THE BIOLOGY, ECOLOGY AND MANAGEMENT OF THE QUANDONG MOTH, *Paraepermenia santaliella* (LEPIDOPTERA: EPERMENIIDAE)

By

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SUMMARY

The native Australian quandong is in increasing demand as a unique Australian bushfood. A major factor reducing the quality of fruit is damage caused by larvae of the quandong moth, also a native species, about which little was known. Commercial growers presently use frequent applications of a broad-spectrum insecticide to try to manage the pest. The aim of the study was to detail the biology and life history of the moth and to investigate management strategies that would enable growers to manage the pest in an economically and environmentally sustainable program.

Regular sampling was conducted at two field sites in South Australia to determine the seasonal cycle of the quandong moth, the damage caused and to survey for natural enemies. There are three to four generations of the quandong moth in South Australia each year. The summer generations occur during flowering and larvae feed on the reproductive parts of the quandong flowers but because the natural shedding of flowers is high, damage during this period is not significant. The autumn-winter generation occurs during fruit development and larvae feed on the kernel and seed coat of developing fruit causing fruit to drop from trees. However, a large proportion of dropped fruit is not damaged by the quandong moth. The spring generation occurs during fruit maturity and larvae feeding on the flesh of quandong fruit cause the most severe damage. This damage directly reduces the quality of fruit at harvest, and in severe cases fruit may be completely unsuitable for consumption.

Several species of parasitoid wasps were reared from eggs and larvae of the quandong moth throughout South Australia. The parasitoids included *Trichogramma*, an egg parasitoid,

Chelonus, an egg-larval parasitoid and *Dolichogenidea*, a larval parasitoid. It is possible that all species collected are native and undescribed and their potential for use in biological control of quandong moth has not been evaluated.

Developmental rates at various temperatures were used to calculate degree-day estimates for the egg, pupal and adult stages of the moth. The estimates, along with field incidence were used to construct a model using Dymex to examine the number and timing of the generations of the moth. Although several assumptions were made during model construction, the model demonstrated the importance of the variation in nutritional status of the quandong throughout the year and the importance of accurate spray timing.

Yield loss assessments were used to determine economic injury levels and action thresholds for the quandong moth. Sequential sampling for eggs of the quandong moth was investigated and the results indicated it is not feasible to obtain precise population estimates for the quandong moth. Until more accurate action thresholds are developed, fixed sample size plans based on presence/absence counts are the most viable option.

Insecticide trials were conducted in the field to examine spray timing and investigate alternative insecticides. None of the spray regimes or alternative insecticides significantly reduced the incidence of larvae or severity of damage compared to the unsprayed control. High degrees of variation in larval incidence, inaccuracy of spray timing, variability among trees and variation in assessment dates may have influenced the results of the insecticide trials.

Monitoring, insecticides, conserving natural enemies and good orchard hygiene should all form part of an integrated pest management program for the quandong moth. Monitoring for eggs of the moth and only employing insecticides when the moth is present will result in more judicious use of broad-spectrum insecticides. Reduced use of insecticides will conserve the populations of generalist and specific natural enemies of the moth and decrease the risk associated with sustained and frequent use of broad-spectrum insecticides. The inclusion of insecticides that are specific to moths will also aid in conserving populations of beneficial insects and restore the ecological balance where it has been disrupted by sustained use of broad-spectrum insecticides.

DECLARATION

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

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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"At the time of my visit a beautiful creek of excellent water, and shaded by fine gum trees, ran purling amongst the hills....., We found plenty of native peaches here, but they are so soon attacked by a kind of caterpillar, or maggot, that it is difficult to meet with a ripe specimen that has not been commenced on by one of these tiresome insects. The peach is about as good as any fruit as any of the wild fruits of the colony..... and has been made the means, occasionally, of sustaining life for days together when no other fruit was to be found."

Austin, 1863

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AQIA	Australian Quandong Industry Association
ASN	Average Sample Number
C	Celsius
C.I.	Confidence Interval
g	gram
ha	hectare
IGR	Insect Growth Regulator
IPM	Integrated Pest Management
IRM	Insecticide Resistance Management
km	kilometre
L	litre
LBAM	Light brown apple moth
L:D	light:dark
m	metre
mL	millilitre
mm	millimetre
OC	Operating Characteristic
SD	Standard Deviation
SE	Standard Error

1. INTRODUCTION

The quandong, *Santalum acuminatum* (R.Br.) A. DC. (Santalaceae) is a native Australian fruit occurring naturally in the arid regions of all the mainland states of Australia (Cribb and Cribb, 1974). Over the last decade, the quandong has been grown commercially and is primarily marketed as a unique Australian bush food. There is huge potential for developing both domestic and international markets. As of November 1999, there were approximately 45 000 quandong trees in plantation in Australia, with 20 000 of those producing fruit. The net harvest of quandongs in 1999 was 25 tonnes of dried de-stoned fruit, consisting of four grades valued at approximately $1.4 million. As the majority of the trees in plantation are not yet producing fruit, a large quantity of fruit is still sourced from trees in the wild (Gordon-Mills, 2000). In order to expand existing markets, and develop new markets for their product, growers need to be able to produce consistent supplies of high quality fruit (A. Beale, pers. comm.). One of the major factors that reduces the quality of fruit is damage caused by larvae of the quandong moth, *Paraepermenia santaliella* Gaedike (Lepidoptera: Epermeniidae). The Australian Quandong Industry Association (AQIA) has identified management of the quandong moth as the industry's primary research need (AQIA, pers. comm.). Currently, fruit grown and harvested for commercial use is graded according to the level of larval damage and frass in the flesh and the presence of quandong moth larvae (AQIA, pers. comm.). Undamaged fruit brings the highest return on the market, and often fruit with a small amount of quandong moth damage is unable to be sold as a premium product (A. Beale, pers. comm.). Prior to this study, no research had been conducted on the biology, ecology or phenology of the quandong moth and management techniques had not been investigated. In the absence of any knowledge of the biology or population dynamics of the quandong moth, and without any

monitoring techniques, growers have been forced to use broad-spectrum insecticides to reduce the damage caused by the quandong moth.

1.1 TAXONOMY AND DISTRIBUTION

The quandong moth, *Paraepermenia santaliella* Gaedike (Lepidoptera: Epermeniidae), was first described in 1968 from a specimen collected from a quandong tree 30km East of Ouyen in Victoria, Australia, on the 28th of November 1966 (Gaedike, 1968). The quandong moth belongs to a small family, the Epermeniidae, in one of the two sub-families, the Epermeniinae. There are five genera and 21 species of Epermeniidae that occur in Australia, with five Australian species in two genera classified in the Epermeniinae. The genus *Paraepermenia* contains only *P.santaliella* (Nielsen et. al., 1996).

The distribution of the quandong moth follows that of the quandong tree, throughout western New South Wales, western Victoria, South Australia and southern Western Australia. The quandong moth has only been reported from quandong (Common, 1990a).

1.2 HOST PLANT

The quandong, *Santalum acuminatum* (R.Br.) A. DC. (Santalaceae) is native to the arid regions of all the mainland states of Australia (Cribb and Cribb, 1974) (Figure 1.1), and has a high tolerance for drought and salinity (Sedgley, 1982a). Quandong trees will grow and bear fruit in semi-arid conditions and under irrigation. Trees can reach 5-6 m in height, and take between 3 and 5 years to begin bearing fruit (Sedgley, 1982a). Flowering occurs from October to March,

with fruit ripening in the following August to November depending on the region in which the tree is located. The floral anatomy was described by Sedgley (1982b). The fruit is spherical, 20-30 mm in diameter and has a large pitted stone (Figures 1.2 and 1.3). The skin of the fruit can vary from red to yellow and the flesh from white to yellow (AQIA, pers. comm.).

Both the flesh and the nut of the quandong are edible and have been consumed by Australian aboriginals for many years (Black, 1965; Cribb and Cribb, 1974). Australian aboriginals also used the kernel for medicinal purposes (Black, 1964). Grant and Buttrose (1978) reported a high content of vitamin C in the flesh of quandongs, almost twice that of oranges on an equal fresh weight basis. The flesh may be consumed raw, but fruit from some trees has an acidic taste and sweetness can vary greatly between trees (Grant and Buttrose, 1978). When harvested the fruit are generally halved, de-stoned and the flesh is either processed immediately, or stored as a dehydrated or frozen product.

Commercially, the fruit is either sold as a dried product or processed into jams, chutneys, chocolate bars and fruit leathers. In addition, several quandong meads are available and more recently, a rich quandong liqueur has been produced (Gordon, 1995).

The kernel is approximately 20% protein and 70% oil (Grant and Buttrose, 1978; Rivett et. al., 1984) and is edible, but contains methyl benzoate that may produce an unpleasant aftertaste (Loveys et. al., 1984). Australian aboriginals used the kernel for the medicinal properties. Santalbic acid is a major constituent of the oil (Rivett et. al., 1984) and is active against gram-positive bacteria and several pathogenic fungi (Jones et. al., 1995).

Like other members of the Santalaceae, the quandong is a partial root parasite. Quandong roots produce a pad-like growth on contact with other plant roots and tissues from the quandong root enter the host root and develop a direct connection (Grant and Buttrose, 1978). Transfer of carbon, water, ions and compounds with insecticidal properties from the host plant to the quandong has been demonstrated (Tennakoon et. al., 1997; Loveys et. al., 2001). Quandongs have a wide host range, including *Eucalyptus*, *Acacia* and *Casuarina* species, as well as other native Australian trees, and native and introduced perennial grasses and pasture species (Grant and Buttrose, 1978). In commercial orchards, many trees are planted with strawberry clover, *Trifolium fragiferum* L. (Leguminosae), lucerne, *Medicago sativa*, L. (Leguminosae) kikuyu grass *Pennisetum clandestinum* Hochst (Poaceae) and *Myoporum* sp. (Myoporaceae), a native creeper, as hosts (AQIA, pers. comm.). Some growers have experimented with hardy Australian and South African flowering plants as hosts, which can also be used for supplementary cut flower production (R. Schaefer, pers. comm.).

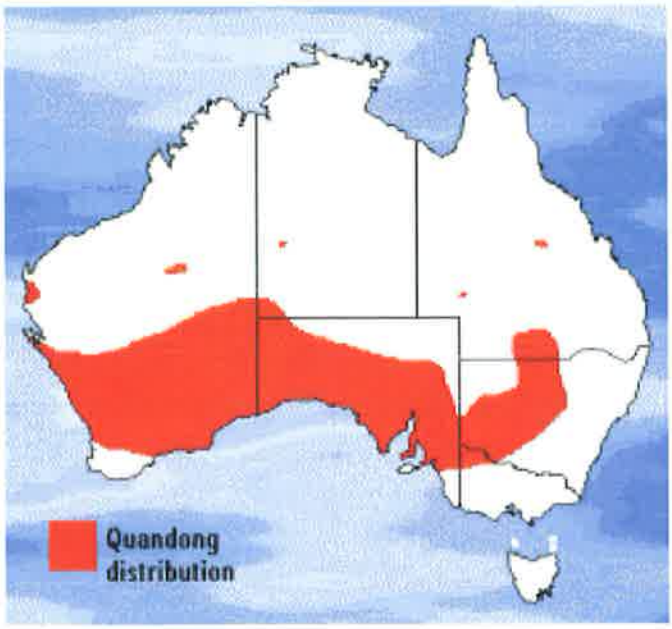


Figure 1.1: Natural distribution of quandong trees in Australia

Figure 1.2: Mature quandong fruit on tree

Figure 1.3: Cross-section of mature quandong fruit showing seed and flesh

1.3 GENERAL BIOLOGY

There are only very brief descriptions of the quandong moth published by Common (1990a) and it is the only research that had been done on the moth prior to this study. Other information presented in this section is based on observations made by P.T. Bailey (pers. comm.) prior to the commencement of this project.

1.3.1 The Egg

The egg is ovoid and flat, approximately 0.5mm in length, with raised ridges on the surface of the chorion (Common, 1990a). Preliminary observations of the eggs indicated they are laid singly, are white when first laid, changing to a pale brown as they mature (P.T. Bailey, pers. comm.).

1.3.2 The Larva

Neonate larvae are approximately 1mm long, yellow to white and burrow into fruit and feed concealed for the majority of their development. Mature larvae are approximately 6mm long and pink to red (P.T. Bailey, pers. comm.). Feeding by larvae of the quandong moth reduces the yield and quality of fruit by damaging the flesh, leaving frass in fruit and allowing secondary fungal infections to cause rotting of fruit (AQIA, pers. comm.).

1.3.3 The Pupa

The quandong moth pupates in a silk cocoon. The site of pupation in the field has not been determined but the general habit of the family is for pupation to occur in leaf litter (Common,

1990a). Pupae of the Epermeniidae have lateral pits on segment 9 that are not present in other families. A peculiar trait of the family is that the pupa is not protruded from the cocoon at adult emergence (Common, 1990a).

1.3.4 The Adult

Adults of the Epermeniidae are small moths with narrow wings and a wingspan of 8-20mm. The adults are distinguished by dense bristles on the hind tibia and tufts of scales that project from the inner margins of the forewings (Common, 1990a). Adult quandong moths are grey with several black horizontal stripes on the forewings and are approximately 6mm long (P.T. Bailey, pers. comm.).

1.4 PEST STATUS

The quandong moth was first described in 1968, but was documented by European explorers in the 1860's heavily infesting quandong fruit in South Australia (Austin, 1863). The major reason for the lack of research to date is that it is only in recent times that the quandong moth has become a pest of any economic importance. Prior to the first attempts to commercialise the quandong fruit, the presence of quandong moth larvae and the associated damage was considered part of the 'quandong experience' by people who collected fruit from wild or backyard trees. Commercialisation of the fruit has prompted the market to demand fruit free of insects and damage.

There are many examples of native Australian insects that have become pests of cultivated crops. Most are polyphagous species and their pest status has resulted from a host shift to introduced and economically important crops. For example the light brown apple moth, *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae) on pome fruit and grapevines (Geier and Briese, 1981), lucerne seed web moth, *Etiella behrii* Zeller (Lepidoptera: Pyralidae) on lucerne (Austin et. al., 1993) and the grape vine moth *Phalaenoides glycine* Lewin (Lepidoptera: Noctuidae) on grapevines (Cordingley, 1980). Even on native plant species that have been developed as a commercial crop, such as macadamia, the two primary lepidopteran pests are native and polyphagous (Jones, 1994). The situation with the quandong moth is unique amongst horticultural crops in Australia, as it is the commercialisation of the plant that has resulted in the moth becoming a pest.

1.5 INTEGRATED PEST MANAGEMENT

Pest management in many crops has evolved from over-reliance on insecticides to an integrated approach, combining a number of compatible management strategies (Talekar and Shelton, 1993; Gray and Luckmann, 1994; Prokopy and Croft, 1994). Over-reliance on broad-spectrum insecticides can lead to many adverse effects including resurgence of pests, detrimental effects on beneficial insects, outbreaks of secondary pests, insecticide resistance and environmental contamination (Metcalf, 1994). Integrated pest management (IPM) can be defined as a system of pest management where "all available techniques are evaluated and consolidated into a unified program to manage pest populations so that economic damage is avoided and adverse side effects on the environment are minimised" (National Academy of Sciences, 1969).

Developing an IPM program for a pest species is dependent upon knowledge of the biology, ecology and economic impact of the pest. Studies of these aspects will identify any vulnerable stages in the lifecycle of the pest, periods in which vulnerable stages are found in the field and the damage caused to various stages of the host plant. Studies of the pest must be interpreted in the context of other factors that influence pest density and damage, including biotic factors such as growth, reproduction, nutrition and mortality and abiotic factors such as climate (Luckmann and Metcalf, 1994). The biology and ecology of the pest will influence the choice of management strategies (Luckmann and Metcalf, 1994). Techniques that are available for use in IPM include insecticides, biological control, cultural methods, resistant plant varieties and in more recent years, genetically modified organisms (Luckmann and Metcalf, 1994). In addition to economics, the environmental and social impact of the program must be evaluated. Communication and education are critical to acceptance and implementation of any IPM program (Luckmann and Metcalf, 1994).

1.5.1 Chemical Control

There are a variety of insecticides available for control of lepidopteran pests, ranging from broad-spectrum organophosphates to specific insect growth regulators (Metcalf, 1994). Several steps can be taken to ensure the integration of insecticides into a pest management program is both effective and sustainable, including the use of selective insecticides and improving the timing of applications (Metcalf, 1994). AQIA has a temporary registration for growers to use dimethoate with a with-holding period of 14 days (AQIA, pers. comm.). Dimethoate was selected for use against the quandong moth because it has systemic properties and relatively short environmental persistence. Observations by growers suggest it

is effective against quandong moth (AQIA, pers. comm.), although there are no quantitative data on the efficacy or residual effects of dimethoate. Spraying with dimethoate is not based on a structured monitoring program and thus timing varies greatly between orchards. Many growers do not begin spraying until late in the growing season, when fruit is beginning to mature (D. Matthews; L. Otto; C. DeLaine, pers. comm.). Other growers apply an initial spray at flowering and subsequent sprays mid-season as fruit begin to mature (L. Otto, pers. comm.). Some other growers have adopted a monthly program of sprays, beginning at flowering (P. Prenzle, pers. comm.). Another broad- spectrum organophosphate insecticide, chlorpyrifos has been used by some growers in an "off-label" manner, (B. Powell; B. McNamara, pers. comm.) but dimethoate is the most commonly applied insecticide (AQIA, pers.comm.).

1.5.2 Biological Control

For native species of pests conservation and augmentation biological control are the most appropriate tactics (Hoy, 1994). Conservation biological control is reliant on protecting existing populations of natural enemies whereas augmentation relies on increasing populations of natural enemies through release of mass-reared individuals (Hoy, 1994). As a native species there is a range of specific and generalist natural enemies that attack the quandong moth. Although several parasitoid wasps had been reared from immature stages of the quandong moth, they had not been identified prior to this study (P.T. Bailey, pers. comm.). There are several generalist predators present on quandong trees, including species of Coccinellidae (Coleoptera), Chrysopidae (Neuroptera) and Mantodea (P.T. Bailey; L. Otto; D. Matthews; C. DeLaine, pers. comm.). As part of an integrated approach, the efficacy of

biological control is somewhat dependent on modifying insecticide spray schedules to protect all natural enemy populations, whether existing or introduced (Bartlett, 1964; Hull and Beers, 1985).

Some quandong growers initially intended to run organic orchards, free of insecticides (C. DeLaine, pers. comm.) or using only approved organic controls such as *Bacillus thuringiensis* (Bt) (R. Jacobs, pers. comm.) with little success. In the absence of any quantitative data, field observations have indicated that Bt is not particularly effective for control of quandong moth (R. Jacobs, pers. comm.). This is likely to be due to a combination of inaccurate spray timing, the concealed feeding behaviour of larval quandong moth and the detrimental effects of sunlight and rainfall on Bt (Behle et. al., 1997). Bt relies on the pest species ingesting treated surfaces and therefore would require an accurate monitoring program to predict when larvae would be active on the surface of the fruit. Once ingested, the gut pH of the target species can also influence the efficacy of Bt preparations (Gringorten et. al., 1992; Wilson and Benoit, 1993), a factor that has not been investigated for quandong moth.

1.5.3 Host plant resistance

Some varieties of quandong seem to have inherent resistance to attack by quandong moth (B. Powell; R. Jacobs, pers. comm.). This may be due to physical or chemical properties of the varieties, or associations with host plants. Anecdotal evidence from some quandong growers suggests that trees growing near *Melia azedarach* (L.) trees suffer less insect attack, particularly from quandong moth (Loveys et. al., 2001). There is however, no baseline data on the susceptibility of fruit on different quandong trees and no proof that the quandong trees

in question are actually hosting on *M.azedarach*. Further, the effect of insecticidal compounds on taste has not been determined, nor has the mammalian toxicity. Plant resistance is often a compromise between those characteristics that are desirable for human use, and those that deter insect pests from infesting the plant. In an established orchard of quandong trees, resistant varieties are unlikely to be of use, but for newly planted orchards resistant varieties may have a role in an IPM program.

1.5.4 Cultural control

With cultural control techniques, the environment of the pest is altered to render it less favourable for pest survival and reproduction (Dent, 1991). Cultural control is reliant on accurate and detailed information on the biology and ecology of the pest species. For perennial crops, cultural control methods can include destruction of crop residues, pruning and thinning, and manipulation of fertilising and watering regimes (Dent, 1991). Prior to this study, little information was available on the ecology of the quandong moth and therefore cultural control strategies had not been employed.

1.6 RELATED LEPIDOPTERA

There are 11 species of *Gnathifera* Gaedike in the subfamily Ochromolopinae (Epermeniidae) that occur in Australia, ranging from Queensland to South Australia and Tasmania. *Gnathifera* species are found on the native cherry, *Exocarpus cupressiformis* G. (Santalaceae) (Common, 1990a). In the subfamily Epermeniinae, there are 4 species of *Epermenia* Hubner, including *Epermenia exilis* Meyr that feeds on the boxthorn, *Bursaria spinosa* Cav. (Pittosporaceae)

(Common, 1990a). None of these species are of agricultural importance. In Europe and America larvae of other species in the Epermeniidae feed within seeds, fruit or flower buds of their hosts plants and some leaf mining activity has also been recorded (Gaedike, 1968).

1.7 OTHER INSECT FAUNA ON QUANDONG TREES

Preliminary survey data and previous publications have reported several other insect species inhabiting quandong trees. The following have been found feeding on leaves; a leaf webbing lepidopteran larva (P.T. Bailey; AQIA, pers. comm.); adults and larvae of a chrysomelid (AQIA, pers. comm.); an eriophyiid mite (P.T. Bailey, pers. comm.); a black ornamental scale (P. Gullan, pers. comm.); a gall forming cecidomyiid (AQIA, pers. comm.). On flowers and fruit there were mite species (P.T. Bailey, pers. comm.) and a fungal rot (AQIA, pers. comm.). Additionally, there are coccinellid and chrysopid species that are likely to be predators of the quandong moth and the other insects on the trees. The presence of other insects and fungi on quandong trees is an important consideration in an IPM program.

1.8 AIMS AND SIGNIFICANCE

The aims of this study were: to examine the biology of the moth, providing a description and measuring the duration of all stages in the lifecycle; to examine the seasonal history of the moth, particularly the location of each stage in the field and the number of generations each year and their interaction with the developmental stages of the quandong tree; to examine monitoring strategies for targeting controls; to survey any quandong trees demonstrating resistance to the quandong moth and examine physical characteristics that may be imparting

resistance; to examine the timing of insecticide applications and trial alternative management strategies; to survey natural enemies, other beneficial insects and potential secondary pests.

Research on the quandong moth is of value from both a natural history perspective and as a study in pest management. Historically, the development of IPM programs for many pests has occurred only after collapse of all other management strategies, particularly those reliant on frequent insecticide usage (Luckmann and Metcalf, 1994). In a developing industry, quandong growers have the advantage of foresight, and many growers are already aware of the dangers of over-reliance on a single pest management tactic. Too many lessons have been learned in other cropping systems for the quandong industry to repeat the same mistakes. The ultimate aim of the study was to examine the prospects for developing an IPM program for the quandong moth that would enable growers to produce a truly unique Australian food in an economically viable and environmentally sustainable way.

2. GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

This section outlines a number of the general materials and methods used throughout this study. Methods that were specific to a particular experiment are outlined in the relevant section.

2.2 CULTURE ROOM

A constant temperature room set at 24°C with a photoperiod of 14:10 L:D was used as a culture room throughout the study. All insect rearing occurred in the culture room.

2.3 REARING JARS

Both large and small jars were used throughout the study. The jars were modified to permit aeration of the contents and prevent the development of high levels of relative humidity. Holes were made in the lids of the jars with a heated metal ring, 43mm in diameter for the large jars, and 24mm in diameter for the small jars. A piece of fine mesh with a diameter 10mm larger than the hole was glued on the outside of the lids using Kwik Grip® (Selley's Pty. Ltd.). In particular the large jars were used as pupation containers (Section 2.6) and the small jars in temperature and development studies (Section 4.3.1). The jars were also used to collect and rear other insect fauna from quandong trees.

2.4 EGG HATCHING

When eggs were collected from flowers or fruit in the field, or from mating cups (Section 2.9) they were transferred to tubes to hatch. The lids were cut from 2.0 mL Eppendorf® tubes, eggs placed inside the lid and then the top of the tube replaced. Tubes were labeled and stored inverted in the culture room. The airtight environment of the tubes maintained relatively high humidity to prevent desiccation of eggs. The detached lids could easily be placed under the stereo microscope to check the development of eggs and any newly hatched larvae. Since at 24°C there was a five to six day delay until hatch, eggs were not placed directly onto media or fruit because fungal contamination would often develop in the interim and the eggs would have to be moved to a fresh food source. Hatching eggs in vials ensured they were only handled once and decreased the chance of injury during handling.

2.5 LARVAL REARING

At the outset of this study the aim was to establish a laboratory culture of the quandong moth so that mass rearing of the moth could be conducted. Despite repeated attempts to develop an artificial media on which to rear larvae of the quandong moth, rearing was only successful on fresh fruit. Attempts to develop an artificial media are outlined in Appendix 1. The methods used to rear larvae on fresh fruit in the culture room are outlined in this section.

To reduce the onset of fungal contamination, fruit were surface sterilised by immersion in a 10% solution of White King®, active ingredient 42g/L sodium hypochlorite, for two minutes and rinsed three times in sterilised de-ionised water. Neonate larvae were transferred to pieces of sterilised quandong flesh that were approximately $10mm^2$. The pieces of fruit were then

placed in individual cells of a Bioassay Tray© and sealed with Pull n' Peel Tab© re-sealable adhesive covers (C-D International, Inc. Pitman, N.J. U.S.A.). The Bioassay Trays© consisted of a large plastic tray comprising eight sections with 16 wells in each section. For use in this study, each tray was cut into individual 16 well sections for ease of handling, as larvae in wells were often checked under a stereo microscope. The larvae were checked every two to three days and fruit pieces replaced when dehydrated, or fungal contamination was developing. This method of rearing was more successful than with artificial media, but was by no means an ideal method, and mass rearing was not achieved with either technique.

2.6 COLLECTION OF MATURE LARVAE AND PRE-PUPAE

Pupae less than 24 hours old required for pupal development studies were obtained by the following method. Infested fruit were collected from or beneath various trees at each of the field sites and placed in a single layer in 5.0L, rectangular, transparent plastic containers 290x200x95mm, lined with paper towel. A section of the plastic lid with dimensions 20mm less than the dimensions of the lid was removed using a scalpel, and a rectangle of fine mesh was glued over the hole. The mesh lid prevented larvae from escaping and maintained a relative humidity of approximately 45%, which delayed the onset of fungal contamination of fruit. The fruit were held in the culture room at 24°C for two to three weeks. During this time mature larvae emerged from fruit as they were ready to pupate. Mature larvae and pre-pupae were commonly found spinning cocoons in two main sites within the containers. The first site was on the lid, in the gap in between the top of the container and the lid and the second site was underneath the paper towel in the base of the container. Containers were checked every two days and mature larvae and pre-pupae removed and placed in large jars

(Section 2.3) lined with paper towel. Pupation cups were checked daily and newly formed pupae, less than 24 hours old, were removed and used in studies as required.

2.7 EMERGENCE CAGES

When adults were required for longevity or single mating pair studies, mature pupae were sexed and placed in separate emergence cages for adult eclosion. Modified 3.0L canisters (Prestige® Australia) were used for adult emergence cages. For aeration, holes 63mm in diameter were made with a heated metal ring in the sides, top, and back of the canisters. The holes were covered with fine mesh glued in place. A mesh sleeve was also fitted to the opening of the canister and held in place with a rubber band. The sleeve allowed easy access to moths inside the canister and when knotted also prevented moths from escaping. Adults in emergence cages were supplied with a feeder as described in section 2.8.

2.8 ADULT FOOD SOURCE

A diluted honey solution of 10% honey in de-ionised water with 0.5% sorbic acid to prevent fungal contamination, was used as a food source for adults. The honey solution was given to adults either on a saturated piece of dental roll (Faulding Dental, Adelaide) or in a feeder. For mating cups, a 40mm long piece of dental roll was saturated in honey solution and placed in the bottom of the cup. In emergence cages, honey was supplied in a feeder that consisted of a small 5mL vial (Selby Biolabs) with a plastic stopper and a 55mm length of dental roll. A hole was cut in the stopper just larger than the diameter of the dental roll (10mm). Approximately 10mm of the dental roll was pushed through the hole with the rest remaining below. The vial was filled with honey solution and the lid of the vial replaced so that only

10mm of the dental roll was protruding out of the vial. This type of feeder remained effective as long as there was honey solution in the vial and capillary action constantly drew the honey solution to the extruded part of the dental roll. Adults were able to land on the rim of the vial and feed on the honey solution. The feeder was used when honey solution was required for a longer period than could be supplied by a saturated dental roll.

2.9 MATING CUPS

The cups used for mating and oviposition were clear plastic tumblers (Deeko®), capacity 225mL. Flowers, developing fruit and mature fruit were used as oviposition substrates in mating cups. Small vials were filled with water and the top covered with masking tape. Holes were punched in the masking tape and the stem of the flower or fruit was positioned so it was immersed in the water. The vials were fixed in the mating cups with double-sided tape. A 30mm long piece of dental roll saturated with honey solution was also provided. For single mating pair studies one male and one female were placed inside cups. For mass rearing work, three to four moths of each sex were placed in cups. The cups were covered with a square of mesh fixed with a rubber band and left in a natural photoperiod at ambient temperatures until moths died. When eggs less than 24 hours old were required for studies cups were checked daily. Otherwise eggs were removed every two to three days and the dental dam re-saturated with honey solution as required.

2.10 FIELD SITES

The field sites were chosen because they had a previous history of regular infestation by the quandong moth and were not sprayed with any insecticides. The first field site selected was a minimally maintained orchard at Quorn, South Australia (Figure 2.1). The orchard was planted from seeds collected from the southern Flinders Ranges and Yorke Peninsula in South Australia, and from around Perth in Western Australia. At the time of sampling, there were approximately 150 trees in the orchard, all about 20 years old. As the trees were planted from a diverse source of seed, there was variation amongst the trees in fruit size, colour and shape, and leaf size and shape. Yield also varied greatly, with some trees never fruiting, or very light fruiting each year and others with regular heavy fruiting. From the trees in the orchard, 11 were selected that had a history of heavy fruiting in each year (Brian Powell, pers. comm.). The climate at Quorn consists of hot dry summers with a moderate winter and an average rainfall of 350mm per annum (Appendix 2, Figures 1 - 3). Natural rainfall in the orchard was supplemented with regular irrigation from a bore.

The second field site selected was at Sedan, South Australia (Figure 2.1). This site was a council reserve of approximately one hectare, containing approximately 300 trees of varying ages. It is likely that there would be much less genetic variation amongst these trees as many of them are situated in small groves with new trees germinating from seed dropped from a few established trees. There are also several groves in which new trees have originated from suckers of established trees. No fruiting history could be obtained for this site, so trees were selected on the basis of the number of flowers on the trees when being examined and the prevalence of quandong seeds underneath the trees from the crop in the previous year.

Thirteen trees were originally selected, but between eight and 10 were sampled on a regular basis depending on the fruit set each year. Overall, temperatures throughout the year at Sedan are lower than at Quorn, but the average yearly rainfall at Sedan is higher at approximately 450mm p.a. (Appendix 2, Figures 4 – 6).

Periodically, fruit from trees at other sites in South Australia were collected, primarily to obtain insects for laboratory rearing and for a natural enemy survey. Samples were taken from trees at Whyalla, Port Augusta, Woomera, McLaren Flat, Paringa, Cummins and Coffin Bay, South Australia. The majority of those trees sampled were wild with a few in urban and rural backyards.

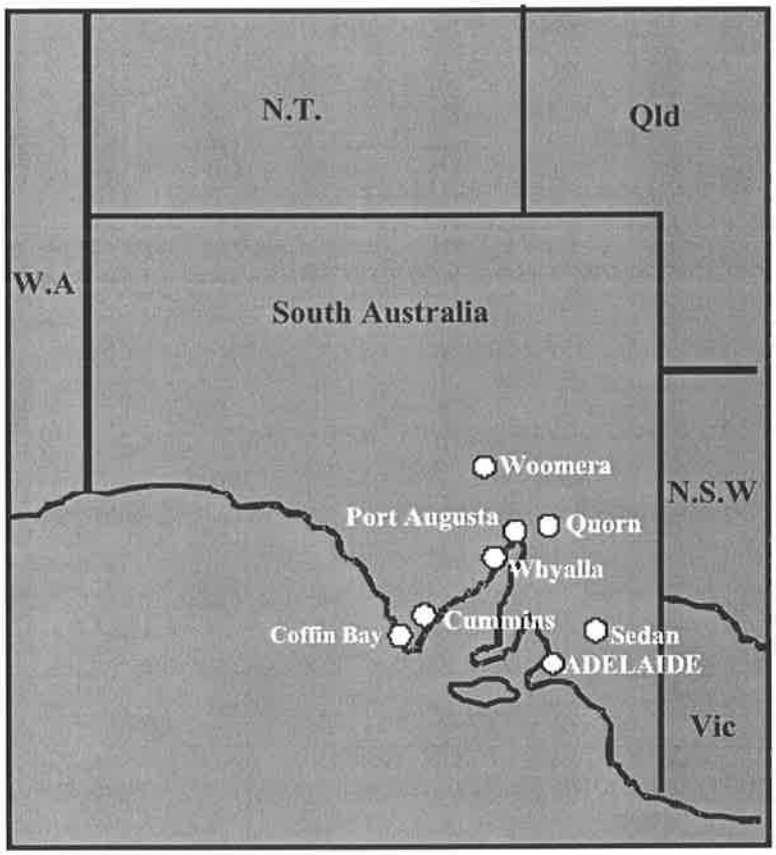


Figure 2.1: Map of South Australia showing location of field sites with quandong trees.

3. LIFE STAGES OF QUANDONG MOTH

3.1 INTRODUCTION

Detailing the biology of the quandong moth is critical to developing a management program for the species. Descriptions of all stages in the life cycle of the moth will enable growers to confidently identify the pest in their orchards and be assured the management strategies they apply are targeting the damaging species. Biological investigations are also important to determine the behaviour of the mobile stages of the moth and to identify periods when various management strategies may be most effective. Several laboratory experiments were conducted to investigate the developmental biology of quandong moth. Developmental times presented in this section are for temperatures in the culture room only, while developmental times at a range of constant temperatures are reported in Chapter 4.

Prior to this study the number and description of the instars of quandong moth had not been reported. For pest management it is important to determine the total number of instars and to identify the most damaging stages. Daly (1985) provides a review of methods used for instar determination in insects and cites many studies in which some techniques were found useful and others not. The two basic methods used to determine instars are: (i) inspection of a frequency distribution of measurements of a structure, frequently head capsule width, and (ii) inspection of a bivariate plot of mean instar sizes against the presumed instar number (Daly, 1985). Often the interpretation of a frequency distribution is not straightforward due to the presence of overlapping peaks caused by sampling errors, or environmental or genetic variation (Floater, 1996). Modifications can be made to aid in interpreting frequency

distributions with overlapping peaks, such as computing a moving average (Austin et. al., 1993), fitting a series of normal distributions to the data (Caltagirone et. al., 1983) or various other statistical methods (Got, 1988; Beaver and Sanderson, 1989; McClellan and Logan, 1994). Bivariate plots can be used as both a check to determine if any instars were missed in field collections, and as a growth curve (Daly, 1985). The Brooks-Dyar rule applies where growth follows a regular geometric progression such that each successive stage increases in size by a constant rate (Brooks, 1886; Dyar, 1895; Hutchinson and Tongring, 1984).

The habit of the family Epermeniidae is to pupate in leaf litter (Common, 1990a), but the pupation site for the quandong moth has not been reported. Pupation site has been exploited in the management of various lepidopteran pests. For example, Kaya (1984) used corrugated paper trunk bands to trap pre-pupae of the codling moth and then applied a suspension of an entomogenous nematode to the trunk bands as a control strategy.

For many Lepidoptera, female pupae and adults are larger than males (Daiber, 1979c) and in some cases size can be used to distinguish between the sexes in the pupal stage. For adults, markings on the wings may also be useful in sex discrimination (Geier and Briese, 1981). Investigations were conducted to determine if females would oviposit on artificial substrates in the laboratory, with the aim of incorporating laboratory oviposition in a mass rearing technique.

Prior to this study it had not been reported if female quandong moths used tactile cues, odours or a combination of both to select oviposition sites. Preliminary observations indicated that a

suitable oviposition site was an essential requirement for the moths to oviposit and unlike some moths, quandong moths do not release their egg load just prior to death. An artificial oviposition substrate would facilitate laboratory oviposition trials both by decreasing the reliance on field collected material as well as standardising the oviposition substrate, thereby removing some of the variation in oviposition studies.

3.2 MATERIALS AND METHODS

Eggs collected on fruit in the field were examined under a stereo microscope at 40x magnification and the length and width of 102 eggs was measured with an eyepiece micrometer. Eggs laid in the laboratory were observed at various times from the day of oviposition to the day of hatch and the physical changes recorded. The location of eggs laid on flowers and fruit in the field and laboratory was noted. In many instances the chorion of the egg remained fixed to the substrate after the larva had hatched, and both hatched and unhatched eggs were recorded and described during assessment of field samples.

The duration of the larval stages was observed in the laboratory at 24°C using fresh, semi-mature quandong fruit as a food source. Larvae were reared from laboratory laid eggs. The head capsule widths of 956 larvae collected from field sites at Quorn and Sedan, South Australia were measured under a stereo microscope fitted with an eyepiece micrometer, at a magnification of 40x. To ensure first instars of the quandong moth had not been overlooked, 50 field-collected eggs of the quandong moth were incubated until they hatched. Likewise, to ensure final instars of the quandong moth had not been overlooked, the head capsules shed by larvae when moulting to the pupal stage were also collected and measured. The head capsule

widths of the newly emerged larvae from eggs in the laboratory were within the range of those assigned to the first instar from field samples. In addition, the discarded head capsules of final instars collected from cocoons in the laboratory were within the range of head capsule widths of mature fourth instars collected from the field. A frequency distribution of head capsule widths was compiled and the growth ratios between successive instars calculated to determine if the data fitted the Brooks-Dyar rule for geometric progression of growth (Dyar, 1895; Hutchinson and Tongring, 1984).

Observations were made on neonate larvae in the laboratory to examine behaviour during hatching from eggs and searching for a feeding site. The observations began with 30 eggs and concluded when larvae had burrowed completely inside fruit. The prevalence of cannibalism in first instar quandong moth was also investigated. On five occasions, 20 eggs were placed inside a hatching tube (Section 2.4) and left to hatch. Once the larvae had hatched, they were left without food to determine if cannibalism would occur.

An experiment was conducted to examine the behaviour of mature larvae searching for pupation sites. Preliminary observations were conducted to examine the responses of mature larvae to Tangle-Trap® (The Tanglefoot Company, Grant Rapids, MI, U.S.A.), and to determine if the material altered the behaviour of the larvae. Five wandering mature larvae were collected from large containers of extra fruit (Section 2.6) and placed inside individual rings of Tangle-Trap® 20mm in diameter on a clear piece of plastic. Within 10 seconds, all five larvae became entangled in the Tangle-Trap®. Larvae began walking immediately after they were placed in the centre of the ring and did not appear to be deterred by the presence of

the Tangle-Trap® until they became entangled and unable to move. The observations were repeated four times on 20 larvae and the same response was seen for each larva. Therefore, it was concluded that Tangle-Trap® did not deter the larvae from walking on the surface, but once in contact with it, they could walk no further.

During mid-October 2000, the period of peak infestation of quandong fruit with mature larvae, 14 stems each bearing two fruit were collected from trees at Quorn. Fruit were chosen from trees on which other fruit were infested to increase the likelihood that mature larvae were collected inside the fruit. Once back at the laboratory, the stems were immersed in water in a vial that was placed in a plastic tray with dimensions of 165mm x 105mm x 40mm. The inner base of the tray was coated with Tangle-Trap®. Beginning at the point where the stem meets the fruit, approximately 25mm of each stem was also coated with Tangle-Trap® (Figure 3.1).

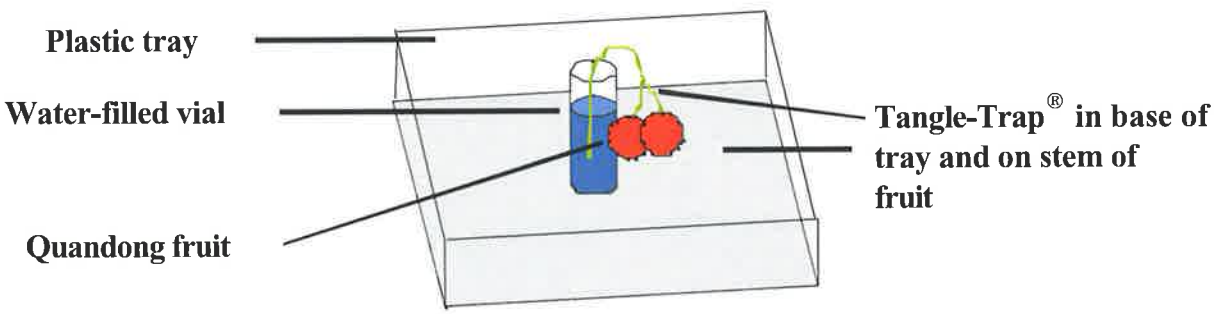


Figure 3.1: Design of experiment to observe activity of mature larvae seeking pupation sites.

Tangle-Trap® was placed on the stems of fruit to trap any larvae that attempted to crawl out of the fruit and use the stem to reach their pupation site. The inner base of the tray was

coated with Tangle-Trap® to trap larvae that dropped directly from fruit on a silk thread at the point where they contacted the surface of the tray. The trays were held in the culture room at 24°C, 14:10 L:D and observed daily. The number of larvae that emerged from a fruit was recorded and the location of each larva was classified as either directly below fruit, on the stem or elsewhere in the tray. A heterogeneity chi-square test was applied to determine if the data from trays were homogeneous and could be pooled for further chi-square analysis (Statistix®, Analytical Software, Tallahassee, Florida, U.S.A.).

The weights of pupae from Quorn and Sedan were measured using a Microbalance LM-600 (Beckman RIIC Limited). At 24 hours old pupae were still soft and were easily damaged by handling, so pupae were weighed when 48 hours old. After weighing, each pupa was placed in a separate 2.0mL Eppendorf tube, labeled with the sample number and the field site from which it was collected as a mature larva. The pupae were then held at 24°C for adult eclosion when the sex of the adult was determined. The lengths of pupae were measured using a stereo microscope fitted with an eyepiece micrometer at 10x magnification. Two-way analysis of variance was conducted to determine if there were any differences between the lengths and weights of male and female pupae from the two field sites (Statistix®, Analytical Software, Tallahassee, Florida, U.S.A).

During the first year of sampling, attempts were made to collect pupae of the quandong moth in the leaf litter below trees. During the peak period of larval incidence in fruit at Quorn from September to November 1998, two leaf litter samples were taken beneath the canopy of those sample trees (n = 11) that were fruiting, over four sampling periods. At Sedan from

September to November 1998, two leaf litter samples were taken from those sample trees (n = 13) that were fruiting, over three sampling periods. At each site, the top 30mm of leaf litter inside a 0.25m^2 quadrat was removed. The first sample was taken from a random location underneath the tree, with the second sample taken from a point on the opposite side of the tree. Thus, 72 and 60 samples were collected from Quorn and Sedan, respectively. Samples were placed in plastic bags that were tied loosely but were not airtight. Once back at the laboratory, the samples were placed into individual 5.0L, rectangular, transparent plastic containers (290mm x 200mm x 95mm) that were aerated via a section of mesh glued to the lid and the base lined with paper towel. Samples were held in the culture room and observed daily over a period of two to three weeks for emergence of adult moths or parasitoids. Following the observation period, the leaf litter was searched for pupal cases. Additional observations on the pupation site of the quandong moth were made throughout the study during the course of field and laboratory work, including examination of tree trunks.

The sex of adults was determined by placing moths into a clear plastic vial and observing genitalia under the stereo microscope. The lengths of male and female moths were measured under a stereo microscope from head tip to wing tip using an eyepiece micrometer at 10x magnification. A two sample t-test was conducted to determine if there was a significant difference between the lengths of male and female moths (Statistix®, Analytical Software, Tallahassee, Florida, U.S.A).

An experiment was conducted to examine whether feeding influenced the longevity of unmated adults of the quandong moth. Males and females were examined separately. The experiment

was designed as a two-way ANOVA to examine if there was an interaction between the sex of moths and the presence of food. Male and female moths no older than 24 hours were collected from emergence containers and placed in small jars with the sexes kept separate. The four treatments were unfed males, unfed females, fed males and fed females. Fed moths were provided with a honey feeder (Section 2.8). The jars were placed inside an airtight 3.0L canister at 24°C and a constant relative humidity of 75 ± 5% (mean ± SD) was maintained inside each canister using a saturated solution of sodium chloride (Winston and Bates, 1960). The jars were examined daily and when dead moths were observed, they were recorded and removed from the experiment. The honey feeders were topped up as required to ensure a constant food source was available. The data were analysed with a two-way ANOVA (Genstat 5, Version 4.1 Rothamstead Experimental Station, England).

A number of artificial oviposition substrates were presented to females, including fans of paper towel, cardboard, wax film and greaseproof paper, excised calyces of quandong fruit and dehydrated pieces of quandong fruit, as well as dehydrated pieces of other fruits such as apricot, apple and pear. Cardboard fans treated with a quandong solution were also prepared by rehydrating 5g of dried de-stoned quandong fruit overnight in 50mL de-ionised water to produce an aqueous solution of quandong. Fans of cardboard were then dipped into the solution, allowed to air dry and then placed in mating cups (Section 2.9). The fans of all the various materials were either fixed to the side of mating cups with double-sided tape or suspended from the top of the cup by cotton. The substrates were chosen to represent a range of textures, with the folds in the fans providing sheltered oviposition sites and the quandong extract providing the odour of the host plant. Male and female quandong moths

were placed either in mating cups either in single pairs, or in groups of three or four pairs with various substrates for oviposition.

A mating chamber that has been used for diamondback moth (M. Keller, pers. comm.) was also trialed and consisted of a glass cylinder 150 x 20 mm. An oviposition stimulant was placed on the end of each of two corks, covered with a small square of fine mesh and sealed at each end of the chamber with a cork. The oviposition stimulants were quandong flowers, dehydrated fruit and leaves and filter paper that had been soaked in the aqueous quandong extract. Moths were placed inside the chamber, with a dental dam soaked in honey solution. Therefore, moths had the odours of the host plant in the chamber, could oviposit onto the mesh that was covering the end of the cork and then eggs could be easily handled after oviposition. Three or four moths pairs of males and females were placed inside each chamber, with 23 chambers trialed.

A trial was conducted to examine the fecundity and fertility of individual females. In the first batch consisting of 10 cups, one female and one male no older than 24 hours were placed in a mating cup with a feeder (Section 2.8). In the second and third batches of 15 and 19 cups, respectively, one female and two males were used to decrease the chances of male infertility or incompatibility with the female preventing mating and/or oviposition. Two fruit were provided as oviposition sites, in case for some reason, one of the fruits was an unsuitable oviposition site. The fruit were checked before being placed in the cups to ensure there were no eggs already present. Cups were checked once daily, the number of eggs laid on each fruit was recorded and the eggs were removed from the fruit and placed in hatching tubes (Section

2.4). Feeders were topped up with honey solution as required. Eggs in hatching tubes were checked daily for hatch to record the fertility of the eggs. Cups were maintained until the female died. All trials were conducted over a period from mid June to late August. Five of the females in the individual oviposition trial that did not oviposit were dissected on day 5 to examine if eggs were present.

3.3 RESULTS

3.3.1 The egg

The egg is oval, with a cream coloured and textured chorion when first laid (Figure 3.2). All the eggs observed in this study had an oval shape with a rounded surface, in contrast to Common's 1990 description of them as being flat. After approximately three days at 24°C the colour changed to pale yellow. One day before hatching, the head capsule of the developing embryo became visible through the chorion as a darkened area at one end of the egg. Infertile eggs developed to yellow but then desiccated and the embryo did not develop any further. The chorion of hatched eggs was translucent white with a textured surface, retained the oval shape and the hole at one end through which the neonate larva emerged. The mean length and width ± 95% C.I. of eggs was 0.35 ± 0.004mm and 0.20 ± 0.003mm (n = 102), respectively. In the culture room at 24°C, eggs took an average of 6.2 ± 0.3 days (mean ± 95% C.I.) to hatch.

Quandong moth eggs were observed on flowers, newly formed, semi-mature and mature fruit in the field. On open flowers, eggs were primarily laid next to or under the anthers and were

mainly found late in the flowering period, when no nectar remained in flowers. Eggs were also observed on the stems of unopened flower buds and concealed in between the flower bud and the remnants of the bract. When female moths were given flower buds in the laboratory and opened flowers for oviposition sites, they laid eggs on the same parts of the flowers as found in the field.

On fruit, eggs were laid in the calyx, next to the remnants of the anthers and also in the crevices between the perianth lobes. Females seemed to prefer trees bearing fruit with closed calyces that provided protection to the developing eggs. Of the 6,714 fruit examined throughout the three years of field sampling at Quorn and Sedan (Appendix 3, Table 1 –2), only six fruit had eggs laid in a location other than the calyx. The oviposition sites were exit holes made by mature larvae or in scars on the surface of the fruit. Similar to the calyx, these oviposition sites provided more protection to the developing larvae than an exposed calyx, or the bare surface of fruit. Quandong moths lay their eggs in loose aggregations. On some fruit there was only one batch of eggs centered around an anther, whereas on other fruit there were up to three aggregations around three of the anthers and others in the crevices between the perianth lobes. It was not possible to determine if the batches had been laid by a single female, or by several individuals. Up to four eggs were laid on a single flower (Figure 3.3) and up to 46 eggs on a single fruit (Figure 3.4).

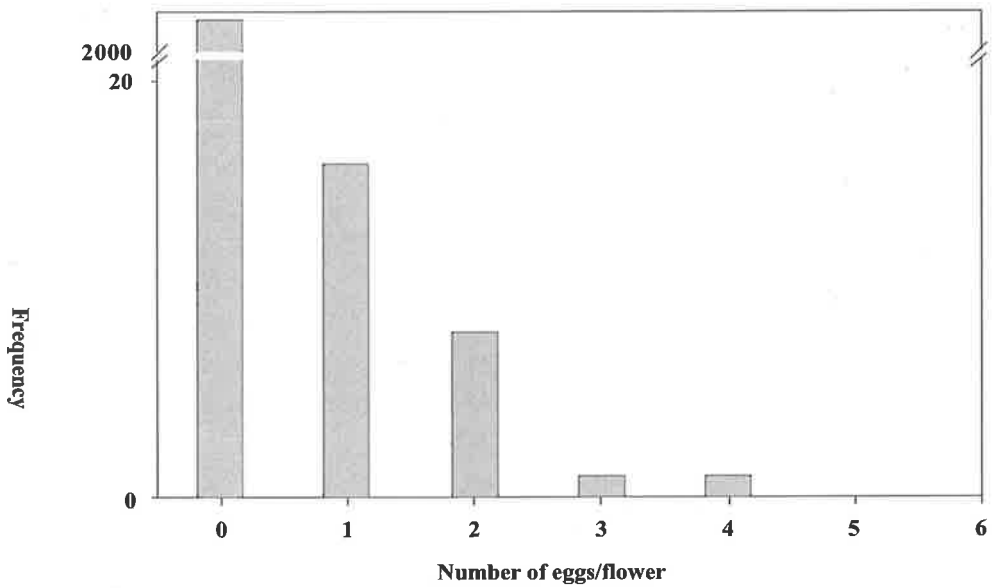


Figure 3.3: Number of quandong moth eggs on flowers collected from Quorn and Sedan 1998-2000.

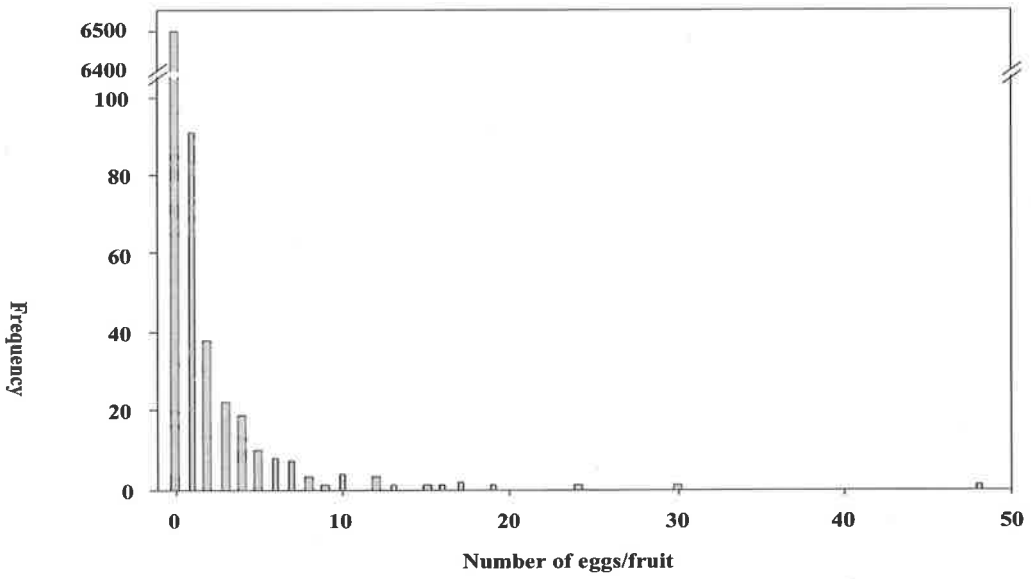


Figure 3.4: Number of quandong moth eggs on fruit collected from Quorn and Sedan 1998-2000.

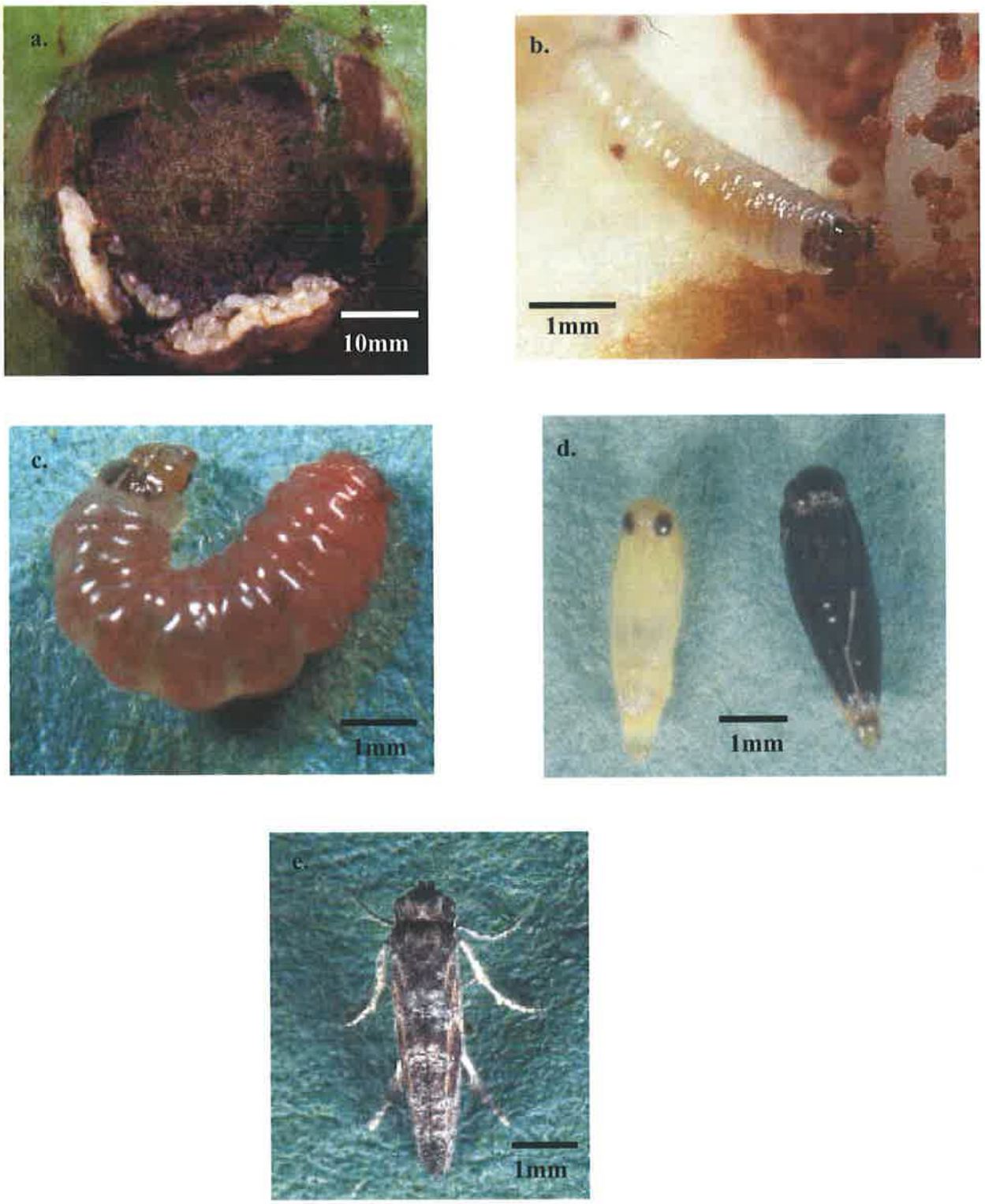


Figure 3.2: Stages of quandong moth (a) eggs in calyx of quandong fruit, (b) 2nd instar in fruit, (c) 4th instar, (d) pupae, immature left, mature right, (e) adult female.

3.3.2 The larva

The determination of the number of instars of the quandong moth is not straightforward, as there is considerable overlap between the head capsule widths of several instars. A 3-point moving average was computed to differentiate the boundaries between the instars. The mean head capsule widths were then calculated as the average of the two minima for each instar (Austin et. al., 1993). The results indicate that there are four instars of the quandong moth (Figure 3.5). The growth ratio, the ratio of each successive instar to the previous declines in successive instars and the data do not fit the Brooks-Dyar Rule (Table 3.1).

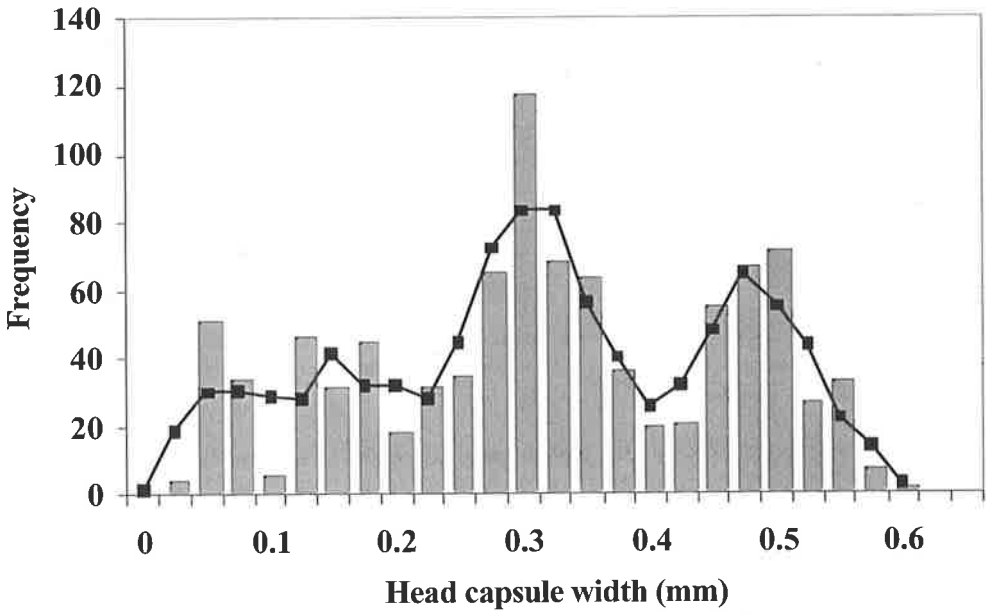


Figure 3.5: Frequency distribution of head capsule widths of quandong moth larvae (n = 956) collected from Quorn and Sedan, South Australia from July 1997 to November 2000. Line represents 3-point moving average.

Table 3.1: Head capsule widths and growth ratios of larval quandong moth

Instar Number	n	Mean head capsule width ± SE (mm)	Min	Max	Growth ratio
1	118	0.08 ± 0.05	0.025	0.125	-
2	134	0.18 ± 0.05	0.125	0.225	2.25
3	411	0.31 ± 0.09	0.225	0.4	1.72
4	293	0.5 ± 0.1	0.4	0.6	1.61

The first three instars are pale yellow with a dark brown head capsule (Figure 3.2). The early fourth instar is grey with a pale brown head capsule, and the late fourth instar is red with a pale brown head capsule (Figure 3.2). The observations on the duration of the larval stages began with 12 larvae, however only four were reared to the pupal stage with most dying in the first instar having never established on fruit. The average duration of the larval stages, from the first instar to pupation was 19.3 ± 0.94 days (mean ± 95% CI). Larvae were collected from the field in all instars and laboratory rearing was attempted throughout each year. The majority of larvae collected were active and made attempts to establish a feeding site, although the majority did not succeed. No evidence of diapause was seen in any larvae collected.

Of the 30 eggs used in observations of neonate larval behaviour, hatching and larval activity were observed in 12. Emergent larvae chewed a hole in one end of the egg but did not feed on the chorion after hatching. The first instar travelled on the surface of the fruit away from the calyx. Ten of the 12 larvae began feeding within five minutes of hatching. The two remaining larvae disappeared from the fruit after more than two hours of wandering on the surface of fruit. The entrance holes of first instars into fruit were less than 1mm in diameter. The distance of the larval entrance holes from the centre of the calyx was 6.7 ± 0.7mm (mean ±

95% C.I.). In observations made of the 10 feeding larvae, the larvae ingested the first mouthful of food. Within approximately 15 minutes of commencing feeding, the larvae began to deposit frass on the surface of the fruit, and all had completely burrowed into the fruit within two hours.

There was no cannibalism by first instars even in the extreme circumstances of hunger to which larvae were subjected. On every occasion, all larvae in the hatching tube were dead 24 hours after hatching, and the dehydrated bodies of all 20 larvae could be seen inside the tube. These observations also demonstrated that neonate larvae could not survive for 24 hours without a source of food.

From the 14 trays that were set up examining the activity of mature larvae, 84 fourth instars emerged in 12 of the trays. The heterogeneity chi-squared analysis indicated that the data were homogeneous, so the data were pooled and the subsequent chi-square showed that the number of larvae in each location differed significantly from the null hypothesis of equal frequencies ($\chi^2 = 81.93$, $P < 0.001$, $df = 2$). The majority of larvae were found directly below either of the two fruit in the tray (Figure 3.6). Seventeen of the 84 larvae that emerged from the fruit were found elsewhere in the base of the tray. Of the 17 larvae found elsewhere, 12 were trapped in the Tangle-Trap® at the base of the vial. Of the remaining five larvae, three were trapped directly below the point of a leaf, indicating they had crawled from the fruit to the edge of the leaf and then dropped to the surface. Three larvae were discovered quite some distance away from the fruit and the vial, one of which was almost 80mm away.

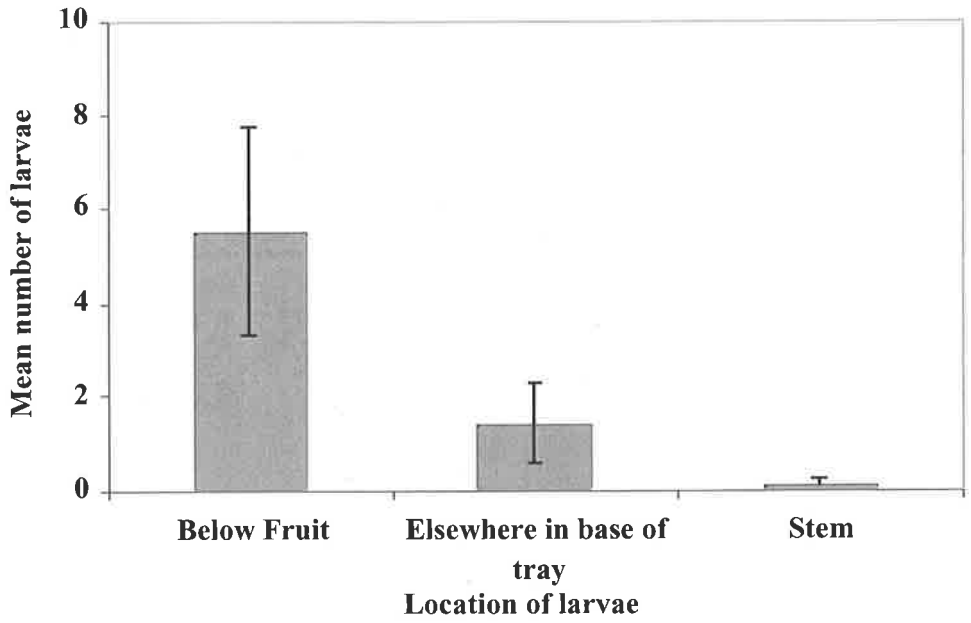


Figure 3.6: Location in which fourth instar quandong moths were trapped when emerging from fruit in search of pupation sites (n=84). Bars are 95% confidence intervals.

This experiment indicates that larvae drop directly from the fruit to the ground to pupate and do not descend via the trunk. Some fruit were in contact with the side of the vial and larvae were able to crawl from their exit point, to the vial and down to the tray. As a consequence, 12 of the 17 larvae found elsewhere in the tray were trapped directly at the base of the vial, where they contacted the TangleTrap® Additionally, some stems were left with some leaves attached, which were in contact with the surface of the fruit. This may explain the presence of larvae trapped directly below the point of a leaf.

3.3.3 The pupa

When first formed, pupae are light brown with green wings, later progressing to a light brown colour and finally darkening to a deeper brown in the two days before eclosion (Figure 3.2). At 24°C the pupal stage lasts an average of 9.0 ± .0.2 days, mean ± 95% C.I. The genital

pores of mature pupae were examined to determine their sex. Sexing was only possible with mature pupae, as the genital pores were not visible on pupae in the first few days of development. Females had a v-shaped structure and males had a raised knob area in their genitalia on the penultimate abdominal segment.

Analysis of variance indicated that neither pupal sex nor the site from which pupae were collected had a significant effect on the length or width of pupae (Table 3.2 - 3.3). The mean length of pupae was 3.55±0.05mm and the mean weight was 1.78±0.08mg (mean ± 95% C.I., Table 3.4)

Table 3.2: ANOVA table for two-way analysis of variance for length of pupal quandong moth

Source	DF	SS	MS	F	p
Site	1	0.06551	0.06551	0.61	0.4369
Sex	1	0.00619	0.00619	0.06	0.8106
Site*Sex	1	0.03235	0.03235	0.30	0.5843
Residual	55	5.87375	0.10680		
Total	58	5.97780			

Table 3.3: ANOVA table for two-way analysis of variance for weight of pupal quandong moth

Source	DF	SS	MS	F	p
Site	1	0.16425	0.16425	3.57	0.0641
Sex	1	0.00309	0.00309	0.07	0.7966
Site*Sex	1	0.00661	0.00661	0.14	0.7061
Residual	55	2.53059	0.04601		
Total	58	2.70454			

Table 3.4: Morphometrics of pupal quandong moth from Quorn and Sedan, 2000.

Site	Pupal Length (mm ± 95%C.I.)			Pupal Weight (mg ± 95% C.I.)		
	Male	Female	Mean	Male	Female	Mean
Quorn	3.58 ± 0.14 (n = 14)	3.61 ± 0.10 (n = 17)	3.55 ± 0.05 (n = 59)	1.79 ± 0.18 (n = 14)	1.82 ± 0.16 (n = 17)	1.78 ± 0.08 (n = 59)
Sedan	3.50 ± 0.05 (n = 12)	3.49 ± 0.11 (n = 16)		1.78 ± 0.13 (n = 12)	1.71 ± 0.18 (n = 16)	

A total of six moths, three males and three females, emerged from the samples of leaf litter collected from beneath trees (Table 3.5). No pupae were detected on tree trunks from either Quorn or Sedan during the same period. Although spider egg sacs were often found underneath the tape bands used to identify trees, there was no evidence that quandong moth larvae had pupated there.

Of the 6,714 fruit examined over the course of field sampling at Quorn and Sedan, (Appendix 3, Table 1 – 2) one pupa was found inside the calyx of each of two fruit collected from the tree and two collected from the ground. Pupae were also found inside the kernel of fruit in which the seed coat had already hardened in two fruit from the tree and 10 fruit from the ground. A dead moth was found inside the kernel of one fruit collected from the ground.

In large pupation containers in the laboratory, pupae were found in two main locations; underneath the paper towel in the base of the container and in the groove where the lid sat on

the base of the container. In all cases, the pupae were found inside a cocoon and the pupa was not protruded from the cocoon when adults emerged.

Table 3.5: Number of male and female quandong moths that emerged from leaf litter samples collected at Quorn (n = 18) and Sedan (n = 20). Dates in brackets are the day that moths emerged.

Date leaf litter sample collected	Quorn		Date leaf litter sample collected	Sedan	
	Male	Female		Male	Female
14 August 1998	0	0	17 September 1998	0	0
2 September 1998	0	1 (19 Sept)	15 October 1998	1 (22 Oct)	0
13 October 1998	1 (21 Oct)	1 (22 Oct)	4 November 1998	0	0
28 October 1998	1 (30 Oct)	1 (3 Nov)			
Total	2	3	**Total**	1	0

3.3.4 The adult

At rest, adult quandong moths sit with both their wings and their antennae parallel against their body and with their body in an upright resting position. The moths are grey, with several black stripes running horizontally across their wings and bronze coloured markings running laterally (Figure 3.2). There is individual variation in the horizontal stripes, which are less distinct on some moths than on others. On some moths three or four stripes were well-defined and on others only one stripe could be seen clearly. The variation in the number of stripes was not sex specific. The bronze markings were more prevalent on the dorsal surfaces

of females, and on males were confined mainly to the lateral areas. The posterior edge of both sets of wings was fringed with hair-like scales.

Female moths are significantly larger than males ($T = 2.82$, $p = 0.0056$, $df = 114$). The mean length (± 95% C.I.) of male moths was 4.53 ± 0.06mm ($n = 60$) and female moths was 4.68 ± 0.09mm ($n = 49$).

Male quandong moths were distinguished by a pair of claspers at the tip of the abdomen. In females, the claspers are absent and the genitalia is primarily hidden inside the abdomen, but the tip of the ovipositor of the female was often visible. In some instances, females were seen probing the surface of the vial with their ovipositor. Like many Lepidoptera, female quandong moths have a flexible ovipositor that allows them to deposit eggs into the sheltered oviposition sites that they favour.

Only food had a significant effect on the longevity of adult quandong moths (Table 3.6), increasing the survival time of both males and females by a factor of approximately three (Figure 3.7). Unfed males lived for an average of 5.3 days ($n = 28$), whereas fed males lived for 17.2 days ($n = 18$). Similarly, unfed females lived for an average of 6.3 days ($n = 30$) and fed females for 18.2 days ($n = 28$). There was no significant difference between males and females for either treatment, fed or unfed.

Table 3.6: ANOVA table for two-way analysis of variance of longevity of adult quandong moths at 24°C in the laboratory

Source	df	SS	MS	F	p
Sex	1	30.1192	30.1192	1.70	0.1950
Food	1	4241.79	4241.79	239.70	< 0.001
Sex*Food	1	0.06248	0.06248	0.00	0.09527
Residual	100	1769.60	1769.60		
Total	103	6041.57			

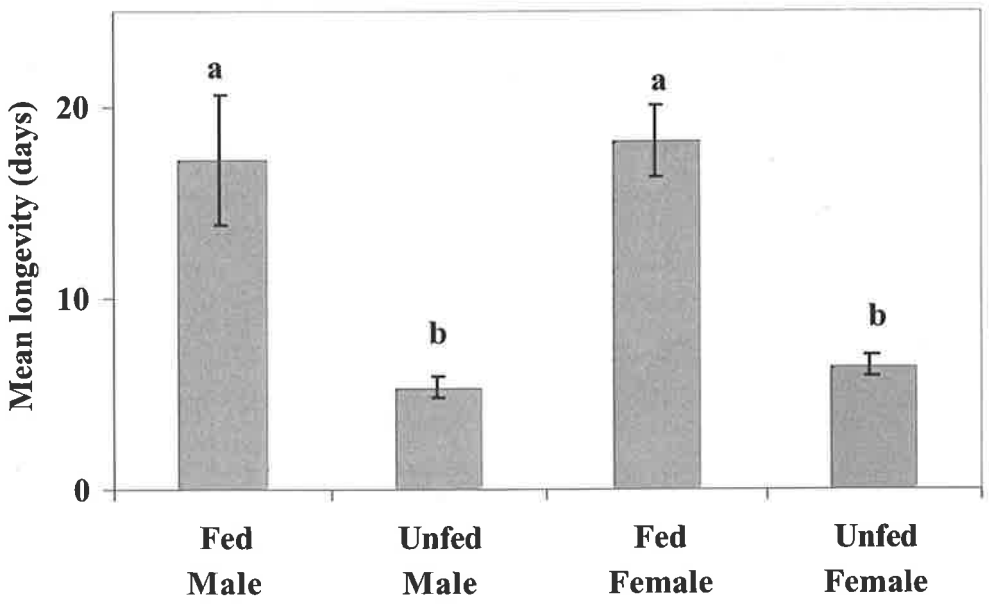


Figure 3.7: Mean longevity of male and female quandong moths when fed or unfed at 24°C and 75% relative humidity. Letters denote significance as determined by two-way ANOVA ($p < 0.001$). Bars are 95% confidence intervals.

Eggs were laid in only one of the 52 cups set up with non-host substrates. Two infertile eggs were laid on a cardboard fan that had been soaked in the aqueous extract of quandong. No eggs were laid in the glass chambers.

Of the 44 females used in individual mating cups only four laid any eggs (Figure 3.8). Of those that laid eggs, one female laid only 2 eggs in her lifetime and both of those were infertile. As the pattern of oviposition was not similar to the other 3 females that laid primarily larger clutches of fertile eggs, the 2 infertile eggs were excluded from the data. There was no oviposition in the remaining 40 cups. Females began ovipositing two days after they emerged and oviposition continued for their lifetime under laboratory conditions (Figure 3.8). They laid up to 94 eggs in a lifetime and viability of eggs exceeded 80% (Table 3.7).

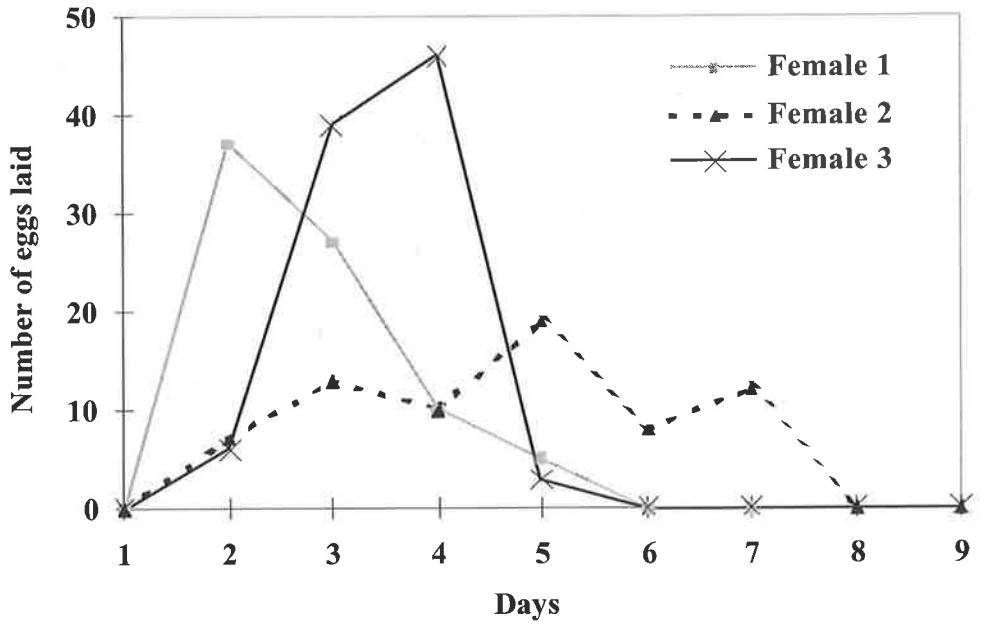


Figure 3.8: Oviposition on quandong fruit by three female quandong moths in individual oviposition trials in the laboratory.

Table 3.7: The fecundity and viability of eggs of female quandong moths in individual oviposition trials in the laboratory.

Female	Total number of eggs laid per female	Egg Viability (%)
1	79	81.0
2	69	82.4
3	94	88.7

Most of the eggs were found in masses larger than 5 with a maximum mass size of 46. As fruit were checked for eggs only once every 24 hours individual masses could not be discriminated and it is possible that large masses consisted of several smaller batches laid in distinct oviposition events in the 24 hour period. The longevity of mated females in the individual oviposition trials was 8.3 ± 1.3 days, (mean ± 95% C.I., n=3). In the five females that were dissected on day 5, 12.6 ± 2.7 mature eggs (mean ± 95% C.I.) were found in the abdomen.

When handling adults it was noted that they often exhibited a 'play dead' behaviour when disturbed, a defensive adaptation common to many insects. The adults were extremely placid and easily handled in the laboratory.

3.4 DISCUSSION

There were a large number of single eggs found on fruit throughout the years of sampling at both sites. The question then raised is why are so many single eggs found, particularly when field collections indicated that the majority of single eggs are infertile? Either females laid

single infertile eggs, or the single eggs are remnants of a larger clutch of eggs from which all the others have hatched and fallen from the fruit. In many instances, hatched eggs were found at the same time as unhatched eggs, indicating that hatched eggs often remain attached to the fruit, so it is unlikely that the single infertile egg remains from a larger clutch. It is more likely that single infertile eggs are a result of oviposition after unsuccessful mating, or when mating has not occurred at all.

The Brooks-Dyar rule has proven to be practical for many Lepidoptera (Gaines and Campbell, 1935; Caltagirone et. al., 1983; Floater, 1996) but not for all (Got, 1988; Jobin et. al., 1992; Van Schagen et. al., 1992). Many authors have reported a negative correlation between instar number and growth ratio of lepidopteran larvae, as is the case with quandong moth, which contradicts the Brooks-Dyar rule (Jobin et. al., 1992; Van Schagen et. al., 1992; McClellan and Logan, 1994). Daly (1985) reported that the growth ratios of 28 species of Lepidoptera were in the range 1.27 – 1.72. For the quandong moth, the ratio of growth in the second instar to that in the first instar is 2.25. This result is viewed with some caution as it involves the two smallest instars, with the greatest difficulty for measurement of head capsules and of which the least number were measured.

The behaviour of the neonate larva is critical to management of the quandong moth. As eggs are laid on flowers or fruit neonate larvae do not have to travel long distances to locate their food source. Larvae begin feeding and burrow into the fruit relatively quickly after emerging from eggs. Geier (1963) reported that neonate larvae of codling moth, *Cydia pomonella* Linnaeus (Lepidoptera: Tortricidae), spent between a few minutes and two hours searching

the surface of a fruit for an entry point. Entry times were short when codling moth larvae entered the fruit at an existing damage site. Like quandong moth, codling moth larvae also took approximately two hours to penetrate the fruit completely. If moths are to be targeted for control while in the larval stage, management strategies must be timed to correspond to the period when neonate larvae are hatching from eggs.

Although larvae of both quandong moth and codling moth feed in protected sites inside fruit, the location of oviposition is different. Some female codling moths lay their eggs on fruit, but the majority lay their eggs on leaves, primarily on the upper surface (Geier, 1963). Therefore, some neonate codling moth would have to travel further than others to locate a fruit and begin feeding, making them more susceptible to natural enemies and contact insecticides than larval quandong moth. It is possible that not all the larval quandong moth that hatch from eggs on a single fruit will penetrate that fruit. Due to crowding, some larvae may travel further than others to find an alternative fruit on which to begin feeding. The maximum number of larvae found in any fruit sampled throughout the study was 12, with the majority of those being younger than fourth instars. Unlike neonate codling moth larvae, quandong moth larvae do ingest the first mouthful of food, and therefore it is unlikely that neonate quandong moth larvae would avoid stomach poisons as does the codling moth.

The observations on neonate larvae demonstrated that they begin to search immediately for a food source, and in most cases, begin feeding with five minutes of hatching. Dispersal from the site of hatching is a strategy used by larvae of many species of insects to avoid parasitism

as some parasitic wasps use hatched eggs and the presence of host frass to locate a host (Quicke, 1997).

The pupation sites of Lepidoptera are varied, as are the methods via which larvae reach their pupation site. Some Lepidoptera pupate in or near their feeding site (Hardy, 1982; Ireson and McQuillan, 1984) while others crawl from their feeding site to the branches or trunk of the tree to pupate (Wearing, 1975). Others crawl or drop on silken threads to the soil or leaf litter below trees to pupate (Daiber, 1979c; Austin et. al., 1993). When a mature quandong moth larva feeding inside a fruit on a tree is ready to pupate, it chews an exit hole in the fruit, and drops to the ground. If the fruit has already dropped from the tree, the larva probably chews an exit hole and crawls directly to the leaf litter to pupate. The use of silk threads was never observed in larvae of the quandong moth. The current results suggest that few, if any, larvae pupate on the tree or descend to the ground via the trunk. Therefore, management techniques employing trunk banding and entomogenous nematodes as used for larval codling moth (Kaya et. al., 1984), will not be effective for the quandong moth. The fourth instars of the quandong moth then reach their pupation site by dropping directly to the ground. In some Lepidoptera, several different pupation sites are used by the different generations during the year (Cordingley, 1980; Hardy, 1983; La Croix and Thindwa, 1986), but for the quandong moth the pupation site appears to be the same for all generations.

The low incidence of adults emerging from leaf litter samples collected in the field indicated that it was not an effective method of sampling pupae of quandong moth. Although it is commonly accepted that many sampling techniques greatly underestimate the size of the

population (Southwood, 1978), leaf litter sampling was unlikely to be an adequate method for estimation of pupal abundance and density. There are several possible explanations for the failure of population estimates gained from leaf litter samples to adequately represent the population. Pupal predation, by ants and other arthropods may reduce pupal numbers. It is also possible that larvae pupated deeper than 30mm into the leaf litter. The level of 30mm was chosen because it was the average depth of leaf litter at the base of trees and Common (1990a) had previously reported that the habit of moths in the Epermeniidae was to pupate in leaf litter. Pupal parasitism cannot explain the low numbers of moths emerging from pupae in leaf litter samples as no parasitoids were captured and the leaf litter was searched for any remaining pupae. Quandong trees are not deciduous, leaf drop occurs sporadically throughout the year, and removing leaf litter from beneath trees for the duration of this study would have greatly altered the ecology of the trees.

Calyces of fruit were examined on every sample date when searching for eggs and on only a few occasions were pupae found in the calyx of a fruit. Pupation inside the kernel of a fruit was only observed on two occasions throughout the study and in both cases the seed coat of the kernel had already hardened, making it impossible for an emerged moth to leave the kernel. In these few cases, the larvae must have become trapped inside the kernel while still feeding and therefore had no option but to pupate in the feeding site.

It appears that quandong moths prefer to construct their cocoons in a sheltered site and whether the surface is soft and textured or hard and smooth was not as important. No pupae

were ever found on the walls of the pupation containers, as it was a very exposed surface with no crevices in which to hide.

The experiment with fed and unfed adult quandong moths showed that not only are quandong moths able to feed, but that the longevity of fed moths is significantly increased compared to unfed moths. Some adult Lepidoptera, such as species in the subfamily Arctiinae (Noctuidae) and family Xyloryctidae have a very reduced or vestigial proboscis and are unable to feed (Bailey, 1978; Common, 1990b). In this study, female quandong moths did not live for longer than males at 24°C. There was a high degree of variation within the treatments in the experiment, which could account for the discrepancy with results found later in the study where females are shown to live longer than males over a range of temperatures (Section 4.4.).

The pre-ovipositional period of the quandong moth was similar to that recorded for other Lepidoptera (Etman and Hooper, 1979; Daiber, 1980; Hardy, 1983). Female quandong moths were extremely discerning with oviposition sites. On only one occasion a moth laid eggs in a location other than quandong flowers or fruit and in that case the eggs were infertile. Many researchers have reported oviposition of Lepidoptera on various artificial substrates in the laboratory, ranging from materials such as cheesecloth (Shearer et. al., 1995) and velour (Wilson and McClurg, 1986) to waxfilm (Trudel et. al., 1995). Others have used extracts of the host plant on a textured substrate (Elsey and McFadden, 1981). However, there are Lepidoptera that are strictly host specific and have not been induced to oviposit on any artificial substrates, such as the grape berry moth, *Endopiza viteana* Clemens (Lepidoptera: Tortricidae) (Clark and Dennehy, 1988) and a *Yponomeuta* species (Hora and Roessingh,

1999). Like the quandong moth, these two species are monophagous and the host selection process begins with oviposition on the host plant. For quandong moth, an artificial oviposition substrate would greatly facilitate laboratory rearing but a suitable substrate was not identified during this study.

Once on a potential oviposition substrate, both tactile and olfactory cues are important to stimulate oviposition in Lepidoptera. Stimuli from the host are perceived with the antennae (Ramaswamny, 1988; Delisle et. al., 1989) and there is evidence to suggest that stimuli are also received via the tarsi (Qui et. al., 1998). In Lepidoptera the ovipositor has been found to have both phytochemical sensillae (Valencia and Rice, 1982; Marion-Poll et. al., 1992; Qui et. al., 1998) and mechanoreceptors, where acceptance of a site is based mainly on structural cues from the substrate (Valencia and Rice, 1982; Marion-Poll et. al., 1992). In a monophagous *Yponomeuta* species, Hora and Roessingh (1999) found that it was primarily the contact cues that were responsible for host discrimination which may explain why quandong moths presented with flowers or fruit underneath a mesh cover in mating chambers did not oviposit. Presumably, the olfactory cues would be present in the chamber but the tactile cues would not. Many times, when examining female quandong moths in vials, in the absence of an oviposition substrate, they were observed probing the surface of the vial with their ovipositor. Both Hora and Roessingh (1999) and Spencer (1996) observed probing behaviour only once an oviposition stimulant had been encountered. In the quandong moth it appears that an initial stimulant is not as important in inciting probing behaviour. Renwick and Chew (1994) suggest that within the Lepidoptera, plant surface texture is more important to moths, and this is also true of the quandong moth where texture seems critical to host discrimination.

Olfactory stimuli may also be important and it is likely that a complex combination of cues is required to stimulate oviposition.

For monophagous insects, oviposition site selection by the female is critical for maximising the chance of their offspring becoming established. It is unlikely that a neonate larva could find a host if laid on a non-host plant, and many neonate larvae will not feed or are unable to complete development on non-host plants (Hora and Roessingh, 1999). Thus, females maintain monophagy via host selection. However, suitable oviposition sites probably cannot explain the lack of oviposition by females in the oviposition trials. The majority of the fruit provided to those females was sourced from an uninfested quandong tree from which fruit had been used successfully in the previous studies to stimulate oviposition. In most cases, two fruit were provided for each female and fruit with closed calyces were specifically selected for the trials. In the cups with only one female and one male, it could be reasoned that mating was unsuccessful between the pair. Even so, cups in later batches that contained one female and two males were no more successful than those with a single pair. The lack of oviposition could have been due to any number of external factors such as unfavourable temperatures or photoperiods, or disturbed behavioural patterns.

4. TEMPERATURE AND DEVELOPMENT

4.1 INTRODUCTION

The concept of degree-day accumulation is the fundamental basis for population models examining temperature-dependent development. Although the developmental rates of insects at high or low temperatures are often non-linear, across an intermediate temperature range, such as those actually experienced by insects in the field, the rates are linear. This relationship is used to establish theoretical threshold temperatures (Arnold, 1959) and estimate thermal constants (Campbell et. al., 1974) on which population models are based.

Inability to establish a laboratory culture of the quandong moth meant that studies were solely reliant on field collected material. This limited the number of individuals available in the various stages, and so sample sizes in the following studies are not as large as they would have been had a mass rearing technique been available. As larvae were unable to be reared on an artificial media in the laboratory, and it was difficult to keep fruit free of fungal contamination even at room temperature, the studies on temperature and development were only conducted on eggs, pupae and adults of the quandong moth.

The different threshold temperatures, developmental rates and optimum temperatures of the various stages of the quandong moth have implications for any phenological model used to predict development of the moth. To ensure the predictions are as accurate as possible, different parameters must be used for the different stages of the moth.

4.2 EGGS

4.2.1 Materials and Methods

Eggs no older than 24 hours were collected from mating cups (Section 2.9). Fifteen eggs were placed in small Petri dishes, 55mm in diameter. Eggs were maintained in constant temperature cabinets with temperatures of 15, 19, 24, 31 and 33°C, and a photoperiod of 14L:10D. The average temperature over the course of the experiment was monitored with a Tinytalk 0023® datalogger (Gemini Data Loggers, West Sussex, England). Moistened filter paper in the base of the Petri dishes maintained the relative humidity near 100%. The dishes were checked daily and any hatched larvae recorded and removed. The median day to eclosion was calculated for each temperature and the data analysed using the Population Model Design System (PMDS), Version 6.3. PMDS is a FORTRAN based program that concurrently fits functions to developmental data and determines the function that best fits a particular data set (Logan, 1988).

4.2.2 Results

Measured mean temperature values were used in the analysis for all stages rather than the set temperatures (Table 4.1). Not all of the eggs hatched, leaving between 9-15 eggs for each temperature. The best fit for the developmental data for eggs was an Exponential T_b function, based on the criterion of maximum adjusted coefficient of determination (Kvalseth, 1985).

Table 4.1: Temperature and relative humidity in incubators set at various temperatures.

Set Temperature (°C)	Mean temperature recorded ± SE (°C)	Mean relative humidity recorded ± SE (%)
15	13.8 ± 0.01	80 ± 0.03
19	18.9 ± 0.01	80.5 ± 0.1
24	23.9 ± 0.03	79.4 ± 0.04
28	27.3 ± 0.02	76.2 ± 0.06
32	30.9 ± 0.05	75.3 ± 0.1
33	33.1 ± 0.02	76.0 ± 0.06
36	35.3 ± 0.01	78.2 ± 0.09

The equation for the Exponential T_b, an exponential form modified for a low temperature threshold is:

$$\text{Developmental Rate} = [e^{\rho(T-Tb)}] - 1,$$

where _ is the slope, T the actual temperature and T_b the lower temperature threshold. The observed values were a good fit to those predicted by the Exponential T_b model (Figure 4.1). The PMDS output gave the following parameter estimations; slope = 0.008 and T_b = 4.2°C. Degree-day estimates were made over the linear portion of the developmental rate for eggs. Asymptotic 95% confidence intervals were calculated for all linear regressions (Zar, 1996).

The lower developmental threshold for eggs, determined by rearranging the equation for the linear regression when the development rate was equal to zero (Onstad et. al., 1985) was 4.5°C. The data indicate that the upper threshold for eggs is somewhere above 33°C. The number of degree-days above 4.5°C required for eggs to hatch, estimated from the reciprocal of the slope of the regression line over the linear portion of the curve, was 121 (Figure 4.2).

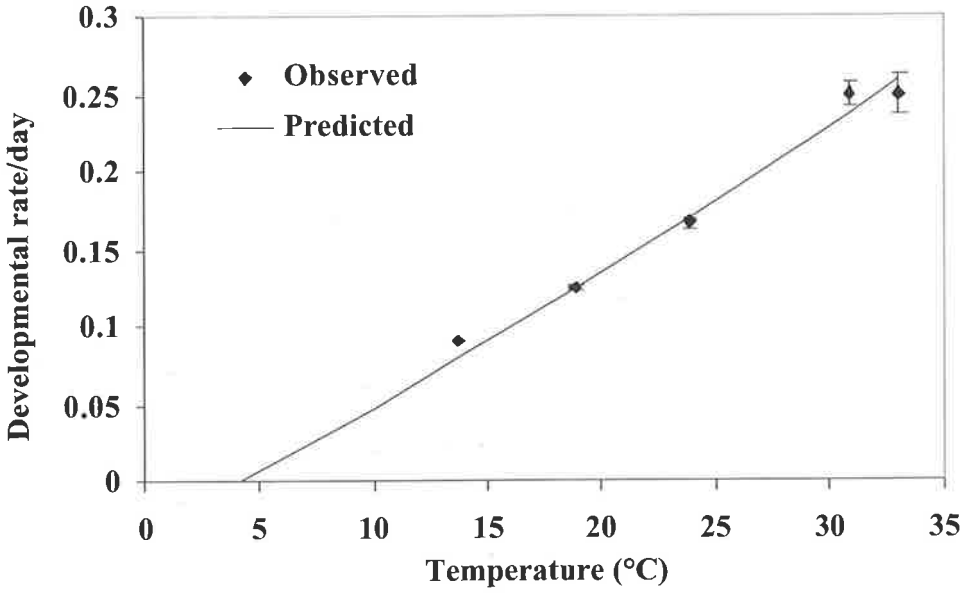


Figure 4.1: Comparison of observed and predicted values for the Exponential T_b model fitted to developmental rate data for eggs of the quandong moth using PMDS. Bars are 95% confidence intervals.

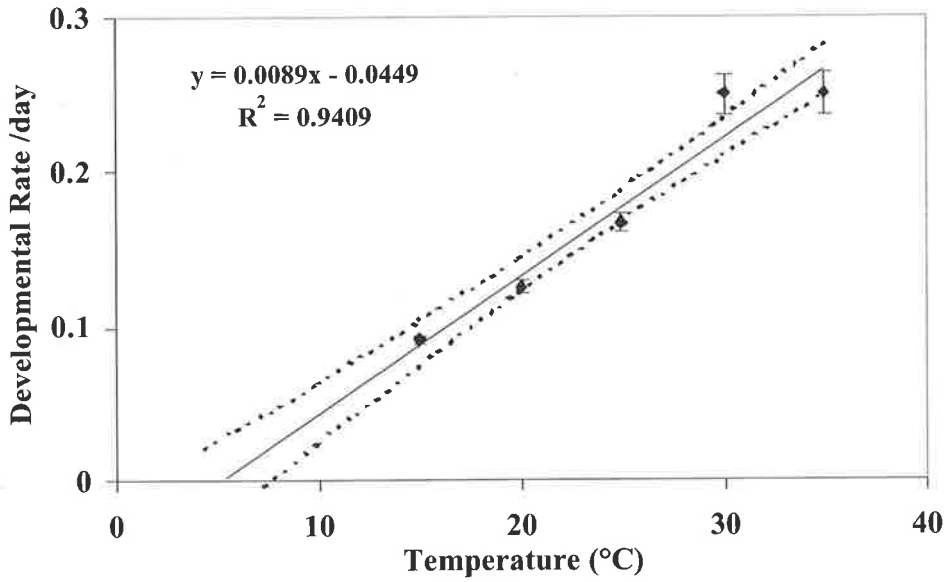


Figure 4.2: Linear regression of developmental rates for eggs of the quandong moth against temperature. Bars are 95% confidence intervals. Broken lines are 95% confidence intervals for the regression line.

4.3 PUPAE

4.3.1 Materials and Methods

Pupae no older than 24 hours were collected from pupation containers (Section 2.6) and put in small jars with a maximum of 30 pupae per jar. The jars were then placed inside an airtight 3.0L canister (Prestige® Australia). A constant relative humidity of approximately 75% was maintained inside each canister using a saturated solution of sodium chloride (Winston and Bates, 1960). The canisters were held in cabinets set at temperatures of 15, 19, 24, 28, 32 and 36°C. The average temperature over the course of the experiment was monitored as for eggs, and relative humidity data recorded with a Tinytalk 0302® datalogger (Gemini Data Loggers, West Sussex, England). Jars were checked daily for eclosion of adults. Adults were removed as they eclosed and their sex was recorded. A two-way ANOVA was conducted to determine if there was an interaction between sex and temperature or if the main effects of sex and temperature were significant (Statistix®, Analytical Software, Tallahassee, Florida, U.S.A). The median day to eclosion was calculated for each temperature and the function that best fitted the data was determined using PMDS (Logan et. al., 1976).

4.3.2 Results

Relative humidity varied between 75.3 and 80% at the various temperatures (Table 4.1). Only temperature had a significant effect on the development rate of quandong moth pupae (Table 4.2).

Table 4.2: Two-way ANOVA of pupae to determine the effects of sex and temperature on developmental rate.

Source	DF	SS	MS	F	p
Sex	1	0.324	0.324	0.29	0.5913
Temp	4	10796.3	2699.07	2408.45	< 0.0001
Sex*Temp	4	2.216	0.554	0.49	0.7428
Residual	240	268.96	1.12067		
Total	249	11067.8			

The developmental rates for males and females were pooled and the best fit determined using PMDS was the Stinner model, based on the criterion of maximum adjusted coefficient of determination (Kvalseth, 1985) (Figure 4.3). The equation for the Stinner model is:

$$\textbf{Developmental Rate} = \frac{C}{(1+e^{k_1+k_2T})} \quad \textbf{for } T < T_{opt} \textbf{ and}$$

$$\textbf{Developmental Rate} = \frac{C}{(1+e^{k_1+k_2(2*T_{opt}-T)})} \quad \textbf{for } T > T_{opt},$$

where C is the asymptote of the function, k_1 and k_2 are constants, T_{opt} is the temperature at which the development rate is at a maximum and T is the actual temperature (Stinner et. al., 1974). The PMDS output gave the following parameter estimations; C = 0.21, k_1 = 3.57, k_2 = -0.16 and T_{opt} = 29.3°C.

As with eggs, the development rate of pupae was regressed against temperature over the linear portion of the development rate (Figure 4.4). The lower development threshold for pupae was 8.1°C. The upper development threshold determined by the point at which pupal

development rate peaked (Brunner and Rice, 1984), was 29.3°C. The number of degree-days above 8.1°C required for eggs to hatch was 139.

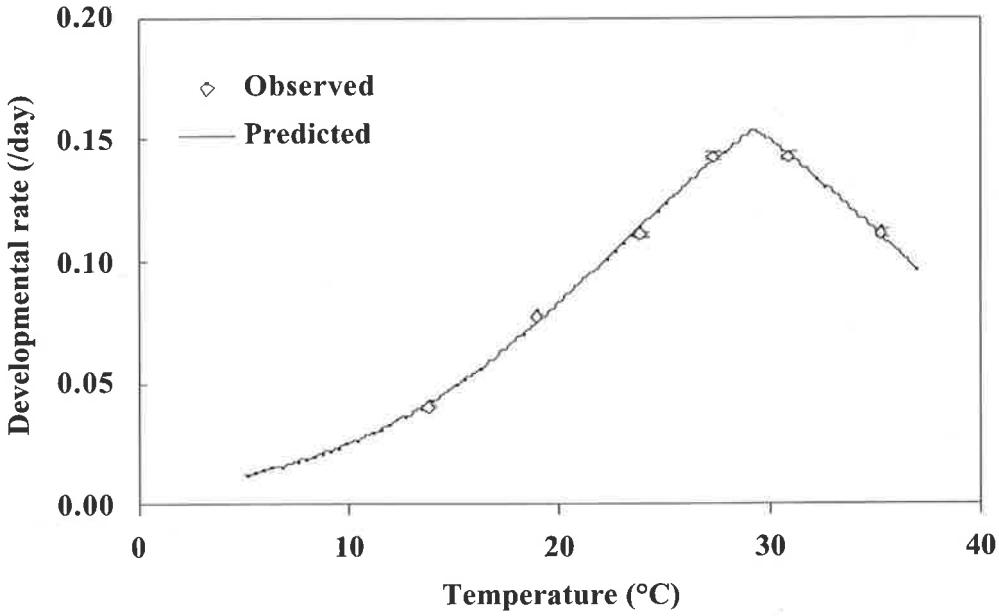


Figure 4.3 Comparison of observed and predicted values for the Stinner model fitted to developmental rate data for pupae of the quandong moth using PMDS. Bars are 95% confidence intervals.

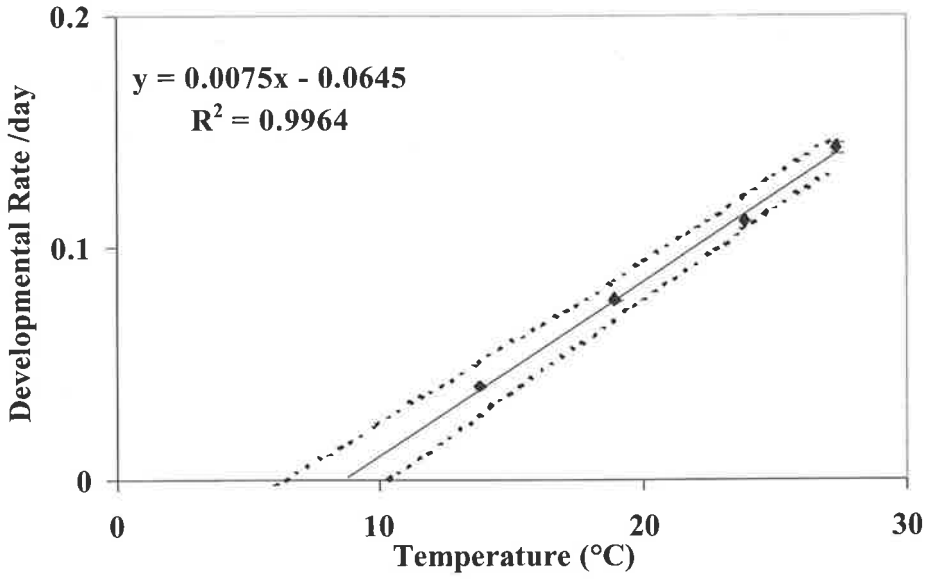


Figure 4.4: Linear regression of developmental rates for pupae of the quandong moth against temperature. Bars are 95% confidence intervals. Broken lines are 95% confidence intervals for the regression line.

4.4 ADULTS

4.4.1 Materials and Methods

The longevity of unmated and unfed male and female moths was determined at temperatures of 15, 19, 24 and 28°C. Moths no older than 24 hours were collected from emergence cages and placed into small jars with 5-10 individuals in each jar. The jars were placed inside airtight canisters and the relative humidity was maintained at approximately 75% (Section 4.3.1). Temperature and relative humidity data were measured the same way as for pupae (Table 4.1). Individual ANOVAs were conducted for each temperature because replication was disproportionate between temperatures (Statistix®, Analytical Software, Tallahassee, Florida, U.S.A). Development functions were also fitted to the data for the rate of ageing for adults using PMDS.

4.4.2 Results

At each temperature female quandong moths lived for significantly longer than males (Figure 4.5, Table 4.3). As the sex of moths had a significant effect on longevity at various temperatures, the rate of ageing of males and females was analysed separately using PMDS and development functions fitted to the data. For males an observation at 32°C and for females an observation at 36°C which had not been replicated for the opposite sex were included when fitting functions in PMDS.

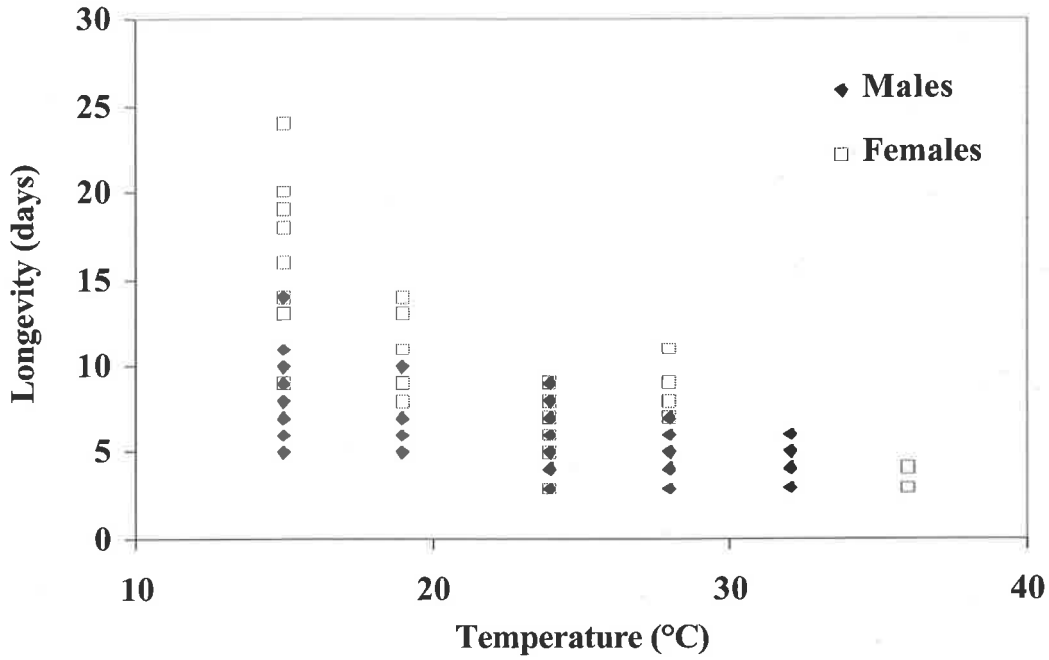


Figure 4.5: Longevity of male and female quandong moths at various constant temperatures and relative humidity of 75%.

Table 4.3: Statistics for individual analyses of variance conducted to determine if sex had a significant effect on the longevity of adult quandong moths over a range of temperatures.

Temperature (°C)	ANOVA statistics		
	F	p	df
15	28.23	<0.001	1
19	16.82	0.007	1
24	6.83	0.0115	1
28	21.12	0.003	1

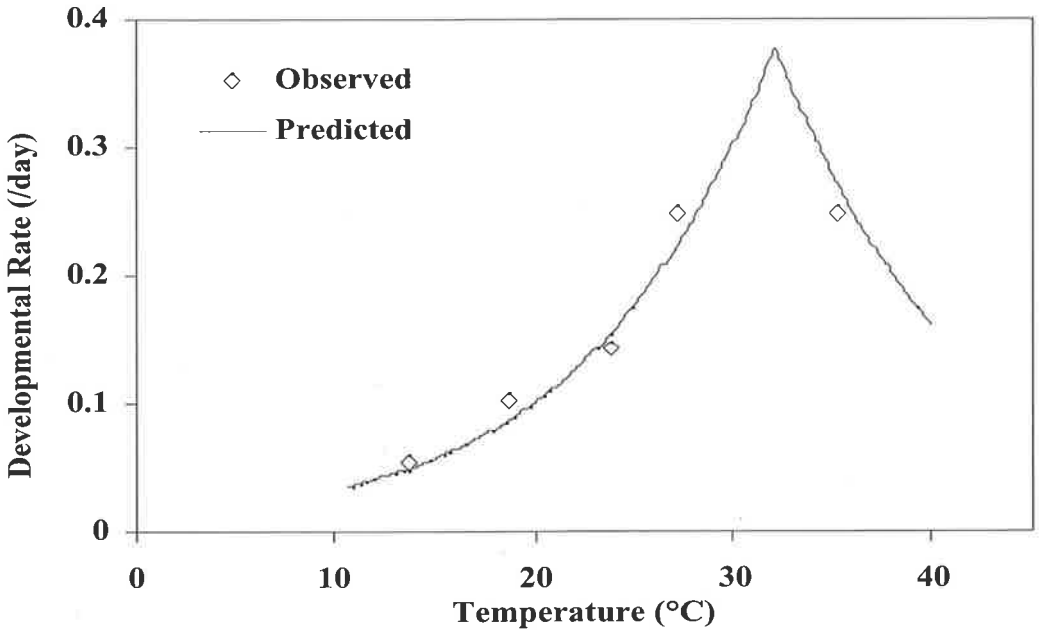


Figure 4.6 Comparison of observed and predicted values for the Stinner model fitted to median developmental rates of female quandong moths using PMDS. Bars are 95% confidence intervals.

For adult females the Stinner model was determined to be the best-fit using PMDS, based on the criterion of maximum adjusted coefficient of determination (Kvalseth, 1985) (Figure 4.6). The PMDS output gave the following parameter estimations for females, C = 4.45, k_1 = 6.07, k_2 = -0.12 and T_{opt} = 32.1°C. For males, the best fit determined using PMDS was the linear model, based on the criterion of maximum adjusted coefficient of determination (Kvalseth, 1985) (Figure 4.8). The equation for the linear model is:

$$\textbf{Developmental rate} = \rho(T - Tb),$$

where _ is the slope and T_b is the x intercept, or base temperature and both are determined from standard linear regression. The PMDS output gave the following parameter estimations for males, _ = 1.43 and x = 0.0085.

Degree-day estimations for female and male quandong moths were made over the linear portion of the developmental rate (Figure 4.7 and 4.8).

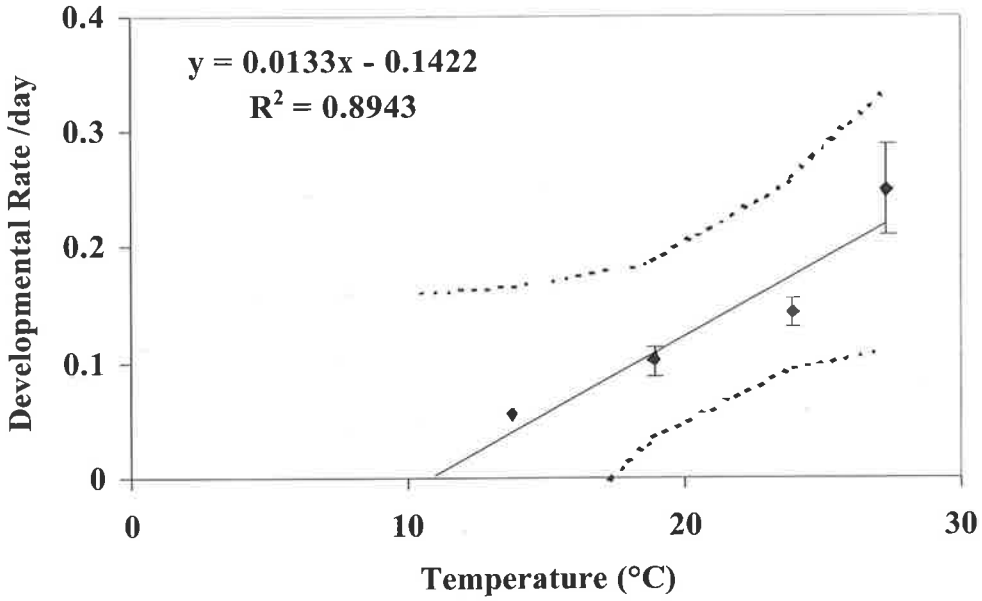


Figure 4.7: Comparison of observed and predicted values for the linear model fitted to median developmental rates of female quandong moths using PMDS. Bars are 95% confidence intervals. Broken lines are 95% confidence intervals for the regression line.

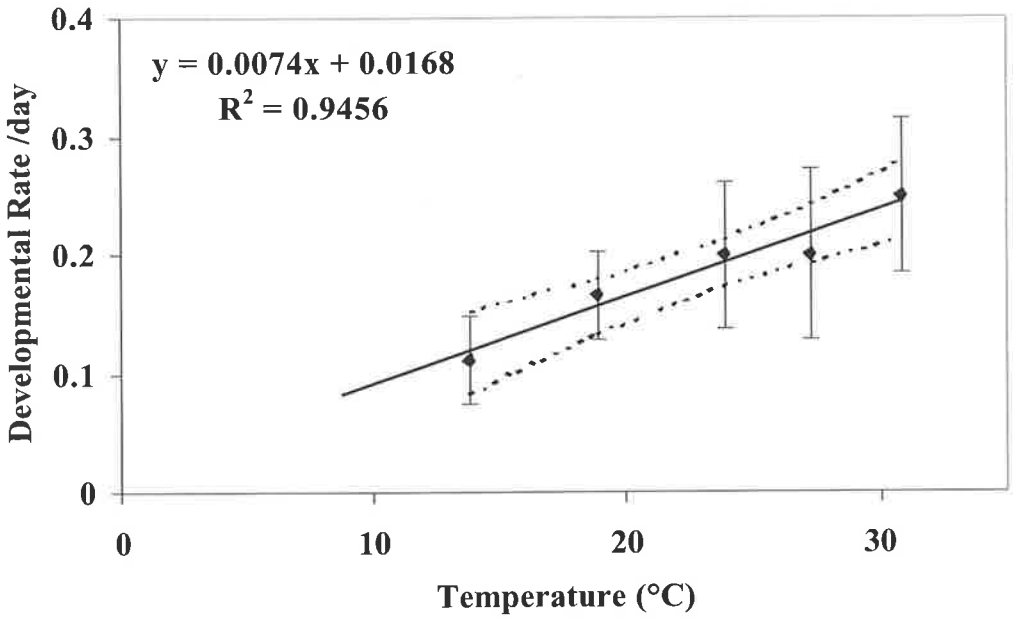


Figure 4.8: Comparison of observed and predicted values for the linear model fitted to median developmental rates of male quandong moths using PMDS. Bars are 95% confidence intervals. Broken lines are 95% confidence intervals for the regression line.

4.5 DISCUSSION

Each stage of the quandong moth has a different lower threshold, optimum temperature and developmental rate. An upper threshold was only detected for pupae. Lower thresholds were not determined for the adult stage as they do not represent activity or reproductive thresholds. Sex did not have a significant effect on the longevity of the pupal stage but it did have an effect in the adult stage. In the absence of food, adult females lived for significantly longer than males. However, when food was available, there was no significant difference between the sexes. The increased longevity of females compared to males in the absence of food could be attributed to egg resorbtion or a greater fat storage capacity in female moths.

Many authors have reported that the different stages of an insect may have evolved different threshold temperatures, and the different thresholds provide an advantage to that stage (Hanula et. al., 1984; Gangavalli and Aliniazee, 1985; Roltsch et. al., 1990). Gangavalli and AliNiazee (1985) reported that the overwintering fourth instar larva of the oblique banded leafroller, *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae) has a lower threshold than that of the fifth or sixth instars. The fourth instar is present when the temperatures are lower than what later instars would normally experience. However, the diapausing third instar has a significantly higher threshold than any of the other instars and the authors suggest that this is a mechanism to delay development until temperatures begin to increase and conditions are more favourable.

Although eggs of the quandong moth have a lower developmental threshold than pupae the species is not univoltine. For differing thresholds to be an advantage, stages within different

generations would need to have different thresholds compared to those within other generations. For example, eggs present during the summer generation would benefit from having a higher upper threshold than that of the winter generation so that hatching could occur over a wider range of temperatures during summer. Further, pupae of the winter generation would benefit from having a lower base threshold. The studies on adults showed different lower threshold temperatures for males and females. There was a marked difference between the thresholds due to the large amount of variation in the data, however, different thresholds for adults are not uncommon. Daiber (1980) reported that the lower threshold temperatures for adults of the false codling moth, *Cryptophlebia leucotreta* Meyr (Lepidoptera: Tortricidae) were 8.0°C and 9.5°C for males and females, respectively.

As the temperature increases so does the rate of ageing of adult quandong moths. At 36°C, unfed females lived for 3.7 days as compared with 17 days at 15°C. Many authors report that oviposition in Lepidoptera increases with temperature (Daiber, 1980; Mason and Mack, 1984; Mack and Backman, 1986; Leather, 1994), in some cases up to a threshold at which oviposition ceases (Mason and Mack, 1984; Leather, 1994). If oviposition does occur at high temperatures, it is likely there is no lag before the commencement of mating and oviposition as seen at lower temperatures. Further, at extreme high temperatures the rate of egg development will cease at an upper threshold, and the desiccation of eggs will also be increased. At low temperatures, females generally live longer than those at higher temperatures, but the rate of oviposition is greatly reduced at lower temperatures and the day on which oviposition commences is delayed (Daiber, 1980; Sands et. al., 1991; Leather, 1994). Extreme temperatures affect both the onset and the rate of oviposition and the development of eggs,

such that average temperatures in the range of 15-20°C are likely to be most favourable for maximising the fecundity and fertility of the quandong moth.

5. LIFE HISTORY AND DAMAGE

5.1 INTRODUCTION

The generation of the quandong moth that damages mature fruit has been familiar to quandong growers and consumers for many years. High larval densities are regularly reported in mature fruit harvested from commercial orchards, wild groves and backyard trees. Prior to this study, quandong fruit had been the only recorded host of quandong moth, but P.T. Bailey (pers. comm.) observed eggs of the quandong moth on fruit of the sandalwood tree, *Santalum spicatum* (R. Br.) A. DC. (Santalaceae). The quandong moth must survive up to four months, from November to March in most regions, without fruit, and the presence of an alternative host or a period of diapause had not been recorded. Regular field sampling was conducted for the entire duration of the project, beginning in July 1997, and concluding at the end of the fruiting cycle in November 2000, with the aim of determining the number and timing of the generations of the moth. The incidence and severity of the damage caused by the quandong moth was also investigated, with the aim of determining the most damaging generation and developing an economic injury level for the moth in quandong orchards.

Inter-tree variation in the phenology of budburst is a common phenomenon in many deciduous species (Hunter, 1992; Quiring, 1994). Although quandong trees are not deciduous, the summer generation of quandong moth is restricted by the phenology of fruit development. Larvae do not feed on leaves, therefore the period between the availability of mature fruit in spring and presence of buds suitable for larval feeding in summer is analogous to budburst in a deciduous species. Larvae are only able to begin feeding on buds once the buds reach a certain

stage of development, and once flowers have opened they are not suitable for feeding again until they begin to swell to form young fruit. Thus, there are two periods early in the development of fruit where larvae are restricted in the availability of feeding sites. Later generations of the moth are not restricted by tree phenology, as larvae are able to feed on fruit as soon as they begin to form right through to mature fruit. Several authors have reported that the trees that break budburst earliest also suffer the highest rate of damage by certain insect herbivores (Hunter, 1992; Quiring, 1994). Other authors have found no correlation between budburst and insect damage (Crawley and Akhteruzzaman, 1988; Watt and McFarlane, 1991). This relationship was therefore investigated for the quandong moth.

5.2 MATERIALS AND METHODS

5.2.1 Sample unit

The larvae of each generation of the quandong moth feed inside flowers or fruit so it was necessary to change the sample unit throughout the year from unopened flower buds and flowers through to developing and mature fruit. Samples were collected from field sites (Section 2.10) at random throughout the years, and the stage of development of the sample unit was recorded.

A major limitation of within-sample precision was leaving sufficient flowers or fruit to sustain sampling from the same trees throughout the entire year. During the periods of highest abundance of quandong moth larvae, up to 12 mature larvae/fruit were collected and the relative variation (RV) (Pedigo et. al., 1972) was 23.8%. During times of lower incidence, the

RV was much higher because of variation within, as well as among, trees. However, the sample size could not be increased due to the risk of not being able to sample throughout the entire year.

Quandong growers had noted that immature fruit dropped from the trees during May-July, following fruit set (AQIA pers. comm.). Prior to this study it was not known whether this phenomenon was due to a period of natural thinning or occurred as a result of feeding by larval quandong moth. Therefore, fruit samples were taken from both the trees and ground beneath the trees, as soon as fruit began to drop and for as long as fruit were present on the ground. The period of fruit drop was investigated further at Quorn in 2000. On two consecutive sampling dates during fruit drop, six trees that were not used for regular field sampling, that had both dropped fruit and fruit intact on trees were selected. From each tree, 20 fruit were collected from the ground and 20 from the tree. These samples were taken back to the laboratory and assessed in the same way as a regular sample (Section 5.2.3). Samples of fallen fruit were also collected from a tree at the Waite Campus of Adelaide University from which larvae of quandong moth had never been collected. The incidence of larvae and damage of the quandong moth in a sample of 100 fallen fruit was assessed.

Sample units were selected at random from around the entire tree and up to a height of 2.2m. On each sample date, a maximum of 8 flowers or fruit was collected from trees and a maximum of 10 fruit was collected from the ground beneath. There were times near the end of fruiting or during the start of fruit drop when the number of fruit available was less than the number

required and on all samples dates the number of fruit collected from both trees and the ground was recorded. Samples were collected at 2-3 weekly intervals.

5.2.2 Collection, transport and storage of samples

Quandong fruit is very susceptible to spoilage caused by secondary fungal outbreaks, so the method of transport and storage of samples was designed to minimise the build-up of humidity in the sample. Vials, 16x75mm, with mesh inserted in the lids for aeration were used to transport flowers to the laboratory. Flowers were placed into vials, with one vial for each tree. Fruit samples were collected into paper bags and samples were stored for a maximum of three days at 4°C before being assessed.

5.2.3 Assessment

The developmental stage of each sample unit was categorised using a rating system developed after preliminary observation of all stages of quandong fruits and flowers (Table 5.1). Fruit diameter was measured using a caliper with measurements taken from the centre of the fruit at the widest point, perpendicular to the stem.

A damage rating system was developed after preliminary observations of the type of damage sustained by flowers and fruit whereby the location of damage was divided into three regions of flowers and three regions of fruit. On flowers, damage was assigned to the anthers, the stem or the central disc of the flower (Figure 5.1). With fruit, damage was assigned to the kernel, the seed coat, or the flesh (Figure 5.1). In some cases damage was present in several

areas in a sample unit. The severity of damage was assessed using a categorical rating system, ranging from 0 – 5, where 0 was equal to no damage, 1 was minimal damage, 2 was 25%, 3 was 50%, 4 was 75% and 5 was the entire area of a particular location damaged.

Flowers and fruit were examined for eggs of the quandong moth under a stereo microscope and the number of both unhatched and hatched eggs was recorded for each sample unit. Where possible, unhatched eggs were removed and placed into hatching vials (Section 2.4) for rearing in the culture room.

As larval quandong moths are concealed for the majority of their development, destructive sampling was necessary to assess the damage caused to flowers and fruit. If the sample unit was an unopened flower bud, with undefined calyx sections, the bud was sliced longitudinally with a scalpel and examined under the microscope. If the bud was unopened, but had defined calyx sections, the stem of the bud was held with forceps and a probe used to force open the calyx sections. Following that examination, the bud was sliced longitudinally and re-examined for any damage to the stem. If the sample unit was a developing fruit with a soft seed coat, the fruit was sliced longitudinally four times with a scalpel to yield eight sections of fruit that were then examined for damage. The tissue around any larva or damage was then dissected further to determine the extent of the damage. This technique was used as standard, however it was possible that some first instars and their damage could have been over-looked because of their small size. Once the seed coat had hardened the fruit could not be cut. Instead, the flesh of fruit was removed either by making one single cut around the circumference of the fruit and peeling the flesh away, or when the flesh was not loose, cutting the flesh off in

several smaller sections. The damage in the flesh was then assessed. To examine the damage to the kernel and seed coat the seeds were constricted in a vice until a large crack appeared, generally in the centre of the seed. The seed was then removed from the vice, a knife inserted into the crack and the seed prised open into two sections in which damage was assessed.

At the same time as damage assessments were made, the sample units were examined for the presence of larvae of the quandong moth. When a larva was detected, it was removed from the fruit, its head capsule width recorded, and the instar determined. The larva was then placed in a well of a rearing tray with fresh fruit to attempt to rear it to the adult stage and capture any parasitoids.

Regression was used to determine the relationship between damage and the different instars of the moth and between damage and the number of fourth instars (Statistix®, Analytical Software, Tallahassee, Florida, U.S.A.).

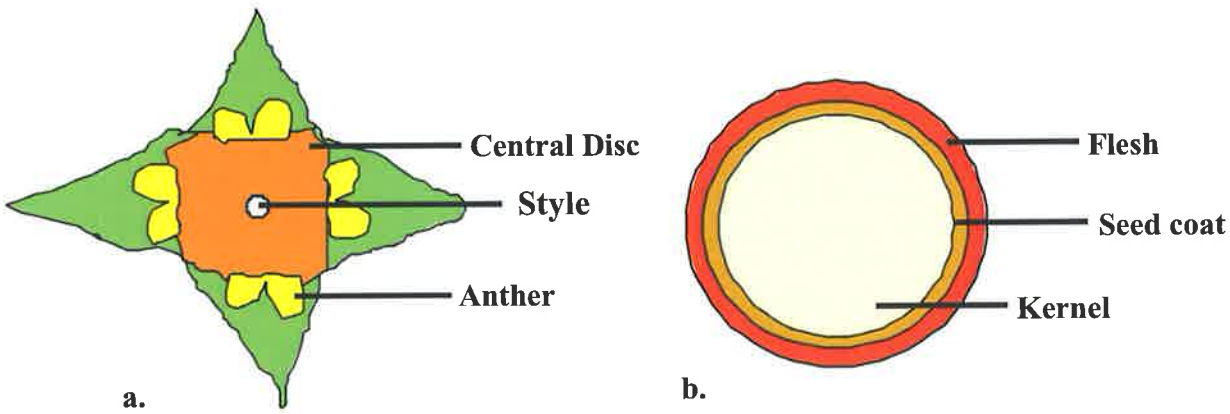


Figure 5.1: Anatomy of quandong flower(a) and mature fruit (b).

Table 5.1: Rating system used to assess developmental stage of quandong flowers and fruit

Rating	Description
1	Buds approximately 1mm in diameter, sections of calyx not distinguishable, bract remnants remaining
2	Buds approximately 1mm in diameter, sections of calyx becoming defined
3	Buds approximately 2mm in diameter, calyx sections all clearly defined
4	Buds approximately 3mm in diameter, flower beginning to open
5	Buds approximately 3mm in diameter, calyx sections all open, anthers, stigma clearly visible, central disc bright yellow or bright red, fresh nectar inside
6	Flower has been open for some time, no fresh nectar visible, anthers and stigma reduced in fleshiness, central disc faded from bright red or bright yellow to brown or pale orange
7	Bud beginning to swell at base 3-5mm in diameter, central disc dark brown, calyx sections may be parallel to fruit
8	Base elongated and swelled to 6-15mm, central disc hardened and brown, fruit green, very shiny
9	Fruit green, 16-25mm in diameter
10	Only stem red (applies to fruit with fleshy stem only), fruit 16-25mm in diameter
11	Up to one quarter of the fruit coloured, generally red but can be yellow to orange
12	One quarter to a half of the fruit coloured
13	One half to three quarters of the fruit coloured
14	Three quarters to whole fruit coloured

5.2.4 Mean and Variance relationships

Means and variances were calculated for eggs of the quandong moth on flowers and fruit for each sample date. The relationship between the mean and variance was examined using Taylor's power law (Taylor, 1961) and Iwao's regression (Iwao, 1968). The equation for Taylor's power law is:

$$s^2 = ax^b,$$

where s^2 is the variance, x is the mean and a and b are coefficients determined by nonlinear regression (SAS Institute Inc., 1982). The coefficient b is a measure of aggregation. Iwao's regression uses Lloyd's mean crowding statistic, x^* (Lloyd, 1967) which is estimated from means and variances by:

$$x^* = x + \left[\left(\frac{s^2}{x}\right) - 1\right],$$

and each x* is regressed against the sample mean such that:

$$x^* = a = bx.$$

In Iwao's regression, b is a measure of aggregation and a is the index of basic contagion. A value of a equal to 0 and b equal to 1.0 indicates random dispersion (Iwao, 1968). The degree of aggregation for eggs was determined and the optimum sample size calculated for a range of means using the formula:

$$N = \frac{s^2}{(E^2 x^2)},$$

where E is the required level of precision as a proportion of the mean (Karandinos, 1976), set at 20% for the purposes of this exercise.

5.2.5 Tree phenology and damage

The inter-tree variation in the phenology of flower bud development was examined and the influence on the damage caused by quandong moth analysed. Analysis of variance was used to detect any differences in bud development between trees. The relationship between tree phenology and larval feeding was examined using the average stage of development for each tree. Regression analyses were conducted for the samples collected during mid December, the period where buds are becoming large enough for larvae to begin feeding. Comparisons were only made at Quorn because of the large degree of inter-tree variation at this site compared with trees at Sedan.

5.2.6 Resistance

The assessments of fruit damage made during regular field sampling were analysed to determine if any trees displayed evidence of resistance to quandong moth. Only the data from Quorn were analysed because the trees displayed the most variability in infestation and physical characteristics. The data were collated from the seven trees that had mature fruit present in each of the three years. The period in which flesh damage occurred was late August to late October in each year at Quorn. The average damage rating across this period was analysed with a two-way ANOVA. The factors of tree and year were used, with the aim of determining how individual trees were affected and if the level of damage was dependent on the year.

5.3 RESULTS

5.3.1 Life History

The peaks in larval abundance indicate that there were three, possibly four generations of quandong moth at Quorn and Sedan in each year (Figure 5.2 and 5.3). In the first two years of observations at Quorn there was at least one, possibly two summer generations during the flowering period of quandong trees. However, during the following year of field sampling, no generations were detected during flowering. In each year the summer generations were followed by an autumn-winter generation that began with a period of oviposition in mid to late April. The spring generation followed, with oviposition during late August to late September, giving rise to the larvae that feed on the flesh of mature fruit. Similarly, a summer generation was detected in each year at Sedan, followed by a period of oviposition in mid-late April to begin the autumn-winter generation. Oviposition in September marked the beginning of the spring generation, again corresponding to the period of fruit maturity. Due to a lack of fruit on the trees at Sedan in 1999, sampling ceased mid-June and re-commenced when flowers were present on trees in mid-November. The trends in egg and larval abundance are similar at Quorn and Sedan, with peaks evident during flowering, fruit development and fruit maturity in most years. At both sites, the peaks in larval abundance traced a progression through each of the four larval instars indicating that the peaks represented distinct generations of the moth (Appendix 4, Figures 1 – 2). There were never any periods in the year when any instar ceased developing as would be seen if the moth had a period of larval diapause.

Larvae of the summer generations primarily fed singly in unopened flower buds (Figure 5.4). A total of 2,275 flowers were sampled from Quorn and Sedan (Appendix 3, Table 1 – 2). Of

those, 98 had at least one hole made by a larval quandong moth with no, one or two larvae, while 82 of the 98 flowers had holes but no larvae and 78 had only one hole. Seven of the 10 larvae feeding inside flowers with holes were third instars. This result indicates that immature larvae may initially feed with up to three larvae in the one flower, but eventually competition for space and food seem to drive some or all of the larvae from the flower. In the 13 flowers in which fourth instars were feeding, nine were found inside unopened flowers that had no holes, suggesting that the larvae had completed development to that point inside that flower bud. Third and fourth instars were also found together inside flowers with holes suggesting that two larvae are capable of reaching maturity while feeding inside the same flower bud, but the frequency distributions (Figure 5.4) indicate that it is not the norm. It is likely that competition between larvae for food and space over-rides the risks involved with seeking out a new feeding site.

Larvae of the winter generation also primarily fed alone but on one occasion two fourth instars were found inside a developing fruit (Figure 5.5). In the spring generation, many larvae fed alone, but a large proportion fed with other larvae of the same instar, with up to a maximum of 11 fourth instars in a mature fruit (Figure 5.6). With instars combined, the maximum number of larvae found feeding in a single fruit was 12 on the 21st September 2000, and comprised four first instars, five second instars and three third instars. It is possible that a single larva could feed on several flowers or fruit in a lifetime, however this was extremely difficult to quantify.

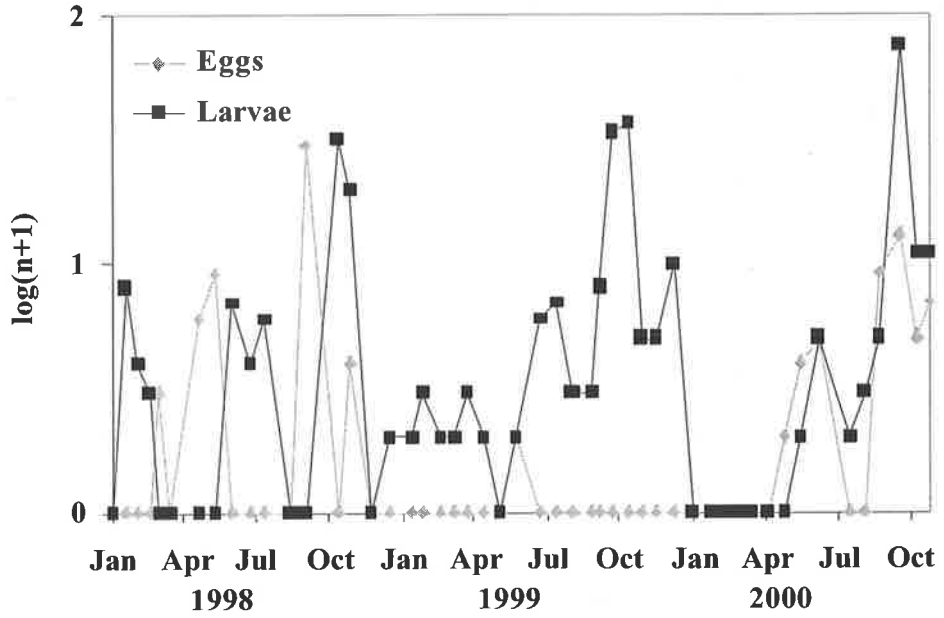


Figure 5.2: Number of eggs and larvae of the quandong moth on each sample date at Quorn, 1998-2000.

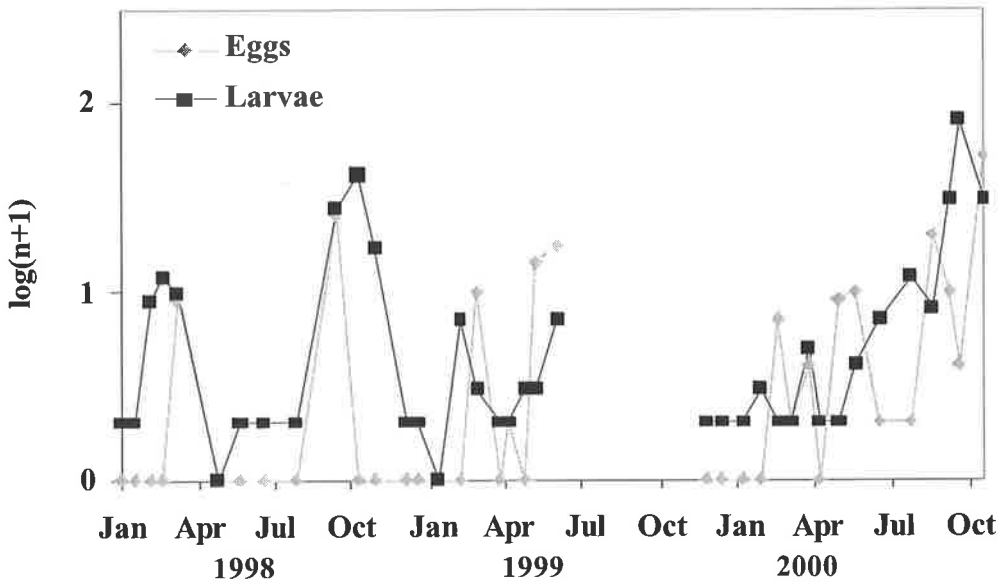


Figure 5.3: Number of eggs and larvae of the quandong moth on each sample date at Sedan, 1998-2000.

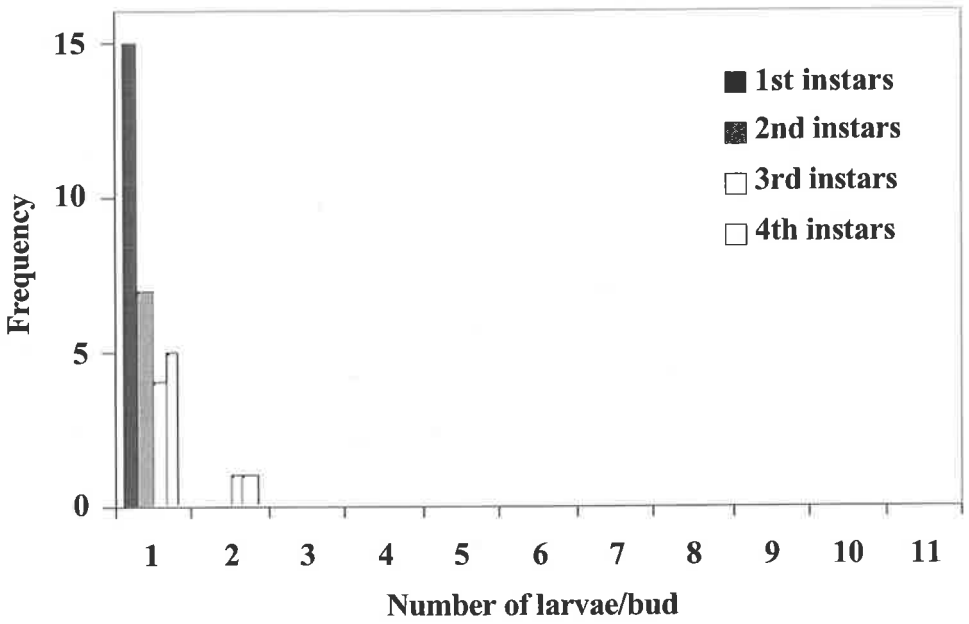


Figure 5.4: Frequency distribution of larvae for the summer generation of the quandong moth at Quorn, 1998-2000.

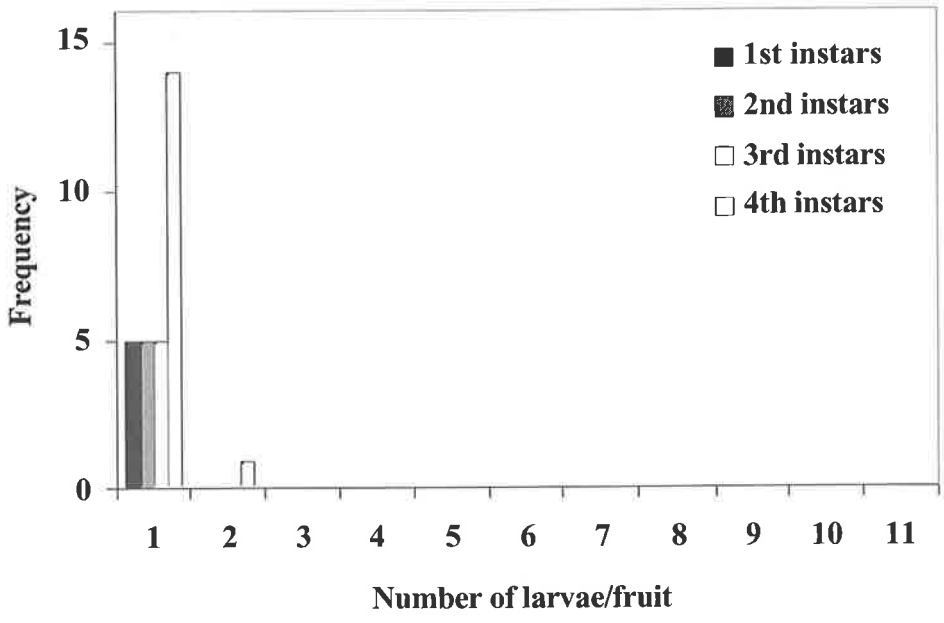


Figure 5.5: Frequency distribution for larvae for the autumn-winter generation of the quandong moth at Quorn, 1998-2000.

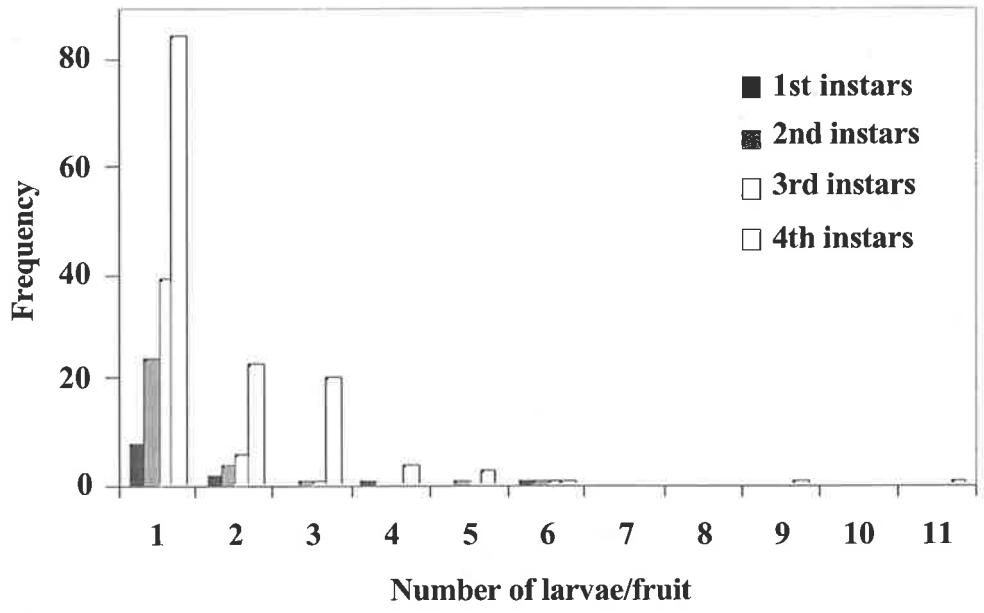


Figure 5.6: Frequency distribution of larvae for the spring generation of the quandong moth at Quorn, 1998-2000.

Various methods of trapping quandong moths were investigated although none were successful. Details of the attempts are outlined in Appendix 4.

5.3.2 Mean and Variance Relationships

Initial regressions were calculated for flowers and fruit separately and comparison of slopes of the regression lines indicated they were not significantly different (T = 0.51058, df = 25) (Zar, 1996) (Appendix 4, Figure 3 - 4). Therefore the data were combined for further analyses (Figure 5.7). Taylor's power law best described the relationship between the sample mean and variance. The coefficients determined by nonlinear regression were a = 19.4206 and b = 2.0472. As the mean increased, the number of samples required to achieve an estimate within 20% of the mean also increased (Table 5.2).

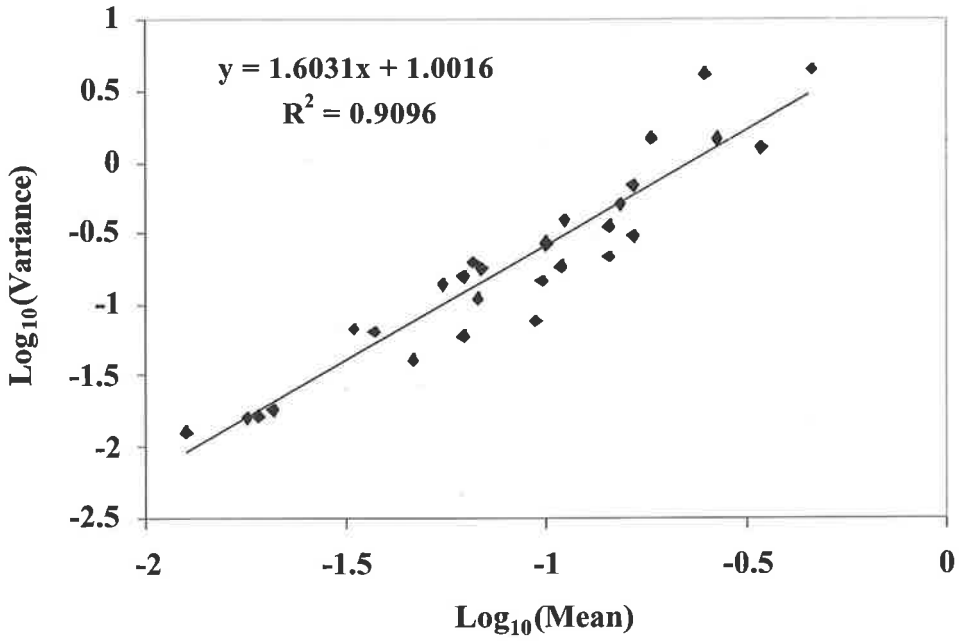


Figure 5.7: Regression of log(mean) against log(variance) for eggs of the quandong moth on quandong flowers and fruit at Quorn and Sedan, 1998-2000.

Table 5.2: Variances calculated from Taylor's Power Law[1] for density of quandong moth eggs on flowers and fruit. Sample sizes calculated for 20% precision level.

Mean	Variance	Sample size
0.05	0.042	422
0.1	0.174	436
0.15	0.400	444
0.2	0.720	450
0.25	1.137	455
0.3	1.651	459
0.35	2.264	462
0.4	2.976	465
0.45	3.787	468
0.5	4.699	470
0.55	5.711	472
0.6	6.825	474
0.65	8.040	476
0.7	9.357	477
0.75	10.777	479
0.8	12.299	480
0.85	13.924	482
0.9	15.653	483
0.95	17.485	484
1	19.421	486

[1]Coefficients derived from non-linear regression (SAS Analytical software); a = 19.42, b = 2.05.

5.3.3 Location of damage

The location of damage caused by quandong moth larvae to quandong trees differed throughout the year. In flowers, larvae primarily fed on the anthers and the central disc of the flower and only inside unopened flower buds (Figure 5.8 – 5.9). There is a period from late November to mid January where the transition of flowers to fruit set is occurring. During this time the majority of flowers are open but have not begun to swell to form young fruit. Larvae are not able to feed on open flowers because they do not provide a secure feeding site, particularly for those in the final instars. Feeding inside the stem of fruit was rare throughout the study.

In developing fruit, damage was mainly located in the kernel and the seed coat of the fruit. Once the seed coat hardened in mid-winter, the damage was restricted to the flesh of maturing fruit (Figure 5.10 – 5.11). Wherever they feed, larvae leave frass that remains around their feeding site. In flower buds, whole buds were found filled with frass where fourth instars were feeding. In developing fruit, the kernel was often filled with frass and in the flesh of fruit, the pattern of larval feeding was evident by the trail of frass left by larvae as they fed. Larvae are able to utilise whichever stage of flowers or fruit is available on quandong trees throughout the year, reducing yield and leaving behind frass that reduces the quality of mature fruit (Figures 5.12 – 5.15).

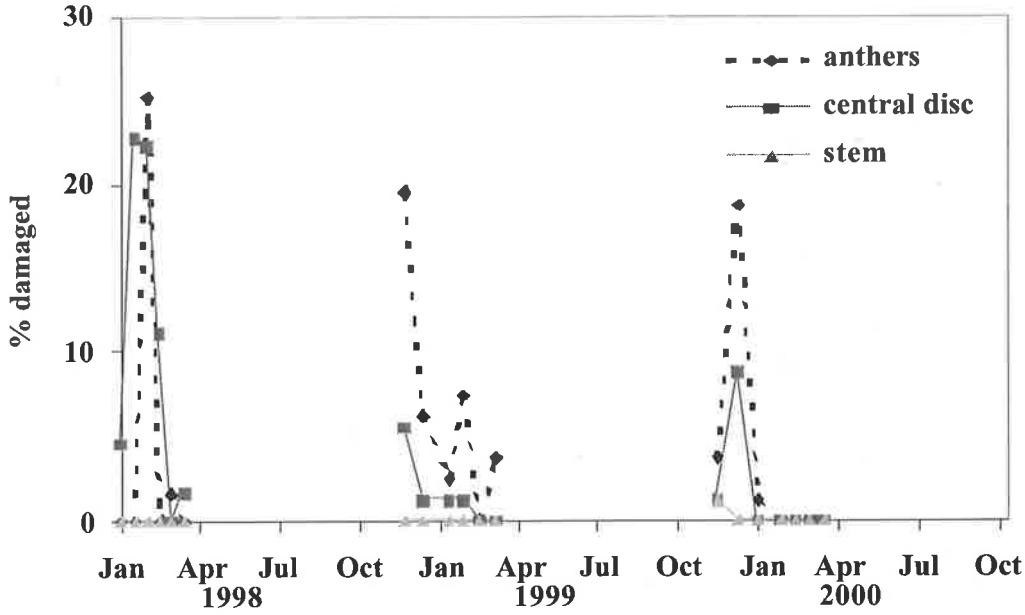


Figure 5.8: Location of damage to quandong flowers at Quorn, 1998- 2000.

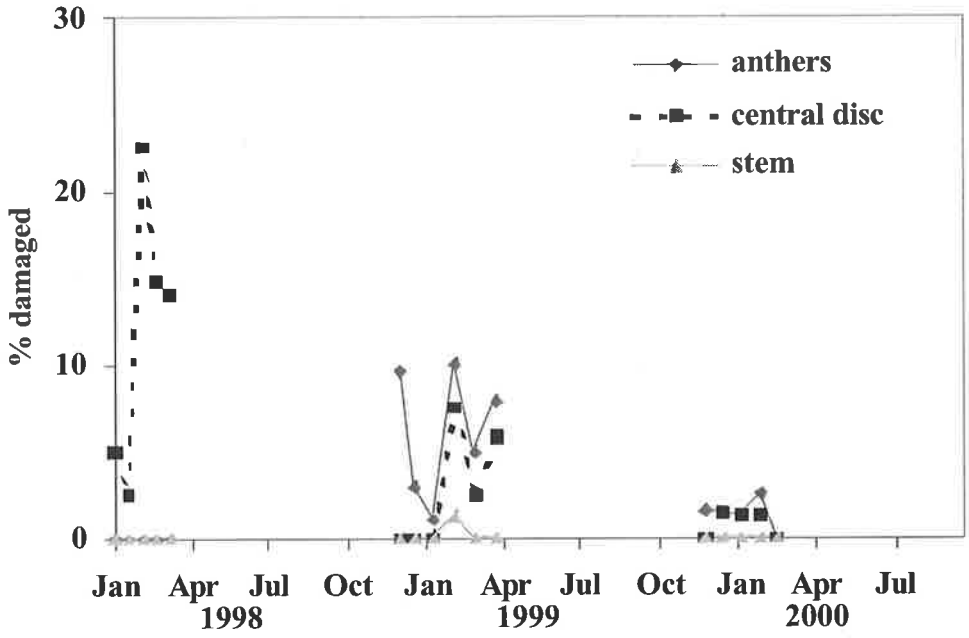


Figure 5.9: Location of damage to quandong flowers at Sedan, 1998- 2000.

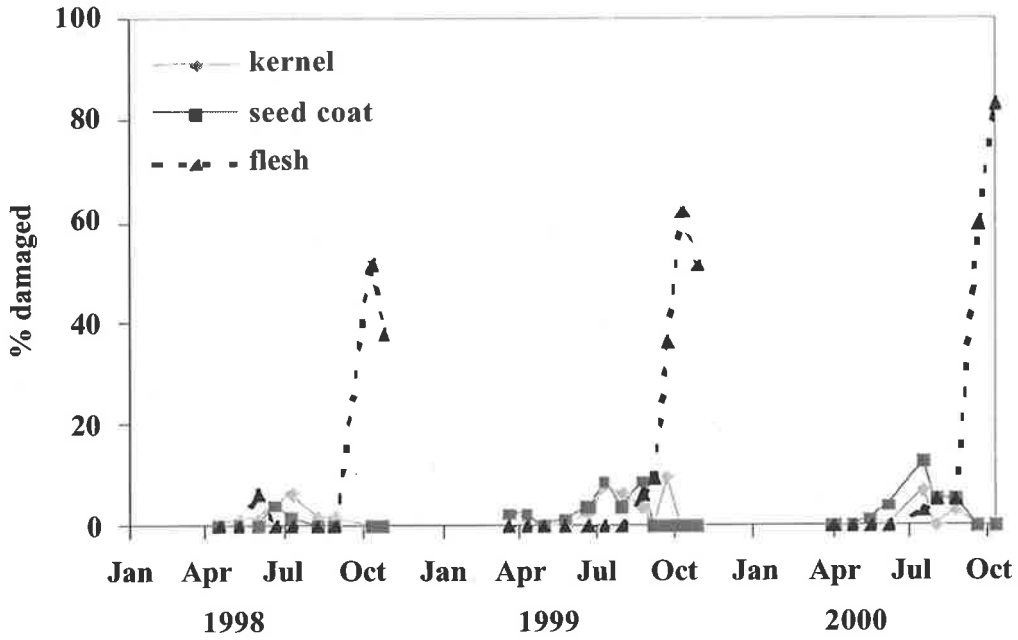


Figure 5.10: Location of damage to quandong fruit at Quorn, 1998- 2000.

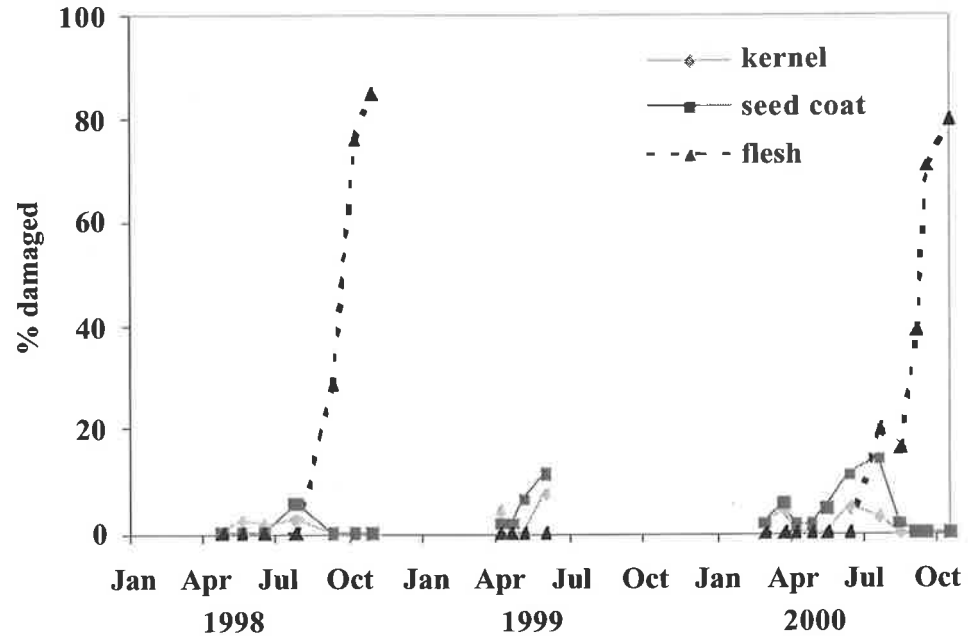


Figure 5.11: Location of damage to quandong fruit at Sedan, 1998- 2000.

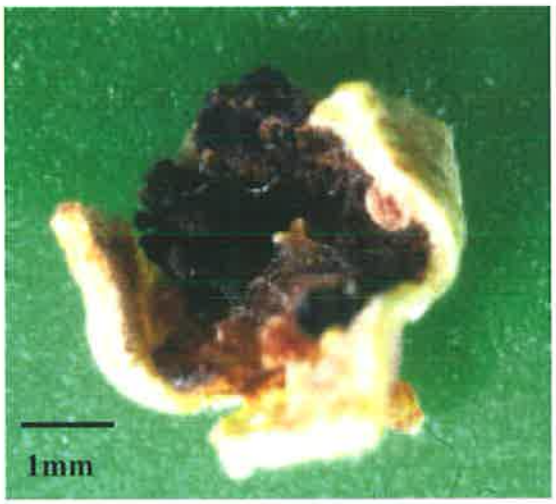


Figure 5.12
Damage to flowers. Bud was unopened when sampled. Anthers and central disc have been eaten out. Bud is full of quandong moth larval frass.

Figure 5.13
Top: undamaged immature quandong fruit. Bottom: Damage to kernel of quandong fruit caused by feeding by quandong moth larvae.

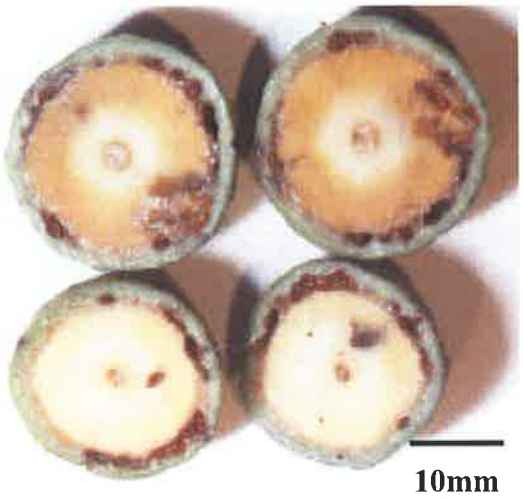


Figure 5.14
Damage to seed coat of immature quandong fruit by quandong moth larvae. Seed coat damage occurs before it has hardened.

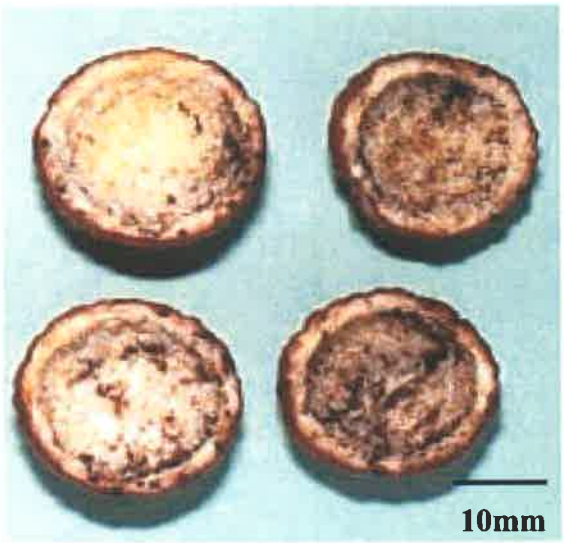


Figure 5.15
Damage to mature quandong fruit caused by quandong moth larvae. Darkened regions are larval frass.

5.3.4 Fruit Drop

During the period from May to late July there was a higher incidence of larval quandong moth in dropped fruit compared to fruit remaining on the trees (Figures 5.16 – 5.17). The damage in immature fallen fruit was primarily located in the seed coat and kernel. In contrast, the damage to mature fruit from the ground was primarily in the flesh with very little seed coat or kernel damage (Figures 5.18 –5.19). Although the hardened seed coat prevented larvae from feeding in the kernel of mature fruit, all seeds were cracked to see if damage done by the winter generation of larvae was present when fruit were mature. These results demonstrate that the majority of fruit damaged internally do not reach maturity. An average of 60% of the fruit that dropped from the trees for both sites in each year had not been damaged by larvae of the quandong moth (Figures 5.18 – 5.19).

The results of extra samples of fruit collected at Quorn in 2000 show that the incidence of both larvae and damage was greater in fruit collected from the ground compared to those collected from the trees (Figure 5.20). However, like the data collected throughout the years from regular sampling, no more than 40% of the fallen fruit were damaged by larval quandong moth and of those that were affected, the damage was primarily to the seed coat and kernel.

Samples from the tree and the ground of an uninfested tree at the Waite Campus indicated that none of the fruit were infested. Fruit drop had occurred prior to the hardening of the seed coat and in the absence of any feeding by the quandong moth or damage of any other nature.

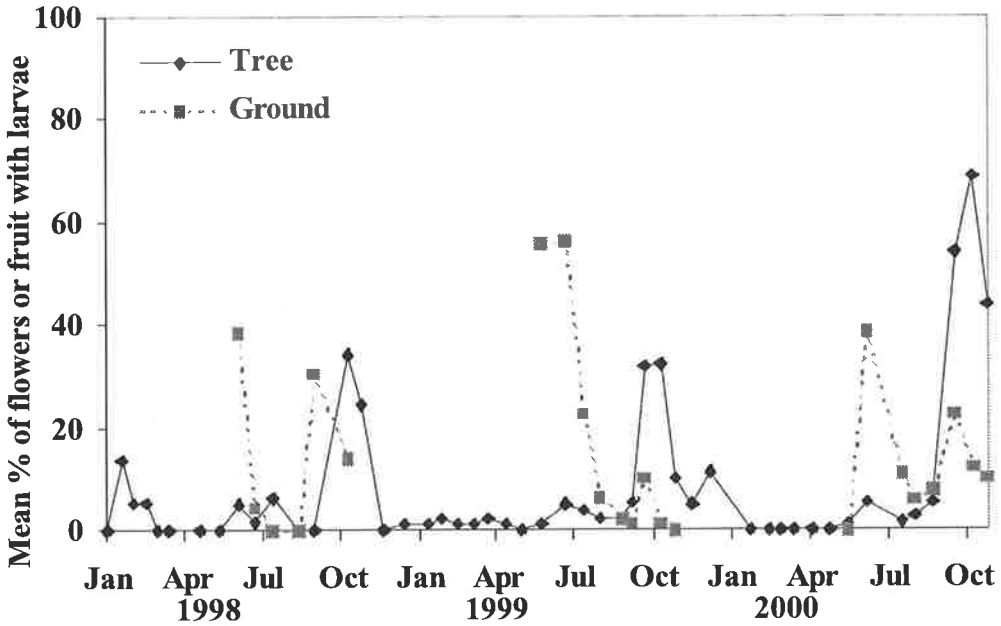


Figure 5.16: Incidence of quandong moth larvae in fruit collected from trees and the ground beneath at Quorn, 1998-2000.

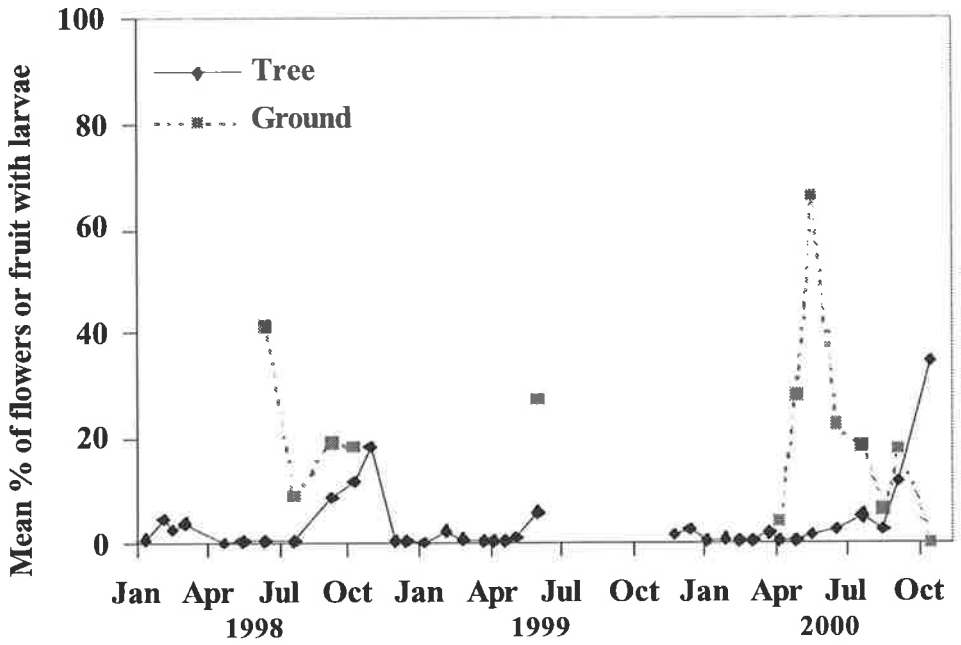


Figure 5.17: Incidence of quandong moth larvae in fruit collected from trees and the ground beneath at Sedan, 1998-2000.

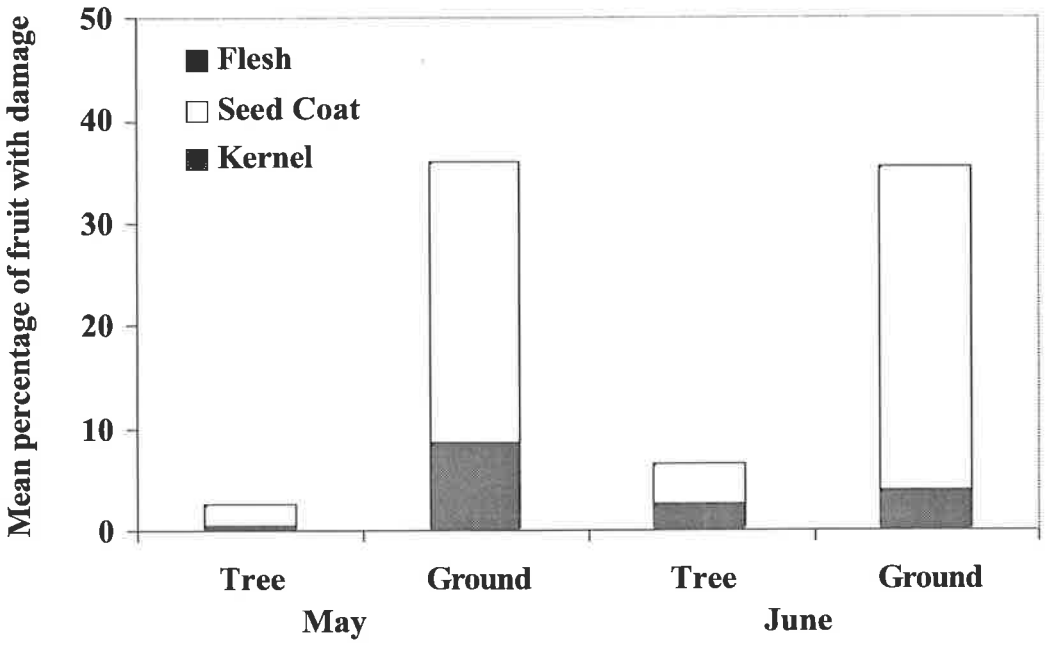


Figure 5.18: Location of damage to quandong fruit from trees and the ground mid-year at Quorn, 2000

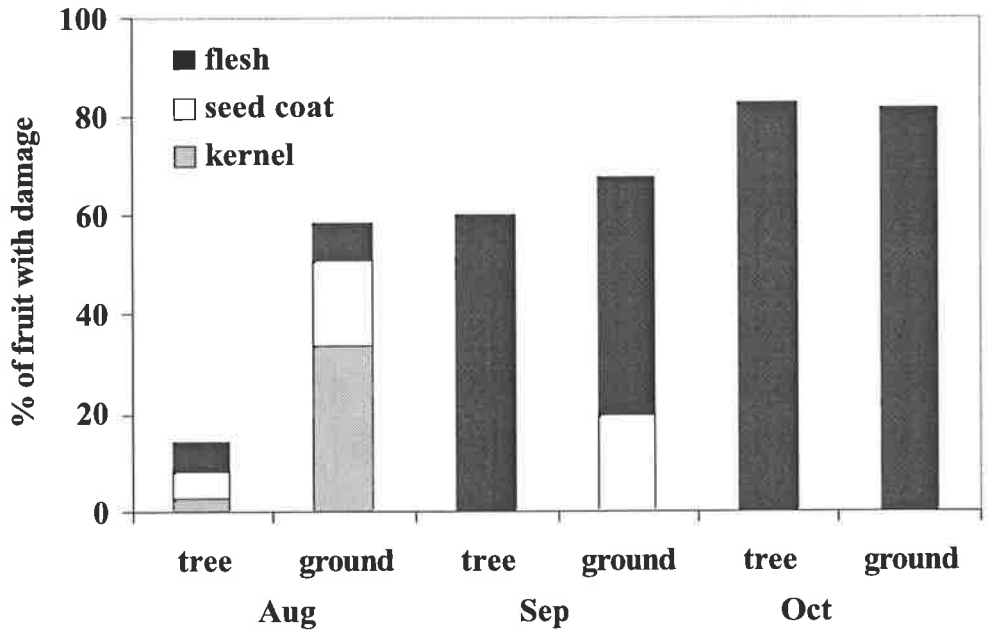


Figure 5.19: Location of damage in mature quandong fruit from trees and the ground at Quorn, 2000

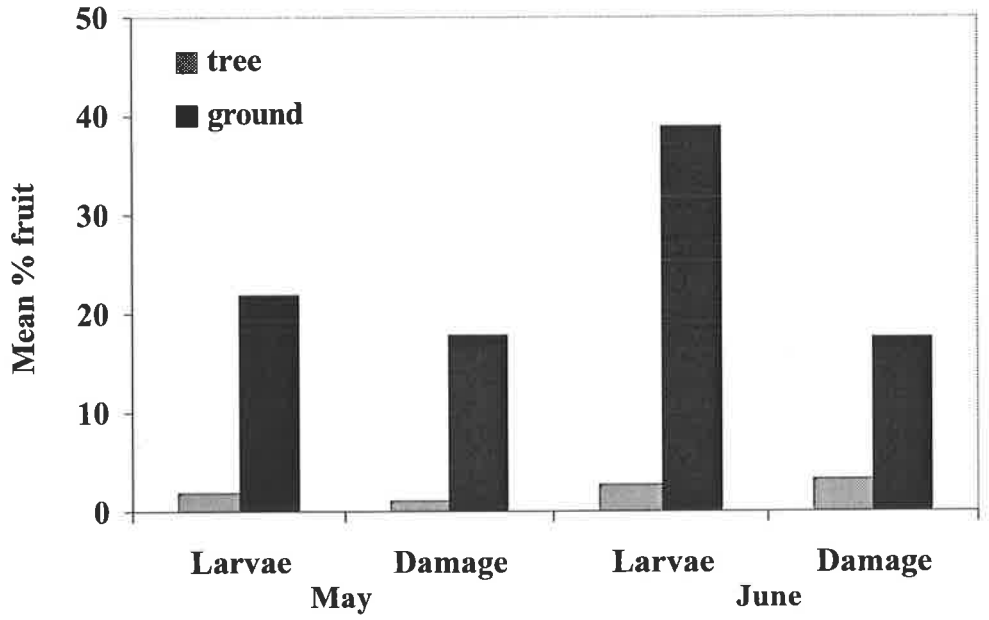


Figure 5.20: Percentage of fruit with quandong moth larvae or damage collected from trees and the ground at Quorn, 2000.

Extra samples of fallen fruit were regularly collected and put into pupation containers in the laboratory (Section 2.6). The emergence of mature larvae from fruit in these containers generally lasted from 5 – 10 days following collection, and the majority of larvae that emerged pupated. In the laboratory, fruit degraded due to dehydration and fungal contamination, restricting the duration of larval emergence. Laboratory estimates indicated that at 24°C the entire larval stage takes approximately 20 days (Section 3.3.2). In the field, fruit drop occurs during winter when temperatures are lower and the onset of dehydration and fungal contamination could be delayed. Therefore it is likely that during the autumn-winter generation, the majority of larvae actually drop from the tree inside fruit, complete their development to the fourth instar and then chew their way out of the fruit to pupate in the leaf litter at the base of trees.

5.3.5 Tree phenology and damage

Quandong trees are active all year and there is rarely a period during a normal fruiting year in which flowers or fruit are not present on trees in some stage of development (Figure 5.21). The only exception is when a tree fails to set any fruit and then the tree will have no fruiting structures from early March to early November.

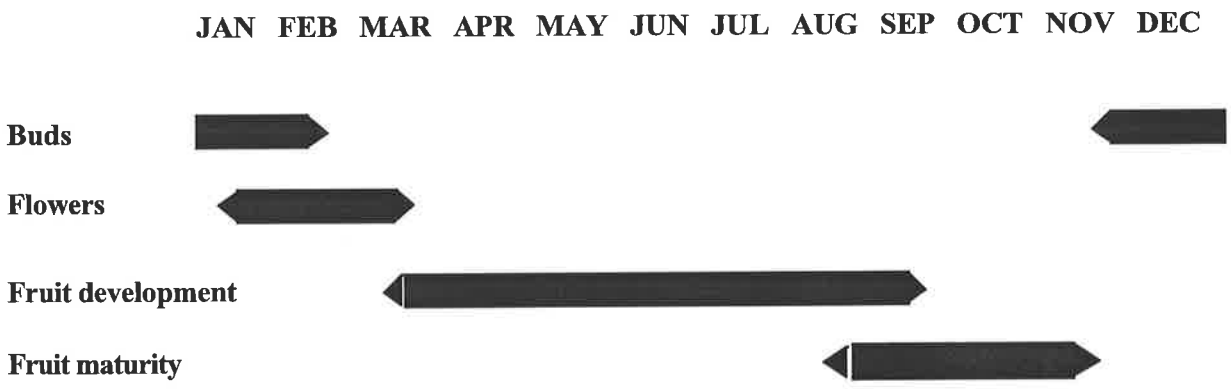


Figure 5.21: Generalised phenology of fruit development on quandong trees in South Australia.

There is often a large amount of variation among individual trees in the onset of flowering and fruit set, particularly in orchards with trees as variable as at Quorn that have been established from seed. There was a significant difference between the stage of development of the trees in every year (Table 5.3). In 1997 and 1998 tree 6 was the most advanced, in 1998 significantly more advanced than any other tree on that sample date. In 1999, tree 8 was significantly less advanced than all other trees with no significant difference between any of the other trees.

The percentage of flower buds damaged by larvae of the quandong moth was significantly correlated with bud development at Quorn in 1997 (r^2=0.5641, p=0.0077) and 1998 (r^2=0.5556, p=0.0133), but not in 1999 (r^2=0.1845, p=0.2153) (Figure 5.22). The more

advanced the development of buds prior to opening, the higher the percentage damaged by the quandong moth.

Table 5.3: Mean development stage of quandong flower buds in December at Quorn, 1997-99.

Tree	Mean Development Stage[1]		
	17 Dec 97	17 Dec 98	10 Dec 99
1	3.0 cde	3.0 b	3.0 a
2	2.75 de	3.0 b	3.0 a
3	2.25 e	3.0 b	3.0 a
4	3.75 abc	3.0 b	3.0 a
5	3.25 bcd	3.0 b	2.875 a
6	4.25 a	4.25 a	3.5 a
7	4.0 ab	3.0 b	3.25 a
8	3.0 cde	2.75	2.0 b
9	3.0 cde	3.0 b	3.0 a
10	3.0 cde	2.75 b	3.0 a
11	3.0 cde	3.0 b	3.0 a
ANOVA statistics	**F=11.8, p<0.0001, df=10**	**F=22.56, p<0.0001, df=9**	**F=7.88, p<0.0001, df=9**

[1]Development stage recorded using Table 5.1

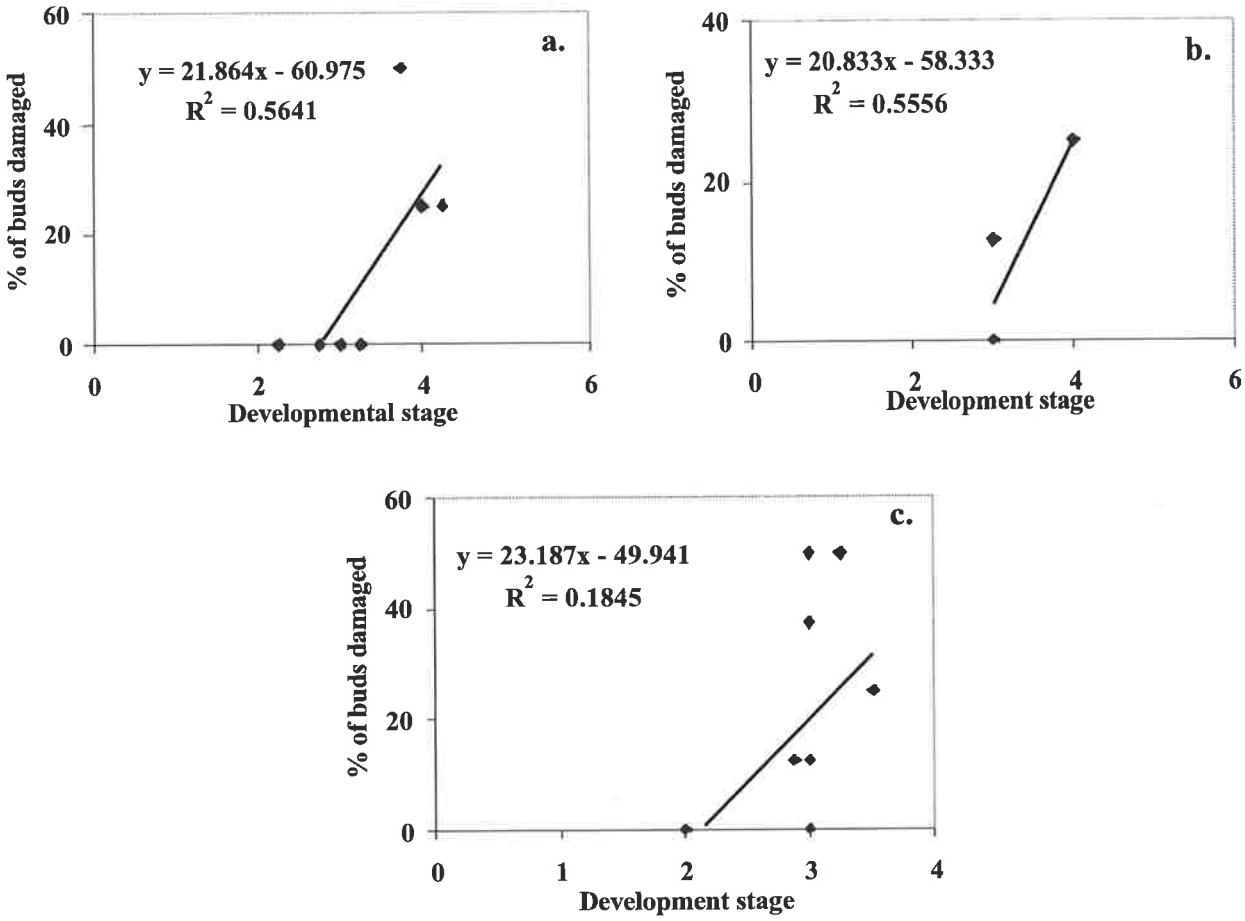


Figure 5.22: The relationship between the development stage of quandong buds and damage to buds at Quorn on (a) 17th December 1997, (b) 17th December 1998 and (c) 10th December 1999.

5.3.6 Resistance

There was no conclusive evidence of resistance to quandong moth in any trees examined at Quorn. Neither the tree, nor the year, were significant factors in the level of damage to mature fruit ($p > 0.05$). None of the trees had a significantly lower level of damage than any other.

5.4 DISCUSSION

Three to four generations of the quandong moth were present on quandong trees each year with some overlap among generations, particularly during the summer months. Each peak in larval abundance consisted of a progression through all the instars. The timing of generations was similar in each year, with each generation feeding on a different stage in the development of fruit. Both the behaviour of larvae reared in the laboratory (Section 3.3.2) and the trends in egg and larval abundance (Section 5.3.1) indicated that there was no diapause by any stage of the moth. A lack of diapause suggests the seasonality of the quandong moth is under direct temperature control. Quandong trees occur naturally in the arid regions of Australia, areas that are not typically subjected to harsh winter conditions. Although many temperate species of insects undergo diapause, where conditions are less extreme, direct control of seasonality by temperature is often feasible (Danks, 1987). Similarly, light brown apple moth generally has up to four generations each year but does not undergo diapause because winter conditions are mild throughout its distribution (Geier and Briese, 1981). *Heliothis punctigera* H_bner (Lepidopteara: Noctuidae) undergoes facultative diapause in the pupal stage and the percentage diapause varies greatly among regions. In the northern regions of Australia *H.punctigera* is active all year round (Zalucki et. al., 1986).

Larvae of different instars may be found feeding in the same fruit. The occurrence of multiple mature larvae in fruit was generally when larvae were feeding in the flesh of mature fruit. The high density of larvae in some fruit in the spring generation may be associated with the nutritional status and the size of mature fruit. Warm temperatures during the period of fruit

maturity promote rapid larval development and high larval densities lead to a high level of damage in many mature fruit.

Analyses indicated that for egg abundance, Taylor's Power Law could best describe the relationship between the mean number of eggs/sample unit and the variance. Overall, the variances for egg data were large and therefore the sample sizes required to maintain estimates within 20% of the mean were also quite large. Monitoring such a large number of fruit for eggs is unlikely to be practical in quandong orchards, however growers are unlikely to be concerned with precision and presence/absence sampling may be suitable for making management decisions (Chapter 8).

Larvae feed on the anthers and central disc where the style of the flower is located. Any damage to the reproductive structures of the flower is likely to prevent pollination. However, the majority of flowers will never set fruit because of a high level of natural shed of flowers, so damage by quandong moth larvae during flowering is unlikely to reduce the potential yield of trees. The autumn-winter generation of the moth feeds on the kernel and seed coat of immature fruit and the spring generation on the flesh of mature fruit. Larvae reduce the quality of the flesh of fruit by their presence, the feeding damage they cause and the frass they leave behind. Larvae did not attempt to remove frass from their feeding site as is seen in other Lepidoptera such as the codling moth (Geier, 1963).

The results for fruit drop have implications for the management of the quandong moth. Larvae of the autumn-winter generation feed in the kernel or seed coat of quandong fruit and

very few of those damaged fruit reach maturity. Thus, larvae of this generation have the potential to cause high levels of damage by reducing the yield of trees. However, because only a maximum of 40% of fruit that drop are damaged by the quandong moth, feeding by larvae may not necessarily cause yield loss. Yield loss will only occur if fruit drop caused by larval feeding is additive to the rate of drop due to natural thinning or other causes such as wind or stress. It is common in many species of plants to produce a much greater number of flowers or young fruit than the plant is ever able to mature. Plants can then compensate for herbivory by reducing the rate of natural thinning of flowers and young fruit (Crawley, 1983). In this way, feeding by larval quandong moth may determine which fruit drop but not cause loss of yield. A similar phenomenon was reported for damage to macadamia by *Cryptophlebia* species (Jones, 1994). This study also suggested that larval feeding may determine which nuts drop, but not cause yield loss unless feeding damage is greater than the natural drop of nuts. Drop of immature quandong fruit was high even in the absence of damage by larval quandong moth or of any other kind. Jones (1994) reported a similar high rate of drop of immature nuts in the absence of damage by *Cryptophlebia* larvae. From a population perspective, even if larvae of the quandong moth do drop inside fruit that are aborted from the trees, many larvae are still able to complete development and will form part of the spring generation that attacks the mature fruit. During the period of fruit maturity, the fruit sampled from the trees generally had as much or more damage than that sampled from the ground. At this time, fruit drop because they are ripe irrespective of any damage that they have sustained.

It is difficult to determine if fruit drop mid-year represents economic damage. If the level of infestation by quandong moth is high, the fruit drop caused by larval feeding may exceed that

of the natural rate of drop. In this study, the proportion of dropped fruit that was undamaged always exceeded the damaged so it is unlikely that economic damage results. However, the survival of larvae of the autumn-winter generation is important for the population dynamics of the quandong moth as they give rise to the damaging spring generation.

The relationship between tree phenology and larval damage indicated that the trees that are most advanced have higher levels of larval damage than the less advanced trees. Intuitively, there would seem to be no evolutionary advantage to early development when it results in severe damage from insect herbivores. Natural selection is more likely to favour an even spread of development rates for buds, as is the case with the less genetically diverse population of trees at Sedan. As mentioned previously, the trees at Quorn were planted from a very diverse collection of seed. In the population from which those seeds were sourced such variation in development would be less common and development among trees would be more synchronised. The uniformity of flowering and fruit set among trees is increasing in orchards, through grafting and clonal techniques (AQIA, pers.comm.).

For univoltine species of Lepidoptera, the hatch of eggs is often synchronised to some phenological stage in the development of the host plant, so that larvae are able to take advantage of a particular resource (Turgeon, 1986; Lawrence et. al., 1997). In some cases there is a distinct window in which larvae are able to develop, or in which they can gain a significant advantage in some aspect of development, such as size or fecundity (Watt, 1987). Although each generation of the quandong moth is able to exploit a different stage in the development of fruit, the window of opportunity for the summer generation is relatively small compared with that for developing and mature fruit. Larvae do not feed on foliage so the

phenology of bud development is analogous to budburst in a deciduous tree. Damage to the stem of any flower buds was rare and when it did occur, only first or second instars were responsible. This suggests that larvae are unable to mature once flower buds have opened and larvae must develop to the final instar inside buds that are yet to open. Oviposition and egg hatch must be timed to the period when the majority of flower buds are unopened or larvae would be forced to move from buds once they have opened to other unopened buds. Such movement would be reliant on variation in budburst on individual trees as movement between trees is unlikely. During flowering larvae take approximately 20 days to develop through all instars (Section 3.3.2). The period in which buds are large enough to accommodate larvae prior to budburst is generally six to seven weeks, which if egg hatch has occurred early enough, would be ample time for larvae to mature. The period between the end of fruiting and the time that flower buds become large enough for larvae to feed may be a synchronising event for the first summer generation. Tree phenology has been demonstrated as a synchronising event in some phytophagous insects (Quiring, 1994) and trees having the most advanced phenology supported the highest levels of insect damage (Hunter, 1992). However, other factors such as age of trees (Quiring, 1994), spatial distribution of trees (Hunter, 1992) and temperatures (Dewar and Watt, 1992) can influence the synchrony between tree phenology and activity of the insect herbivore.

As with many crops, with quandongs there is a trade-off between the desired characteristics and those that impart resistance. The severity of quandong moth damage across years was consistent at Quorn, and no trees had consistently low levels of damage in each year. Observations suggested that the trees that were infested had thick flesh and closed calyces,

however speculation can only be made on a few physical characteristics of fruit. Analysis of tree phenology showed that the trees that develop buds earliest are more heavily attacked by the quandong moth in the bud stage. The advanced development may contribute to higher levels of damage throughout the whole year. Although not significant at the 5% level, tree 6 had the highest mean damage rating in buds and in mature fruit. Infestation may not necessarily be related to a physical characteristic that imparts susceptibility, but be influenced by the phenology of individual trees. If a particular tree is infested first and provides ample nutrition to the individuals on the tree throughout their lifetime and that of their progeny, there may be no reason for an individual to leave the tree, particularly if the distance between trees is large (Hunter, 1992).

It is now recognised that quandong seeds do not necessarily fruit true to the parent tree and grafting has become common in both established and new orchards (AQIA, pers.comm.). Grafts are selected on the basis of a number of characteristics, such as flesh and skin colour and thickness, qualities that are desirable for human consumption that are often also the characteristics favoured by the quandong moth. Further research is needed to determine if any specific characteristics can be identified that impart resistance to the quandong moth without sacrificing the quality desired by quandong growers and consumers.

6. CHEMICAL CONTROL

6.1 INTRODUCTION

To date, the only management strategy that has been used for the quandong moth in quandong orchards is regular applications of a broad-spectrum organophosphate insecticide. Frequent use of a broad-spectrum insecticide over the long-term is not a sustainable or desirable option for management of pests of perennial tree crops (Furness, 1983; Metcalf, 1994). Non-target effects of broad-spectrum insecticides may include outbreaks of secondary pests, adverse effects on beneficial insects, insecticide resistance and environmental contamination (Metcalf, 1994). However, insecticides will continue to be a valuable management tool for insect pests, providing their use is judicious and their limitations are recognised (Metcalf, 1994).

The Australian Quandong Industry Association has had a temporary registration to use dimethoate for control of quandong moth in orchards since 1997. Dimethoate, an organophosphate insecticide was primarily selected for use against larvae of the quandong moth because it has systemic properties and could target larvae feeding inside fruit (Tomlin, 1997). However, dimethoate undergoes rapid degradation in the environment, is highly toxic to birds, fish, aquatic invertebrates and bees, and spraying during flowering when bees are active is not recommended (Tomlin, 1997). The broad-spectrum of activity of dimethoate means it is also harmful to beneficial insects such as the predators and parasitoids of quandong moth. Due to the previous lack of research into the biology, ecology and phenology of quandong moth, many growers were reliant on calendar applications of insecticides, or haphazard monitoring. Although the systemic action of dimethoate facilitates calendar

application, because efficacy is not reliant on targeting exposed larvae on the surface of fruit, the maximum effects will only be achieved if larvae are caught early to limit damage. Increasing the selectivity of insecticides could reduce the adverse effects of insecticide use in quandongs (Metcalf, 1994). Selectivity of insecticides can be increased physiologically by the use of chemicals that are specific to Lepidoptera, such as IGRs, and ecologically through knowledge of the ecology of the pest (Metcalf, 1994).

Studies on the life history of the quandong moth indicated three to four generations of the moth in each year (Section 5.3.1). From a management perspective the most damaging generations are the autumn-winter and spring generations that feed on developing fruit and mature fruit, respectively. In order to refine the use of insecticidal management practices, two field trials were conducted, the first to examine spray timing with the currently used insecticide dimethoate, and the second to investigate alternative insecticides with potential for use against the quandong moth. The aim of the spray timing trial was to examine if a program of sprays accurately timed to the start of the generations would manage quandong moth populations as effectively as monthly applications. Increasing the accuracy of spray timing would increase the efficacy of applications of dimethoate and decrease the adverse effects, such as selection pressure for resistance, by reducing the number of applications (Metcalf, 1994). As sustained and frequent application of dimethoate is not feasible in the long-term, alternative insecticides with potential for use against the quandong moth were investigated for rotation with, or replacement of dimethoate.

6.2 MATERIALS AND METHODS

Two field trials were conducted at a commercial orchard of quandong trees at Whyalla, South Australia. The orchard was planted in seven blocks with two blocks of approximately 40 trees each used in the trials. Although the trees were planted in rows, there had been a lot of seedling death in the orchard and, as the grower had since moved to better yielding grafted varieties in other blocks, the trees that died had not been replaced. In addition, many of the trees in the blocks were very low yielding and therefore not able to be used in field trials in either year. Trees used in the trials were selected primarily on the basis of potential yield to ensure that there would be adequate fruit to sustain sampling throughout the season.

6.2.1 Spray timing

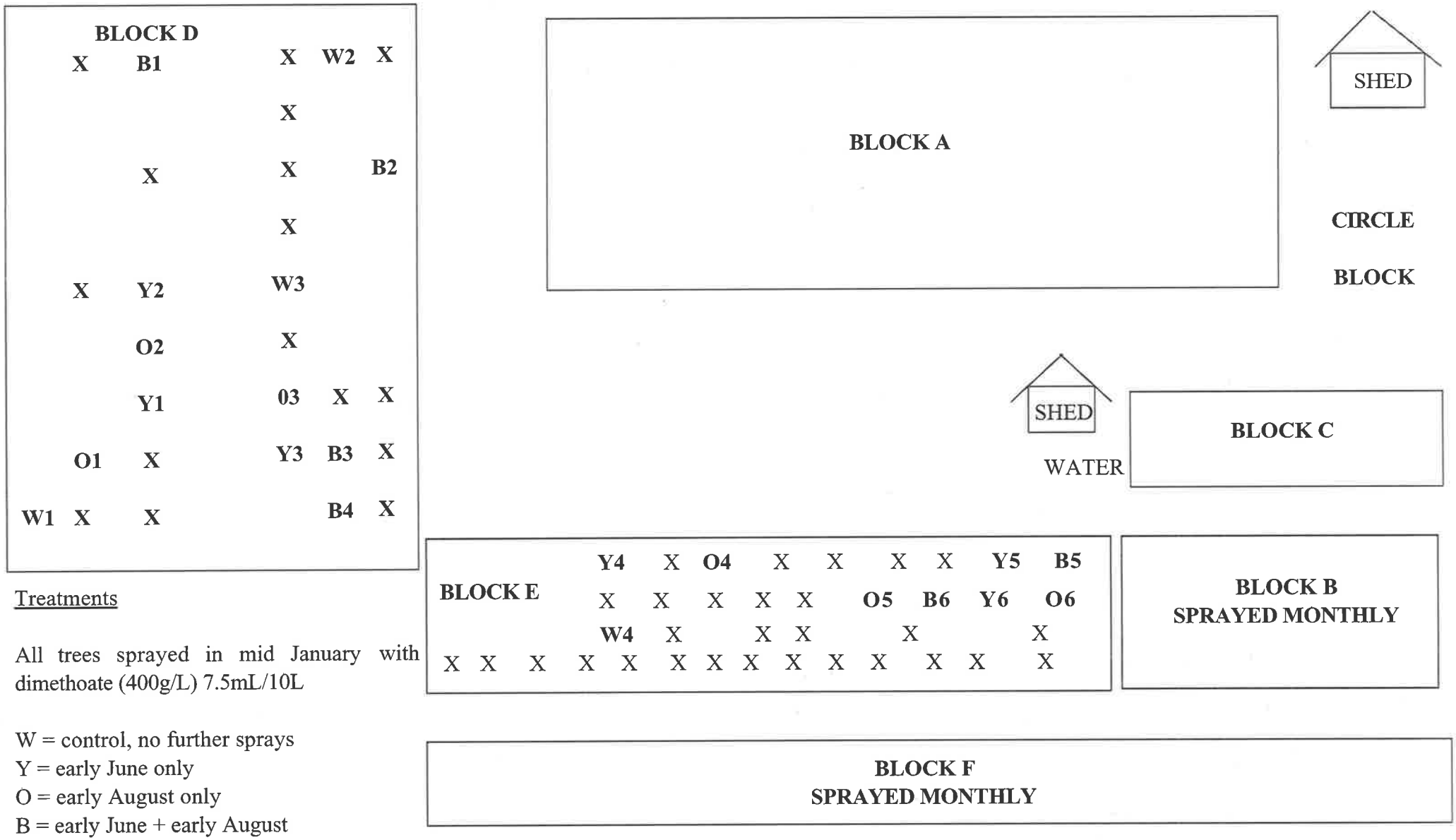
The spray timing experiment was designed as a randomised complete block design with the factor being spray timing. A blocked design was used because of the high degree of variability in the yield of trees and the physical characteristics of fruit and leaves. Dimethoate is an organophosphate insecticide with systemic properties and has both contact and stomach action against larvae (Tomlin, 1997). The grower initially sprayed all trees with dimethoate in mid-January and then the treatments were applied throughout the remainder of the year. Single tree replicates were used, with trees randomised in six blocks each with six replicates (Figure 6.1). The four treatments were sprays applied in early June only, early June and early August, early August only and an unsprayed control. All sprays were dimethoate at 75ml/100L, applied with a Solo® Knapsack sprayer to run-off. The early June and early August sprays were aimed to target the start of the autumn-winter and spring generations of the quandong moth, respectively. Samples were taken from trees on 30th January and 10th

March and prior to the first spray on the 2nd June, and assessed in the laboratory. Although no eggs or larvae were detected in fruit on 2nd June the spray was applied as a precautionary measure as some eggs or larvae of the quandong moth may have gone undetected. The timing of the spray applied for the autumn-winter generation was also based on the previous year of data at Quorn where eggs of this generation were found in mid May. The next treatment was applied in early August and a sample taken on the 19th August as the fruit on some trees began to mature. Subsequent samples were taken from trees as fruit ripened, on the 10th and 24th September and the results were pooled for analysis of mature fruit. All samples were assessed by the methods outlined in Section 5.2.3. Both the mean numbers of larvae per fruit and variation in the severity of damage among treatments were analysed. Data were analysed with two-way ANOVA and power analysis was used to interpret the results (JMP®, Version 4, SAS Institute, 2000).

6.2.2 Alternative insecticides

The field trial examining alternative insecticides was also conducted using a randomised complete block design (Figure 6.2). The insecticides trialed were fenthion as Lebaycid® (75mL/100L), a broad-spectrum organophosphate with both contact and stomach action against larvae, fenoxycarb as Insegar® (20g/100L) an insect growth regulator (IGR) with ovicidal and morphogenetic action, and tebufenozide as Mimic® (8.6g/100L), an insect growth regulator specific to Lepidoptera that is active against all larval stages. Dimethoate (75mL/100L) was also included as an industry standard. All insecticides were applied three times throughout the trial. The first spray was applied on 15th February after detecting immature larvae in a pre-spray sample. The second spray was applied on 19th May and

samples of fallen fruit were collected where possible from this date until the end of the trial. The third spray was applied on the 25th August after eggs were detected in a pre-spray sample. The sprays were timed to the beginning of the second summer generation, the autumn-winter generation and the spring generation of the quandong moth with an unsprayed control. Mature fruit were harvested from late August to mid October and the results were pooled for analysis of the incidence of larvae and the severity of damage. Data were analysed with two-way ANOVA and Tukey's test to compare treatment means. Power analysis was used to aid in interpretation of the results (JMP®, Version 4, SAS Institute, 2000).



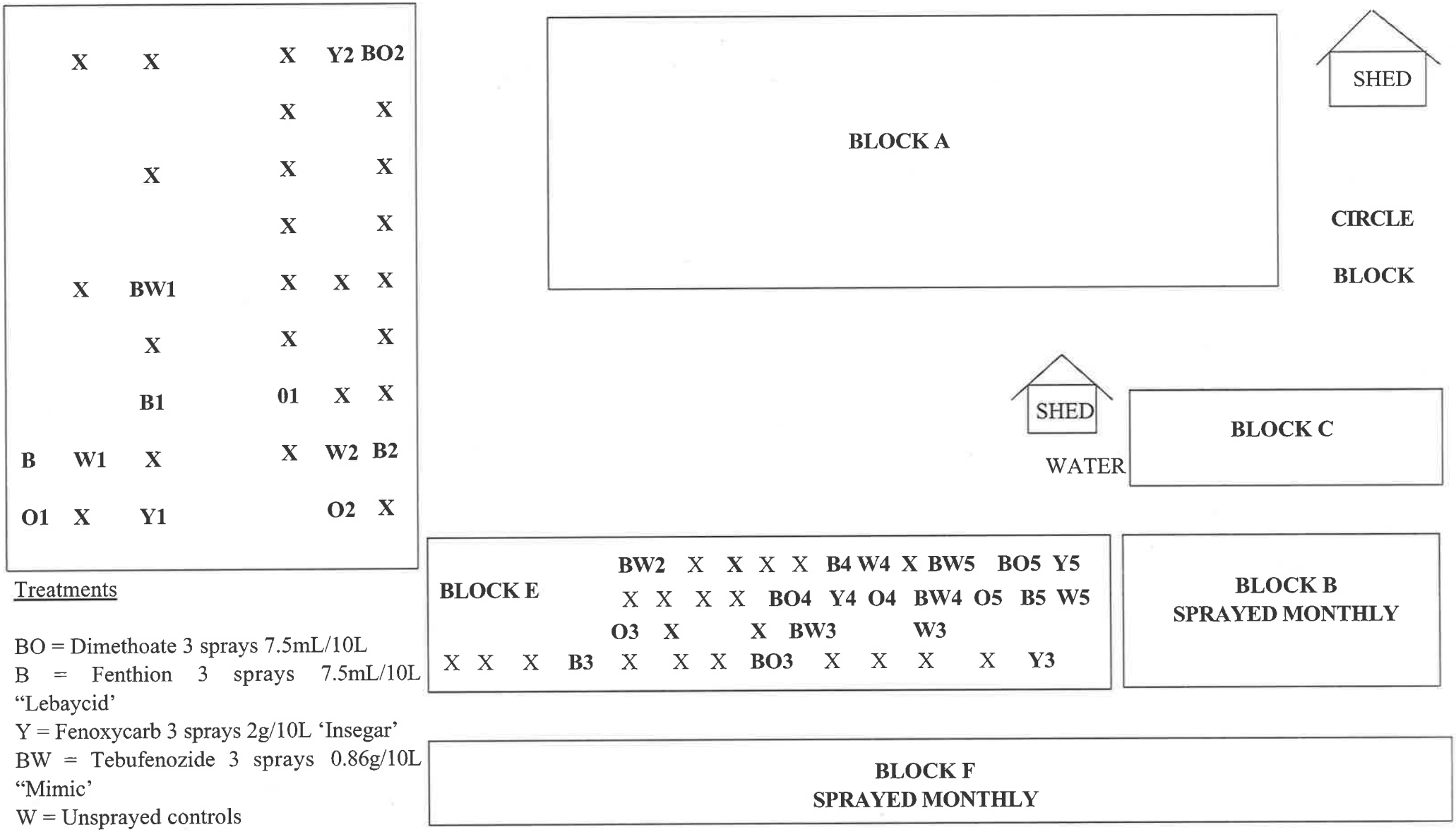
Treatments

All trees sprayed in mid January with dimethoate (400g/L) 7.5mL/10L

W = control, no further sprays
Y = early June only
O = early August only
B = early June + early August

X = quandong tree not used in trial due to inadequate fruit set

Figure 6.1: Outline of spray timing trial at Whyalla, South Australia, 1999.



Treatments

BO = Dimethoate 3 sprays 7.5mL/10L
B = Fenthion 3 sprays 7.5mL/10L "Lebaycid'
Y = Fenoxycarb 3 sprays 2g/10L 'Insegar'
BW = Tebufenozide 3 sprays 0.86g/10L "Mimic'
W = Unsprayed controls
O = Dimethoate monthly 7.5mL/10L
X = quandong tree not used in trial due to inadequate fruit set

Figure 6.2: Outline of alternative insecticide trial at Whyalla, South Australia, 2000.

6.3 RESULTS

6.3.1 Spray timing

The results of the spray timing trial indicated that neither the block nor the treatment factors had a significant effect on the number of larvae or the severity of damage ($p>0.05$; Table 6.1). There was a high degree of variability in the data, indicated by the root mean square errors of 0.24 and 0.31 for number of larvae and the severity of damage, respectively. Analysis indicated that the power associated with the largest effect size for larvae and severity was 73.5% and 90%, respectively. The power analysis also indicated that the least number of samples required to be confident that a significant result did not happen by chance alone was 19. The actual sample size for the trial was 22, suggesting that the sample size was sufficiently large enough to be confident in the results.

Table 6.1: Mean number of quandong moth larvae/fruit in spray timing trial with dimethoate at Quorn 1999.

Treatment	Mean number of larvae/fruit ± 95% C.I.
June only	0.18 ± 0.17
August only	0.27 ± 0.37
June + August	0.13 ± 0.13
Unsprayed control	0.08 ± 0.06

6.3.2 Alternative insecticides

There was no significant effect of either the block factor or the treatment on the number of larvae or the severity of damage in the flesh of fruit ($p>0.05$; Table 6.2). As with the spray timing trial, there was a high degree of variation in the data collected for the alternative insecticide trial. The root mean square errors were 0.22 and 0.23 for number of larvae and severity of damage, respectively. Analysis indicated that the power associated with the largest effect size for both larvae and severity was over 95%. The power analysis also indicated that the least number of samples required to be confident that a significant result did not happen by chance alone was 16. The actual sample size for the trial was 25, again suggesting that the sample size was sufficiently large enough to detect differences if in fact they were significant. Several larvae in fruit collected from trees sprayed with tebufenozide were deformed.

Table 6.2: Mean number of quandong moth larvae/fruit in alternative insecticide trial at Quorn 2000.

Treatment	Mean number of larvae/fruit ± 95% C.I.
Fenthion	0.06 ± 0.11
Fenoxycarb	0.22 ± 0.27
Tebufenozide	0.03 ± 0.05
Dimethoate	0.07 ± 0.11
Unsprayed control	0.37 ± 0.32

When fruit drop began in 2000 all dropped fruit was collected from beneath trees. Analysis of variance showed that there was no significant difference between treatments in terms of the

number of fruit dropped from trees throughout the experiment. The overall drop of fruit throughout the trial was low in all treatments.

6.4 DISCUSSION

The results of the spray timing trial indicated that the spray regimes employed did not reduce the incidence of larvae or the severity of damage more than the unsprayed control. Similarly, the results of the alternative insecticide trial indicated that no insecticide reduced the incidence of larvae or severity of damage significantly more than the unsprayed control. Although the differences in the number of larvae/fruit among treatments in the alternative insecticide trial were not statistically significant, it is likely that some may actually be biologically meaningful. For example, the mean number of larvae/fruit for the control was equivalent to more than one in three fruit infested, whereas the mean for the tebufenozide treatment was equivalent to one in forty fruit infested (Table 6.2). If a single larva in a fruit is considered enough to warrant intervention with management strategies the difference may actually be important from a grower's perspective.

Failing to reject the null hypothesis of no difference between treatments does not necessarily prove that there is no difference. It simply means that there is "not sufficient evidence to conclude that it is false" (Zar, 1996). With field trials in particular, there are many known and unknown biotic and abiotic factors that may influence the result. The accuracy of spray timing is a major consideration. Although regular egg monitoring was conducted to identify the start of generations, in the spray timing trial the initial spray was applied as a precautionary measure because eggs of the generation had not been detected on several

subsequent sampling dates. The application may have not reached eggs or larvae. Similarly, the spray for the second generation was based as much on timing of generations in the previous years as it was on the presence of eggs. In the alternative insecticide trial two of the three sprays were applied when eggs or young larvae were detected, however it is possible that these incidences did not represent the peak in the incidence of the stage. Mis-timing applications by as little as two or three days could mean that vulnerable stages such as eggs or neonate larvae are not affected. Increasing the intensity of egg monitoring to be sure that eggs or young larvae are present in the orchard would help to ensure that the generations had been targeted and the results of the trial are in fact accurate.

Tebufenozide is an IGR that is highly specific to Lepidoptera with low toxicity to non-target organisms (Tomlin, 1997). The action of tebufenozide is primarily expressed through ingestion by larvae and it is effective against all instars. Tebufenozide initiates a premature lethal moult and explains the presence of several deformed dead larvae found in fruit treated in the trial. However, applications must be timed to correspond with the activity of neonate larvae as mature larvae are concealed. In addition, because the chemical mainly operates through ingestion, thorough coverage is essential. Coverage can be increased by using high spray volumes or by adding surfactants (Matthews, 1979). Further investigation of the effect of tebufenozide is warranted. Laboratory bioassays would be particularly informative and could not be conducted because of the lack of a laboratory population of the quandong moth.

Fenoxycarb is also highly reliant on accurate spray timing. It is possible that eggs of the quandong moth were not contacted and then a subsequent spray did not reach fourth instars

to prevent pupation. In the lightbrown apple moth (LBAM) fenoxycarb is both ovicidal and morphogenetic (Ebert and Henderson, 1991). The ovicidal action is most effective when LBAM eggs are laid onto treated foliage. Once LBAM larvae have hatched, they are not vulnerable to fenoxycarb again until the fifth instar, and will not pupate if contact does occur during this instar. For quandong moth, final instars are unlikely to be affected by fenoxycarb as they are inside fruit. The ovicidal action of fenoxycarb is likely to be more important for the quandong moth because fourth instars will have already caused a substantial amount of damage to fruit. Activity against neonate LBAM also explained the higher efficacy of tebufenozide compared to fenoxycarb in trials in apple orchards (Valentine et. al., 1996). However, the potential for fenoxycarb against eggs of the quandong moth may be worthy of further investigation as failure to contact eggs could have confounded the relative inefficacy reported in this trial.

Fenthion is highly toxic to birds and, like dimethoate, has a broad-spectrum of activity (Tomlin, 1997). It is not ideal for inclusion in an IPM program for many of the same reasons that dimethoate is not ideal, such as lack of selectivity that can cause secondary pest outbreaks. However, fenthion may have a role in reducing the selection pressure for resistance if the only alternative is sustained and frequent usage of dimethoate. Once again, further research is required before any recommendations could be made regarding its use.

The timing of assessment could influence the accuracy of results, particularly with the number of larvae/fruit. For this reason, two assessment dates over the period of fruit maturity were pooled to decrease the chance that larvae or their damage would be missed. The overall

density of larvae in the trees that were assessed was low and the variance high. Although power analyses indicated that sufficient samples had been taken to detect significant differences increasing the sample size would reduce the variability in the data particularly when the density of larvae is so low. Increasing the sample size would also increase the power associated with the analyses (Zar, 1996).

It has been suggested that the fruit that larvae of the quandong moth favour are also those that are favoured for human consumption (B. Powell, pers. comm.). The trees on which the trials were conducted were those not favoured for commercial use by the grower and may have also been unfavourable to the quandong moth. The trees that were used for the trials were also highly variable, which is the reason a randomised complete block design was used for the trials. The trees were planted from seed and although they were mainly of the same age, differences in yield and vigour and the inherent variation in the physical and chemical characteristics of fruit could have influenced the results of the trials. Using grafted trees that are more uniform both in genetics, yield and vigour could help to reduce some of the variability in the data. Such trees are also likely to be those that produce the most commercially viable fruit.

In many instances it is likely that several of the monthly protective sprays applied by growers throughout the year are ineffective because they completely miss larvae, or do not reach larvae early enough to prevent damage. Not only are the cover sprays in a calendar program often not reaching the intended target, there can also be collateral damage to non-target organisms and associated problems with secondary pests, resistance and environmental

contamination (Metcalf, 1994). There are also associated economic and labour costs that are wasted on inaccurately timed applications. Onstad et. al. (1985) reported that one well-timed spray reduced larval density and damage of the oblique banded leafroller equally as well as several cover sprays. Timing sprays to when eggs or young larvae of the quandong moth are present in the orchard will increase the efficacy of sprays and may also reduce the number of sprays applied throughout the year.

Development of resistance in the pest population is a major concern voiced by many quandong growers. Resistance is well documented in cropping systems with a history of over-reliance on broad-spectrum insecticides, including the diamondback moth, *Plutella xylostella* Linnaeus (Lepdioptera: Plutellidae) in crucifers (Talekar and Shelton, 1993), cotton bollworms, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), *Helicoverpa armigera* H_bner (Lepidoptera Noctuidae), tobacco budworm, *Heliothis virescens* H_bner (Lepidoptera: Noctuidae) (Adkisson, 1971; Eveleens, 1983) and cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Metcalf, 1980) in cotton, and lightbrown apple moth, *Epiphyas postvittana*, Walker (Lepidoptera: Tortricidae) in apples (Lloyd et. al., 1970). The cotton leafworm has evolved resistance to over 20 insecticides across all classes and in some cases resistance developed within two years (Metcalf, 1980). Similarly, in many countries the diamondback moth has developed resistance to every synthetic insecticide used against it (Talekar and Shelton, 1993). Once resistance has developed, cross resistance and multiple resistance then further compound the resistance problem. Cotton leafworm, tobacco budworm and diamondback moth have all evolved multiple resistance across almost all classes of insecticides (Metcalf, 1994).

Insecticide resistance management (IRM) has become a critical component of many IPM programs and has most often been implemented as a curative rather than preventative strategy (Forrester, 1990; Metcalf, 1994; Heisswolf et. al., 1997). Resistance management can include reducing the frequency of applications, alternating insecticides, monitoring the efficacy of insecticides to detect evidence of resistance in the insect population and refraining from using mixtures of insecticides (Metcalf, 1994). Other important considerations are alternative host plants, the number and timing of generations of the pest, the mobility of the pest and other key pests in the system (Forrester, 1990). The Australian cotton industry began implementing IRM in the early 1980's as a curative strategy for pyrethroid resistance and as a preventative strategy for organophosphate and carbamate resistance (Cox and Forrester, 1992). The IRM strategy for cotton is based on restriction of the usage of pyrethroids to one generation of *H.armigera* and *H.punctigera* each year. The restrictions apply to all crops in the region that are hosts for *Helicoverpa* species, such as sorghum, lupins and sunflowers, because *Helicoverpa* is highly migratory (Forrester, 1990). Endosulfan usage is restricted to early and mid-season usage in cotton only, as alternative insecticides are available for late season use in cotton, but not in the other host crops (Forrester, 1990). Forcing growers to rely on expensive alternative insecticides is likely be detrimental to acceptance of an IRM strategy (Forrester, 1990). The cotton IRM strategy is logical and relatively simple so growers are able to understand and appreciate the benefits, and flexible between seasons so that new technology can be integrated (Forrester, 1990). Implementation was also facilitated by the presence of an existing IPM program.

Ideally, IRM should be a preventative strategy (Forrester, 1990) and the quandong industry has not yet reached the crisis point that necessitated curative IRM in the cotton system. Insecticide resistance in species such as *Helicoverpa* has developed in an environment of intense selection pressure. In the quandong system, selection pressures can vary among orchards. For example, some growers are only spraying once or twice in a season and in other cases growers are spraying monthly, exposing up to four generations of the moth to the dimethoate each year. However, there are several aspects of the quandong system that help to reduce selection pressure. Generally, only the larval stage of the moth is exposed to dimethoate, the adults are not migratory and there are often large distances between quandong orchards. Further, the quandong moth breeds only in one host plant. Although at this stage there is no evidence of resistance developing in the population, it is important to note that there are no data on the baseline susceptibility of quandong moth to dimethoate. Monitoring for resistance will begin with the vigilance of growers and management of resistance must be a priority for all growers because once it has developed it rarely remains confined to a single production region (Talekar and Shelton, 1993; Frisbie et. al., 1994). Ultimately, IRM is a "social technology" and acceptance by growers is highly dependent on communication between researchers, extension personnel and growers (Forrester, 1990).

Further research into the potential for IGRs in management of the quandong moth is required. Although specific IGRs are ideal for inclusion in IPM programs their use in the quandong industry may be restricted because many orchards are small-scale and the industry is in the fledgling stages of development. Economics are a major consideration, along with the research required for registration of new insecticides and access to new insecticides and application

equipment. However, for growers who have the resources to use more selective insecticides, IGRs when well timed and applied thoroughly, could provide a viable option.

7. BIOLOGICAL CONTROL

7.1 INTRODUCTION

Biological control can be defined as the use of natural enemies to reduce pest populations (Hoy, 1994). Natural enemies of insects include parasitoids, predators and pathogens. Augmentation and conservation biological control are the biological control strategies most commonly employed for management of native pests (Hoy, 1994). Augmentation involves supplementing the existing populations of natural enemies with enemies reared in laboratory populations. Augmentative releases may be inoculative for season long control of a pest, or inundative for immediate suppression of the pest population (Hoy, 1994). Conservation biological control involves preserving and protecting existing populations of natural enemies through the modification of agricultural practices. The enemies employed in conservation biological control may be native or established foreign species, or stem from populations of augmentative releases (Hoy, 1994).

There are a number of factors that influence the way that biological control is used and the potential for effective management of a pest species. The origins of the pest and the presence and effectiveness of existing natural enemies are major considerations (Hoy, 1994). The quandong moth is a native pest and therefore is likely to have both specific and generalist natural enemies that are established, but may be only partly effective in suppressing pest populations. As part of an integrated approach, the efficacy of biological control is largely dependent on minimising insecticide spray schedules to protect existing or introduced populations of natural enemy populations (Bartlett, 1964; Hull and Beers, 1985).

Surveys were conducted in orchards and wild groves of quandong trees across South Australia to collect natural enemies of the quandong moth, particularly specific parasitoid wasps. Other mortality agents such as diseases were also identified during the study.

7.2 MATERIALS AND METHODS

7.2.1 Parasitoids

Percentage parasitism was estimated from eggs and fourth instars that were collected during regular field sampling and reared in the laboratory. Egg parasitoids were collected as they emerged from eggs in hatching vials. Egg-larval and larval parasitoids were collected from larvae reared on fresh fruit. Cumulative percentage parasitism was calculated by dividing the cumulative number of parasitoids by the cumulative number of hosts collected on each sample date. Percentages were calculated for the spring generation as estimates of this type have been shown to more accurately represent the level of parasitism within a generation than peak or average values (Van Driesche, 1983). Abundance of the quandong moth during other generations was too low to examine parasitism. Other instars collected during regular field sampling were reared individually in the culture room and mortality factors identified. Any parasitoids that emerged were collected and preserved in 70% ethanol for identification. Hyperparasitoids were also collected as they emerged. Surveys were conducted at the regular field sites at Quorn and Sedan, and the trial site at Whyalla, as well as at Cummins and Coffin Bay on Eyre Peninsula, several locations in the Riverland and in the far north of the state at Woomera.

7.2.2 Predators

Throughout the regular field sampling the activity of other insect species on the trees was observed. Species that were known to be generalist insect predators were collected and catalogued.

7.2.3 Disease

Where possible during attempts at laboratory rearing, larvae that died due to a suspected disease were recorded. The symptoms of the disease were documented and a causal agent suggested. Records of death due to disease were only taken from larvae collected in the fourth instar.

7.3 RESULTS

7.3.1 Parasitoids

The most common species of parasitoid wasp reared from the final instar of the quandong moth were two species of Braconidae (Table 7.1). The most widely collected parasitoid was a *Chelonus* (*Microchelonus*) sp., the nominal genus in the subfamily Cheloninae. It is a solitary, egg-larval parasitoid that emerges from the fourth instar of the quandong moth just before it pupates. The genus has not been thoroughly revised for Australia which made identification to species level impossible (A.D. Austin, pers. comm.). The next most commonly collected wasp was a *Dolichogenidea* sp. in the subfamily Microgastrinae. It is a solitary, larval

parasitoid and also emerges from the fourth instar just before the moth larva pupates. A species of *Trichogramma* was also collected from eggs of the quandong moth. It is likely that all of the above species are native and previously undescribed (A.D. Austin, pers. comm.). A larval ectoparasitoid was also collected and identified as *Euderus* sp. in the family Eulophidae (J. La Salle, pers.comm.). A *Perilampus* species (Hymenoptera: Perilampidae) (J. LaSalle pers. comm.) that is commonly a hyperparasitoid of braconids and ichneumonids was collected at several sites. Voucher specimens of all parasitoids collected in this study were deposited in the Waite Insect and Nematode Collection at the Waite Campus of Adelaide University.

Due to difficulties with larval rearing, parasitism estimates could only be made from low numbers of larvae (Table 7.2). Incidence of egg parasitism was low throughout the study (Table 7.3). In the spring 2000 generation at Quorn, the cumulative percentage parasitism for *Chelonus* sp. and *Dolichogenidea* sp. combined reached a maximum of 6.8% on 27th Oct (Figure 7.1). For *Trichogramma* sp., the maximum was also recorded on 27th October at 8.3% (Figure 7.2). For *Chelonus* sp. and *Dolichogenidea* sp. in the spring generation at Sedan, the cumulative percentage parasitism was at a maximum of 6.3% on 26th September and then declined on 23rd October (Figure 7.3). For *Trichogramma* sp. the maximum was also recorded on 26th September at 7.7% and like egg-larval and larval parasitism, decreased thereafter (Figure 7.4).

Table 7.1: Parasitoids of quandong moth collected from orchards and wild stands of quandong trees in South Australia.

Species	Quorn	Sedan	Whyalla	Port Augusta	Coffin Bay	Cummins
			Primary parasitoids			
Endoparasitoids						
Braconidae						
Chelonus sp.	x	x	x	x	x	x
Dolichogenidea sp.	x	x	x	x	x	x
Trichogrammatidae						
Trichogramma sp.		x	x	x		
Ectoparasitoids						
Eulophidae						
Euderus sp.		x		x		
			Hyperparasitoids			
Perilampidae						
Perilampus sp.	x	x		x		

Table 7.2: Number of fourth instars of the quandong moth reared and number diseased or parasitised by *Chelonus* sp. and *Dolichogenidea* sp. at Quorn and Sedan during the spring generation in 2000.

Sample date	Number of 4th instars reared	Number of 4th instars diseased	Number of 4th instars parasitised	
			Chelonus	*Dolichogenidea*
		Quorn		
21-Sep-00	27	9	0	0
12-Oct-00	31	7	1	2
27-Oct-00	16	3	1	1
		Sedan		
12-Sep-00	4	0	0	0
26-Sep-00	16	11	1	0
23-Oct-00	10	1	0	0

Table 7.3: Number of eggs of the quandong moth reared and number parasitised by *Trichogramma* sp. at Quorn and Sedan during the spring generation in 2000.

Sample date	Number of eggs reared	Number parasitised
	Quorn	
25-Aug-00	8	0
21-Sep-00	16	0
12-Oct-00	3	0
27-Oct-00	9	3
	Sedan	
22-Aug-00	24	0
12-Sep-00	11	0
26-Sep-00	4	3
23-Oct-00	50	0

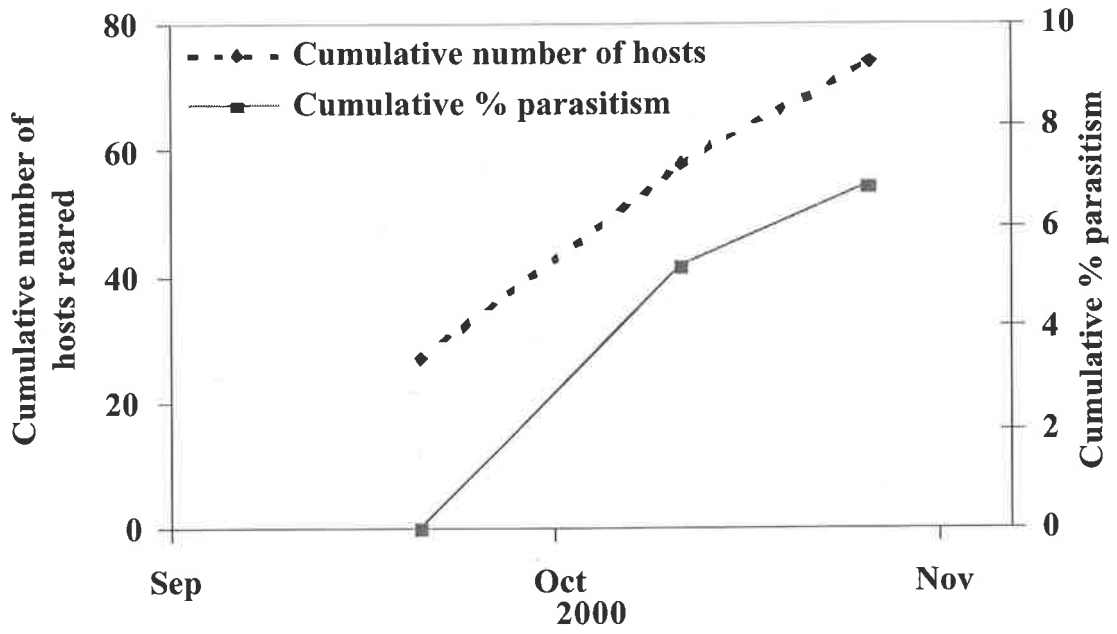


Figure 7.1: Cumulative number of 4th instar quandong moth reared and cumulative percentage parasitised by *Chelonus* sp. and *Dolichogenidea* sp. in the spring generation at Quorn in 2000.

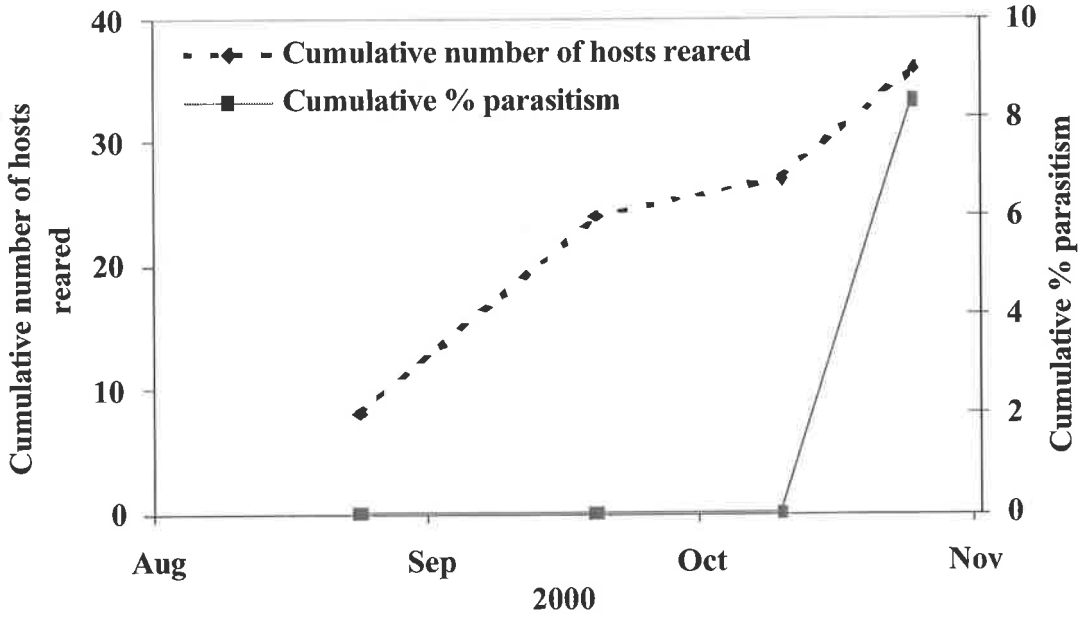


Figure 7.2: Cumulative number of eggs of the quandong moth reared and cumulative percentage parasitised by *Chelonus* sp. and *Dolichogenidea* sp. in the spring generation at Quorn in 2000.

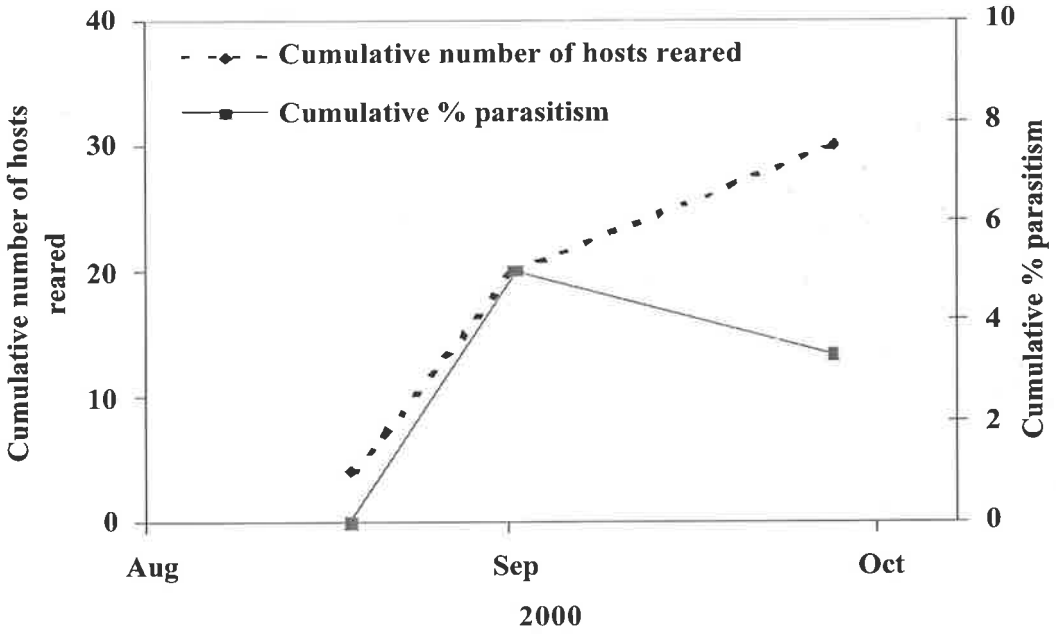


Figure 7.3: Cumulative number of 4th instar quandong moth reared and cumulative percentage parasitised by *Chelonus* sp. and *Dolichogenidea* sp. in the spring generation at Sedan in 2000.

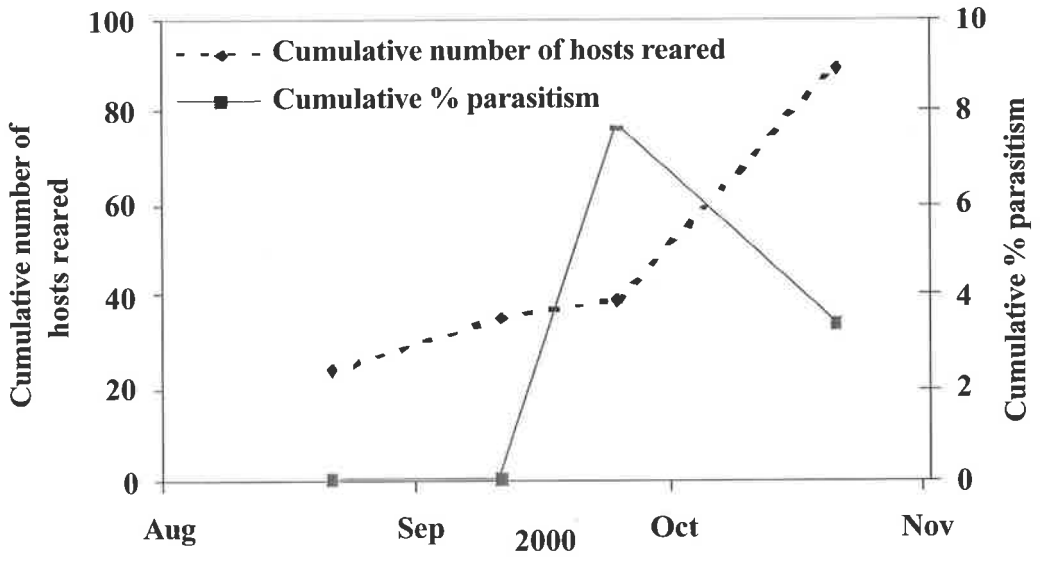


Figure 7.4: Cumulative number of eggs of the quandong moth reared and cumulative percentage parasitised by *Chelonus* sp. and *Dolichogenidea* sp. in the spring generation at Sedan in 2000.

7.3.2 Predators

Predation on eggs or larvae of the quandong moth was not observed directly in the field, but several generalist predators were observed on quandong trees at various times of the year. These included adults and larvae of the spotted ladybird, *Harmonia conformis*, (Coleoptera: Coccinellidae), adults and larvae of green and brown lacewings (Neuroptera), several species of flower spiders (Araneae: Thomisidae) and the bird-dropping spider, *Celaenia* sp. (Araneae: Araneidae).

7.3.3 Disease

Disease symptoms were apparent in some fourth instars and infected larvae ceased feeding, became sluggish and blackened, and in many cases body tissues liquified and the blackened liquid exuded from the body. The symptoms appeared consistent with a bacterial disease but the possibility of a viral agent has not been discounted (Lacey and Brooks, 1997). Symptoms generally appeared after several days in the laboratory and were primarily apparent in fourth instars although two third instars were also symptomatic. Of all the fourth instars collected in the spring generation and reared in the laboratory, only one was symptomatic on the day assessments were made. On the same sample date, two third instars also showed symptoms of the disease.

In the fourth instars of the spring generation at Quorn in 2000 that were reared in the laboratory, 25.7% died with disease symptoms. In the spring generation at Sedan, 46.2% of those reared in the laboratory died with disease symptoms (Table 7.2).

7.4 DISCUSSION

In eggs and larvae that were individually reared to estimate parasitism, few individuals were successfully reared to the pupal stage and therefore very few parasitoids were collected. The cumulative percentage parasitism for the spring generation increased at Quorn and decreased at Sedan with the overall maxima similar for the egg and egg-larval parasitoids. Only one larva was parasitised in those reared from Sedan and the decrease in cumulative percentage parasitism at Sedan may be an artefact of the low numbers detected. Almost three times as many fourth instars were reared from Quorn compared to Sedan, with five times as many parasitoids emerging.

Van Driesche (1983) reported that determining the generational level of parasitism is most difficult with systems that have several parasitoid species, hosts with overlapping generations and parasitoids that oviposit in and emerge from different host stages. In such systems, cumulative percentage parasitism more accurately reflects generational levels of parasitism than peak or average percentage parasitism (Van Driesche, 1983). Many factors can confound estimates of parasitism, such as the phenology of the host and the parasitoid, asynchrony between host and parasitoid generations, and the differences in development and behaviour of parasitised versus unparasitised hosts (Brown, 1946a; Brown, 1946b; McGugan, 1955; Bisabri-Ershadi and Ehler, 1981; Van Driesche, 1983). The quandong moth is a host for which a "stable moment" in its life history has not been identified, i.e. when all hosts are in the susceptible stage and all parasitism has occurred but emergence of hosts or parasitoids has not begun. Such a period greatly facilitates the estimation of generational parasitism (Van Driesche, 1983). For species lacking such a moment, several steps can be taken to ensure that

estimates reflect generational levels of parasitism as accurately as possible (Van Driesche, 1983) however any estimates made during this study are likely to be underestimates. Two stages in the life cycle of the quandong moth were identified as susceptible stages, eggs and fourth instars. For the egg parasitoid, the egg is the stage from which the parasitoid emerges. For the egg-larval and larval parasitoids, the fourth instar is the stage from which the parasitoids emerge. Although sample sizes were greatly reduced by restricting sampling to these two stages only, it was important for the accuracy of the estimates that certain stages be used as indicator stages for parasitism. There were problems in this study, particularly with larval rearing, that greatly restricted the number of individuals available for parasitism estimates. Many larvae died of disease during laboratory rearing, in some cases almost 50%, before parasitism could be detected. Many other larvae seemed to desiccate and die without any symptoms of the disease, probably as a result of failure to establish a feeding site on fresh cut fruit in the laboratory. Due to the prevalence of spoilage in quandong fruit in the laboratory, larvae had to be frequently moved to fresh fruit and in many cases the trauma or injury caused during handling caused permanent disruption to their feeding behaviour. Changes in the behaviour and developmental rate of parasitised hosts can influence the accuracy of estimates of parasitism (Wylie, 1981; Wylie, 1982; Steward et. al., 1990). Larvae were only collected from inside fruit, thus any larvae that had altered behaviour may not have been collected during sampling. In the limited samples available, parasitism did not appear to have altered the developmental rates of eggs or larvae of the quandong moth.

A study by Day (1994) reported that percentage parasitism recorded by rearing, compared to dissection of hosts, was significantly different for hosts in two different insect orders.

Although dissection produced more accurate estimates of parasitism than rearing, in both cases there remains several advantages of rearing hosts (Day, 1994). Particularly with new species of parasitoids, rearing is necessary initially to identify parasitoids and also to detect changes in the behaviour and developmental times of hosts, and to identify diseased hosts (Day, 1994). Dissection requires more technical skill than rearing, but results are obtained more rapidly and the process is not reliant on a larval food source (Day, 1994). For the quandong moth, rearing was necessary initially to identify the species of parasitoids. Dissection of hosts may increase the accuracy of subsequent estimates of parasitism of quandong moth. It is likely that the larvae of the two egg-larval parasitoids would be sufficiently different to enable identification of the individual species during dissection.

Both *Trichogramma* sp. and *Chelonus* sp. attack the quandong moth in the egg stage. Removal of eggs and larvae from the field before parasitism is complete could result in underestimates of generational parasitism. The *Dolichogenidea* sp. is a larval parasitoid and the instar of the quandong moth that is attacked is unknown. A thorough knowledge of both the phenologies of the host and the parasitoid is critical for identifying periods where the generational level of parasitism can be accurately estimated. In this study, the phenology of the host has been recorded over three years and further examination of the phenology would be invaluable. The phenologies of the parasitoids identified in the study have not been examined and would contribute greatly to understanding the synchrony in the host-parasitoid complex and a more accurate determination of the levels of parasitism.

It is clear that even in unsprayed orchards parasitoids are ineffective in suppressing populations of the quandong moth. The data on the percentage parasitism indicated the overall level of parasitism was very low in the spring generation for all parasitoid species. Levels of parasitism are likely to be even lower in orchards where broad-spectrum insecticide usage is frequent (Metcalf, 1994). In blackheaded fireworm, *Rhopobota naevana* H_bner (Lepidoptera: Tortricidae), native to North America, parasitism by native species of *Trichogramma* decreased in cranberry fields where insecticides were used compared to those in unsprayed blocks (Li et. al., 1993). Even in the absence of insecticides, low rates of parasitism of native Lepidoptera by native parasitoids in Australia have been reported. Larvae of LBAM are parasitised by three native parasitoids (Danthanarayana et. al., 1977) and key factor analyses indicated that none of the larval parasitoids were significant mortality factors (Danthanarayana, 1983). Hokkanen and Pimentel (1984) postulated that a long association between a host and natural enemy may lead to the evolution of homeostasis in the system. However, homeostasis does not always explain the inability of native parasitoids to suppress native pests. Parasitism of LBAM by native *Trichogramma* was not a significant mortality factor and asynchrony between the host and natural enemy was considered the most likely explanation (Danthanarayana, 1980a). If homeostasis does exist, economics often determine whether it prevents a parasitoid from being an effective biological control agent (Hokkanen and Pimentel, 1984; Hokkanen and Pimentel, 1989). The macadamia nutborer, *Cryptophlebia ombrodelta* Lower (Lepidoptera: Tortricidae) is parasitised by six native parasitoids, but even those with the highest rates of parasitism were considered ineffective in commercial terms (Sinclair, 1979). While there may be an ecological balance in terms of the host-parasitoid relationship, control is determined purely from an economical viewpoint.

The low rates of parasitism of quandong moth by both egg-larval and egg parasitoids may result from a lack of specificity of the parasitoids. *Trichogramma* species in particular, are often generalists (Hoy, 1994) and the species identified during this study may have hosts other than quandong moth. Further research is required to determine the host range of the parasitoids reared from quandong moth.

Conservation biological control involves preserving and protecting existing populations of natural enemies through the manipulation of agricultural practices. In many systems one of the main factors suppressing the effectiveness of natural enemies of a pest, including parasitoids, is the use of broad-spectrum insecticides (MacLellan, 1972; Luck and Dahlsten, 1975; Talekar and Shelton, 1993). However, even before any human intervention, many insect populations are already above the arbitrary threshold set by humans that defines an insect as a pest. The quandong moth was recognised as a pest as early as the 1860's (Austin, 1863), and the tolerance for damage has lessened even further since commercialisation of fruit production. Although natural enemies alone do not provide adequate control of quandong moth, the adoption of lepidopteran specific insecticides could help to conserve populations of the natural enemies as part of an integrated management program.

If native natural enemies cannot effectively suppress pests even in the absence of widespread insecticide use, then augmentation biological control may be an appropriate strategy (Hoy, 1994). Augmentation could have a role in the management of quandong moth but would be reliant on mass-rearing large numbers of the parasitoid in the laboratory for release and

immediate or season-long control of a pest. As the quandong moth cannot currently be mass reared in the laboratory, its egg-larval and larval parasitoids cannot be mass reared for evaluation either in the laboratory or the field. However, it is possible that the egg parasitoid *Trichogramma* could be reared in the laboratory and the use of an alternative host may greatly facilitate mass rearing (Hoy, 1994). Alternative hosts must be used with caution as there are reports of physical deformities in wasps reared on hosts other than the pest species (Hoy, 1994). Maintenance of genetic fitness in mass reared parasitoids is also a major consideration (Roush, 1990). Due to the fledgling nature of the quandong industry, augmentation with *Trichogramma* sp. is only likely to be feasible if parasitoids could be sourced from an alternative industry, such as cotton.

The majority of the larvae affected by disease only became symptomatic after laboratory incubation, suggesting laboratory conditions contributed to the onset of the disease. Many entomopathogens exist in low levels in populations and it is not uncommon for insect populations to succumb to disease when predisposed to infection under conditions of stress (Smith and Johnson, 1989; Lacey and Brooks, 1997). Laboratory rearing can be especially stressful for insects and is known to accelerate the incubation period of pathogens or to induce symptoms not present in the field. Laboratory stress can also lead to infection by pathogens that are normally saprophytic (Lacey and Brooks, 1997). Although there were many problems with fungal contamination of fruit, symptoms of the bacterial disease were only recorded from larvae on fruit free of contamination. It is difficult to speculate on the effects of the disease in the field population. The disease may remain latent in the population for much of the year but during stressful periods, such as extreme temperatures or crowding, could

cause mortality. Behavioural changes could also explain why symptomatic or dead larvae were rarely detected during regular field sampling. Disease can lead to the cessation of feeding and also dispersal of insects just prior to death (Lacey and Brooks, 1997). It is possible that the disease was present in the field but altered behaviour prevented symptomatic larvae from being sampled along with healthy larvae in regular field sampling.

Many of the larvae collected from the field in the fourth instar were diseased but there is likely to be an equivalent rate of parasitism in diseased and healthy larvae, particularly for the *Chelonus* species. *Chelonus* is an egg-larval parasitoid and so would have already parasitised an individual before any behavioural alterations occur. The instar that *Dolichogenidea* attacks is not known and behavioural alterations may affect rates of parasitism if larvae are infected before parasitism occurs.

8. ACTION THRESHOLDS AND SEQUENTIAL SAMPLING

8.1 INTRODUCTION

Monitoring a pest population is critical if a program of calendar application of insecticides is to be replaced with a "treat when necessary" strategy (Luckmann and Metcalf, 1994). The spray timing trial indicated that spraying monthly with dimethoate was statistically no more effective than the application of two accurately timed sprays (Section 6.3.1). Monitoring is critical to ensure the most effective timing of sprays. The decision to spray is based on the use of an action threshold developed from a knowledge of the relationship between pest density, population growth and the economics of damage (Luckmann and Metcalf, 1994). The economic injury level (EIL), developed by Stern (1959), is the "lowest population density that will cause economic damage". The action threshold (AT) is the pest threshold at which treatment is justified, that is the "threshold at which control measures should be determined to prevent an increasing pest population from reaching the economic injury level" (Stern et. al., 1959).

Of all the stages in the life cycle of the quandong moth, monitoring for the eggs is the most amenable for use in a sampling plan. Monitoring for eggs can be done non-destructively, and requires a hand-lens with a minimum of x10 magnification and a minimum of training to recognise egg masses. Monitoring for eggs enables growers to make management decisions before damage has occurred. In contrast, monitoring for neonate larvae is unlikely to be feasible due to their small size, cryptic nature and the need to destructively sample fruit. The relationship between the mean and variance for eggs of quandong moth in flowers and fruit

indicated that a sample size over 400 was required to achieve precise estimates of mean density (Section 5.3.2). For growers wishing to make pest management decisions, the use of binomial rather than enumerative counts often decreases sample sizes while maintaining an acceptable level of precision in the counts. Sequential sampling based on classifying the proportion of fruit infested above or below the AT reduces the sample size required and can greatly facilitate monitoring compared to fixed sample size methods (Binns and Nyrop, 1992).

8.2 MATERIALS AND METHODS

8.2.1 Damage regressions

The relationship between pest density and damage is an important component in the development of an EIL. When the relationship is not known, as is the case for quandong moth, it is often assumed to be linear (Pedigo et. al., 1986). As experimental population densities could not be established, damage regressions were calculated from data collected during regular field sampling. Data were collated from mature fruit in which only fourth instars were feeding and without larval exit holes. Exclusion of all other fruit ensured that the damage to flesh was caused only by fourth instars. The relationship between the number of larvae in each of these fruit and the mean damage rating to the flesh of the fruit was examined by linear regression. Fourth instars were used for regressions because the likelihood of having overlooked fourth instars in the flesh of fruit was low compared to that of the earlier instars, and ultimately the most damage is done by fourth instars.

8.2.2 Economic Injury Level

Preliminary economic injury levels for the quandong moth were calculated from field data. EILs were only calculated for mature fruit, for which economic damage is a direct result of larval feeding.

The equation used for the EIL after (Ostlie and Pedigo, 1985) was:

$$\text{EIL} = \frac{\text{gain threshold (kg/ha)}}{\text{loss per insect (kg/insect)}},$$

$$\text{where the gain threshold} = \frac{\text{management costs (\$/ha)}}{\text{market value (\$/kg)}}.$$

Economic injury levels were initially calculated as number of larvae/ha and were converted to an estimate of proportion of fruit infested using average yield/ha of newly established orchards. The recommended average planting density for quandong trees is 400 trees/ha. Yields vary greatly among trees and among orchards, but a minimum yield of approximately 150 fruit/tree is a standard the industry has set for new orchards, after around two to three years of fruiting, with a projected yield increase of approximately 10% each year. Thus, approximate yields in new orchards are in the order of 60,000 fruit/ha (R. Jacobs, pers. comm.).

The management cost generally has three components; the cost of the control agent, the cost of application equipment and labour cost (Pedigo et. al., 1986). Currently, in the majority of quandong orchards labour costs are likely to be nominal, as the enterprise is only just becoming commercial for many growers and in many cases began as a hobby. Due to the current small scale of many orchards, the cost of application equipment may also be negligible as knapsack sprayers are commonly used. However, a range of thresholds are presented to

account for the use of tractors and other more sophisticated equipment in some orchards. In 2000, dimethoate could be purchased for approximately $10/L wholesale, up to $60/L when purchased retail. The cost of control was based on field trials conducted at Whyalla using dimethoate and grower's estimates of cost. The cost of applying dimethoate ranges from $5-20/ha depending on the cost of the chemical, the equipment used and the inputs required from the grower (R. Jacobs, pers. comm.).

In 1999, the value of quandong fruit ranged from primary value added products such as jams and sauces at an equivalent of $175/kg, down to processing grade at $35.30/kg (Gordon-Mills, 2000). Undamaged fruit may be sold as a premium vacuum-packed product for up to $90/kg. Often, the damaged areas of fruit are removed and the remainder used for processing. The tolerance for damage to fruit varies greatly depending on the market for which the fruit is produced. For the premium market, particularly for fruit sold uncut, no damage is tolerable and all damage equates to economic loss. However, if growers are producing for the processing market and fruit is cut and graded on site before sale, a higher level of damage is tolerable. Growers have the option to sell fruit to several markets, ranging from the lowest grade processing fruit, to the highest grade premium fruit.

8.2.3 Sequential sampling

The action thresholds used in sequential sampling were based on the EILs determined for quandong moth. The suitability of different action thresholds was investigated and was represented as the proportion of fruit having at least one egg. Upper (T_{upper}) and lower (T_{lower}) stop lines were calculated to facilitate classification of populations on the basis of the

cumulative number of fruit infested (T_n) for each sample size (n). The population level is considered to be above the AT if $T_n > T_{upper}$ and below it if $T_n < T_{lower}$. Where $T_{lower} \leq T_n \leq T_{upper}$ the population level cannot be classified and sampling continues until a decision can be made about the population level relative to the AT (Fowler and Lynch, 1987). Operating characteristic (OC) and average sample number (ASN) functions were used to evaluate the sampling plans. The operating characteristic is the probability of "no intervention", in this case no insecticide application, versus the population density. The average sample number is the average number of fruit required to make a decision at a given population density (Fowler and Lynch, 1987). Equations for the stop lines, OC and ASN curves for the negative binomial distribution were defined by Fowler and Lynch (1987) (Appendix 5).

8.3 RESULTS

8.3.1 Damage regressions

Initially, separate regressions were computed for data from Quorn and Sedan for damage to mature fruit. As would be expected, there was no significant difference between the field sites, the data were therefore pooled and a common regression equation was computed. There was a significant linear relationship between the number of fourth instars of the quandong moth and the damage to the flesh of mature fruit, with the mean damage rating increasing as the density of larvae increased ($r^2 = 0.8315$, $F = 3783.6$, $p < 0.0001$). The equation for the regression line was *damage rating* = 1.313**number of larvae/fruit* + 0.0261. The mean percentage damage caused by a single fourth instar was 25%, and the maximum and minimum were ratings equivalent to 50% and 5% of the flesh of fruit damaged, respectively.

8.3.2 Economic Injury Level

Regression indicated a positive linear relationship between damage and the number of fourth instars of quandong moth (Section 8.3.1). The high degree of variation in the damage caused by a single fourth instar led to the development of a range of EILs. The yield loss per fourth instar was calculated using estimates of damage by individual larvae and the average weights of mature quandong fruit at Quorn in 2000. The average weight of a mature fruit at Quorn in 2000 was 5.05 ± 0.62g (mean ± 95% C.I., n=46). The average yield loss/fruit was 1.26g for the mean, 0.25g for the minimum and 2.5g for the maximum loss caused by a single fourth instar. Conservative, moderate and liberal estimates of the EIL were calculated based on the minimum, mean and maximum losses caused by a single fourth instar, respectively, and assuming a distribution of one larva/fruit. Although the population is generally more aggregated, particularly in mature fruit, the presence of a single individual in a fruit warrants action. The lack of accurate estimates of egg and larval mortality led to the assumption that each fruit infested with at least one egg equates to a fruit infested with at least one fourth instar, so that EILs could be communicated in terms amenable to monitoring. The maximum EILs ranged from the liberal estimate at 13.2% of fruit infested, to the conservative estimate at 1.3% infested (Table 8.1). The highest proportion of fruit infested in the spring generation at Quorn in 2000 was 44%, and many fruit had more than one fourth instar feeding inside. Generally, the peak proportion of mature fruit infested at each site in each year was well above all the EILs calculated in this study.

Table 8.1: Conservative, moderate and liberal estimates of the economic injury level for the spring generation of the quandong moth (percentage of fruit with eggs).

Crop value	Mangement costs ($/ha)			
($/kg)	5	10	15	20
		Conservative[1]		
10	0.3	0.7	1.0	1.3
30	0.1	0.2	0.3	0.4
55	0.1	0.1	0.2	0.2
90	0.0	0.1	0.1	0.1
		Moderate[1]		
10	0.7	1.3	2.0	2.6
30	0.2	0.4	0.7	0.9
55	0.1	0.2	0.4	0.5
90	0.1	0.1	0.2	0.3
		Liberal[1]		
10	3.3	6.6	9.9	13.2
30	1.1	2.2	3.3	4.4
55	0.6	1.2	1.8	2.4
90	0.4	0.7	1.1	1.5

[1]Estimates based on minimum, mean and maximum level of damage caused by a single fourth instar of quandong moth (Section 8.3.1).

8.3.3 Sequential sampling

Action thresholds were set at 12% and 2% of fruit infested, below the maximum EILs for the liberal and moderate EILs respectively. The nominal proportions below and above the AT for calculation of the stop lines were $p_0 = 0.07$ and $p_1 = 0.19$ for the conservative EIL, and $p_0 = 0.01$ and $p_1 = 0.03$ for the moderate EIL.

The parameter k of the negative binomial distribution was calculated by substituting the mean number of eggs/sample unit and the equation for Taylor's Power Law into:

$$k = x^2/(s^2-x),$$

such that,

$$k = x^2/(ax^b-x),$$

where x is the mean, s^2 is the variance, and a and b are coefficients determined by nonlinear regression (Section 5.3.2). Constant parameters in the calculations were k = 0.11653 and the error rates for recommending control when the proportion infested is actually below the AT, α, or above the AT, β (Mo et. al., 2000), were set at 0.1.

The stop lines indicated that for AT = 0.12, approximately 180 fruit would have to be examined before a decision could be made not to spray (Figure 8.1). For AT = 0.02, approximately 960 fruit would have to be examined before a decision could be made not to spray (Figure 8.2). As the AT increased from 0.02 to 0.12, the maximum ASN value increased from 420 to 1,945 with little increase in the risk of incorrect decisions being made, as indicated by the OC functions (Figures 8.1 - 8.2). An AT for the conservative EIL was not examined because the ASN for the moderate estimate was so high, lowering the threshold further would only result in a less practical ASN. Even the most liberal EIL is unlikely to be practical for sequential sampling because of the high input of time required. With sample sizes of up to 420, monitoring of fruit may not be consistent or sustainable over a long period of time. However, at high population densities, decisions to spray could be made with a much smaller sample size and within a shorter period of time.

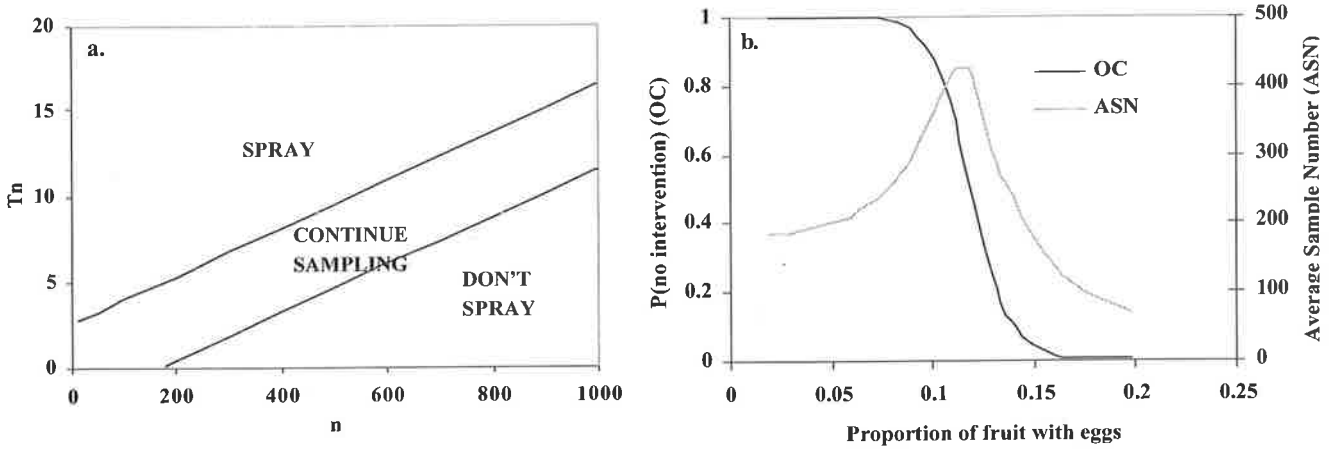


Figure 8.1: (a) Sequential stop lines and (b) operating characteristic and average sample number functions for classifying proportion of fruit with eggs of quandong moth at action threshold of 0.12. Tn = cumulative number of fruit with eggs, n = number of fruit examined.

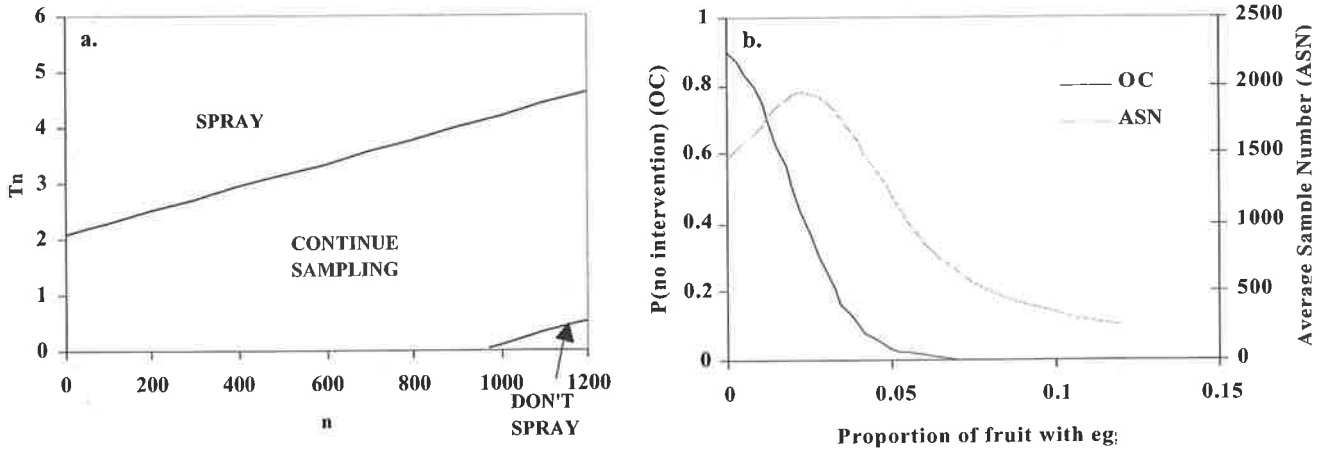


Figure 8.2: (a) Sequential stop lines and (b) operating characteristic and average sample number functions for classifying proportion of fruit with eggs of quandong moth at action threshold of 0.02. Tn = cumulative number of fruit with eggs, n = number of fruit examined.

8.4 DISCUSSION

It is acknowledged that estimates of the EIL for the quandong moth are simplistic and based on assumptions that may not be valid in the field. One major assumption is that a single egg equates to a single fourth instar. In reality, egg and larval mortality, particularly in the neonate stage, is likely to be high and therefore a large proportion of eggs will not reach the damaging fourth instar. It is probable that even the most liberal estimate will be an overestimate, because of the impact of mortality.

Knowledge of the relationship between pest density and yield loss is central to the development of thresholds (Pedigo et. al., 1986). The damage regressions used to formulate EILs in this study were based on naturally occurring densities of the quandong moth at field sites. More accurate estimates of the EIL would require a better understanding of the interaction between the pest and host plant. Establishing artificial population densities may help to reduce some of the variability seen in the field data used in this study.

Conversion of the EILs from number of larvae/ha to proportion of fruit infested also involved assumptions regarding the yield of orchards. These assumptions are likely to be valid only in newly established orchards and variations in older orchards may need to be considered. Further, yield increases in each year should be taken into account.

In this study, labour costs were considered nominal but could vary greatly between orchards. As with any activity there is an input of labour that necessarily cannot be devoted to another activity and thus cannot be discounted. In some cases, traveling to orchards for spraying

would also be significant. As the commercialisation of quandong fruit progresses labour costs may become a significant portion of the cost of management.

Larval dispersal may be an important factor in determining the relationship between the proportion of fruit with eggs and the proportion of fruit infested with larvae. Dispersal induced by crowding may result in an increase in the proportion of fruit with larvae compared to the proportion with eggs from which the larvae originated.

Economic injury levels are dynamic and evolve as knowledge of the pest and the response of the crop increases (Ostlie and Pedigo, 1985). Knowledge of the EIL can evolve from a situation where no decision criteria are available to comprehensive thresholds accounting for the economics of a wide range of management options and crop values (Poston et. al., 1983; Ostlie and Pedigo, 1985). The only recorded EILs for the quandong moth are based on the results of this study. Accuracy of the thresholds will undoubtedly improve as further study is undertaken on the interactions between the quandong moth and the host plant, and the mortality factors that operate in the immature stages.

The end-use of the fruit is a major consideration when determining the EIL for quandongs. Damage to the flesh of mature fruit causes a reduction in quality that equates to a reduction in yield. As the majority of fruit is processed, there is a level of damage that can be tolerated and may be dependent on where processing is to occur. A grower processing on site in a home-based enterprise is likely to tolerate a higher level of damage than a commercial processor who purchases the fruit. In some cases, growers sell fruit to processors or to retail outlets as a

premium uncut prodcut, and therefore must be able to assure the buyer that the quality is high. Thus, despite the associated problems, at this time, a monthly spray regime seems the only way to ensure that fruit remain free of larvae or damage of quandong moth.

The distribution of larvae in mature fruit is aggregated and frequency distributions indicated that fruit in which larvae occurred usually had more than one fourth instar in a single fruit (Section 3.3). In some cases other instars were also present. Thus, it is likely that yield losses in individual fruit will be greater than that caused by a single fourth instar and the presence of a single larva in a fruit is all that is required for management strategies to be implemented.

The regular field sampling conducted at Quorn and Sedan indicated the number and timing of the generations of the moth and also identified the most damaging generation (Section 5.3.1). Although IGRs such as tebufenozide are active against all instars, the quandong moth is only vulnerable for a very short period in its lifecycle because of its concealed feeding behaviour. Even with broad-spectrum insecticides that have systemic properties, maximum efficacy will be achieved if the early larval stages are targeted to minimise feeding damage and non-target effects will be minimised by judicious usage. Thus, if insecticides are to be applied, it is imperative that they are well timed to maximise efficacy and such timing is reliant on monitoring populations. It is likely that the spring generation of quandong moth will always warrant management, and this study examined whether sequential sampling could be used to more accurately time management for this generation. Although at the highest AT a decision not to spray could be made after examining approximately 180 fruit, the average number of fruit required to make a decision when the population density was near the AT was 420.

Although the ASN is only an approximation and actual values may vary (Binns, 1994), an ASN of over 400 is probably impractical for most growers. The ASN for the moderate estimate is much higher because the values of p0 and p1 are necessarily lower, and the difference between them is also smaller compared to those for the higher AT (Jones, 1993).

Problems with sequential sampling will arise where sample sizes are large and the population is at a level that cannot be classified above or below the action threshold. Given the current regime of calendar spraying against quandong moth, growers are likely to be more tolerant of applying what may later prove to be an unnecessary spray, than they will be of failing to apply a spray that leads to damage later in the season (Ring et. al., 1989). When the population cannot be classified relative to the AT, the point at which sampling ceases will be largely determined by the commitment and persistence of the individual grower and their propensity for insecticide use. Although such an arbitrary cut-off point may decrease the accuracy of decisions (Fowler and Lynch, 1987), it is a necessary part of the sampling procedure because of the finite nature of temporal and economic resources. In such a case growers could be advised to make a decision about treatment based on historical pest densities or another measure of pest density, such as trapping data (Mo et. al., 2000).

In some cropping systems growers have become accustomed to monitoring using fixed sample size plans. The use of sequential sampling has been compared to existing sampling plans for pests, to examine the likelihood that growers will adopt the new technique (Hoffman et. al., 1992; Nault and Kennedy, 1996). It may be an advantage that a monitoring technique has not been used previously in quandong orchards, so growers do not have a pre-conceived notion of

the sample sizes required. With an AT of 12% it is probable that the spring generation will be treated at some point, but the results suggest it is not feasible to obtain precise estimates of the proportion of fruit infested using sequential sampling.

At this time, the best recommendation would be for growers to monitor for eggs of the quandong moth, with a sample size of approximately 200 fruit, and to spray when eggs are present. Based on the data from field sampling, the period of oviposition that produces larvae of the spring generation commences in early August to late September, depending on the region. Monitoring should begin prior to this period with insecticides being applied when the eggs are detected or hatching, depending on the activity of the insecticide. According to degree-day estimates, eggs of the spring generation will take approximately 7-14 days to hatch depending on the location and the time of year (Appendix 6, Figure 1 - 6). If a decision is made not to spray, then re-sampling within 7-14 days is recommended. If the majority of egg masses detected are newly laid, ovicides may be applicable, but for more developed eggs, larvicides could be applied to target larvae as they hatch. Spraying only when eggs are present will help to increase the efficacy of sprays, and may also reduce the number of applications each year.

The attitude of the individual grower can never be discounted as a factor in sampling programs. Although the aim of a sampling program should be to optimise both labour input and the accuracy of decisions, the level of commitment will undoubtedly vary amongst individual growers. Such variation will greatly influence the likelihood of adoption of a sampling program. There is no doubt it would be advantageous for growers to become

accustomed to the concept of monitoring as part of an IPM program, even if EILs are in the preliminary stages of development. Then, as more accurate EILs are developed, implementation of improved monitoring programs would be greatly facilitated.

9. MODELING

9.1 INTRODUCTION

Over the last 30 years, the modeling of populations has become an integral part of pest management (Ruesink and Onstad, 1994). Models now play a role both in improving existing pest management programs and in developing new programs. Using data from the ecosystem, in conjunction with climatic and economic data, models can be developed to predict certain events in the system and to evaluate optimal and alternative strategies (Ruesink and Onstad, 1994). It is no longer only the researcher or extension specialist who has access to the technology required for modeling insect populations. The advent of the personal computer has meant that the individual grower is now able to use models to make pest management decisions. Temperature dependent population models can be used both to understand the phenology and to predict events in the life history of an insect.

There were several periods during sampling when eggs or larvae of the quandong moth were not detected. It was not known how much of the variation in the number and timing of generations was due to inherent variability between years and between sites, and how much was due to sampling being wrongly timed or lacking intensity. Modeling was used to examine the developmental times of the various stages of the quandong moth, and the number and timing of the generations.

The estimates of rates of development obtained from laboratory constant temperature studies (Chapter 4), along with the incidence of eggs and larvae in the field, provided input for Dymex professional (CSIRO Publishing Version 1.0, 1999). Dymex is a model of a lifecycle made up of a user-defined number of life-stages and simulates the processes that influence cohorts of individuals throughout their lifecycle. The program consists of a Builder component in which the model is constructed, and a Simulator component in which the model is run, parameters can be manipulated and the outputs are graphed, tabulated or exported to other programs. The program is modular and has components that allow development, mortality, transfer between stages and reproduction to be manipulated by the user. Outputs are presented as the number and age of individuals in a cohort at any given time-step. Meteorological data can be imported and the model can be designed so that physiological time is used to drive the development of individuals using degree-days. Dymex also enables the user to simulate events that affect the development and survival of the population. Such events may be harvest of the host plant or application of insecticides. Events can be set to occur once the population reaches a certain threshold level, or to occur on specific dates. In this study, Dymex was used to simulate the effect of insecticide applications on both the population in the current generation and in the subsequent generation.

Although temperature is the major factor driving the development of insects, nutrition can also influence development times. Several authors have acknowledged that nutrition can influence the developmental rate of insects in the laboratory (Etman and Hooper, 1979; Schroeder et. al., 1986; Zalucki et. al., 1986). Onstad et.al. (1985) noted the different effects on the rate of

development of early season and mid-season leaves consumed by lepidopteran larvae. This phenomenon has also been factored into the model for the quandong moth.

9.2 MATERIALS AND METHODS

The model of quandong moth constructed with the Dymex package was based on a simplified life cycle of the quandong moth; egg-larva-pupa-adult. It was not possible to separate the instars in the model, as developmental data have not been collected for each instar of the quandong moth. Laboratory developmental thresholds and degree-day estimates for eggs and pupae (Chapter 4) were used in the model so that physiological time was used to drive development. Temperature parameters used for larvae were estimates based on the lower threshold for quandong moth eggs because estimates could not be made for larvae. A lower developmental threshold of 5°C was used for larvae. An upper temperature threshold was only recorded for the pupal stage (Chapter 4) and to maintain consistency high temperature inhibition was not included for the simulations in any stage. The laboratory oviposition data for adults was used for the reproduction parameters, with mean fecundity and fertility data halved to account for the 50% of individuals in the population that are male. The simulations are not intended to simulate population dynamics and therefore do not include mortality in any immature stages. Age at adult death was set at 9 days and was based on studies on adult longevity when mated (Section 3.3.4). Values for all parameters used in the model are shown in Table 1, Appendix 6. Daily maximum and minimum temperatures recorded from weather stations at Nuriootpa, 36km north-west of Sedan, and Hawker, 66km north-east of Quorn were used in the model.

9.2.1 Phenology

Simulations were initiated at the start of flowering each year, with meteorological data for each year used as input. In most years of sampling at Quorn and Sedan, eggs or 1st instars were found on flower buds in December producing one or two summer generations, an autumn-winter generation and a spring generation of the moth. To examine whether sampling had accurately detected what was occurring, eggs were put into the simulation on the date that eggs were initially detected on flower buds in the previous year. The simulations were run with data from December to the following December. The simulation was used to determine the earliest date on which eggs of the next generation would be present in the population and the timing of subsequent generations. The larval food source utilised by the various generations of the quandong moth is different throughout the year and this variation was factored into the model using a module similar to that used to input meteorological data. On each date in the simulation, a nutritional component was added to the temperature component so that both were used to drive larval development. Values for nutrition were determined arbitrarily and represented as a proportion of the most nutritious food available to larvae. The most nutritious food source was the kernel, set at 1.0, flowers 0.3 and flesh of fruit 0.7. Based on nitrogen content, the kernel is by far the most nutritious food source available to larvae (Crawley, 1983), but larvae are only able to feed in the kernel until the seed coat hardens. Flowers, particularly the anthers, are high in nitrogen but flower buds are an ephemeral food source with the majority being shed from trees. The effects of nutrition and temperature were combined using a product rule to drive development.

The adaptive significance of different threshold temperatures in different stages of the life cycle of the moth was also examined. Simulations were initiated with 100 eggs either in a single cohort, as 10 eggs/day over 10 days, or 5 eggs/day over 20 days. The convergence within cohorts between stages was then examined.

9.2.2 Insecticide timing

Several combinations of insecticide timing were simulated using the Dymex model using a hypothetical insecticide. The parameters used for the mortality caused by the insecticide and the degradation of its efficacy in the field were assigned arbitrarily. A maximum mortality level of 90% was assigned for the day of application, with effectiveness declining daily using an exponential decay function. The equation for exponential decay is:

$$y = Ae^{-kT},$$

where y is mortality, A is the maximum mortality, T is the time in days and k determines how quickly the insecticide decays. After 2 days, the effectiveness of the insecticide was assumed to be 5%, so the constant k determined by substitution into the equation was 1.45. The effectiveness of monthly applications of the insecticide was compared to applications timed to the beginning of the generations and with subsequent applications at 7, 10 and 14 days after the initial application. All simulations were initiated with a single cohort of eggs.

9.3 RESULTS

Dymex uses daily maximum and minimum temperatures to generate a daily temperature cycle based on a sine curve. The sine curve is divided into 12 two hourly segments, and degree-day

estimates are then determined for each block of two hours. The first aspect of the system analysed was the development times of the immature stages of the quandong moth throughout each year. A separate simulation was run for each year at Quorn and Sedan. As the model operates on a daily time-step, development times are shown in days. The results showed the common pattern of decreased developmental times during summer compared to the cooler winter months, where development takes much longer (Appendix 6, Figures 1 – 6). Overall, the temperatures at Quorn are higher throughout the year than those at Sedan, and this is reflected in the degree-day accumulations and the developmental rates of all stages of the quandong moth (Figures 9.1 – 9.2). Using a base temperature of 4.5°C, an average of 5155 degree-days were accumulated at Quorn and 3693 degree-days at Sedan over the three years for which data were collected. There were no marked fluctuations in the pattern of degree-day accumulation over the years that this study was conducted.

9.3.1 Phenology

The developmental data collected for pupae suggest that there is high temperature inhibition of development for the stage (Section 4.3). However, because upper thresholds were not detected for any other stages, linear models were used for all stages to maintain consistency. The Dymex simulations indicated that there were differences in the number of generations possible at each of the field sites and in each of the years for which the simulations were run. The differences primarily lay in the number of generations completed during summer to early autumn. At Quorn, the simulations indicated that a maximum of four complete generations were possible in each year (Figure 9.3 – 9.5), and three in each year at Sedan (Figure 9.6 – 9.8). In all years there was a partial generation carrying over into the following year. In 1998

and 1999 at Quorn, two complete generations developed from summer to early autumn, whereas at Sedan only one complete generation developed in that period. When development was reliant on temperature alone, up to six generations of the quandong moth could be completed at Quorn and five at Sedan (Figure 9.9). However, when the nutritional component was added to the simulations the predicted timing of generations better approximated that observed in the field.

Dates on which eggs or larvae were detected in field sampling were compared to the simulations. For the majority of the generations, eggs or neonate larvae were observed within 1-2 sample dates of the oviposition dates predicted by the simulations.

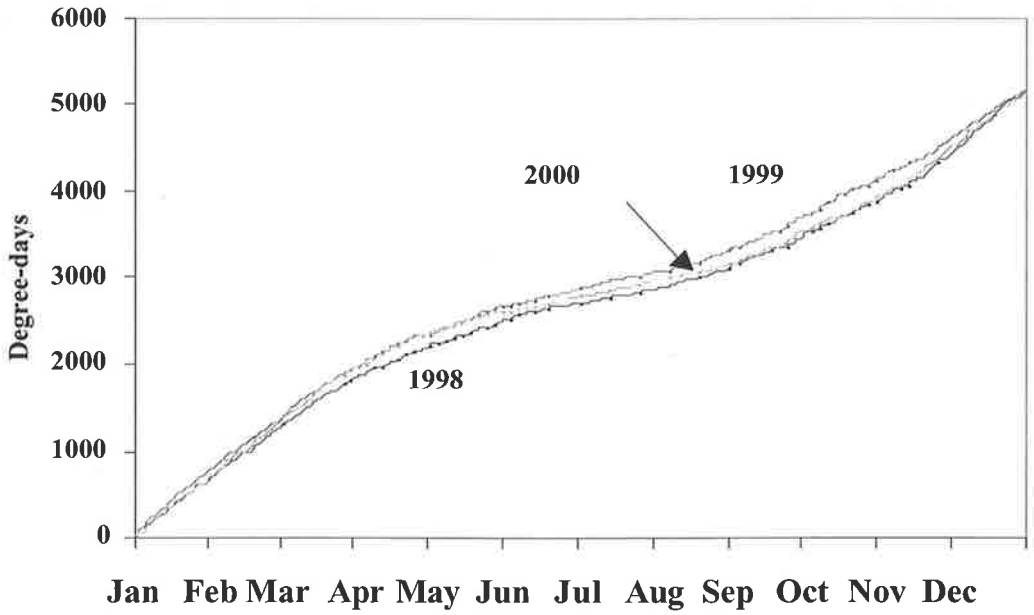


Figure 9.1: Degree-days accumulated above a base temperature of 4.5°C at Quorn 1998-2000

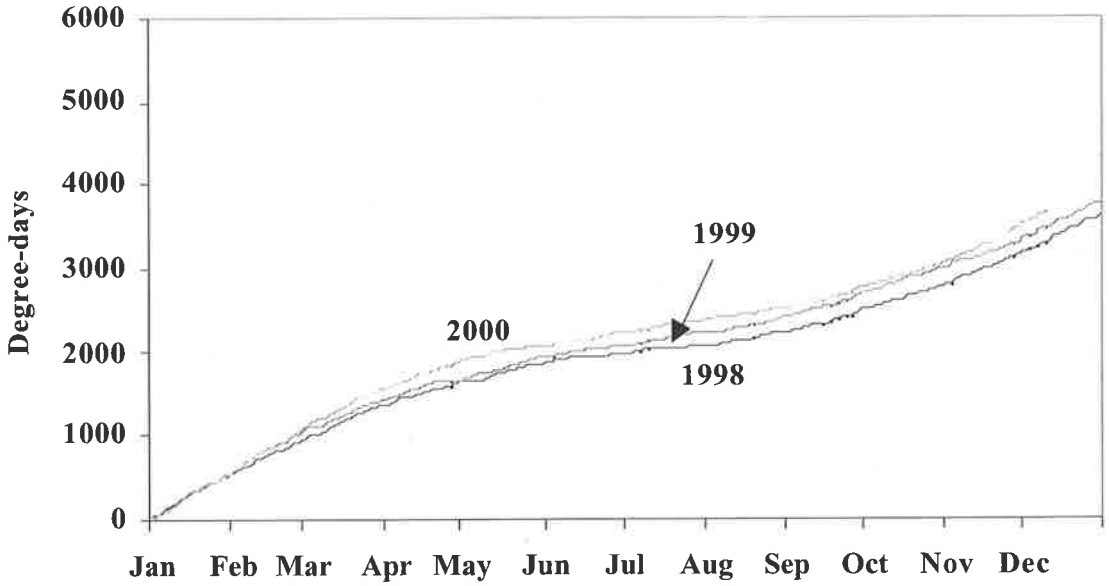


Figure 9.2: Degree-days accumulated above a base temperature of 4.5°C at Sedan 1998-2000

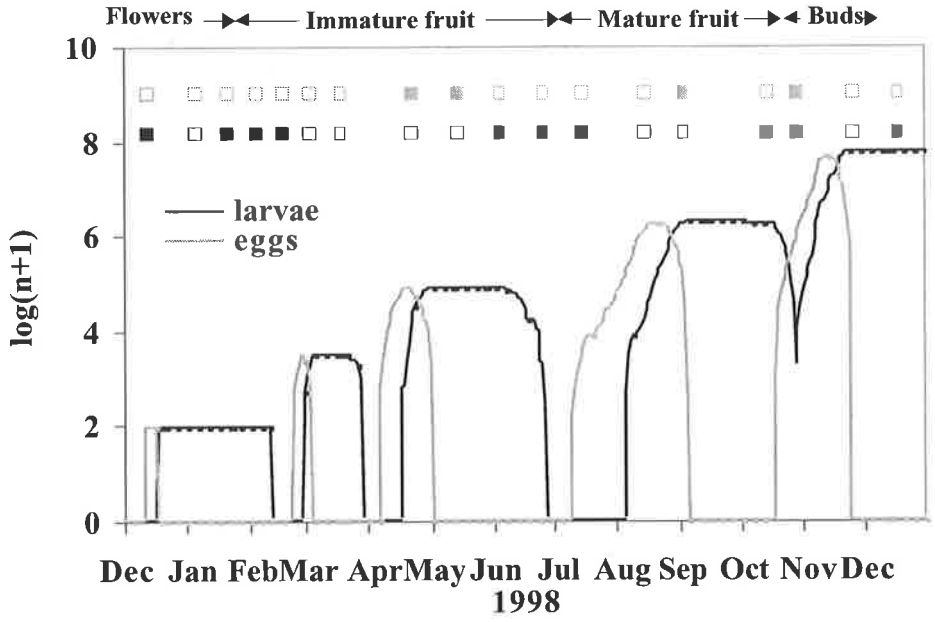


Figure 9.3: Simulation of generations of the quandong moth at Quorn 1997-98. Squares indicate dates of field samples, ■ = presence, □ = absence of eggs or larvae.

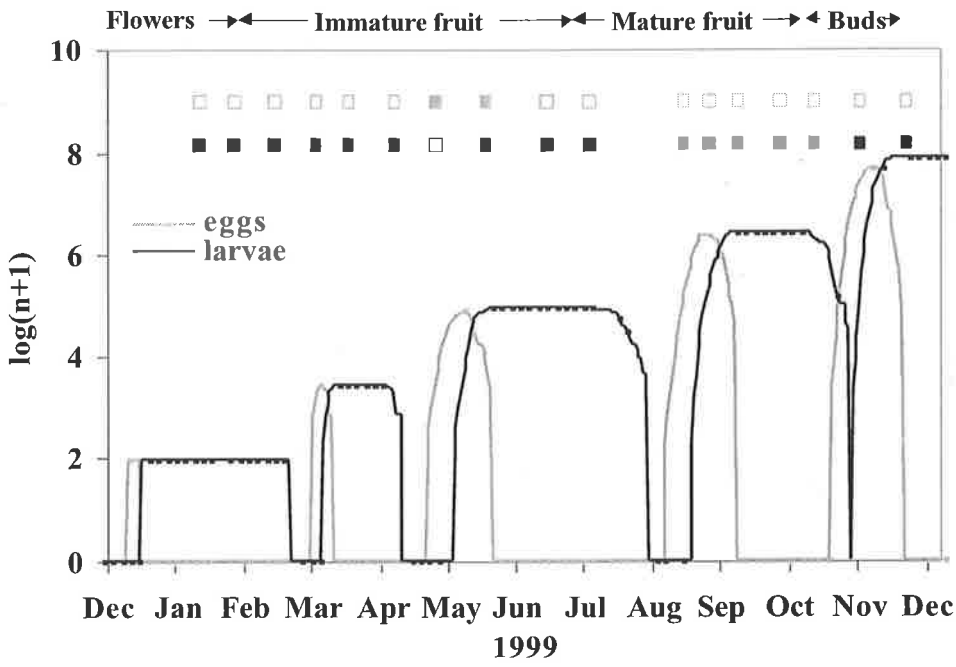


Figure 9.4: Simulation of generations of the quandong moth at Quorn 1998-99. Squares indicate dates of field samples, ■ = presence, □ = absence of eggs or larvae.

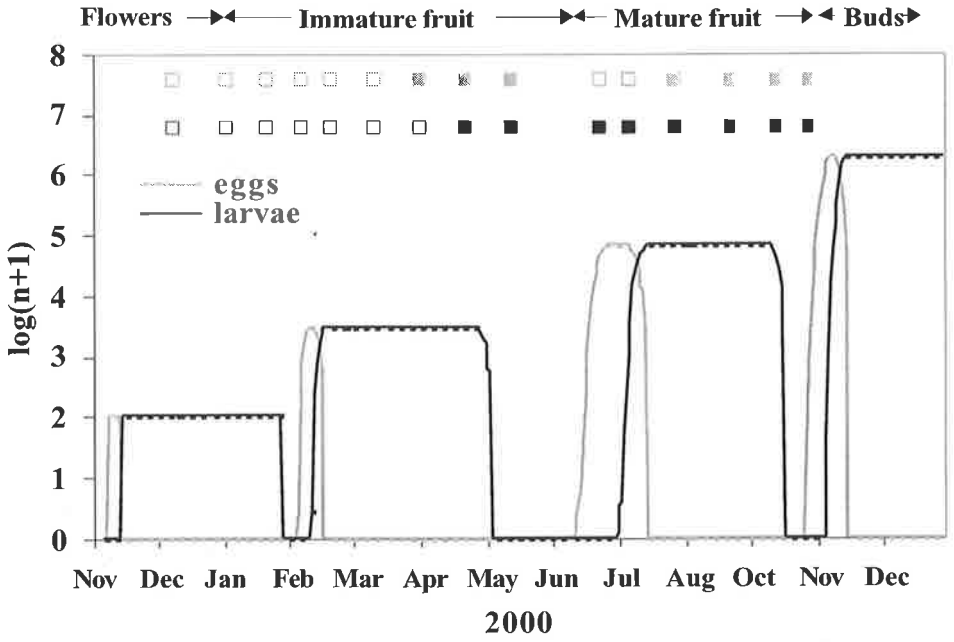


Figure 9.5: Simulation of generations of the quandong moth at Quorn 1999-00. Squares indicate dates of field samples, ■ = presence, □ = absence of eggs or larvae.

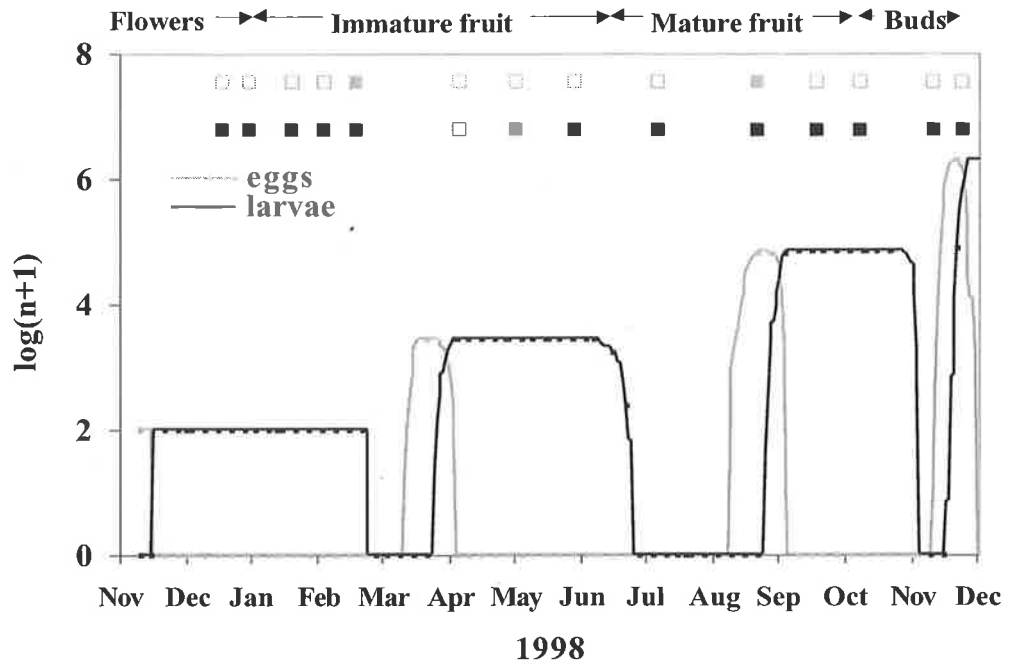


Figure 9.6: Simulation of generations of the quandong moth at Sedan 1997-98. Squares indicate dates of field samples, ■ = presence, □ = absence of eggs or larvae.

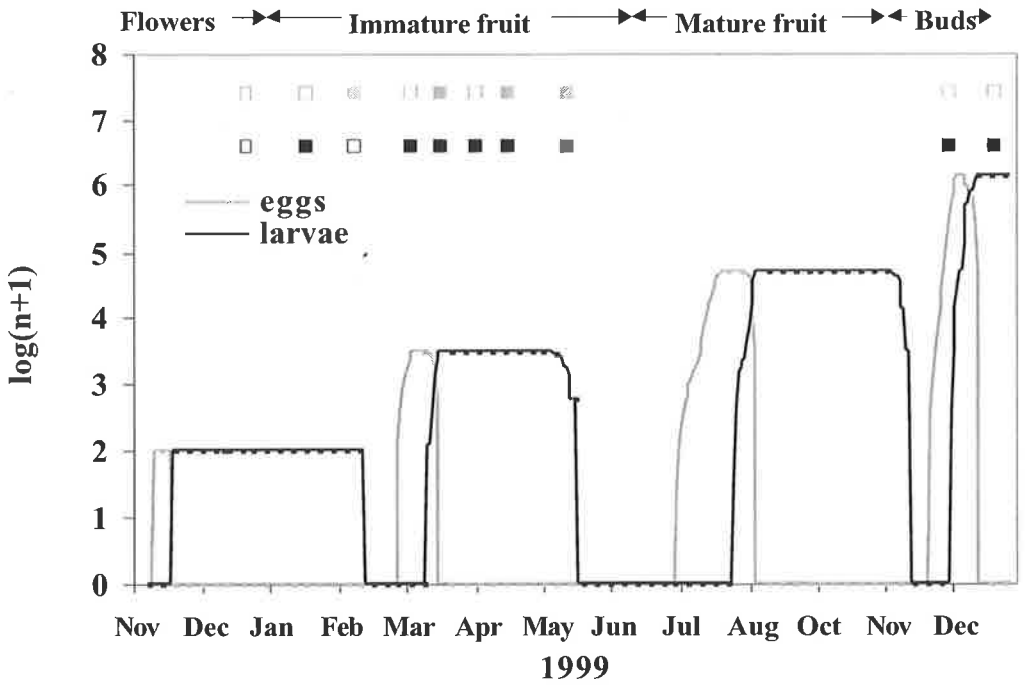


Figure 9.7: Simulation of generations of the quandong moth at Sedan 1998-99. Squares indicate dates of field samples, ■ = presence, □ = absence of eggs or larvae.

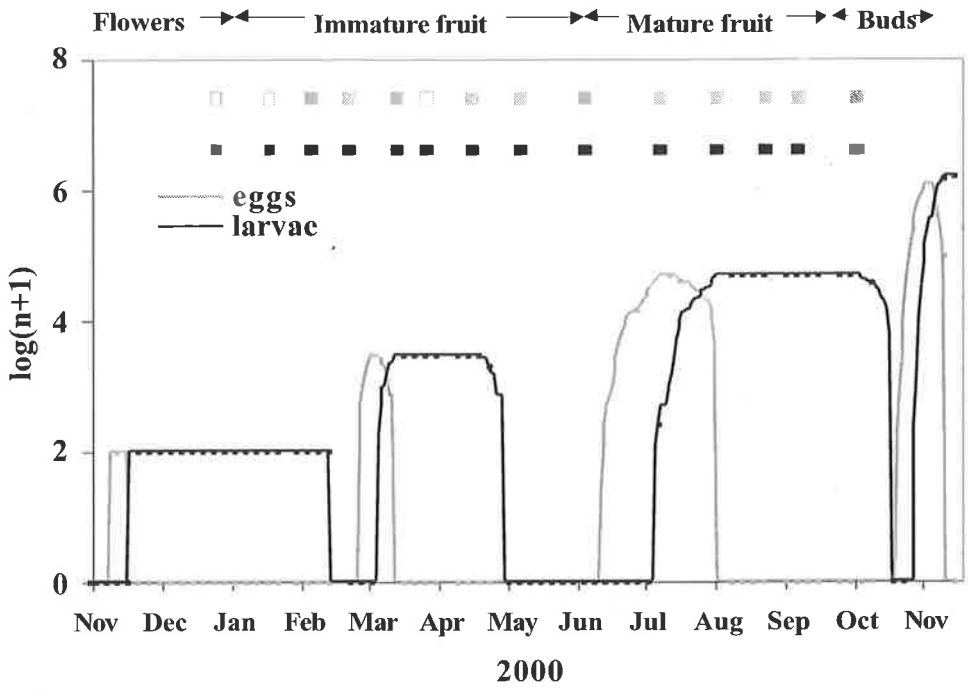


Figure 9.8: Simulation of generations of the quandong moth at Sedan 1999-00. Squares indicate dates of field samples, ■ = presence, □ = absence of eggs or larvae.

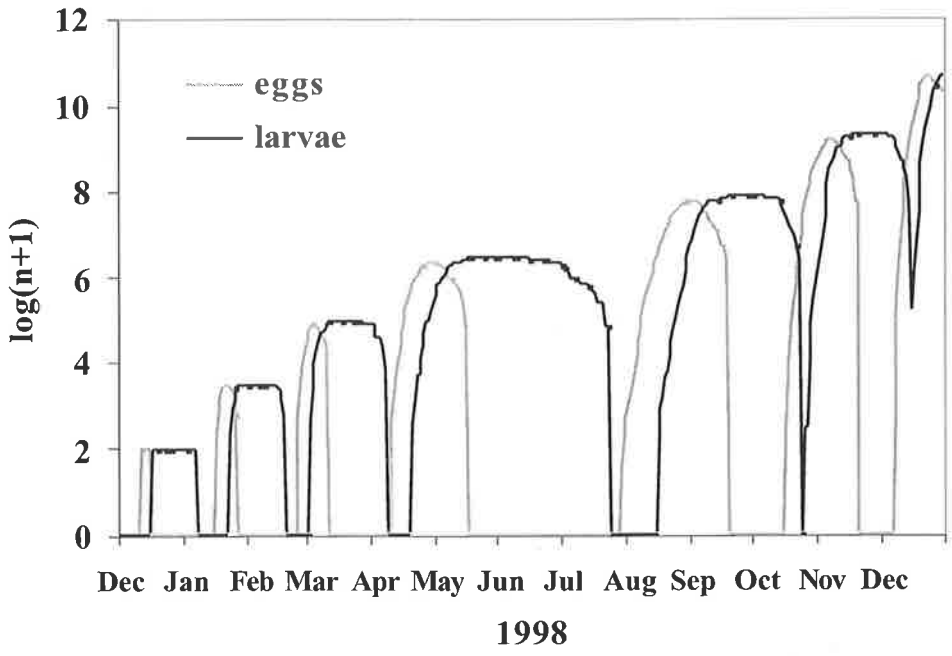


Figure 9.9: Simulation of generations of the quandong moth at Quorn 1997-98 with nutritional component consistent throughout the year.

The results of the simulations with single or multiple cohorts of eggs demonstrated that development was initially widely dispersed in the egg and larval stages, but in the pupal stage all cohorts became temporally converged (Figure 9.10). Initially in the summer generation there was a difference of up to 18 days in the entry to the egg stage, by the pupal stage, the difference was at a maximum of 6 days. In the winter generation, the same phenomenon was evident but cooler winter temperatures meant that dispersion of entry to the larval stage was greater than that in the egg stage. Once again, temporal convergence occurred in the pupal stage, with a difference of 28 days in the larval stage decreasing to 15 days in the pupal stage (Figure 9.11).

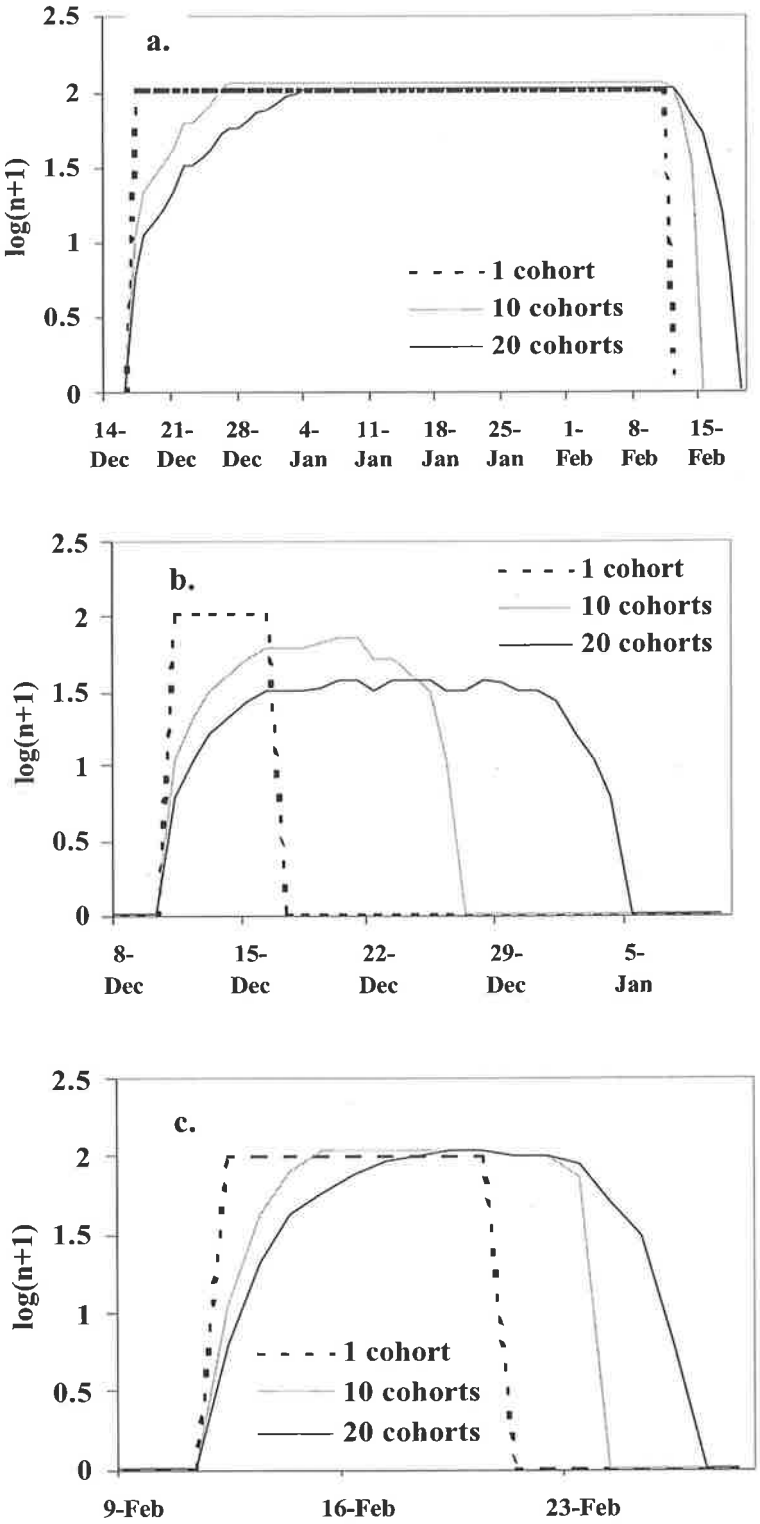


Figure 9.10: Simulation of temporal convergence in development of (a) eggs, (b) larvae and (c) pupae of the summer generation of the quandong moth. Simulations were initiated with 100 eggs oviposited in 1 cohort, 10 cohorts over 10 days or 20 cohorts over 20 days.

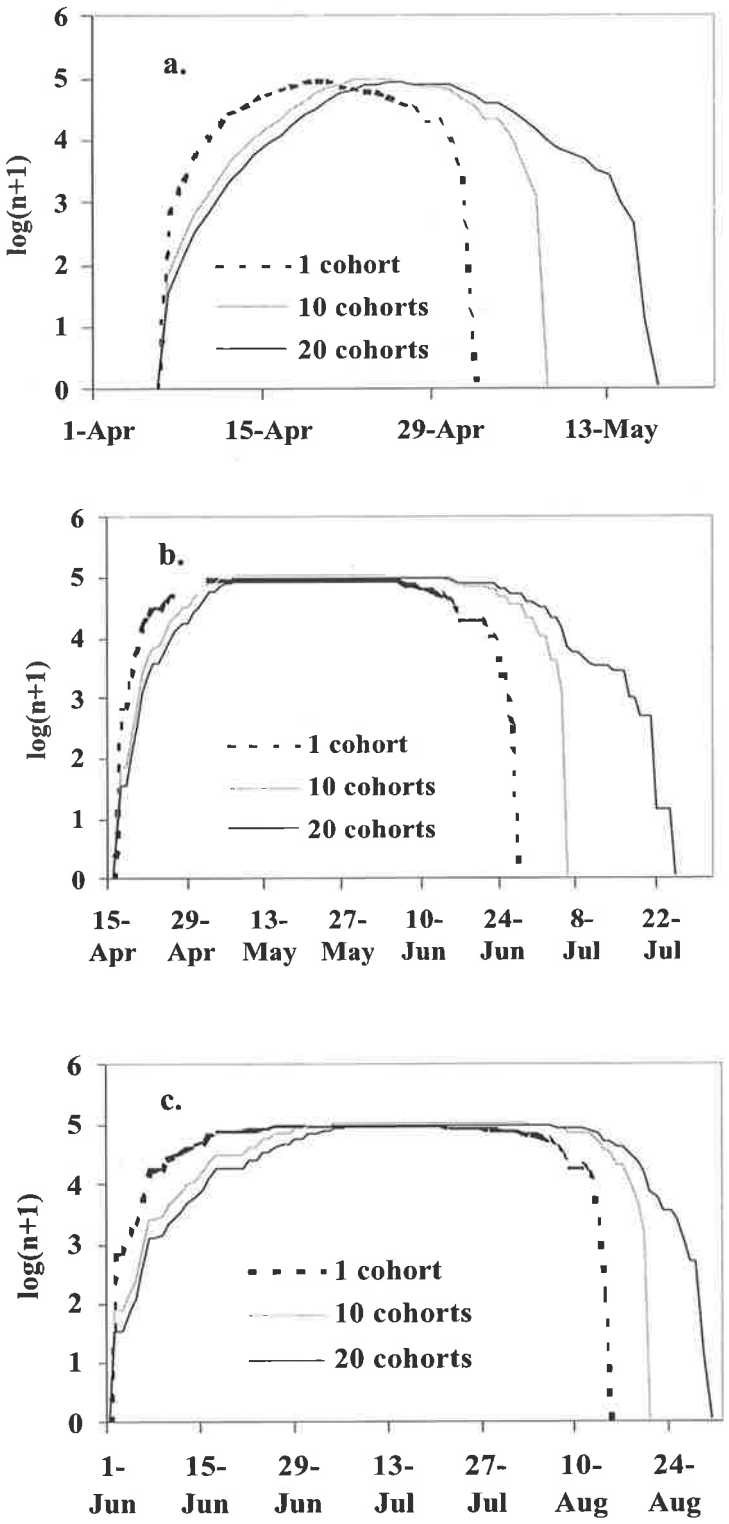


Figure 9.11: Simulation of temporal convergence in development of (a) eggs, (b) larvae and (c) pupae of the winter generation of the quandong moth. Simulations were initiated with 100 eggs oviposited in 1 cohort, 10 cohorts over 10 days or 20 cohorts over 20 days.

9.3.2 Insecticide timing

Of all the insecticide timing scenarios examined the most effective was sprays applied at the beginning of the autumn-winter and spring generation, with follow up applications seven days after the initial spray for each generation (Figure 9.12). The initial applications were timed to the peak in entry to the larval stage. Initial applications made at the beginning of the autumn-winter and spring generations but followed by applications 10 days later had a similar effect on the population as those sprayed at 7 day intervals (Figure 9.13). However, applications 14 days after the initial applications at the beginning of the generations were less effective as the population was able to begin increasing again in the summer generation (Figure 9.14). Monthly applications made on a calendar basis beginning on the 1st April and ending on the 1st September, allowing for a with-holding period of at least two weeks before harvest, were not as effective as the sprays timed to the beginning of the generations (Figure 9.15). Inaccurate timing at the start of generations generated up to a ten-fold increase in population size. The simulation also showed that removing the applications made on the 1st April, 1st July and 1st August did not decrease the efficacy compared to the monthly applications during April to September. Single applications at the beginning of the autumn-winter and spring generations were more effective than any combination of calendar application (Figure 9.16).

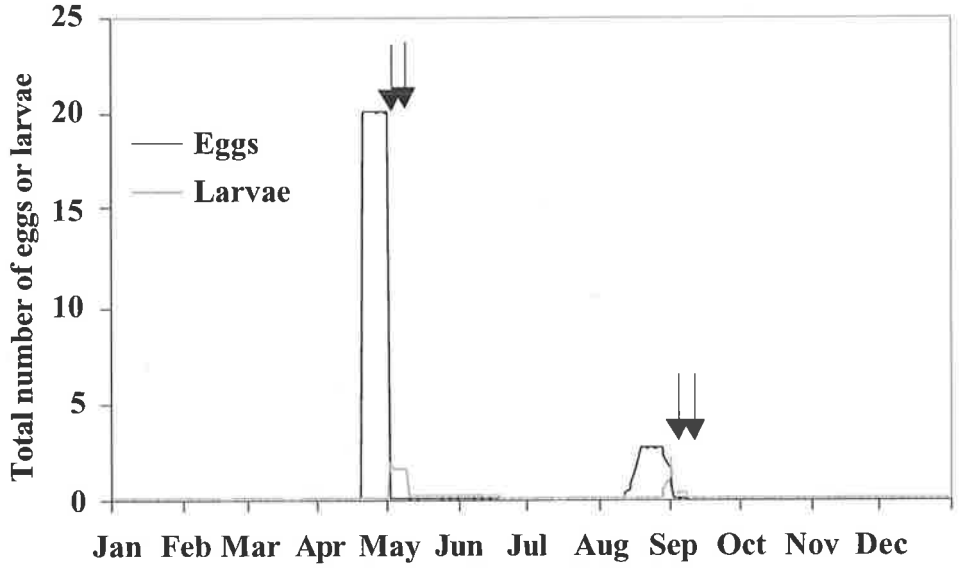


Figure 9.12: Simulation of effect of insecticide applications on population of quandong moth at Quorn in 1998. Arrows indicate insecticide applications on 3rd May, 10th May, 2nd September and 9th September.

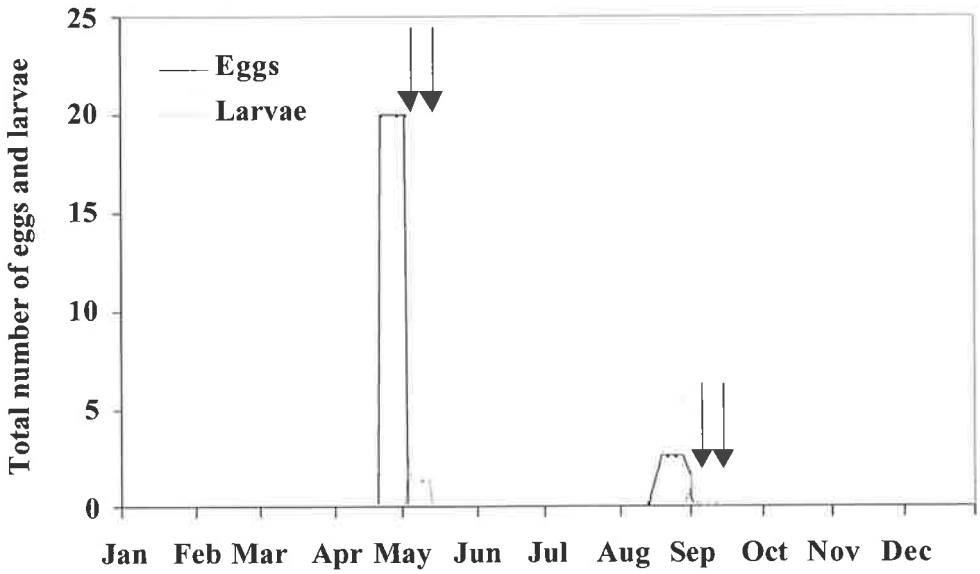


Figure 9.13: Simulation of effect of insecticide applications on population of quandong moth at Quorn in 1998. Arrows indicate insecticide applications on 3rd May, 13th May, 2nd September and 12th September.

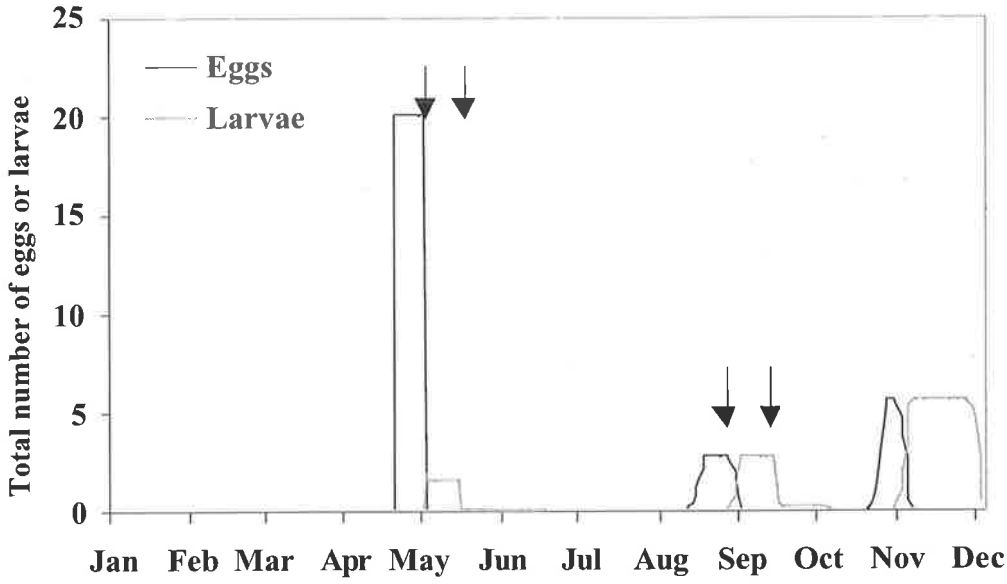


Figure 9.14: Simulation of effect of insecticide applications on population of quandong moth at Quorn in 1998. Arrows indicate insecticide applications on 3rd May, 17th May, 2nd September and 16th September.

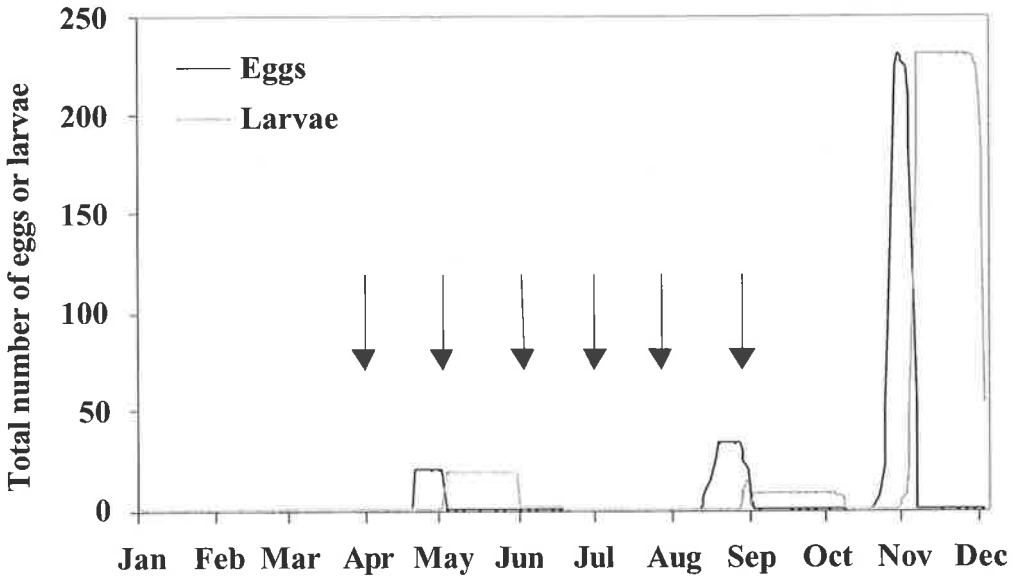


Figure 9.15: Simulation of effect of insecticide applications on population of quandong moth at Quorn in 1998. Arrows indicate insecticide applications on 1st of each month from April to September.

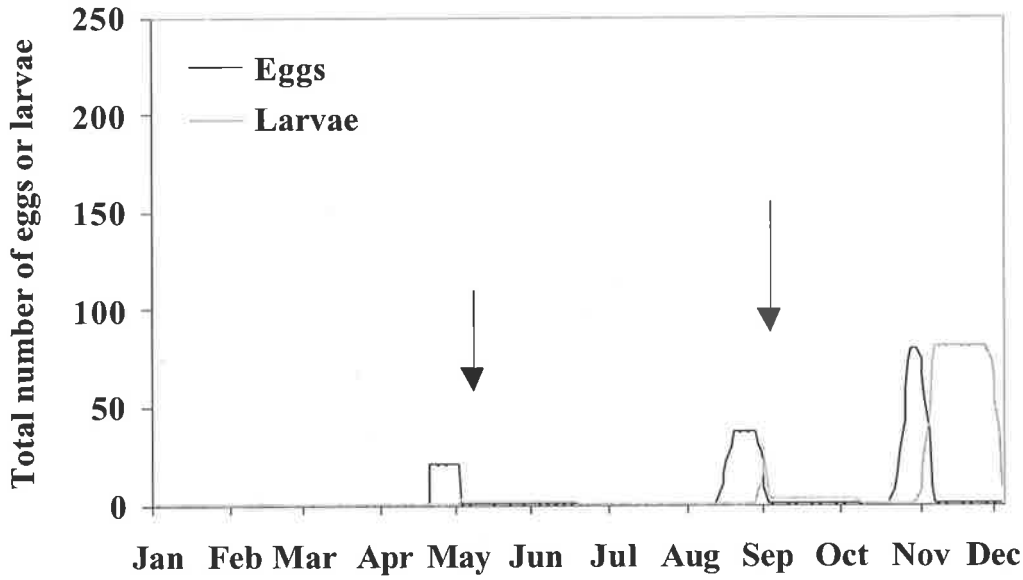


Figure 9.16: Simulation of effect of insecticide applications on population of quandong moth at Quorn in 1998. Arrows indicate insecticide applications on 3rd May and 2nd September, the start of the autumn-winter and spring generations respectively.

9.4 DISCUSSION

The development of all the immature stages of the quandong moth was slowed greatly in the winter and spring generations, compared to those developing during summer. Development of larvae is reliant on both temperature and the quality of the larval food source. The kernel of developing fruit present during winter is likely to be the most nutritious larval food source available for the whole year, but temperatures at that time are less favourable. Although the nutritional component of development was set at an arbitrary level, the combination of nutrition and temperature more accurately simulated the number and timing of generations of the quandong moth than temperature alone. Degree-days alone cannot be used to simulate the development of quandong moth and fluctuations in larval food source are an important

influence on development. The effect of the variation in the nutritional status of the stages in the development of quandong fruit is worthy of further investigation.

The simulations proved useful both to predict generation timing and to explain patterns seen during the year. During 2000 at Quorn, neither eggs nor larvae of the quandong moth were detected in samples from early January to mid April. Simulations based on temperatures and the food source present at the time, demonstrated that a generation was possible during that period. Either the intensity of sampling was not sufficient to detect the generation, or the generation did not occur at that time because of other factors. Mortality may have an important influence on generation timing, particularly during the summer. It is possible that the mortality of the immature stages of the quandong moth is highest during summer due to vulnerability to natural enemies, desiccation due to high temperatures and the ephemeral food source provided by flowers. Many flowers drop from trees and neonate larvae are unlikely to be able to find a new food source if feeding in a dropped flower bud. The only stage for which high temperature inhibition was identified was pupae and delayed development in the pupal stage would delay oviposition of the autumn-winter generation. High temperature inhibition could not be modeled accurately. Other climatic factors such as humidity and rainfall could also influence the development of the moth.

Where discrepancies in developmental times were present between predicted and observed dates, the predicted dates were neither consistently earlier nor later than the observed dates of oviposition in the field. The difficulty of detecting eggs on flowers contributed greatly to the variability between predicted and observed dates. It is common when sampling for different

stages of an insect to over-look and therefore underestimate the incidence of the smaller stages. However, this factor cannot explain the lack of detection of eggs at certain times in each year. Once the eggs were first collected and described, they were easily recognised in the field and even more easily in the laboratory when assessing samples with a stereo microscope. Of all the stages in the development of fruit, eggs were most difficult to detect on flowers as they were laid in smaller masses than in calyces of fruit, and both hatched and unhatched eggs are more likely to be dislodged from flower buds. The variances associated with eggs on flowers and fruit were large (Section 5.3.2) and it is likely that many eggs went undetected. In many instances neonate larvae were found on the same date as eggs and the presence of larvae could then be related to oviposition dates. However, there may have been some sample dates on which eggs had not yet hatched and sampling could then have produced a false negative. Although the values for the nutritional component of development were set arbitrarily, those used result in the most accurate simulation of the occurrence of the stages of the quandong moth in the field.

Daiber (1980) reported a similar phenomenon with simulations of the phenology of generations of the false codling moth. If conditions were close to optimal, the false codling moth could complete five generations each year. However, studies on larval feeding showed that poor food quality could significantly reduce the rate of larval development (Daiber, 1980). Thus it was deemed likely that a maximum of only three generations occurred each year. Much like the false codling moth feeding on peaches, the quandong moth experiences periods of the year when food quality is lower than at other times. Providing larvae in the simulations with the most nutritious theoretical food source throughout the whole year

decreased the development time in most cases by 1-3 days and increased the number of generations each year by one or two. In laboratory studies with other lepidopteran larvae, more favourable diets have increased development rates by a similar magnitude (Etman and Hooper, 1979; Schroeder et. al., 1986).

The quandong moth does not undergo diapause, suggesting that the timing of emergence may be under direct temperature control. Where populations are under direct temperature control, emergence often occurs over a long period of time (Danks, 1987). Different threshold temperatures in the various stages in the life cycle of an insect may be a mechanism to maintain synchrony within generations. Such a mechanism has been demonstrated for a univoltine species of beetle (Bentz et. al., 1991) and has been suggested as the simplest way to ensure synchrony in generations (Danks, 1987). The lower developmental threshold for pupae of the quandong moth is much higher than that of the eggs, and allows cohorts to 'catch up' somewhat to cohorts that have entered the population on an earlier date. The phenomenon was demonstrated for eggs and larvae in the summer generations of the quandong moth. However, in the winter generation, dispersion in entry actually increased from the egg to larval stage because low temperatures during winter greatly reduced the developmental rates of all cohorts. By the pupal stage, dispersion had decreased somewhat, but convergence was not as great as in the summer generation.

In this study, the lower threshold temperature for pupae compared to eggs, can explain convergence in populations. Late instar larvae may also cause convergence amongst cohorts in the same way. Thresholds for larval stages of the quandong moth were set at a constant level in simulations because they could not be recorded experimentally. However, several studies

have provided evidence of variable thresholds within the larval stage. Gangavalli and Aliniazee (1985) reported that the over-wintering fourth instar larva of the obliquebanded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae) has a lower threshold than that of the fifth or sixth instars. The fourth instar is present when the temperatures are lower than those that later instars would normally experience. However, the diapausing third instar has a significantly higher threshold than any of the other instars and the authors suggest that this is a mechanism to delay development until temperatures begin to increase and conditions are more favourable. Similarly, Giese and Casagrande, (1981) suggest that the thresholds for development of eggs of the gypsy moth, *Lymantria dispar* Linnaeus (Lepidoptera: Lymantriidae) may change and degree-day accumulations for a single threshold may not accurately describe development. Unlike the quandong moth, both the obliquebanded leafroller and gypsy moth are univoltine. For differing thresholds to be an advantage for the quandong moth, stages within different generations would need to have evolved different thresholds compared to those within other generations. For example, eggs present during the summer generation would benefit from having a higher upper threshold than that of the winter generation so that hatching could occur over a wider range of temperatures during summer. Similarly, larvae of the winter generation would develop at a faster rate during winter if the threshold were lower.

As Regniere (1987) suggests, it is possible that all the parameters of thermal responses are variable and that variation may even occur amongst individuals within generations. There are several assumptions that have been made during the development of this model that could significantly affect the accuracy of the simulations. The first is that the lower developmental

threshold for larvae is close to the lower threshold for eggs. In studies of many different Lepidoptera, the threshold for eggs is within 1-2°C of that of the larvae (Daiber, 1979a; Daiber, 1979b; Hanula et. al., 1987; Roltsch et. al., 1990; Sands et. al., 1991; Judd et. al., 1994) and it is a reasonable assumption. However, the egg threshold is based on a relatively small number of observations and altering the thresholds for both eggs and larvae has a dramatic effect on development rates, particularly in the cooler months.

In the absence of detailed data, development rates were assumed to be constant across all instars but several studies in other Lepidopteran species suggest otherwise. Development rates of the speckled green fruitworm, *Orthosia hibisci* Guenee (Lepidoptera: Noctuidae) were shown to increase with the instar. First instars developed fastest and fifth instars the slowest (Judd et. al., 1994). It was suggested that rapid development times for first instars were an adaptation to the high risk of mortality in the instar. For fifth instars, it was postulated that slower development rates accompanied by prolonged feeding resulted in an increased chance of surviving the over-wintering period in this species (Judd et. al., 1994). Quandong moth larvae do not have a period of diapause but do over-winter to an extent. Any difference in the developmental rates of instars during winter is more likely to be due to the decrease in temperatures as winter progresses, rather than any specific adaptation to over-wintering. Unlike many insect species that over-winter, for the quandong moth food is abundant and highly nutritious during winter. It is not necessary for the species to adapt to harsh winter conditions and time the entry of the next generation to critical events in the cycle of its host tree, such as budburst. For the quandong moth, constant development across all instars is a

valid assumption, as the species does not rely on the strict regulating factors that allow other species to adapt to periods when conditions are unfavourable.

Parasitism can also alter the development rate of all immature stages of moths (Brown, 1946a; Brown, 1946b; McGugan, 1955). Larvae of the western yellow striped armyworm, *Spodoptera praefica,* Grote (Lepidoptera: Noctuidae) parasitised by *Chelonus insularis* Cresson (Hymenoptera: Braconidae) underwent premature pupation (Bisabri-Ershadi and Ehler, 1981). Disease can also have an influence on the development rate of individuals (Thomson, 1958). Neither parasitism nor disease has been simulated in the model because their effects on development were not investigated in this study. Inclusion may improve the accuracy of the simulations.

A nutritional component was factored into the simulation, however the nutritional levels were set arbitrarily. The simulation would be validated if the nutritional levels of the various stages of flowers and fruit throughout the year could be quantified experimentally. For the quandong moth this would prove a difficult task in the laboratory as abscised flowers and fruit do not keep well and fruiting trees are large.

The simulations of insecticide timing showed the efficacy of accurate spray timing on population suppression, not only in the generation that is sprayed, but also in subsequent generations. A one-day difference in the simulated application date can have a marked effect on population size. Sprays timed at the peak of egg hatch were most effective. Applications made before egg hatch, assuming the insecticide has no ovicidal action will kill emerged larvae

but larvae that emerge in the subsequent days will not be affected. Monthly applications were simulated because several commercial quandong growers use calendar applications of insecticides to manage the quandong moth. The simulations showed that at least three of the sprays applied between April and September were completely ineffective because larvae were not present at the time of application. Such mistimed sprays had no direct effect on the population of the quandong moth but would have detrimental effects on whatever beneficial insects are present during those times as well as wasting both the time and money required to make the applications. The model proved a useful tool for simulating the effects of insecticide applications on the various generations of the quandong moth but field validation is required.

A predictive model could be useful in improving the accuracy of timing insecticide applications for the quandong moth. It may also be used to narrow the window in which monitoring is required for eggs to detect the beginning of generations of the moth. If timing of oviposition in an orchard and the subsequent emergence of the neonate larvae can be modeled accurately, then fewer sprays would be applied and those that are applied would be timed more accurately to prevent establishment of larvae.

10. POTENTIAL SECONDARY PESTS

10.1 INTRODUCTION

Secondary pest outbreaks are common with sustained and frequent use of broad-spectrum insecticides, and often arise when applications of insecticides for one pest disrupt the natural enemies of other insects in the system (Metcalf, 1994). Insects that were once considered minor or occasional pests in the cotton industry became major pests because of widespread and repeated insecticide usage (Adkisson, 1971). Secondary pest problems can also be induced by applications of fertilisers to trees that alter nutrient levels and may lead to outbreaks of mites and aphids (Onstad et. al., 1986). Currently, the quandong moth is the only insect pest consistently causing damage to quandong trees. However, an IPM program for quandong moth must be developed in the context of the other insect species in the system. Particularly with broad-spectrum insecticides, applications for the quandong moth will impact on all susceptible insect species and the long-term effects could be detrimental. This study surveyed quandong orchards throughout South Australia to examine other insect fauna feeding on quandong trees that may have the potential to become secondary pests.

10.2 MATERIALS AND METHODS

Surveys for potential secondary pests were made during regular field sampling for quandong moth. Eggs, larvae or nymphs, depending on the order, of insects feeding on quandong trees were collected from leaves and taken back to the laboratory. Eggs were held in small jars until they hatched and then larvae or nymphs were placed on fresh quandong leaves in a rearing cage. Larvae or nymphs collected in the field were placed directly into cages with fresh

quandong leaves. The leaves were replaced every 2-3 days depending on the requirements of the species or the degradation of the leaves. Parasitoids of these insects were collected during rearing in the laboratory. Only those species that completed development from an early stage on quandong leaves were recorded as potential secondary pests.

10.3 RESULTS

Many of the species of insects found feeding on quandong trees caused incidental damage and their presence in an orchard did not necessarily require intervention. Where possible, identifications were made (Table 10.1), but in some cases a lack of suitable material, high rates of parasitism and problems with adult emergence prevented identification. Most were found at the same site in each year suggesting they were consistently minor pests in the system. The majority of these species fed only on leaves of the quandong tree, and none were found exclusively feeding on quandong fruit in any stage of fruit development. LBAM larvae were found only on quandong trees at Waite, S.A., were only feeding on leaves, and never caused significant damage. The only species found feeding on quandong fruit was the unidentified leaf webbing moth, possibly a tortricid. The larvae of this moth webbed several leaves together and only fed on fruit when their feeding shelter was webbed to a fruit, which was not the norm. Several parasitoids were also reared from the immature stages of these species (Table 10.1). Of the species found, those causing the most damage to foliage were a scale, *Cardiococcus foramifor* (Hemiptera: Coccidae) at Quorn, the leaf case moth, *Hyalarcta huebneri* (Lepidoptera: Psychiidae) at Whyalla and an *Edusa* sp. (Coleoptera: Chrysomelidae) at Sedan. There were three species that were regularly collected at the two main field sites, Quorn and Sedan and at the other sites visited periodically throughout the study, the leaf

webbing moth, the gall midge and the wood white butterfly. Anecdotal reports by growers suggested eriophyiid mites were becoming a problem in some orchards where insecticides were used (P.T. Bailey, pers. comm.). The mites were mainly found on the tips of new shoots, but were not causing significant damage to trees. A fungal pathogen infecting maturing fruit was identified as *Pseudocercospora* sp., which has been previously recorded from quandong trees (B. Hall, pers. comm.).

Table 10.1: Other species of insects damaging quandong trees in South Australia.

Species	Stages	Damaged	Sites	Parasitoids
		Lepidoptera		
Delias aganippe (Pieridae)	eggs, larvae	leaves	Quorn, Sedan, Swan Hill	*Perilampus* sp?
Epiphyas postvittana (Tortricidae)	larvae	leaves	Waite	-
Hyalarcta huebneri (Psychiidae)	larvae	leaves	Whyalla	Tachinid fly
Genduara acedesta (Lasiocampidae)	larvae	leaves	Quorn, Whyalla	-
Spilosoma glatignyi (Arctiidae)	larvae	leaves	Quorn, Coffin Bay	-
Unidentified leaf webbing moth (Tortricidae?)	larvae	leaves, fruit	Quorn, Sedan, Whyalla, Pt. Augusta, Coffin Bay	*Meteorus* sp.
		Hemiptera		
Cardiococcus forammifor (Coccidae)	nymphs	leaves	Quorn	-
Ornamental black scale (Diaspididae)	nymphs	leaves	Waite	-
Unidentified armoured scale	nymphs	leaves	Pt Augusta	-
		Diptera		
Gall midge (Cecidomyiidae)	larvae	stems	Quorn, Sedan, Whyalla, Swan Hill, Cummins, Coffin Bay	collected, not identified
		Coleoptera		
Edusa sp. (Chrysomelidae)	eggs, nymphs, adults	leaves	Sedan	-

10.4 DISCUSSION

There are no reports to date of any serious outbreaks of secondary pests in quandong orchards (AQIA, pers. comm.). Surveys for other insect fauna on quandong trees revealed several insect species feeding on the leaves of trees but none feeding preferentially on fruit. Where secondary pests were identified, such as a scale at Quorn, a chrysomelid beetle at Sedan, and the leaf case moth at Whyalla, the outbreaks were localised at the sites. At the unsprayed sites, the scale outbreak occurred on two trees that had shown signs of stress prior to the outbreak and the chrysomelid outbreaks occurred primarily on a grove of young trees. At Whyalla, an orchard frequently sprayed with dimethoate, the outbreak of the leaf case moth was restricted to an area adjacent to a windbreak of native Australian trees including *Eucalyptus* species that are also a host for the polyphagous leaf case moth (Common, 1991). Further, the block in which the outbreak occurred was not treated with insecticides when the outbreak occurred. The absence of any other species that feed specifically on quandong fruit may explain the lack of secondary pests at this time.

Insecticide usage could not explain any of the secondary pest outbreaks recorded in this study. The outbreaks that had occurred were restricted to one site, and localised on trees within those sites. It is more likely that the condition of trees and the proximity to alternative hosts for polyphagous species were responsible for the outbreaks. Although the majority of the other insects feeding on quandong trees are foliage feeders, there is potential for the disruption of all natural enemies in the system from broad-spectrum insecticide use. Many insects that are now major pests were initially incidental pests in the cropping system (Adkisson, 1971; Onstad et. al., 1986; Frisbie et. al., 1994).

There have been anecdotal reports of eriophyiid mites feeding on the young tips of quandong foliage (P.T. Bailey, pers.comm.). Pest resurgence as a result of broad-spectrum insecticide usage has been reported across a wide range of insect and mite families and a varied range of crops (DeBach and Rosen, 1991). Work by DeBach and Bartlett (1951) on scale and mite species in citrus orchards demonstrated the differential toxicity of broad-spectrum insecticides to pest and natural enemy species. Higher toxicity of insecticides to natural enemies resulted in increases in infestations of the scale and mite pests (DeBach and Bartlett, 1951). The insect and mite species that are currently innocuous in quandong orchards have the potential to develop into major pests because of over-use of insecticides. Development of an IPM program is greatly facilitated if the target species is the sole key pest in the system (Luckmann and Metcalf, 1994). Preventative measures should be implemented in quandong orchards to reduce the risks of pest resurgence and include improving the timing of insecticide applications and increasing the selectivity of insecticides.

11. PROSPECTS FOR IPM

The pest status of the quandong moth is relatively new and has arisen because of commercialisation of the quandong fruit. The tolerance for damage or larvae of the quandong moth in fruit is low, with both producers and consumers demanding high quality fruit free of larvae and damage of quandong moth. In the absence of information on the biology and seasonal cycle of the moth, growers have used a systemic insecticide, often with application at regular intervals. Although research has been conducted on many native Australian species of insects on introduced crops, this is the first study in Australia examining a native food crop and a native pest species. Quandong growers have the opportunity to implement IPM programs early in the development of their industry and therefore could avoid many of the crises that have occurred in other cropping systems because of over-reliance on insecticides.

There is a wide range of methods available for insect pest management that vary greatly in complexity (Luckmann and Metcalf, 1994). Development of an IPM program is reliant on determining how several suitable methods can be integrated to achieve economically viable and sustainable suppression of the pests. The most suitable methods for a particular pest will only be determined after thorough investigations into its biology, ecology and phenology. However, a pest management program does not have to be complete before implementation can begin, and parameters such as the EIL will evolve as knowledge of the interactions between the insect and host plant increases (Luckmann and Metcalf, 1994).

A critical aspect of any pest management program is to ensure that the personnel concerned with managing the pest are able to identify accurately the pest species. Prior to this study,

the majority of quandong growers were only familiar with the fourth instar larva of quandong moth. Descriptions of all stages of the moth have now been compiled to help growers identify the moth in their orchards, particularly in the egg stage (Chapter 3). The way in which the various stages in the lifecycle of the pest interact with the host plant influences the choice and timing of management strategies. All larval stages of quandong moth are concealed inside flowers or fruit (Section 5.3.3) so strategies that rely on direct contact with eggs or larvae, such as IGRs, have to be accurately timed to target exposed eggs or first instars.

An understanding of the phenology of a pest can greatly reduce the labour costs associated with monitoring programs by narrowing the window of time in which monitoring is required (Ring et. al., 1989; Jones, 1995). For the quandong moth, the period between the end of fruiting and the development of flower buds to a stage in which they are suitable for feeding may synchronise the start of the summer generation. Following that, there is no other event in the development of the fruit that could synchronise the generations, as fruit are then suitable for feeding from late February to early November, with some temporal variation, depending on the region. The data collected in this study suggest that there are periods of oviposition in late March to mid May and late July to September that give rise to larvae of the autumn-winter and spring generations, respectively (Section 5.3.1). Emergence over a long period of time is characteristic of populations that are under direct temperature control (Danks, 1987). Thus, the period of monitoring cannot be narrowed and monitoring should commence around late March and continue for the remainder of the season. At this stage, presence/absence monitoring is the most viable option, as the high degree of aggregation in the species means that large inputs of time are required to obtain precise estimates of pest density. Monitoring

intervals of seven to ten days may be necessary in autumn and spring, but during winter the interval could be increased to 14-20 days as the lower temperatures during winter decrease the developmental rate of the moth. In the context of climatic variation and sampling precision, three years of field data could be considered a preliminary examination of the seasonal cycle of the moth. Several more years of data on the seasonal abundance of the moth in the field would further elucidate the number and timing of the generations of the quandong moth. Research in other regions in Australia where quandongs are grown would also be valuable to determine the effects of climatic variation on phenology of the quandong moth and its host tree.

There is often a perception that biological control alone is a permanent solution to suppression of pest populations. However, it is more often the case that biological control is most effective when integrated with other management strategies (Hoy, 1994). Several species of parasitoid wasps that attack the quandong moth were identified during this study. None of the parasitoids effectively suppressed populations of the quandong moth, even in the absence of broad-spectrum insecticide use. Augmentation of natural enemies may be an option for management of quandong moth, but it is reliant on effective rearing methods and investigations into release rates and methods (Hoy, 1994) and the functional and numerical responses in populations (Waters et. al., 1976). Mass rearing of larval parasitoids of quandong moth is restricted by the lack of a suitable artificial diet for the host species, a common problem in many systems (Hoy, 1994). Various species of the egg parasitoid *Trichogramma* have been mass reared and released for control of lepidopteran pests (Hoy, 1994). The lack of an artificial diet for rearing the pest species can be overcome by rearing *Trichogramma* on alternative host species. However, there are reports of parasitoids reared

on alternative hosts being lower in quality than those reared on the pest species (Hoy, 1994). Such factors would have to be investigated for quandong moth before any mass rearing and release program could be implemented. None of the parasitoids collected during this study could be identified to species and their specificity is unknown. For the *Trichogramma* species in particular, more specimens are required to determine if it has been described and if there is potential for use in management of quandong moth.

Insecticides will remain valuable tools in management of quandong moth if their use is judicious and their limitations are recognised. Selective use of insecticides is the key to integration with other management strategies (Metcalf, 1994). The sustained and frequent usage of a broad-spectrum insecticide for control of quandong moth is neither desirable nor sustainable. Selectivity of insecticides could be improved both by replacing routine applications with responsive treatments indicated by monitoring, and by using alternative insecticides with lepidopteran specificity (Metcalf, 1994). Further research is needed to investigate insecticides with potential for use against quandong moth, particularly those with lepidopteran specificity. The field trials examining spray timing and alternative insecticides conducted in this study (Chapter 6) should be repeated with improved timing of applications and less variability in the experimental trees. In addition, examination of insecticides in laboratory bioassays would help to interpret the results of field trials.

It is clear that damage of the spring generation of quandong moth causes loss of both yield and quality (Section 5.3.3). The impact of the autumn-winter generation is less clear. Damaged fruit do drop from trees, but the major proportion of fruit drop occurs naturally (Section

5.3.4). If trees are able to compensate for herbivory by adjusting the rate of natural thinning (Crawley, 1983), then damage during this period may not actually equate to financial loss. Many of the larvae that drop from trees inside fruit may survive to adulthood and give rise the damaging spring generation. Cultural control could have a role during the fruit drop period. Although collecting and destroying fallen fruit is laborious, it may be feasible in some orchards, particularly those that are regularly maintained and small-scale. Further research into the proportion of fruit that drop from infested and uninfested trees would help to determine if damage to immature fruit actually results in financial loss.

Pheromones can have a role in an IPM program through the use of pheromone traps or to disrupt mating. Monophagous pest species that do not disperse over long distances and are the major pest in the system are well suited to mating disruption (Suckling, 1993). The lack of a laboratory culture restricted investigation into the use of pheromones, but the quandong moth appears to be a good candidate for mating disruption. Although pheromone traps using live females trialed in this study were not effective (Appendix 4), factors such as trap design and behaviour of males could have produced a misleading result (Rothschild and Minks, 1977; Wilson, 1984). Research on the presence of a pheromone and subsequent isolation, synthesis and evaluation is required before mating disruption or trapping could be considered in quandong orchards.

As with many research programs, there were several major constraints that restricted the scope and outcomes of this study. Inability to establish a laboratory culture limited almost all aspects of this research, including the effect of temperature on development of the moth,

examination of the relationship between larval feeding and yield loss, the rearing of parasitoids and the investigation of potential insecticides through laboratory bioassays. It is possible that with further research a suitable artificial medium could be developed. In the absence of an artificial food source, cultures may be established inside field cages, but the size of bearing trees would be a major limitation. A field culture would also be restrained by climatic variation with development rates significantly lower at certain times of the year. Glasshouse rearing is unlikely to be feasible because of the large size of bearing trees and the parasitic nature of the trees. Another major constraint, related to the establishment of a field culture, was the proximity of quandong orchards to the laboratory in which research was conducted, and the availability of trees on which to conduct trials. The feasibility of an experimental orchard should be considered by the industry, so that trees would be in close proximity to the research centre and their availability not restricted by the needs of the grower. Artificial population densities could then be established to determine more accurately the effects of feeding by the quandong moth on the yield and quality of fruit.

It is common for human intervention, such as the growing of high yielding monocultures of crops and use of insecticides to induce problems with resurgence of pests and secondary pests (Adkisson, 1971; Luckmann and Metcalf, 1994; Metcalf, 1994). Quandongs are now being grown in orchards with regular watering and fertilisation to increase yields and general plant health. To date, the quandong moth is the only pest that causes major yield losses in quandongs, but there are a number of other insects that feed on the leaves of quandong trees, many of which are native and polyphagous (Chapter 10). Several species were isolated to one orchard and outbreaks were unrelated to previous insecticide usage. There are also reports of

mites becoming a problem in some orchards. Currently, quandong moth is the sole key pest in the system, a major advantage for the development of an IPM program for the moth (Luckmann and Metcalf, 1994). There exists the potential for other species to develop as major pests because of the improved condition, close proximity and uniformity of trees, and the use of insecticides in orchard situations. It is a problem about which quandong growers need to remain vigilant.

In wild stands, quandong trees are often discontinuously distributed. The trees are relatively uncommon but in the regions where they are found trees are usually clustered. It is likely that the small size and fragile nature of the moths limits their ability to fly long distances in search of host trees. They are not migratory, so in their relatively sedentary existence the stand of quandong trees in which they live provides ample nutrition to the population, and migration to new sites is not necessary for survival. Similarly, adults of the light brown apple moth in a favourable orchard will not readily disperse (Geier and Briese, 1981). If quandong moths invade previously uninfested trees, it seems more likely to be because of transport by wind, rather than active flight. Growers with young orchards in which fruiting has not commenced often source fruit from the wild to sell, so the market for their fruit is already established once their trees begin to bear fruit. There is a possibility that transfer of infested fruit could contribute to infestations in new orchards if larvae in discarded fruit are not destroyed. Anecdotal evidence suggests quandong moths are able to infest new orchards within one to two years of the trees beginning to bear fruit, providing there are infested trees within at least 3km (Len Twigg, pers. comm.). Three isolated quandong trees at the Waite campus of the Adelaide University had been fruiting for approximately three years before this project

commenced and remained free of quandong moth for the duration of this study. The nearest infested trees are likely to be more than 5km away. At the field site at Quorn, there was one quandong tree bearing a thickly fleshed yellow fruit that was rarely infested with larval quandong moth despite being less than ten metres away from heavily infested trees. The flesh of the uninfested tree was very acidic and not favoured for human consumption (Brian Powell, pers. comm.). It is somewhat difficult to ascertain if certain trees remain uninfested because they are isolated from infested trees or if they are somehow unfavourable for feeding by larval quandong moth.

Monitoring, insecticides, augmentation of natural enemies and good orchard hygiene could all form part of an IPM program for the quandong moth. Monitoring for eggs of the moth and only employing insecticides when the moth is present at damaging levels will result in more judicious use of insecticides. Reduced use of insecticides will conserve the populations of generalist and specific natural enemies of the moth and decrease the many adverse effects of over-reliance on insecticides. The inclusion of insecticides that are specific to moths will also aid in conserving populations of beneficial insects and help restore the ecological balance where it has been disrupted by sustained use of broad-spectrum insecticides.

APPENDIX 1

Artificial media

To facilitate mass rearing of the quandong moth, attempts were made to develop an artificial media suitable for larvae of the quandong moth but two main problems developed. The first problem was the high susceptibility of quandong fruit to fungal contamination. The second problem was a consequence of the first problem in that larvae appeared to be very sensitive to any antibiotic products used to reduce fungal contamination of the media.

Initial attempts were made to produce an artificial food source consisting of dried quandong, mixed with gelatine or various oils, plus preservatives such as methyl-p-hydroxybenzoate and vitamin C. However, a consistency favourable to larval feeding could not be attained.

Several versions of a media based on a diet for larvae of *Pieris brassicae* (Lepidoptera: Pieridae) (David and Gardiner, 1965) were trialed with different instars of the quandong moth. The initial version had a strong odour, probably due to some of the preservative components. First instars trialed on the first version of the media did not feed at all and were dead within 24 hours. In the next version, the formaldehyde was removed completely and the amount of methyl-p-hydroxybenzoate was reduced. The quantities of every other ingredient were halved, while the amount of ground dried quandong was doubled. Again, first instars were placed on the media, but did not feed and the majority were dead within two to three hours of being placed on the media. Onset of death was much more rapid than would be expected if the larvae had died of starvation. It was concluded that a component of the media was causing irreversible damage, probably through dermal absorption, as no feeding had occurred. Some

first instars were removed from the media after one hour of not feeding and transferred to fresh fruit. The initiation of feeding in these larvae on fresh fruit was almost immediate. For a quandong moth larva, the cues associated with fresh fruit appear to be very strong and this monophagous insect is highly evolved to respond to its one and only food source. Fourth instars were also trialed on the second version of the media. Most of those attempted to feed on the media but then ceased within a few minutes. The mortality seen in first instars was not evident in fourth instars. However, like first instars, the fourth instars began feeding almost instantly when transferred from the media to fresh fruit. The next step in the development of the media was to remove all the preservatives and add sodium ascorbate both as a preservative and also to increase the nutritional value of the media. Six first instars were placed on the media, within one hour, three of those larvae were feeding on the media. However, within two days the media was heavily contaminated with fungi due to the lack of preservatives. It was simply not feasible to dissect media every other day to remove feeding larvae. The likelihood of actually finding early instars in the media was very low, larvae were often injured during the search and disturbing them during feeding often disrupted their feeding behaviour permanently. Version four of the media was developed, which had low levels of preservatives and the vitamin solution was inadvertently omitted, however it was only slightly more favourable than the previous versions.

One of the many difficulties associated with developing an artificial media was to find a suitable balance between the level of antibiotics and the level of fungal control attained. Ideally, eggs or neonate larvae would be placed in cups of the media and left to feed until pupation. At 4°C, the media was able to be stored for two to three months, but once in the

culture room at 24°C, media only remained free of fungal contamination for five to seven days. This period was not long enough for larvae to progress to the pupal stage and dissection of the media to extract mid-stage larvae proved very difficult and was hazardous for larvae. It was also important to stimulate feeding by the larvae, by having the right balance of quandong fruit, vitamins, carbohydrates, protein and salts. It is likely that there are tactile, olfactory or taste cues involved in the stimulation of feeding activity. It is not known whether the absence of a feeding response on artificial media was due to some anti-feedant properties of the media, or due to a lack of stimulants for feeding activity.

APPENDIX 2

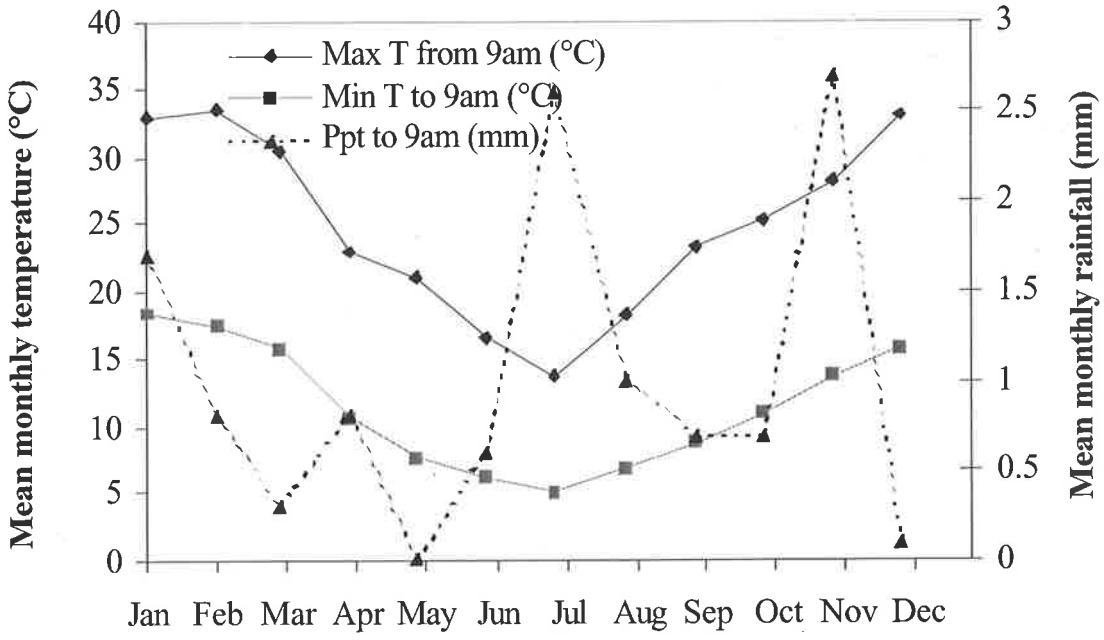


Figure 1: Mean monthly temperature and rainfall data recorded at Hawker in 1998

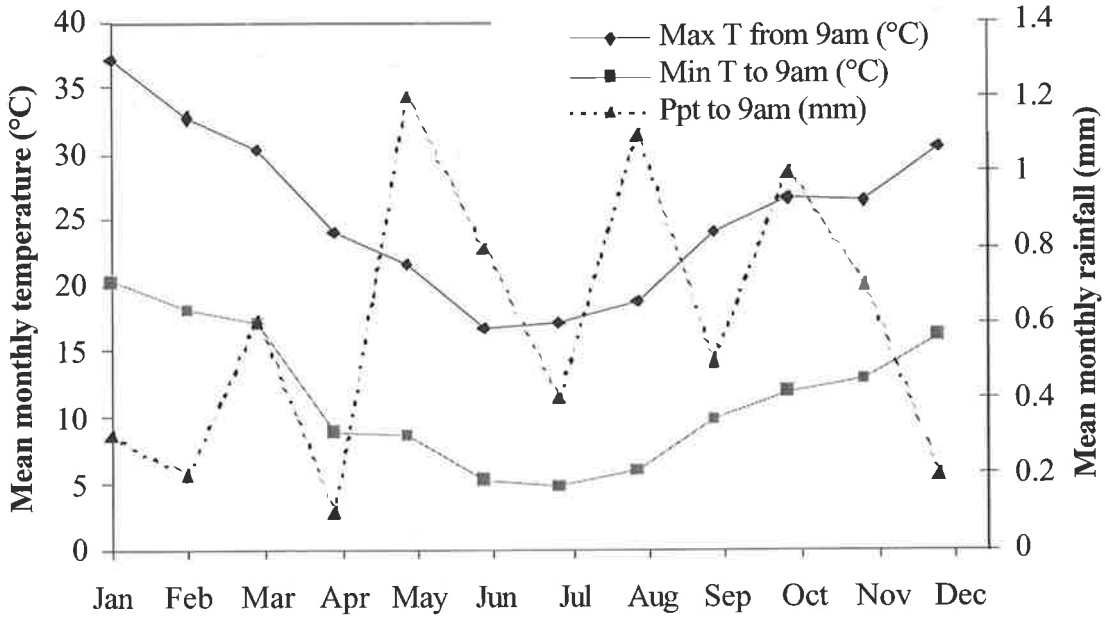


Figure 2: Mean monthly temperature and rainfall data recorded at Hawker in 1999

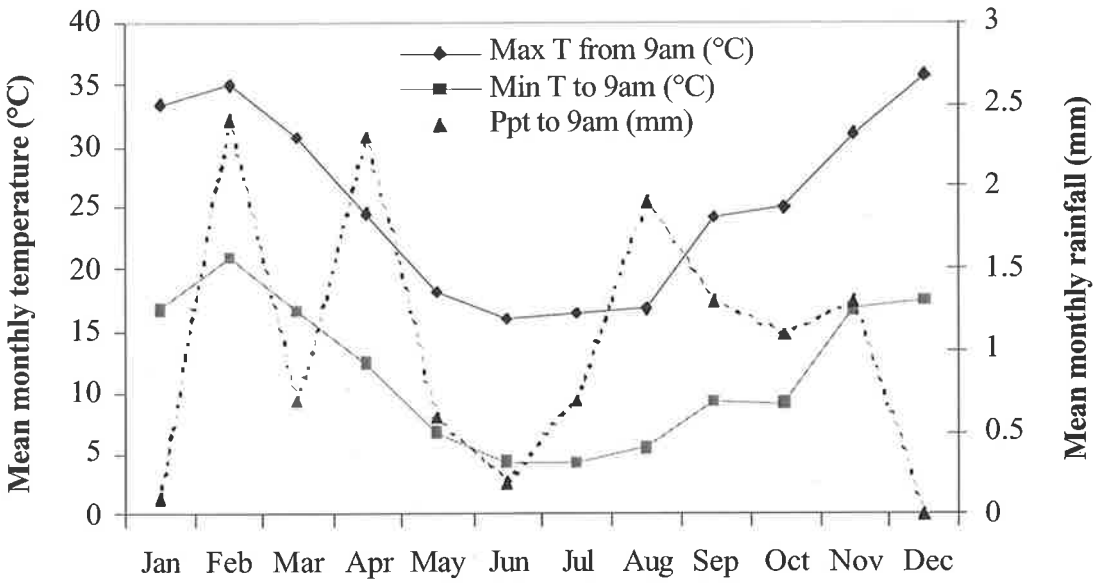


Figure 3: Mean monthly temperature and rainfall data recorded at Hawker in 2000

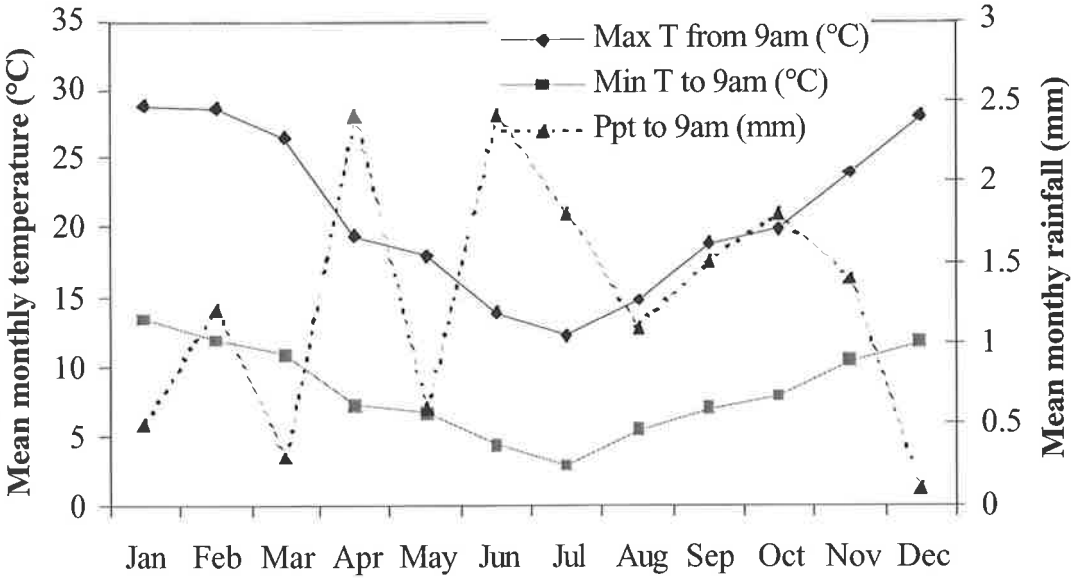


Figure 4: Mean monthly temperature and rainfall data recorded at Sedan in 1998

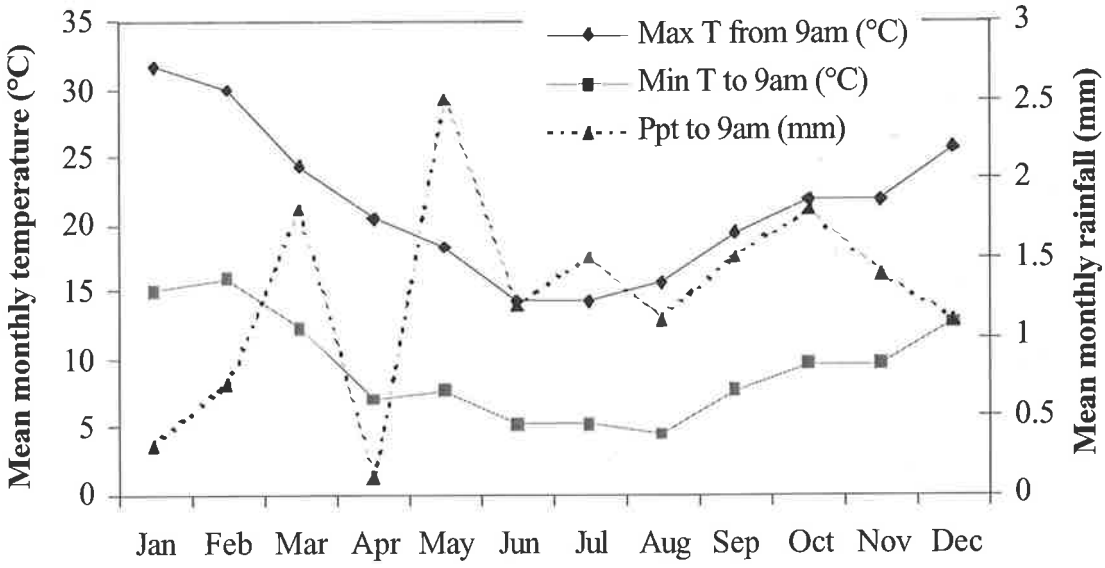


Figure 5: Mean monthly temperature and rainfall data recorded at Sedan in 1999

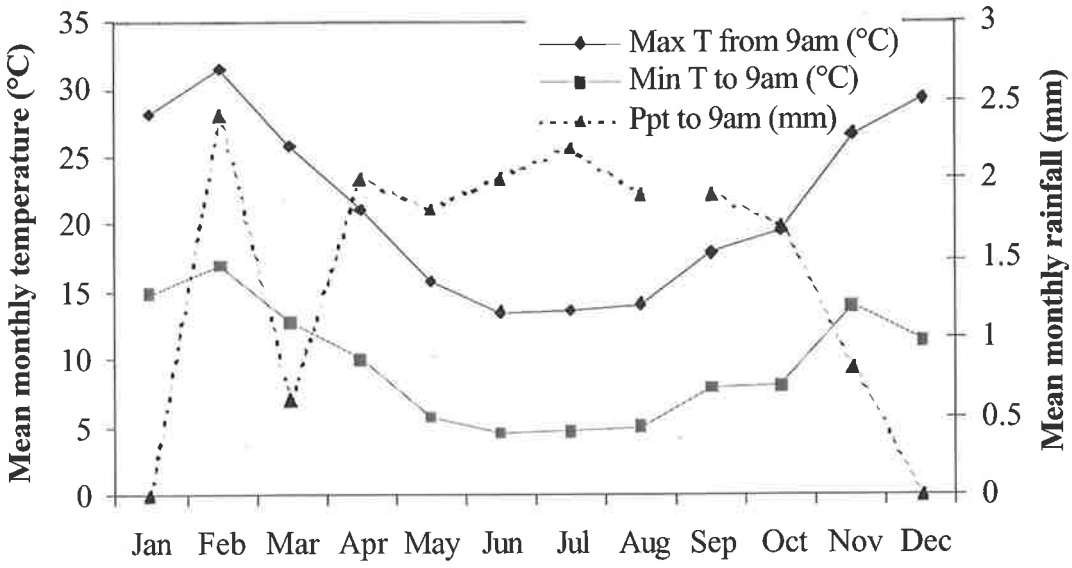


Figure 6: Mean monthly temperature and rainfall data recorded at Sedan in 2000

APPENDIX 3

Table 1: Number of quandong flowers and fruit collected from the trees and the ground at Quorn 1997-2000.

Year	Trees		Ground	Total
	Flowers	Fruit	Fruit	
1997	88	255	488	831
1998	432	608	376	1416
1999	480	888	755	2123
2000	240	791	434	1465
Total	1240	2542	2053	5835

Table 2: Number of quandong flowers and fruit collected from the trees and the ground at Sedan 1998-2000.

Year	Trees		Ground	Total
	Flowers	Fruit	Fruit	
1998	416	538	246	**1200**
1999	387	267	29	**683**
2000	232	597	442	**1271**
Total	**1035**	**1402**	**717**	**3154**

APPENDIX 4

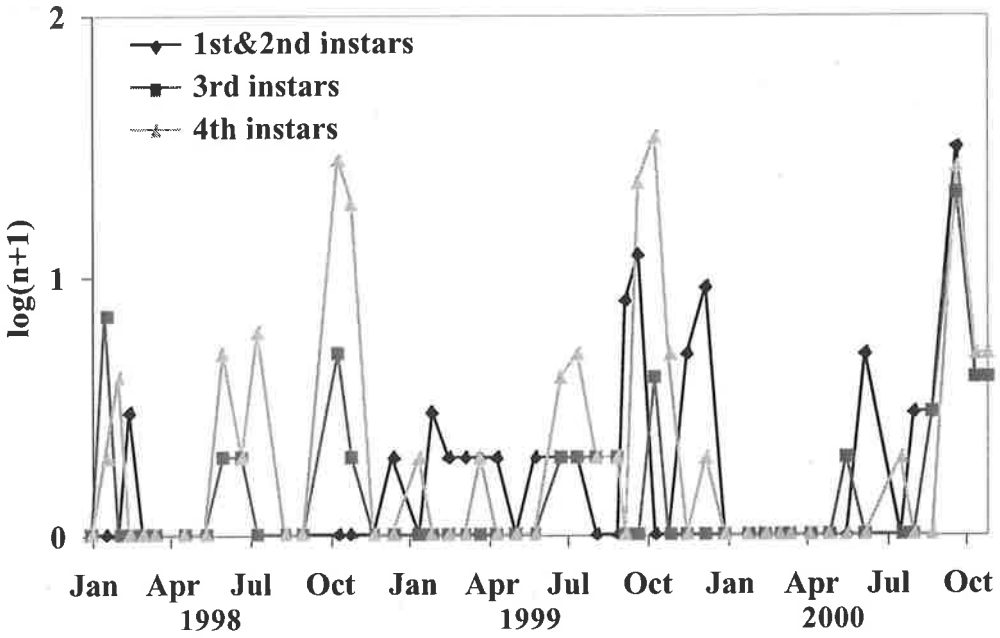


Figure 1: Breakdown of instars of the quandong moth at Quorn, 1998-2000

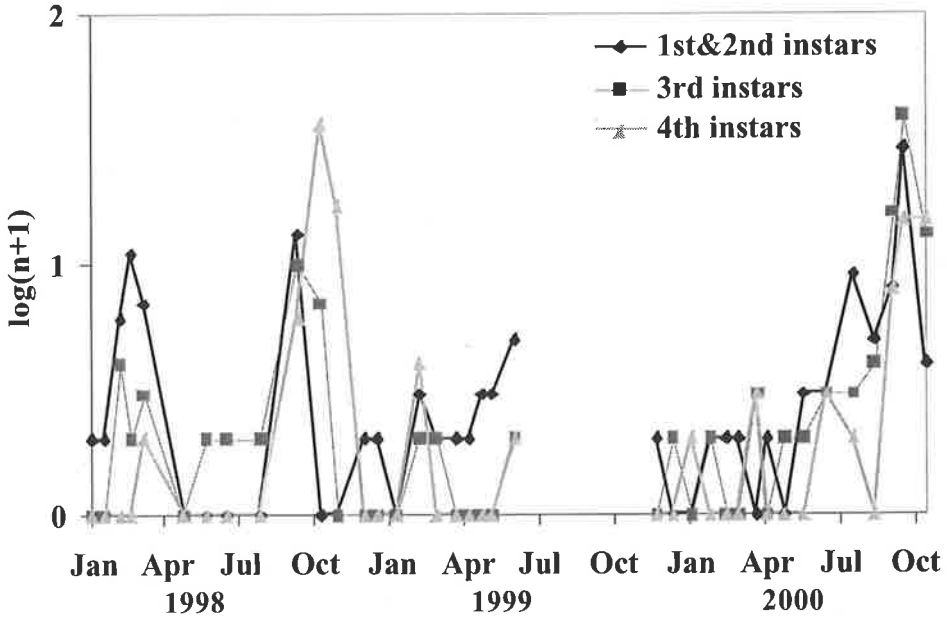


Figure 2: Breakdown of instars of the quandong moth at Sedan, 1998-2000

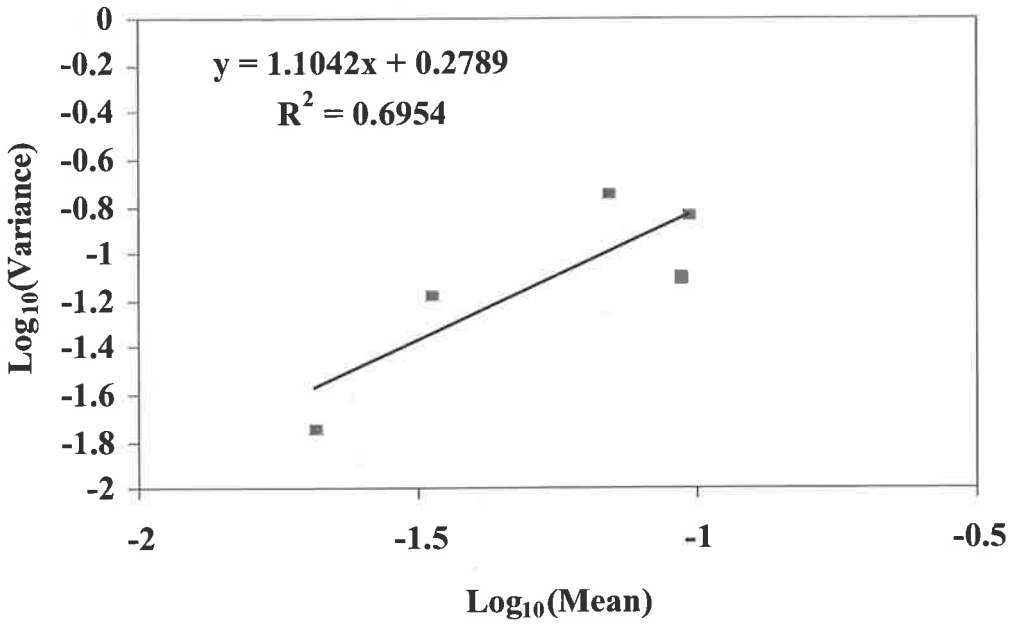


Figure 3: Regression of log(variance) against log(mean) for eggs collected on flowers at Quorn and Sedan, 1998-2000.

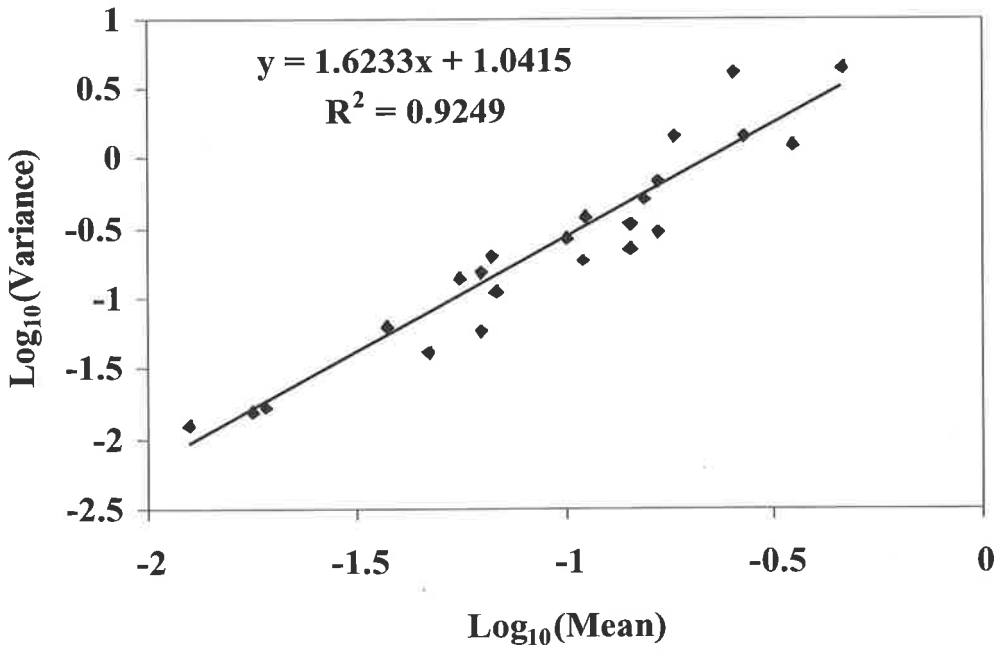


Figure 4: Regression of log(variance) against log(mean) for eggs collected on fruit at Quorn and Sedan, 1998-2000.

Trapping

Several different techniques were used in an attempt to trap adult quandong moths. Sticky traps based on whitefly traps were constructed from milk cartons, consisting of a rectangle of the white, wax coated cardboard 280x95mm, folded in half lengthwise. The blank surface of the cardboard was coated with Tangle-Trap® (The Tanglefoot Company, Grant Rapids, MI, U.S.A.) and the trap was folded with the coating outside. A hole was punched at the top and a twist tie used to affix the trap to trees. The traps were placed two per tree on all sample trees at Quorn and Sedan in 1998-1999 and replaced every 2-3 weeks. Traps from the field were taken back to the laboratory for examination. Sticky traps were successful in catching many small Diptera but very few quandong moths.

Light traps were set-up at Sedan and McLaren Flat during fruit maturity, in late-September and during flowering in mid-January. The trap consisted of a white sheet fixed between two trees, with a Mercury-Vapour lamp hung with rope approximately 300mm in front of the sheet. The light was turned on just prior to sunset and run for 2-3 hours. Insects were observed as they were attracted to the light and those of interest collected into vials for later examination in the laboratory. The time was noted as quandong moths were collected. One male quandong moth was captured at the light trap run at Sedan during flowering. The moth was captured approximately 30 minutes after dusk. No others were captured at either site.

Female quandong moths formed part of live pheromone traps with Tangle-Trap® used to capture any males attracted to a pheromone released by the female. Traps were constructed from milk cartons folded into hollow pyramids. Female quandong moths two days old reared

in the laboratory were enclosed in a square of fine mesh closed with a drawstring of cotton. The moths inside the mesh were placed inside the triangle and fixed with a staple. The inner surface of the trap was coated in Tangle-Trap ®. A hole was punched at the top and a twist tie used to fix traps to the trees. The traps were trialed both at Sedan and at Whyalla during mid-September to late-October 2000. At Sedan, the traps were placed two per tree on trees that had intact fruit. The traps were left out in the field for 7-10 days, then checked for any quandong moths. The females inside were replaced with new two day old females reared in the laboratory, traps cleaned of insects and a new coating of Tangle-Trap® applied. At Whyalla, traps were hung in trees during mid-afternoon and collected the following morning. Traps were placed out one day per fortnight for the following six weeks. No quandong moths were captured on pheromone traps.

APPENDIX 5

The equations for stop lines, OC and ASN curves taken from Fowler and Lynch (1987) were as follows:

$$\text{upper stop line} = \frac{a}{\ln\left[\frac{P_1 Q_0}{P_0 Q_1}\right]}$$

$$\text{lower stop line} = \frac{b}{\ln\left[\frac{P_1 Q_0}{P_0 Q_1}\right]}$$

$$\text{common slope of stop lines} = K\frac{\ln\left[\frac{Q_1}{Q_0}\right]}{\ln\left[\frac{P_1 Q_0}{P_0 Q_1}\right]}$$

$$\text{Operating Characteristic} = \frac{A^{h(P)} - 1}{A^{h(P)} - B^{h(P)}}$$

$$\text{Average Sample Number} = \frac{bL(P) + a[1 - L(P)]}{K \ln + \left[\frac{Q_0}{Q_1}\right] + KP \ln \left[\frac{P_1 Q_0}{P_0 Q_1}\right]},$$

where _ = type I error; _= type II error; $A = \frac{1-\beta}{\alpha}$; $B = \frac{\beta}{1-\alpha}$; $a = \ln(A)$; $b = \ln(B)$; P_0 = nominal proportion below AT; P_1 = nominal proportion above AT; $Q_0 = 1 + P_0$; $Q_1 = 1 + P_1$; K = constant for negative binomial distribution estimated using Taylor's Power Law; L = operating characteristic.

APPENDIX 6

Table 1: Parameters for Dymex simulation model for quandong moth

Eggs		Larvae	
Development		Development	
Lower developmental threshold	4.5°C	Lower developmental threshold	5°C
Developmental function	Linear above threshold	Developmental function	Linear above threshold
Slope	1	Slope	1
Stage transfer		Stage transfer	
Threshold	120.5 degree-days	Threshold	450 degree-days
Transfer function	Linear above threshold	Transfer function	Linear above threshold
Proportion transferring	1	Proportion transferring	1
Pupae		**Adults**	
Development		Mortality	
Lower developmental threshold	8.1°C	Age at death	9 days
Developmental function	Linear above threshold	Mortality function	Step
Slope	1	Proportion dying	1
Stage transfer		Development	
Threshold	138.9 degree-days	Threshold	5°C
Transfer function	Linear above threshold	Slope	1
Proportion transferring	1	Fecundity	
		Number of eggs	30
		Progeny production	
		Commencement of oviposition	2 days
		Oviposition function	Linear above threshold
		Rate of oviposition	0.1

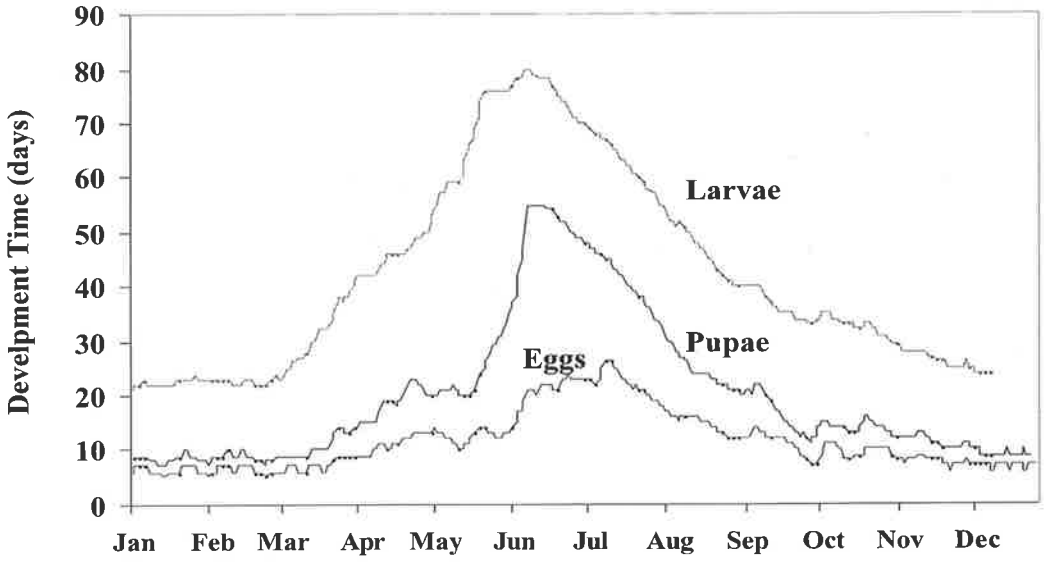


Figure 1: Developmental times of eggs, larvae and pupae of quandong moth at Quorn in 1998

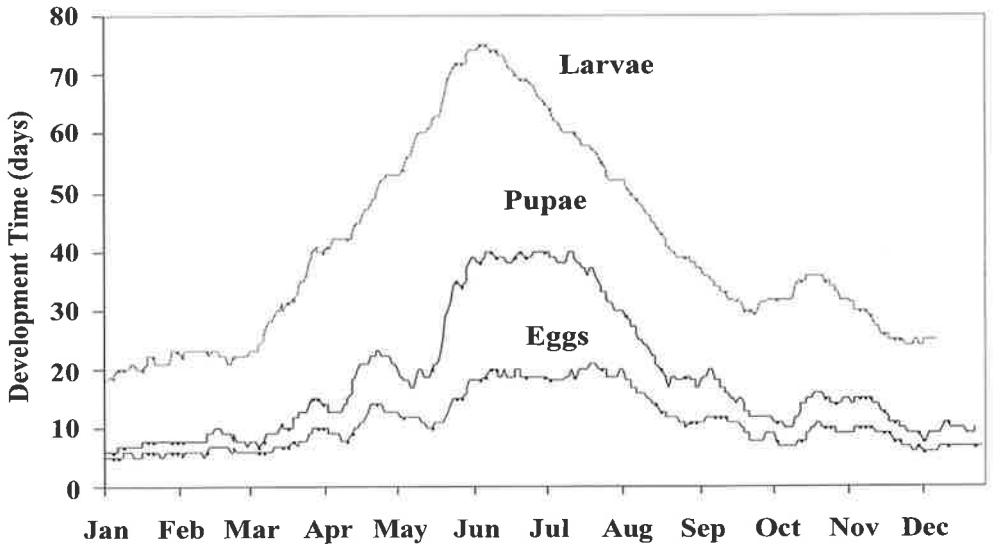


Figure 2: Developmental times of eggs, larvae and pupae of quandong moth at Quorn in 1999.

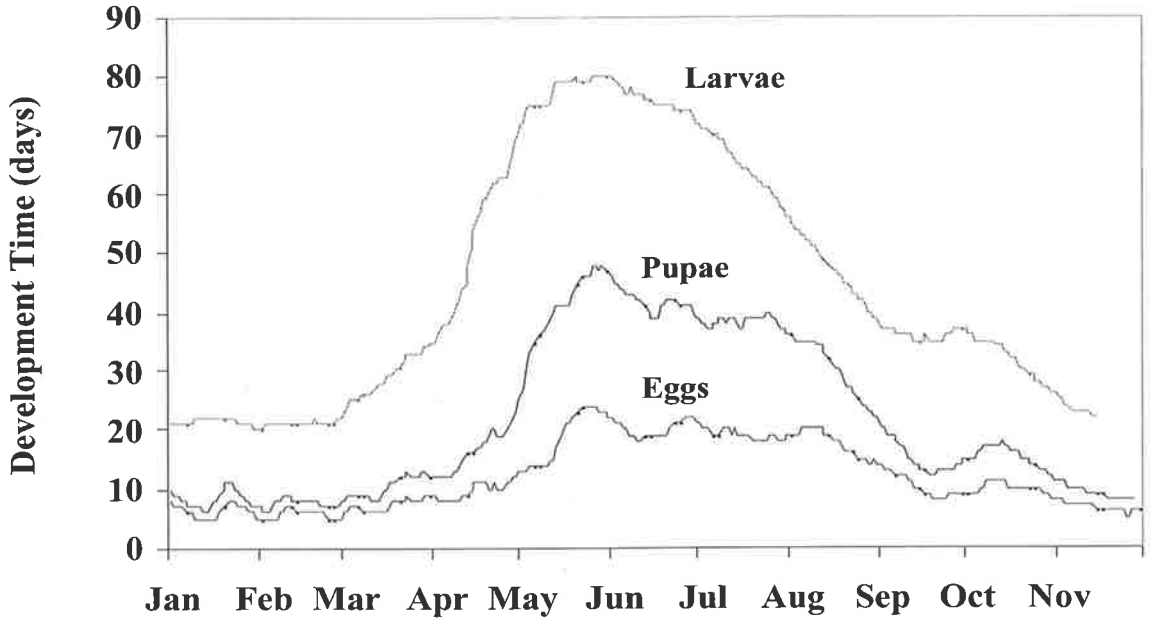


Figure 3: Developmental times of eggs, larvae and pupae of quandong moth at Quorn in 2000.

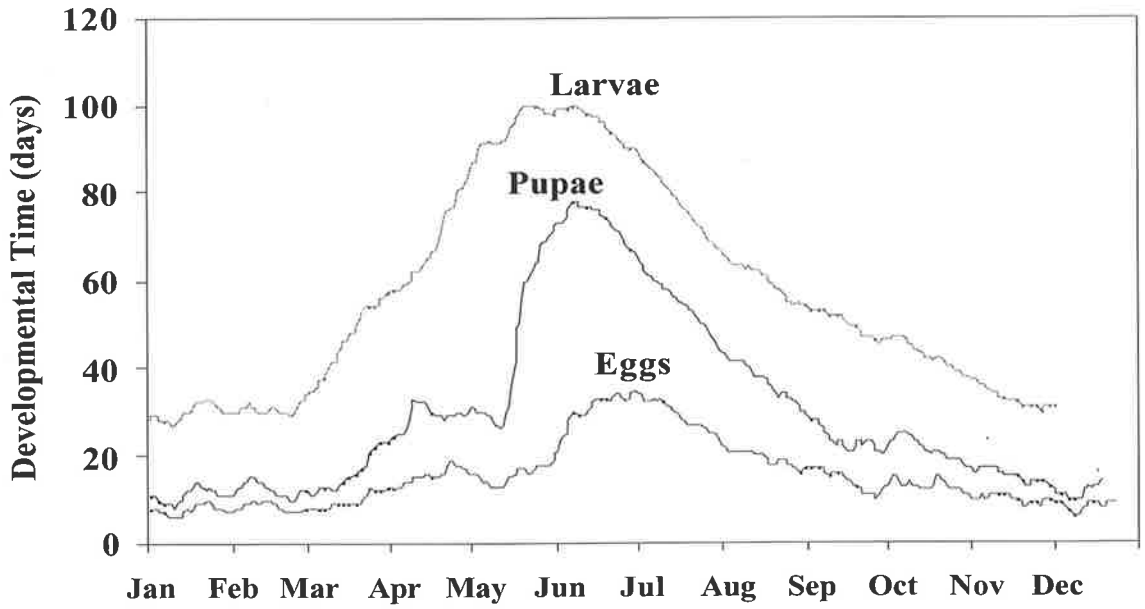


Figure 4: Developmental times for eggs, larvae and pupae of quandong moth at Sedan in 1998.

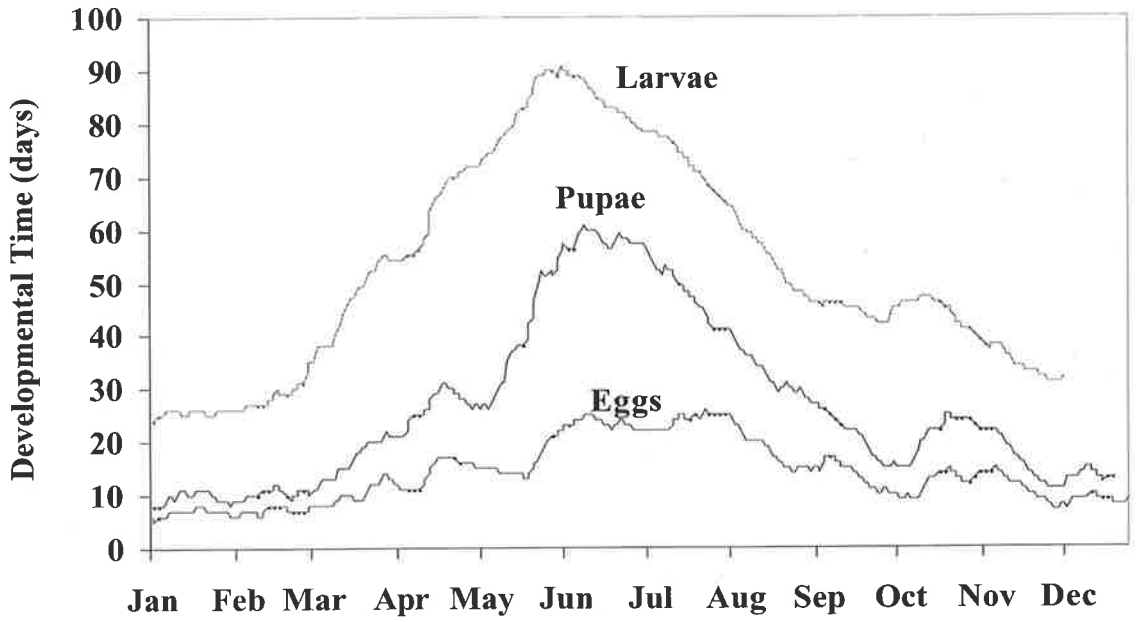


Figure 5: Developmental times for eggs, larvae and pupae of quandong moth at Sedan in 1999.

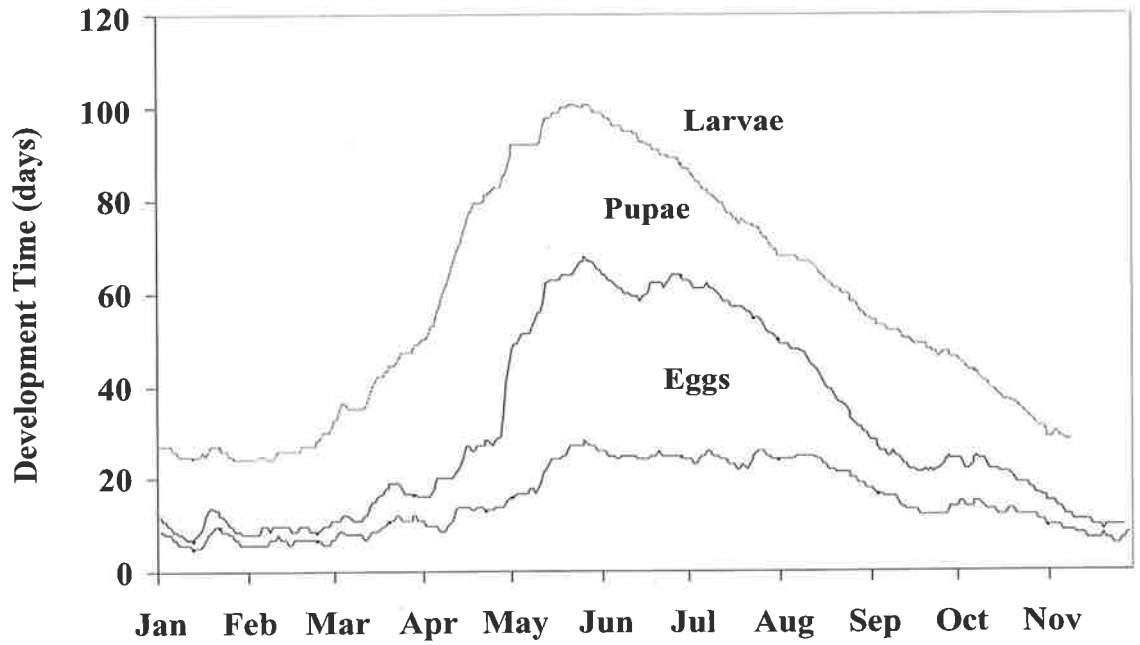


Figure 6: Developmental times of eggs, larvae and pupae of quandong moth at Sedan in 2000.

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