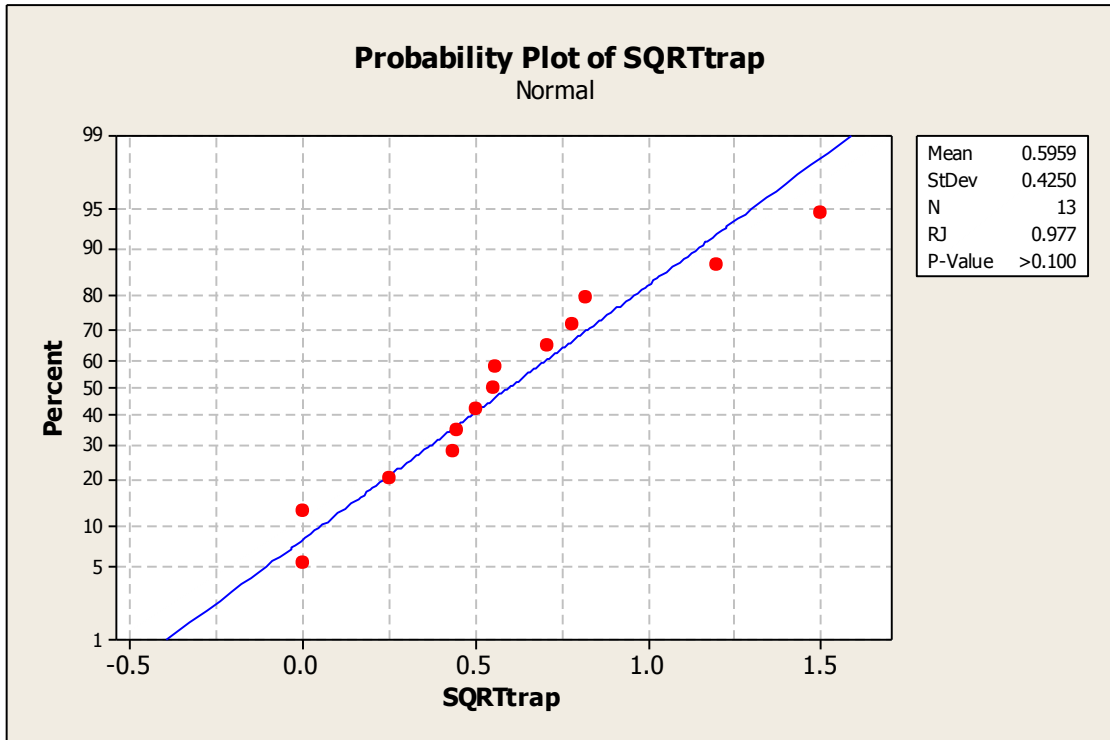
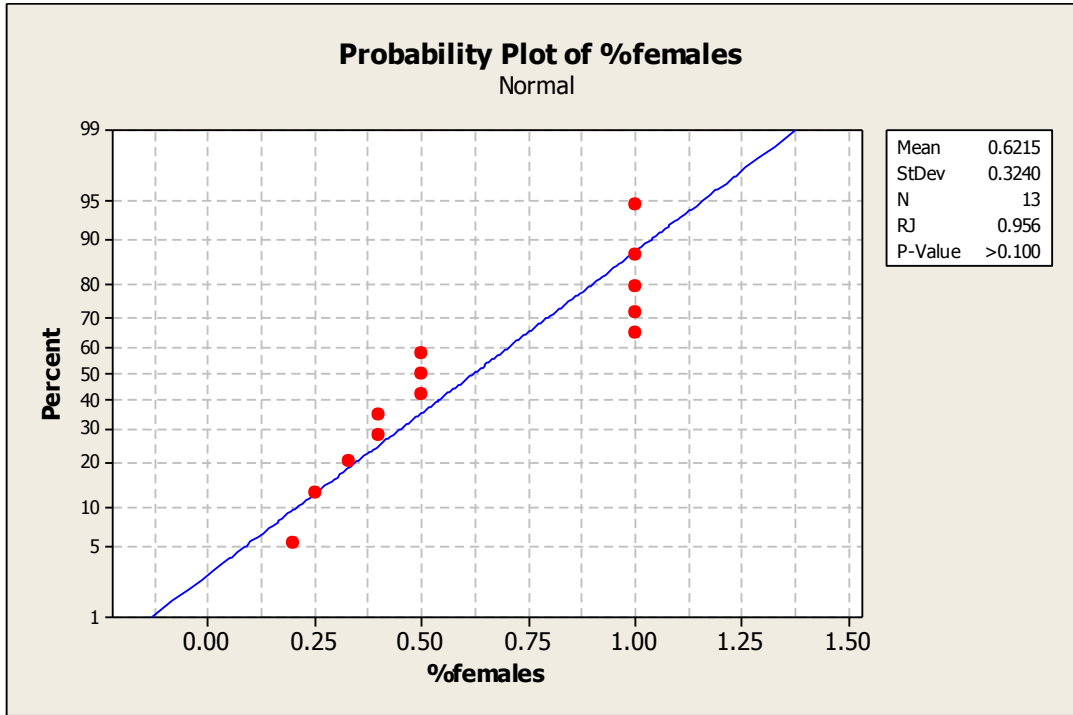
A.2 – Normality tests

The test used is the Ryan-Joiner or Shapiro wilks test in MINITAB (Ryan *et al.* 1992).

Normality was only found in the raw data for %females, so Square root transformations were used and tested for pheromone trap data. From the graphs it is evident the Square root transformation achieved normality allowing for statistical analyses to be conducted.

Note: Normality = $P > 0.100$





A.3 – Analysis of variance for transformed data on Pheromone trap catch numbers versus % of females in the field population

Regression Analysis: SQRTtrap versus %females

The regression equation is
SQRTtrap = 0.661 - 0.105 %females

Predictor	Coef	SE Coef	T	P
Constant	0.6609	0.2741	2.41	0.035
%females	-0.1046	0.3943	-0.27	0.796

S = 0.442498 R-Sq = 0.6% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.0138	0.0138	0.07	0.796
Residual Error	11	2.1538	0.1958		
Total	12	2.1676			

Unusual Observations

Obs	%females	SQRTtrap	Fit	SE Fit	Residual	St Resid
5	0.33	1.500	0.626	0.168	0.874	2.13R

R denotes an observation with a large standardized residual.

B. Brigadoon, Boggabri: Analysis

B.1 - Data Display

Data Display

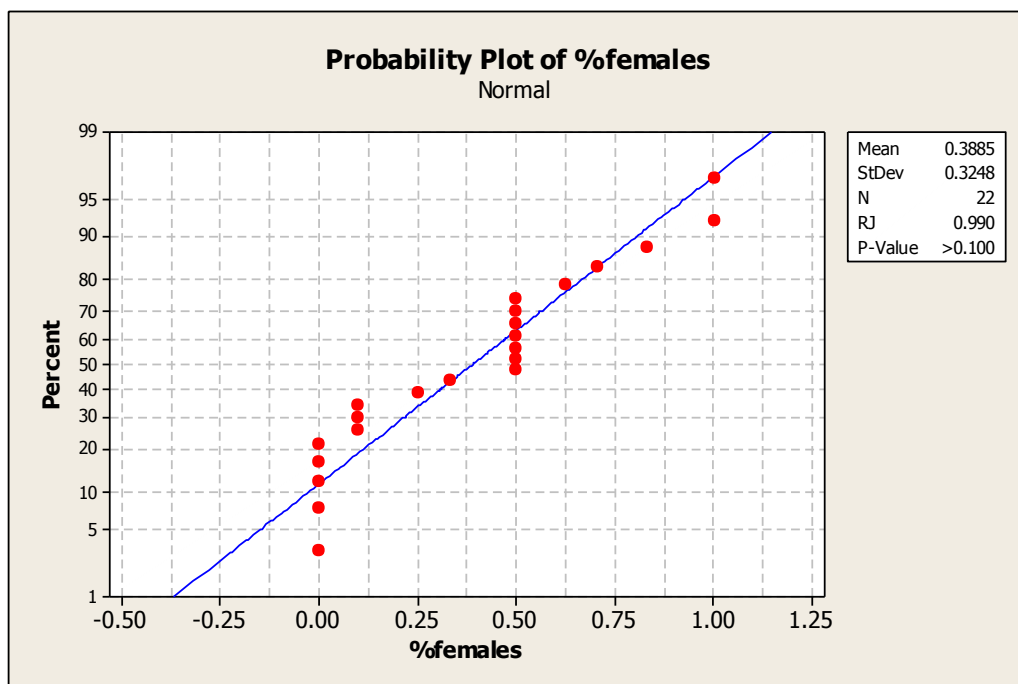
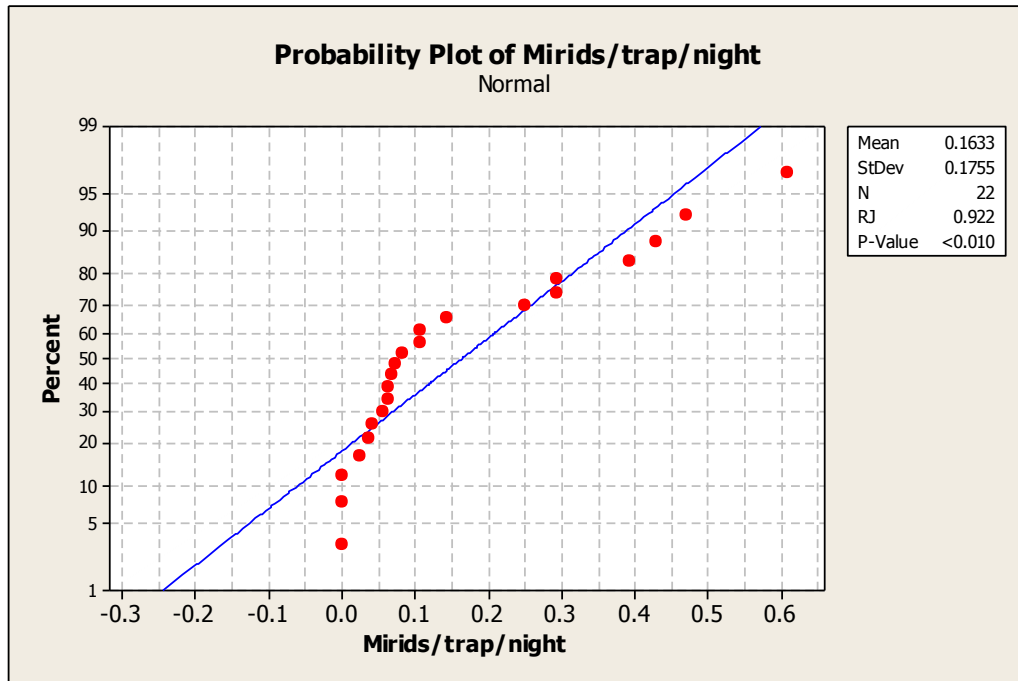
Row	Day	% females	Pheromone	Phero sqrt
1	65	100.000	0.468750	0.684653
2	68	100.000	0.291670	0.540065
3	72	100.000	0.142857	0.377964
4	85	33.333	0.083330	0.288669
5	92	50.000	0.428571	0.654653
6	96	25.000	0.607143	0.779194
7	100	50.000	0.062500	0.250000
8	103	83.333	0.107143	0.327327
9	107	70.588	0.107143	0.327327
10	112	33.333	0.055560	0.235712
11	115	62.500	0.062500	0.250000
12	118	50.000	0.000000	0.000000
13	121	50.000	0.041667	0.204125
14	125	50.000	0.071429	0.267262
15	130	50.000	0.083330	0.288669
16	134	50.000	0.000000	0.000000
17	137	100.000	0.000000	0.000000
18	144	50.000	0.025000	0.158114
19	150	100.000	0.035714	0.188981

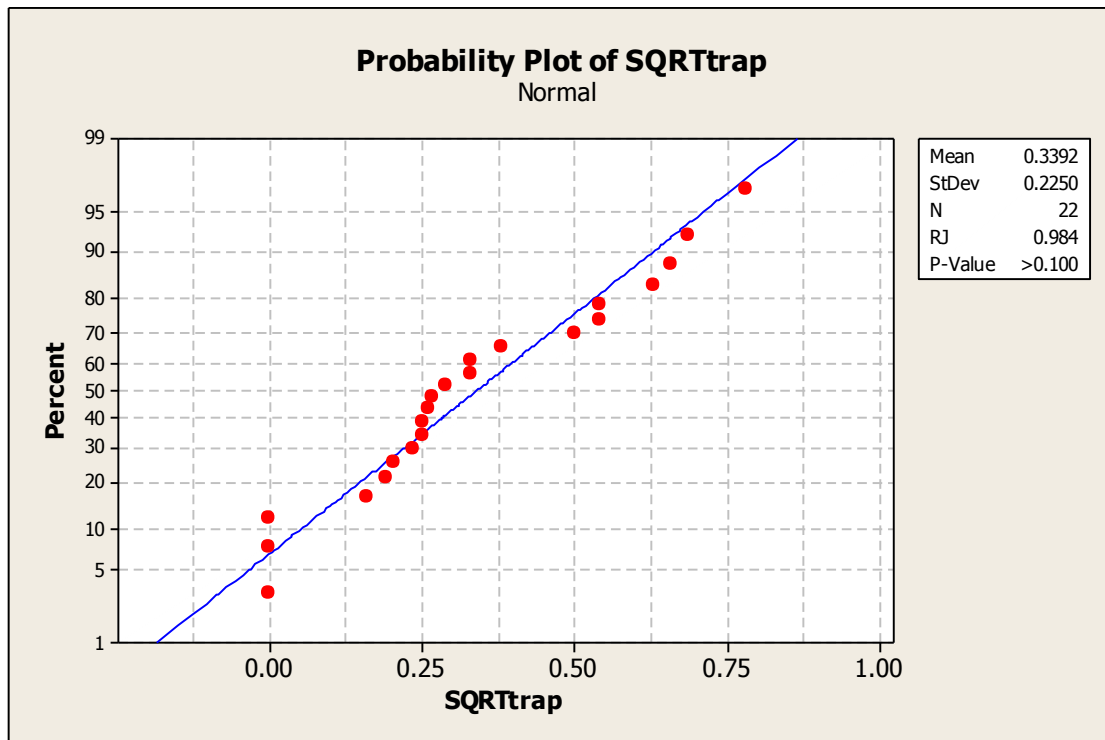
B.2 – Normality tests

The test used is the Ryan-Joiner or Shapiro wilks test in MINITAB (Ryan *et al.* 1992).

Normality was only found for the % of mated females in the raw data so Square root transformations were used and tested for the pheromone trap catch numbers. From the graphs it is evident the square root transformation achieved normality allowing for statistical analyses to be conducted.

Note: Normality = P-value >0.100





B.3 – Analysis of variance for transformed data on Pheromone trap catch numbers versus % of females in the field population

Regression Analysis: SQRTPhero versus %females

The regression equation is
 Phero sqrt = 0.290 + 0.00026 % females

Predictor	Coef	SE Coef	T	P
Constant	0.2901	0.1434	2.02	0.059
% females	0.000257	0.002099	0.12	0.904

S = 0.228692 R-Sq = 0.1% R-Sq(adj) = 0.0%

Analysis of Variance					
Source	DF	SS	MS	F	P
Regression	1	0.00078	0.00078	0.01	<u>0.904</u>
Residual Error	17	0.88910	0.05230		
Total	18	0.88988			

Unusual Observations

Obs	% females	Phero sqrt	Fit	SE Fit	Residual	St Resid
6	25	0.7792	0.2966	0.0965	0.4826	2.33R

R denotes an observation with a large standardized residual

C. Korolea, Goondiwindi: Analysis

C.1 - Data Display

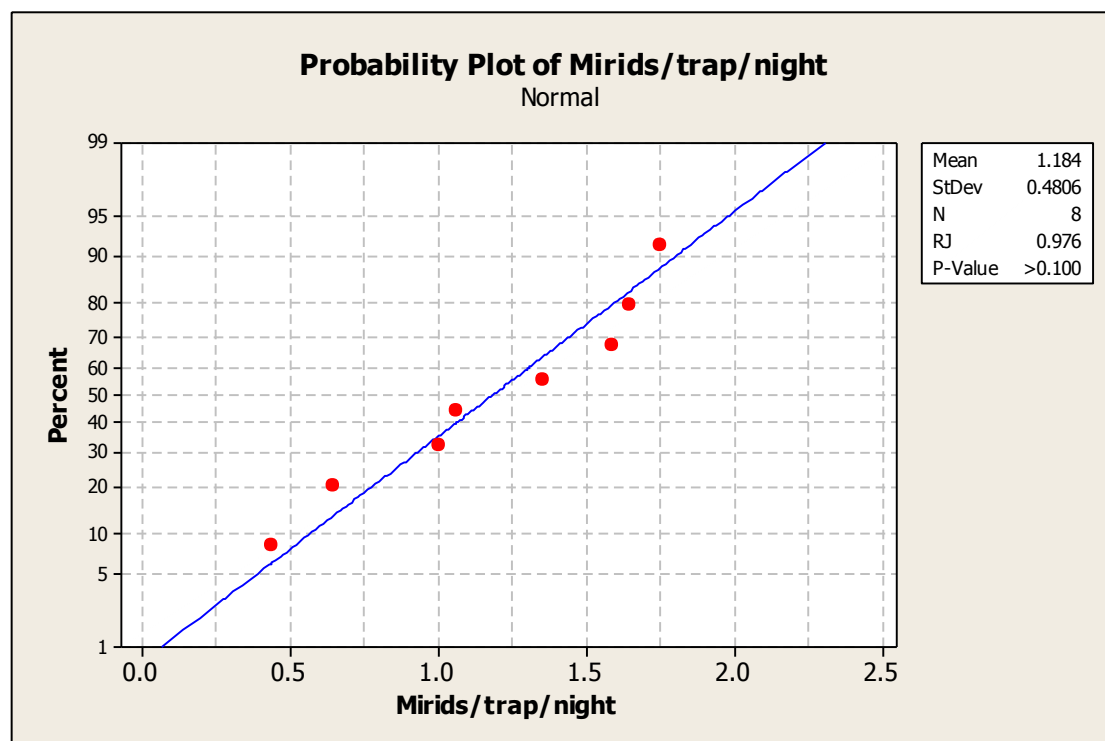
Mirids/trap/night	%females
1.00000	0.4000
0.64286	0.5000
1.06250	0.2500
1.58333	0.5454
0.43750	0.4838
1.75000	0.3571
1.64286	0.7647
1.35000	0.6000

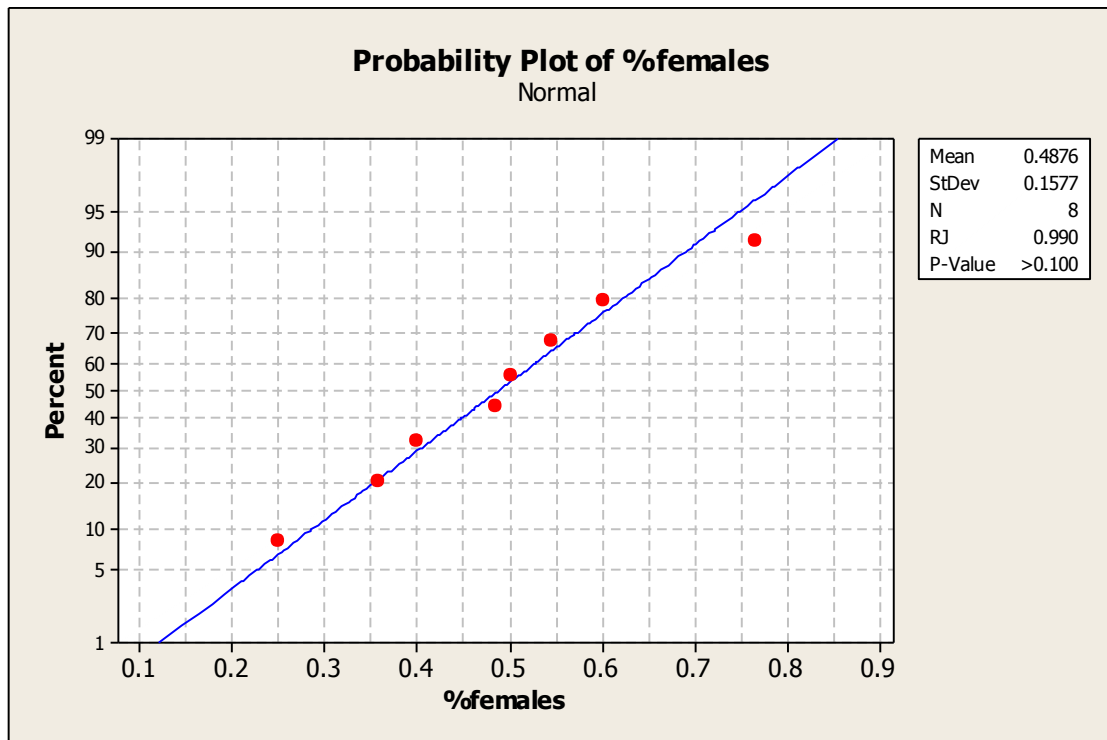
C.2 – Normality tests

The test used is the Ryan-Joiner or Shapiro wilks test in MINITAB (Ryan *et al.* 1992).

Normality was found for the raw data so no transformations were used. From the graphs it is evident that normality was achieved therefore allowing for statistical analyses to be conducted.

Note: Normality = P-value >0.100





C.3 – Analysis of variance for data on Pheromone trap catch numbers versus % of females in the field population

Regression Analysis: %females versus Mirids/trap/night

The regression equation is
 $\%females = 0.388 + 0.084 \text{ Mirids/trap/night}$

Predictor	Coef	SE Coef	T	P
Constant	0.3880	0.1639	2.37	0.056
Mirids/trap/night	0.0842	0.1295	0.65	0.540

S = 0.164631 R-Sq = 6.6% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.01146	0.01146	0.42	0.540
Residual Error	6	0.16262	0.02710		
Total	7	0.17408			

Appendix 5:

Statistical analyses and methodology for Aim 3, Study 3

Pheromone trap catches versus mated females

Question 3: Are pheromone trap catches associated with the mated status of females in the field population?

A. Auscott, Narrabri: Analysis

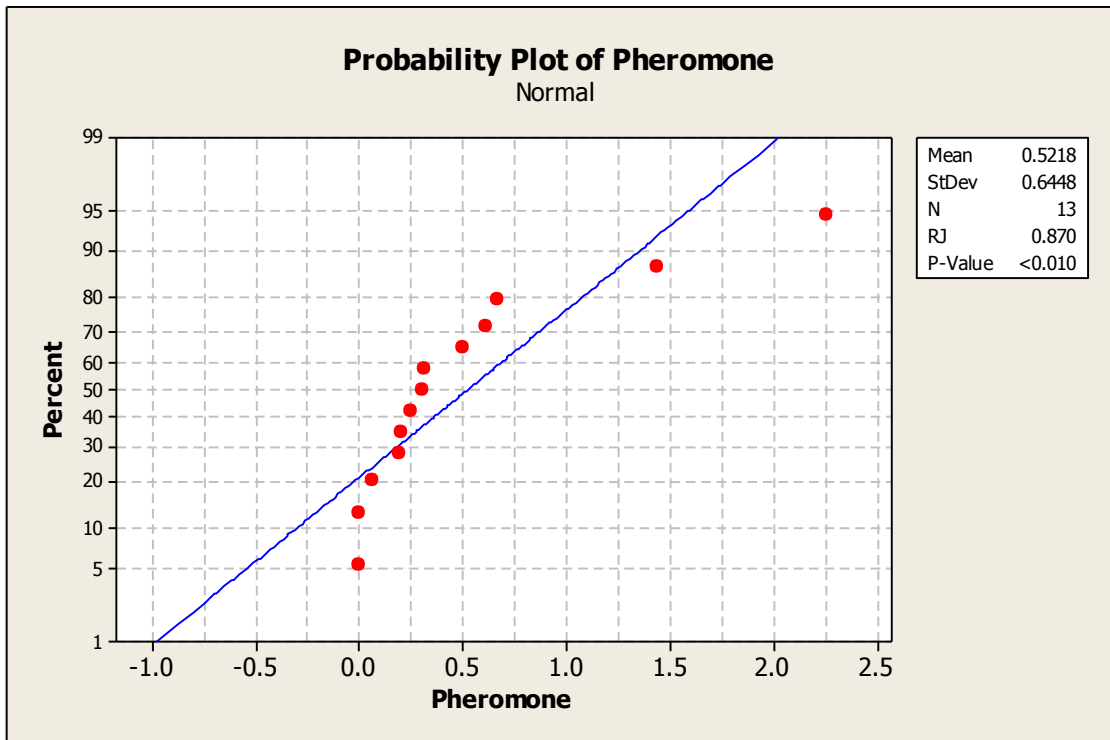
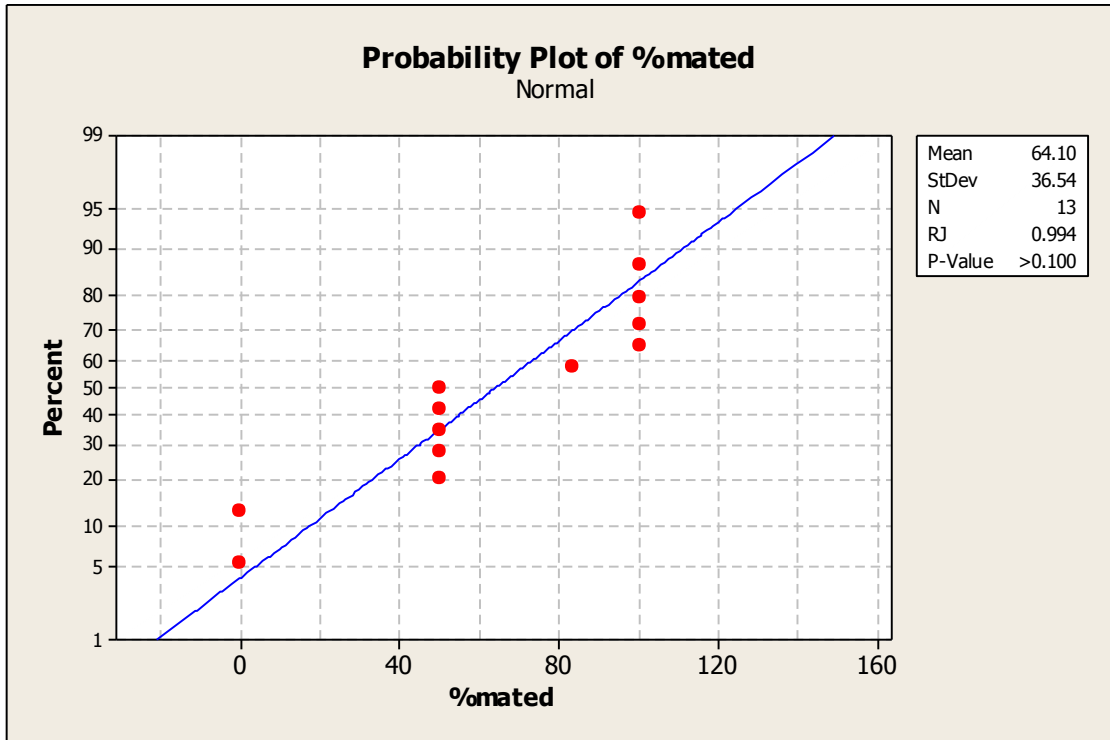
A.1 - Data Display

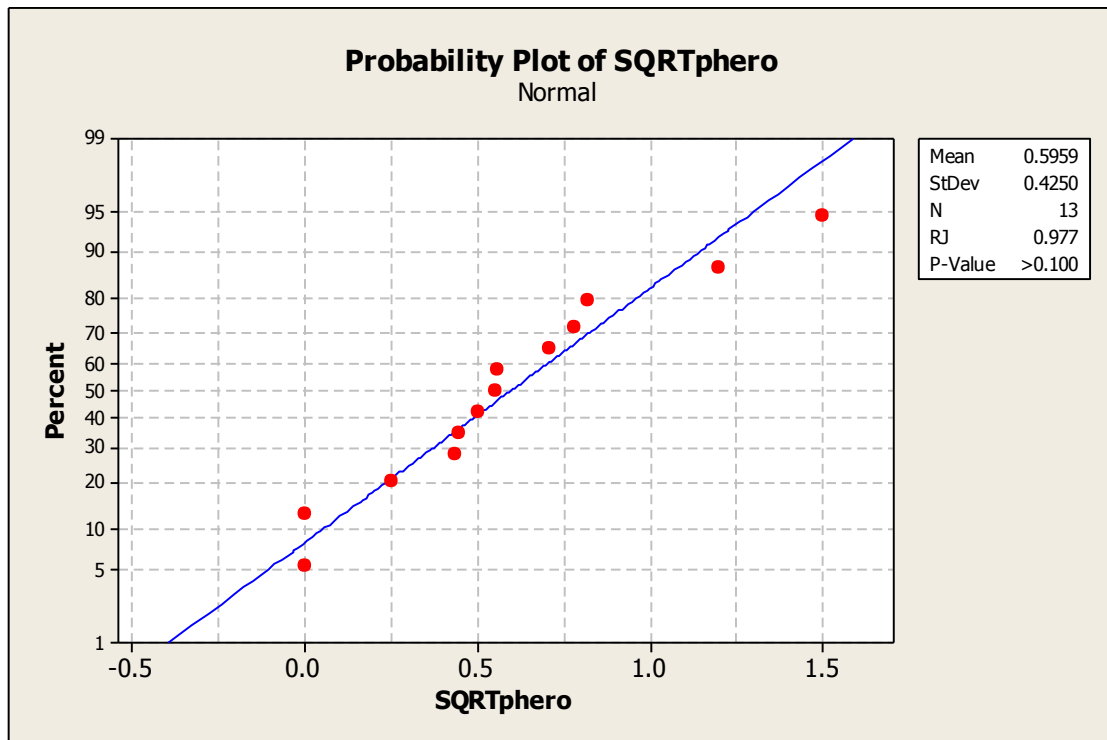
Day	% mated	Pheromone
74	100	0
81	0	0.5
85	50	0.3125
89	100	1.4375
93	0	2.25
96	100	0.667
100	50	0.1875
103	83.33333	0
108	100	0.2
117	100	0.30556
124	50	0.25
131	50	0.6111
148	50	0.0625

A.2 – Normality tests

The test used is the Ryan-Joiner or Shapiro wilks test in MINITAB (Ryan *et al.* 1992).

Normality was only found in the raw data for the % mated data, so Square root transformations were tested for the trap dtat. From the graphs it is evident the Square root transformation achieved normality allowing for statistical analyses to be conducted.





A.3 – Analysis of variance for Log transformed data on Pheromone trap catch numbers versus % of females mated in the field

Regression Analysis: %mated versus SQRTphero

The regression equation is
 $\%mated = 83.0 - 31.7 \text{ SQRTphero}$

Predictor	Coef	SE Coef	T	P
Constant	83.02	17.40	4.77	0.001
SQRTphero	-31.75	24.09	-1.32	0.214

S = 35.4718 R-Sq = 13.6% R-Sq(adj) = 5.8%

Analysis of Variance					
Source	DF	SS	MS	F	P
Regression	1	2185	2185	1.74	0.214
Residual Error	11	13841	1258		
Total	12	16026			

B. Brigadoon, Boggabri: Analysis

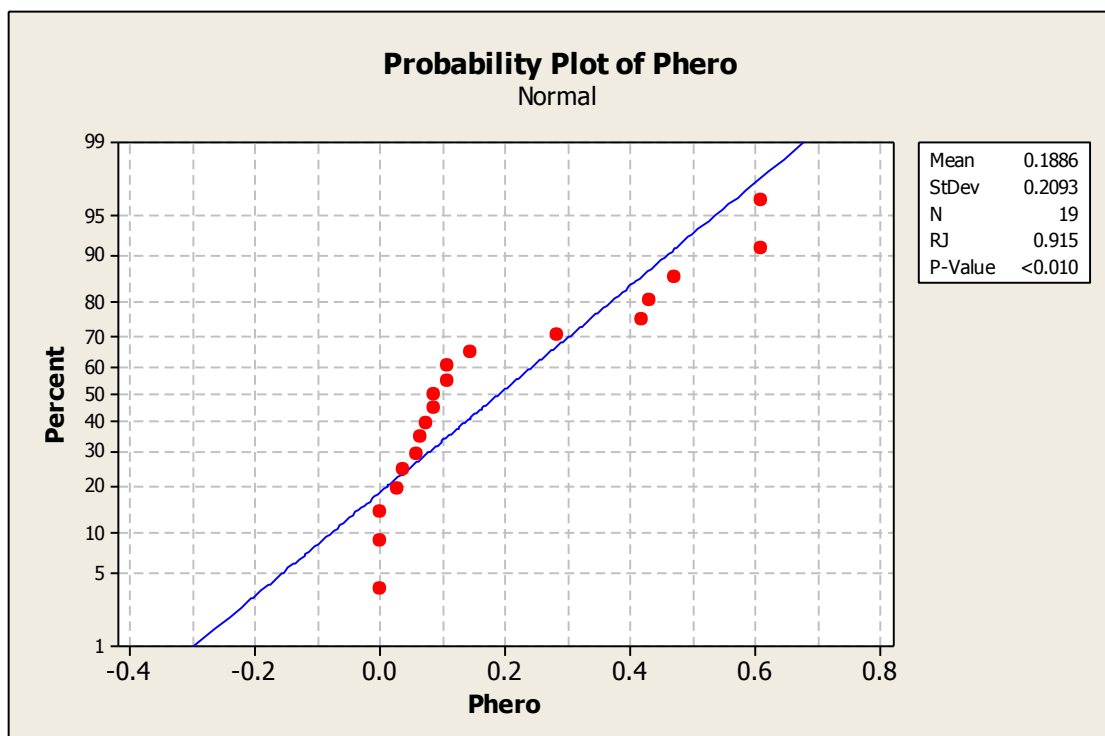
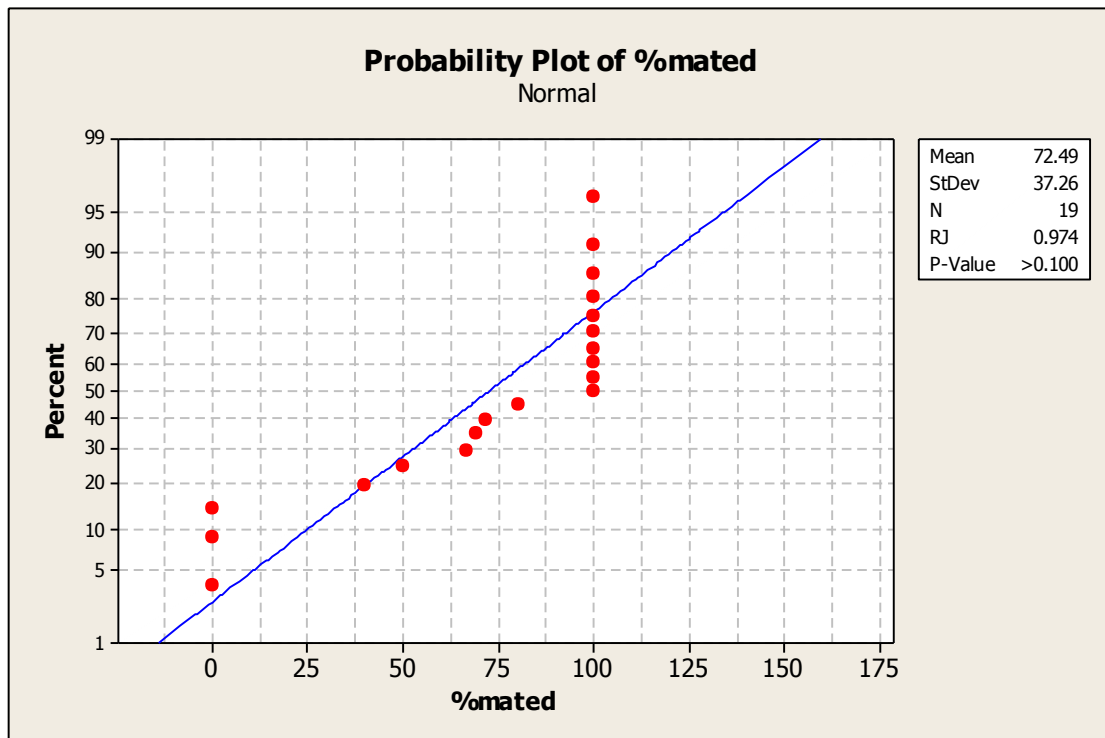
B.1 - Data Display

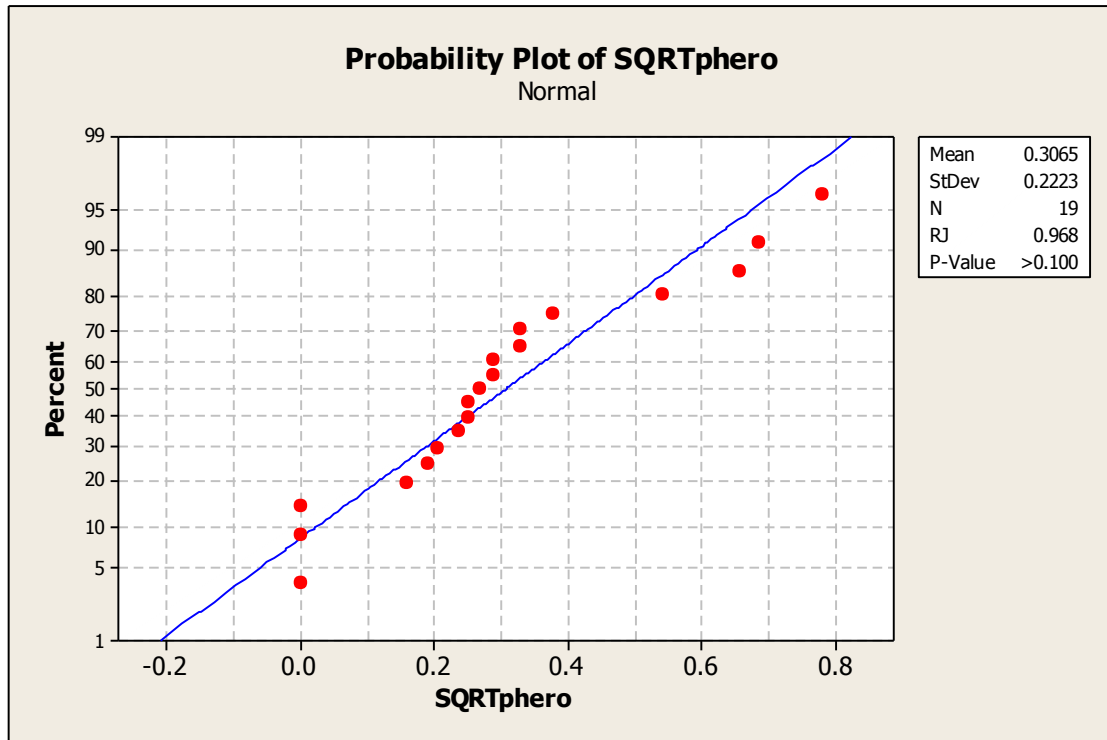
%mated	Phero	SQRTtrap1
100.000	0.468750	0.684653
100.000	0.281670	0.530726
100.000	0.142857	0.377964
100.000	0.083330	0.288669
100.000	0.428571	0.654653
100.000	0.607143	0.779194
50.000	0.607143	0.779194
80.000	0.107143	0.327327
66.667	0.107143	0.327327
100.000	0.055560	0.235712
40.000	0.062500	0.250000
69.231	0.000000	0.000000
71.429	0.416670	0.645500
100.000	0.071429	0.267262
100.000	0.083330	0.288669
0.000	0.000000	0.000000
0.000	0.000000	0.000000
100.000	0.025000	0.158114
0.000	0.035714	0.188981

B.2 – Normality tests

The test used is the Ryan-Joiner or Shapiro wilks test in MINITAB (Ryan *et al.* 1992).

Normality was only found for the % mated females in the raw data so Square root transformations were used and tested for the pheromone trap catch numbers. From the graphs it is evident the square root transformation achieved normality allowing for statistical analyses to be conducted.





B.3 – Analysis of variance for transformed data on Pheromone trap catch numbers versus % of females mated in the field

Regression Analysis: %mated versus SQRTphero

The regression equation is
 $\%mated = 41.3 + 102 \text{ SQRTphero}$

Predictor	Coef	SE Coef	T	P
Constant	41.33	12.12	3.41	0.003
SQRTphero	101.69	32.31	3.15	0.006

S = 30.4806 R-Sq = 36.8% R-Sq(adj) = 33.1%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	9201.7	9201.7	9.90	0.006
Residual Error	17	15794.1	929.1		
Total	18	24995.9			

Unusual Observations

Obs	SQRTphero	%mated	Fit	SE Fit	Residual	St Resid
19	0.189	0.00	60.54	7.96	-60.54	-2.06R

R denotes an observation with a large standardized residual.

C. Korolea, Goondiwindi: Analysis

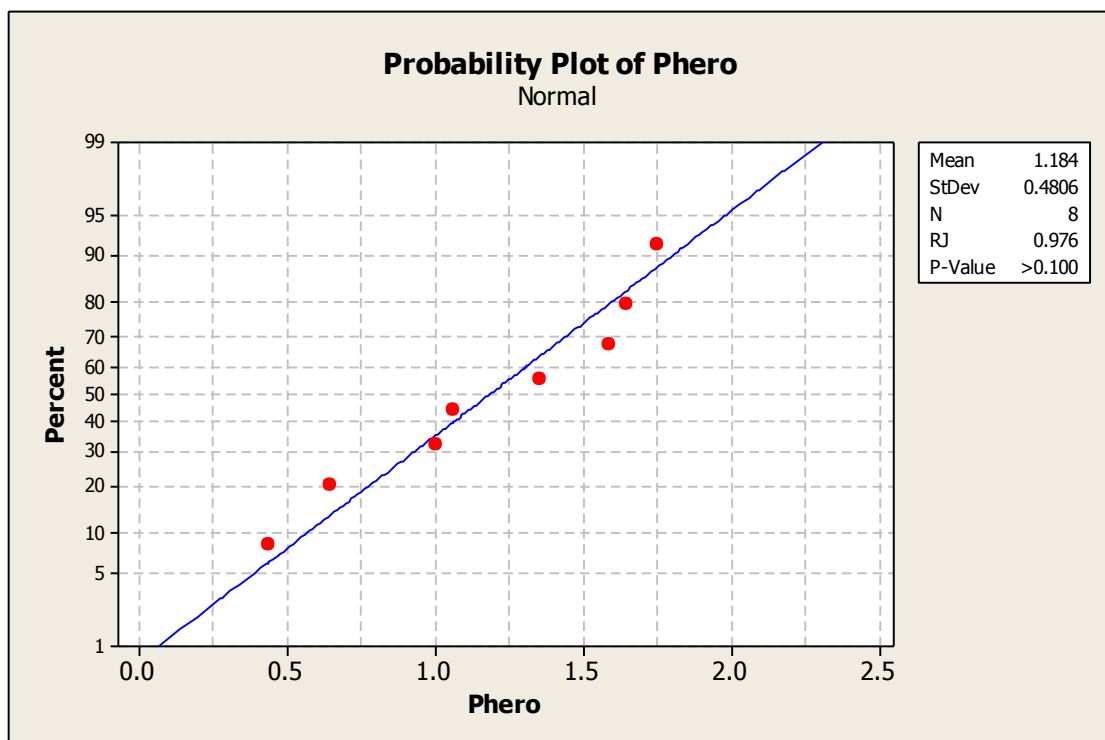
C.1 - Data Display

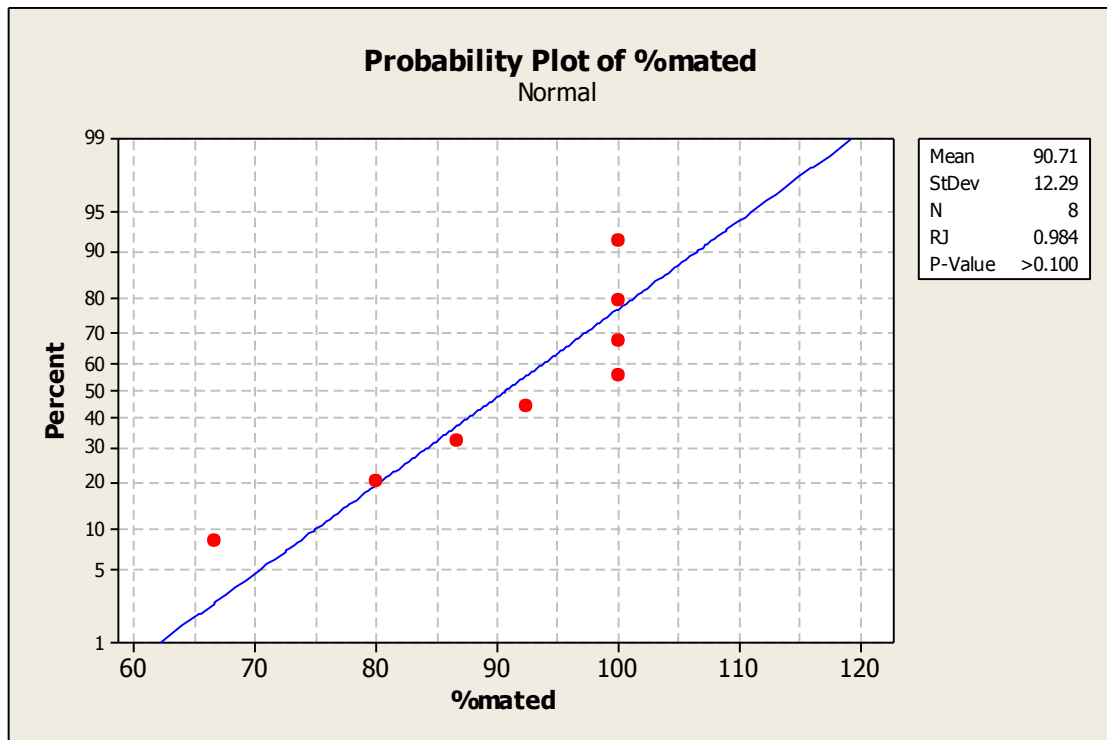
ROW	Day	%mated	Phero
1	57	100.000	1.00000
2	64	100.000	0.64286
3	68	100.000	1.06250
4	71	100.000	1.58333
5	79	86.667	0.43750
6	81	80.000	1.75000
7	88	92.308	1.64286
8	93	66.667	1.35000

C.2 – Normality tests

The test used is the Ryan-Joiner or Shapiro wilks test in MINITAB (Ryan *et al.* 1992).

Normality was found for the raw data so no transformations were used. From the graphs it is evident that normality was achieved therefore allowing for statistical analyses to be conducted.





C.3 – Analysis of variance for data on Pheromone trap catch numbers versus % of females mated in the field

Regression Analysis: %mated versus Phero

The regression equation is
 $\%mated = 98.4 - 6.5 \text{ Phero}$

Predictor	Coef	SE Coef	T	P
Constant	98.36	12.79	7.69	0.000
Phero	-6.47	10.10	-0.64	0.546

S = 12.8403 R-Sq = 6.4% R-Sq(adj) = 0.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	67.6	67.6	0.41	0.546
Residual Error	6	989.2	164.9		
Total	7	1056.9			