

**BIOLOGY OF THE OLEANDER MEALYBUG, *PARACOCCUS
BURNERAE* (BRAIN) (HEMIPTERA: PSEUDOCOCCIDAE)**

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STATEMENT OF ORIGINALITY

I, the undersigned hereby declare that the work presented in this thesis is my own original work and has not previously been submitted in its entirety or in part at any university for any other degree.

Signature.....

Date

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Mealybugs

Mealybugs are tiny, soft-bodied insects which constitute the second largest scale insect family Pseudococcidae (Downie & Gullan 2004). The family comprises approximately 2000 species in 300 genera (Ben-Dov 1994), of which 20 species are pests of cultivated plants in South Africa (Annecke & Moran 1982). In South Africa, approximately 109 species of mealybugs have been recorded from 50 genera (Millar 2002).

Some mealybugs are notorious pests (Kaydan *et al.* 2006) of various fruit crops, field crops and ornamentals (Bartlett & Lloyd 1958; Summy *et al.* 1986; Williams & Granara de Willink 1992; Franco *et al.* 2001 in Wakgari & Giliomee 2003b). They are found throughout the world except in Polar Regions (Cannon 2006), but are most abundant in the tropics and subtropics (Ben-Dov 1994). Their host preference ranges from grasses (Poaceae) to woody plants (Asteraceae) (Ben-Dov 1994; Ben-Dov & German 2003), and more than 20% of pest species might be polyphagous (Miller *et al.* 2002 in Downie & Gullan 2004).

1.2 Economic importance of mealybugs on citrus

Mealybugs cause extensive damage to citrus plantations through their sap sucking activity on leaves and fruits. The honeydew they produce attracts a sooty mould fungus which reduces photosynthesis and blemishes fruit. A study conducted by Hattingh *et al.* (1995), revealed that mealybugs attacking citrus had an important role to play in the incidence of navel-end *Alternaria*

decay in orange fruit caused by the fungus *Alternaria citri*. The honeydew produced by mealybugs is rich in carbohydrates and creates a favourable environment for the fungus to flourish. The formation of a secondary fruitlet on navel oranges, provides an entry route for the fungus into the fruit (Lima & Davies 1984 in Hattingh *et al.* 1995). If stress develops in the navel and calyx regions of fruits, this provides a naturally occurring entry point for the fungus (Wager 1939). Mealybug feeding damage can also create additional entry routes through crawlers (1st instar nymphs) which may act as carriers of spores to the navel openings (Hattingh *et al.* 1995). Fruit skin feeding by both the citrus and oleander mealybugs was reported as being the cause of deep colour development and protrusions at feeding sites (Hattingh *et al.* 1998).

From the 20 pseudococcid species known to attack cultivated plants in South Africa (Millar 2002) at least seven are known to infest citrus with each one of them producing varying levels of economic damage (Wakgari & Giliomee 2003a). Of the seven species observed on citrus in South Africa, *Planococcus citri* is the most widespread and economically destructive. It has also been cited as a serious pest of citrus in Africa (Hattingh *et al.* 1998), Australia (Smith *et al.* 1988; Ceballo *et al.* 1998), the Mediterranean region (Spicciarelli *et al.* 1994; Blumberg *et al.* 1995; Mendel *et al.* 1999), North, Central and South America (Bartlett & Lloyd 1958; Williams & Granara de Willink 1992 in Wakgari & Giliomee 2003b). The citrophilous mealybug, *Pseudococcus calceolariae* (Maskell) and long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) are also common pests of citrus in South Africa, with *P. longispinus* being the most widely distributed (Hattingh *et al.* 1998). However, the citrus mealybug *Planococcus citri* (Risso), the Karoo thorn mealybug *Nipaecoccus vastator* (Maskell) and the oleander mealybug *Paracoccus burnerae* (Brain) are considered to be the most important species of mealybug occurring on citrus in southern Africa (Hattingh 1993). The importance of some of these mealybugs has risen over the years in South Africa such that three of the seven common citrus mealybug pests (including *P. burnerae*) have become quarantine pests for citrus exports to the USA (Wakgari & Giliomee 2003b).

1.3 Host plant range and distribution of *P. burnerae*

The oleander mealybug, *Paracoccus burnerae* is a tropical species indigenous to the Southern and Eastern Africa Subregions. It has also been recorded as far as India (Ben-Dov *et al.* 2001) According to Hattingh *et al.* (1998), *P. burnerae* is found in all citrus growing zones of southern Africa. It was first described by Brain (1915) as *Pseudococcus burnerae*, and then redescribed by De Lotto (1967). Brain (1915) recorded it on six plant species, namely: *Viburnum sp.*, granadilla (*Passiflora edulis*), *Nerium oleander*, *Gleditschia sp.*, *Sida rhombifolia* and *S. longipes* in South Africa. To date, only five species of *Paracoccus* are known to occur in South Africa. Despite its economic importance, there is virtually no information on the reproductive and developmental biology of *P. burnerae*.

1.4 Pest status of the oleander mealybug

According to Hattingh *et al.* (1998), *P. burnerae* only became markedly more prevalent during the early 1990s. Reasons for the increase in prevalence are not yet known. Hattingh *et al.* (1994) in Hattingh *et al.* (1998) describe it as being problematic in Natal and Swaziland where they found that *P. burnerae* more readily colonizes leaves than *P. citri* (citrus mealybug) and causes extensive leaf rolling. *P. burnerae* was until recently the most dominant mealybug species on citrus in some parts of the Eastern and Western Cape Provinces of South Africa (Moore and Kirkman 2005). Reasons for the occasional dominance of *P. burnerae* over the citrus mealybug, *Planococcus citri* are still unknown. The oleander mealybug does not only affect citrus but is also known to attack plants in a wide range of families (Ben-Dov *et al.* 2001).

1.5 Population dynamics of mealybugs

Although most mealybugs including *P. burnerae* reproduce sexually, a few species are parthenogenetic (Downie & Gullan 2004). Both males and females of the oleander mealybug

have to go through three larval stages to reach adulthood. The final larval stage of the male is completed in a cocoon as a so-called pupa (Hattingh *et al.* 1998). The adult female, which is all “mealy” when mature and bears very little resemblance to a typical insect, is neotenic and wingless while the male is an active flyer.

The challenges faced with trying to elucidate seasonal population cycles and events as they unfold in tropical species such as *P. burnerae*, which experience overlapping generations, are very difficult but not impossible. Wakgari & Giliomee (2003b) found almost similar generation times for three mealybug species (*Planococcus citri*, *Pseudococcus longispinus*, *Pseudococcus calceolarie*) reared at the same temperature on citrus. However, *P. citri* was observed to dominate and displace other mealybug species after three to four generations on the same host plant under the same laboratory conditions.

In Southern Australia, Furness (1976) recorded three generations of *Pseudococcus longispinus* on citrus in a year. This is in contrast to observations noted in California where *P. longispinus* bred continuously throughout the year (Clausen 1915 in Furness 1976; DeBach 1949; DeBach *et al.* 1949; Browning 1959). *P. burnerae* populations, besides being multivoltine, are surprisingly able to survive low winter temperatures in cracks found on the bark of trees and inside curled leaves (Hattingh *et al.* 1998). Besides that, very little is known about the seasonal life changes and phenology of *Paracoccus burnerae* populations in the field.

1.6 Significance of temperature on development of mealybugs

The oleander mealybug, just like any other ectotherm organism, is affected by temperature during its development. Temperature has been reported to be the deciding factor affecting the development rate of insects. Previous studies have also shown that temperature affects several biological traits of insects such as sex ratio (Godfray 1994), adult life-span, survival, fecundity and fertility (Singh & Ashby 1985; Yang *et al.* 1994; Dreyer & Baumgartner 1996). When the

temperature is low, development occurs at a much slower rate than at high temperature (Jarosik *et al.* 2004), and subsequently time spent in each developmental stage increases. In reality, the relationship between temperature and development in insects is expected to be linear but can also be sigmoid. However, the effect of temperature on development of *P. burnerae* on citrus still remains unknown.

It has been suggested that linear models are good for estimation of the developmental thermal constant. Both the thermal constant (K) and lower developmental threshold (T_0) have frequently been employed to explain how insect development is dependent on temperature (Aysal & Kivan 2008). They both provide vital information that is used to determine the development rate of an individual (Jarošik *et al.* 2002). Thermal constants are often used to generate predictive models of pest development in diverse environments, including stored products (Subramanyam *et al.* 1990), greenhouses and orchards (Graf *et al.* 1996 in Malina & Praslicka 2008). However, when the model of Briere *et al.* (1999) is applied on temperature versus developmental rate, a nonlinear relationship between rate of insect development and temperature is obtained, and can thus be used to estimate a developmental threshold above which development occurs, the optimum temperature for development and the upper lethal temperature at which mortality occurs.

Developmental rates and life tables are essential tools that can be used for investigating and understanding the impact temperature has on growth, survival, reproduction and rate of increase of an insect population. Life tables are especially important in understanding age dynamics of insect populations studied under controlled laboratory conditions (Aysal & Kivan 2008), prediction of population expansions (Hemerik *et al.* 2004) and in tackling the issue of life expectancy when affected by environmental changes (Pilkington & Hoddle 2007). We can, therefore, rely on them to identify the core mortality factors and to reveal the most susceptible stages for management of the oleander mealybug. Temperature and its effects on rate of development have long been used by entomologists to estimate the “growing degree-days”, which are the amount of heat required for an insect to reach a certain life stage, i.e. egg hatch,

adult flight, etc. Degree-days are very important in predicting and determining treatment timings in proactive crop protection systems. For example, Shelford (1927) in Varley *et al.* (1973) used degree-days to predict adult emergence of the codling moth, *Cydia pomonella* from the pupal stage in spring. He also discovered that these life history events were related to daily climatic data. Forecasts on when the different insect life stages become active provide a cost effective means of reducing insect feeding damage to crops (Murray 2008). This is especially true with insects because some stages such as larvae (crawlers in mealybugs) are more susceptible to pesticides. Degree-day estimates can also be useful in the calculation of the theoretical number of generations an insect is expected to undergo during a specific period (Purcell & Welter 1990 in Pilkington & Hoddle 2006).

1.7 Mass rearing and biological control of mealybugs

For a sound biological control program to be successful in suppression of mealybug populations, food plants that give rise to high population growth rates of mealybugs need to be identified and exploited for mass rearing of pest mealybugs and mass releases of biocontrol agents. Mass rearing of these mealybugs has the advantage of ensuring that a constant supply of breeding hosts for predators and parasitoids is maintained. In this case parasitoids are wasps, which lay their eggs on or in the body of the host. The host eventually dies as the parasitoid's young feed on it to reach maturity. Biological control of mealybugs by mass rearing and mass releases of natural enemies has been found to be successful against a number of pseudococcid pests (Walton & Pringle 2002).

Entomologists have in the past managed to control certain pests by introducing parasitoids into an area or by mass releases of parasitoids present. In this way, they were able to prove the potential which parasitoids have in controlling their hosts. Whereas suitable host plants for mass rearing of mealybug species such as the citrus mealybug, *Planococcus citri*, and the vine mealybug, *Planococcus ficus*, have been identified it has resulted in the mass culturing and mass release of natural enemies (Daane *et al.* 2004; Walton & Pringle 2002; Sagarra *et al.* 2001; Walton & Pringle 2005; Polat *et al.* 2008). However, there is still no documented evidence

about host plant suitability for mass rearing of *P. burnerae* for mass rearing and release of its natural enemies.

Mealybugs have previously been known to be extremely harmful to citrus in many countries where exotic species first established. However, large complexes of natural enemies do exist and are often capable of keeping the mealybug populations under control. Mealybug populations in many citrus growing regions of southern Africa have for a long time been kept below economically damaging levels in the absence of pesticides used for other pests. Prior to the mid 70s, a number of classical biocontrol introductions were effected in South Africa to control mealybugs. During this period, some non-indigenous natural enemies of mealybugs also emerged in the region through unspecified means. However, in the late 70s the onset of organophosphate pesticides led to the disruption of these natural enemies. Their disruption then resulted in outbreaks of mealybug pests. Pesticide sprays delay the build up of natural enemies at the beginning of each growing season on citrus, resulting in mealybug populations getting established on the young fruit (Hattingh *et al.* 1998).

In the absence of crop sprays, a wide range of natural enemies are known to control outbreaks of mealybug species. In a survey of natural enemies associated with mealybug species on citrus in South Africa, Hattingh *et al.* (1994) in Hattingh *et al.* (1998) found *Anagyrus* sp. and *Coccidoxenoides peregrinus* as the most widespread and numerous parasitoid species. As for predators, coccinellids *Nephus reunion*, *Exochomus* spp. and the Australian ladybird, *Cryptolaemus montrouzieri* were recorded. They also found chrysopids and hemerobiids as well as larvae of Diptera and Cecidomyiidae to be important in regulating mealybug population outbreaks.

In their study of natural enemies of three mealybug pests of citrus in the Western Cape of South Africa, Wakgari & Giliomee (2003) found the encyrtid wasps, *Anagyrus pseudococci*, *Anagyrus* sp. and *Coccidoxenoides peregrinus* to be the most important primary parasitoids of *Planococcus citri* and *Pseudococcus calceolariae* while *Anagyrus pseudococci* and *Anagyrus* sp. were the

most dominant parasitoids of *Pseudococcus longispinus*. They also found *Anagyrus* sp. attacking *Pseudococcus viburni* on apple (Wakgari & Giliomee 2004). Walton & Pringle (2004) also observed that *Anagyrus* sp. and *Coccidoxenoides perminutus* were two of the four active parasitoids of the vine mealybug, *Planococcus ficus*, in the Western Cape. *Cryptolaemus montrouzieri*, *Nephus* spp. and *Chrysopa* spp. were recorded predators.

Biological control of mealybug species infesting citrus and other crops in Africa has also been carried out in other parts of the world using similar natural enemies. In southern Australia, the indigenous *Anagyrus fusciventris* was found to be one of the four primary parasitoids of *Pseudococcus longispinus* while its main predators were *Chrysopa* sp. and another coccinellid (Furness 1976). In a study conducted by Bartlett (1957) in California on *Planococcus citri*, the ladybird beetle, *Cryptolaemus montrouzieri*, the brown lacewing, *Sympherobius californicus*, and the green lacewing, *Chrysopa plorabunda*, were among the six predators associated with the citrus mealybug. Another species of parasitoid, *Leptomastix dactylopii* was also associated with this mealybug. The significance of *Leptomastix dactylopii*, as an effective parasitoid of *P. citri* was also pointed out by Smith *et al.* (1988) in Queensland, Australia. In the Western Cape, South Africa, this parasitoid was, however, associated with the vine mealybug, *Planococcus ficus* (Walton & Pringle 2002).

Augmentative releases of *L. dactylopii* and *C. montrouzieri* on *P. citri* were reported to be effective in several Mediterranean countries, *e.g.* Italy, Spain, Greece and Turkey (Franco *et al.* 2004). The augmentation of biocontrol agents with *L. dactylopii* or *C. montrouzieri* has also been tested with positive results in citrus growing areas such as Australia and California (Franco *et al.* 2004). Despite the abundance and diversity of indigenous and exotic biocontrol agents, there is no information on the natural enemies of *P. burnerae*.

The objectives of this thesis are five fold:

To study the biology (fecundity, developmental rate and survival) of *P. burnerae* at five different constant temperatures.

To investigate whether differences in developmental rates are the reason why the oleander mealybug has been outcompeting the citrus mealybug in the Eastern and Western Cape.

To establish the biology of *P. burnerae* under South African climatic conditions in the field.

To determine the species composition and abundance of the natural enemies of *P. burnerae* in order to identify species suitable for its biological control.

To establish the suitability of citrus, butternuts and sprouting potatoes as food sources for mass rearing of *P. burnerae*.

Voucher specimens of *P. burnerae* with accession number HC 7099 have been deposited at the National Collection of Insects, Biosystematics Division, Agricultural Research Council, Roodeplaat, South Africa.

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CHAPTER 2

DEVELOPMENTAL BIOLOGY OF THE OLEANDER MEALYBUG, *PARACOCCUS BURNERAE* (BRAIN) (HEMIPTERA: PSEUDOCOCCIDAE) AT FIVE TEMPERATURES ON CITRUS

2.1 ABSTRACT

The effect of constant temperatures on the development, survival and fecundity of the oleander mealybug, *Paracoccus burnerae* on citrus was determined. Developmental time, rate of development, fecundity and survival were investigated at five constant temperatures and a 16L: 8D light: darkness regime. The rate of development increased linearly with an increase in temperature for the egg, 1st nymphal and pupal stages as well as the entire biological cycle (egg – adult), but was nonlinear for the 2nd and 3rd nymphal stages. Survival decreased with an increase in temperature. *P. burnerae* required 666.7 degree-days above a lower threshold of 8.7°C to complete one generation. The highest mean number of 68 eggs per female was reached at 22°C. A sex ratio of 0.52:0.48 (male:female) was obtained from the life table. The net reproductive rate (R_0) was >1 at all five temperatures, an indication that it is capable of increasing its population numbers despite the high mortality experienced in the 1st and 2nd nymphal stages.

2.2 INTRODUCTION

The oleander mealybug, *Paracoccus burnerae* (Brain) is indigenous to the Southern and Eastern African Subregions. It was first described by Brain (1915) and redescribed by De Lotto (1967). The Pseudococcidae, to which *P. burnerae* belongs, comprises approximately 2000 species in 300 genera (Ben-Dov 1994), of which 20 species are pests of cultivated plants in South Africa

(Millar 2002), with the oleander (*Paracoccus burnerae*), karoo thorn (*Nipaecoccus vastator*) and citrus (*Planococcus citri*) mealybugs being the most important mealybug pests on citrus (Hattingh 1993).

According to Hattingh *et al.* (1998), *P. burnerae* only became rampant during the early 1990s. Reasons for the increase in prevalence of *P. burnerae* are not yet known. However, it is now found in all citrus growing zones of southern Africa and Hattingh *et al.* (1998) describe it as being problematic in Natal and Swaziland. It was also until recently a widespread and dominant pest of citrus in some parts of the Eastern and Western Cape of South Africa (Moore & Kirman 2005). Its presence on citrus fruits results in reduced quality. However, the main concern to growers is that in view of its endemic status in South Africa, the presence of this species on fruit is unacceptable to citrus importing countries, particularly the USA. Due to phytosanitary concerns raised in the fruit export sector, *P. burnerae* has now become one of three mealybug species with a quarantine status (Wakgari & Giliomee 2003). It does not only affect citrus, but is also known to attack plants in a wide range of families (Ben-Dov *et al.* 2001).

Although parthenogenesis is common in mealybugs, *P. burnerae* reproduces sexually. Both the neotenic adult female and the alate male have three nymphal instars. There is no observable sex differentiation in the first two instars, but in the third instar the male has the appearance of a pupa. As an ectotherm and multivoltine pest species, *P. burnerae* populations survive low winter temperatures in the cracks of trees and inside curled leaves (Hattingh *et al.* 1998). In ectotherms, temperature is the dominant factor affecting development rate. When the temperature is low, development occurs at a much slower rate than at high temperature (Jarosik *et al.* 2004), and subsequently time spent in each developmental stage increases. The relationship between temperature and development in insects is often linear but can also be sigmoid. Linear models are good for estimation of the developmental thermal constant (Kontodimas *et al.* 2004). Both thermal constant (K) and lower developmental threshold (T_0) are frequently employed to explain how insect development is dependent on temperature (Aysal & Kivan 2008). However, complex

and theoretically advantageous models have been used to depict the nonlinear relationship between rate of development and temperature (Jones *et al.* 2005). Nonlinear models can be used to estimate optimum and upper lethal temperatures for development, but not the thermal constant (Kontodimas *et al.* 2004). In this study, the nonlinear model of Briere *et al.* (1999) was used to estimate the lower developmental threshold, optimum temperature and upper lethal temperature for development. Preference was given to this model because not only does it describe the relationship between temperature and development, but also attempts to explain the relationship in terms of physiological and biochemical mechanisms (Kontodimas *et al.* 2004).

Life tables and developmental rates are essential tools for investigating and understanding the impact of temperature on growth, survival, reproduction and rate of increase of an insect population. Life tables are especially important in understanding age dynamics of adult populations studied under controlled laboratory conditions (Aysal & Kivan 2008) and in tackling the issue of life expectancy when affected by environmental changes (Pilkington & Hoddle 2007).

The objective of the present study was to determine the developmental biology of *P. burnerae* at a range of constant temperatures as well as to construct an age-specific life table.

2.3 MATERIAL AND METHODS

Laboratory cultures of oleander mealybug were obtained from a colony reared on citrus in the greenhouse at the Department of Botany and Zoology of the University of Stellenbosch.

Fecundity, development time and survival of *P. burnerae* were determined at 18, 20, 22, 25 and 27°C using growth chambers with humidity varying between 60 and 90% and a light:darkness cycle of L16:D8.

Ovipositing females were obtained from the greenhouse and introduced onto potted citrus seedlings. The seedlings were then incubated and the females allowed at least 24 hours to lay eggs before being removed. A maximum of 10 eggs from each female ovisac were retained on each of the five experimental seedlings. The development of each individual was observed under a stereomicroscope and recorded daily. Survivorship of the cohort was followed and recorded until the last surviving individual died.

When females reached adulthood and there were no males available from the same cohort within the growth chamber, they were provided with males from the greenhouse. Once mating was achieved and formation of the ovisac observed, egg counting was conducted daily under a stereomicroscope until no further oviposition took place.

2.3.1 Data analysis

Development time, fecundity and survival data were used to construct a life table and survivorship curves for *P. burnerae*. For the duration of the life span of *P. burnerae*, l_x , the proportion of the cohort alive on day x , and m_x , the mean number of eggs produced by a female on day x , were determined at 18, 20, 22, 25 and 27°C. Both l_x and m_x were used to calculate the net reproductive rate (R_0)

using $\sum_{x=1}^t l_x m_x$, where t = time in days. The mean generation time (T)

was calculated as described by Price (1997). These values were then used to derive the intrinsic rate of natural increase (r_m), using the formula as in Price (1997) where $r_m = [\ln(R_0)]/T$.

The derived r_m value was then used as the first estimate for solving the following equation iteratively (Watson 1964):

$$\sum_{x=1}^t e^{-r_m x} l_x m_x = 1, x=1,2,3,\dots, t \text{ days}$$

where x is age in days, m_x is the mean number of eggs produced by a female during age interval x and l_x is the proportion of females alive at age x .

The iterations were continued until the left-hand side of the equation was within 0.0001 of the right-hand side.

The inverse of the mean developmental times for the egg, 1st nymphal instar, male pupa and the biological cycle (egg to adult) was regressed on temperature resulting in the equation, $1/\text{time} = 1$.

Linear regression was only used for these stages and the biological cycle because the probability of linearity in their regressions was highly significant. The nonlinear model by Briere *et al.* (1999) was used to describe the relationship between rate of development for the 2nd and 3rd nymphal instars as well as the biological cycle.

The following standard thermal indices as defined by Kontodimas *et al.* (2004) were calculated, where appropriate, for each of the 7 developmental models (4 linear and 3 nonlinear):

- *The lower developmental threshold (T_0)*. The minimum temperature at which the rate of development is zero or there is no measurable development. It may be estimated by some nonlinear and by linear models as the intercept value of the temperature axis.
- *The upper lethal temperature (T_L)*. The maximum temperature at which the rate of development is zero or life cannot be maintained for a prolonged period. This is estimated by most nonlinear models.
- *The optimum temperature for development (OT)*. The temperature at which the rate of development is maximum. It may be estimated directly by the equations of some nonlinear models, or as the parameter value for which their 1st derivatives equals zero.

- *The thermal constant (K)*. The amount of thermal energy (degree-days) needed to complete development. The thermal constant (K) or degree-days (DD) can be estimated only by the linear equation as the reciprocal of the slope b , $DD = 1/b$.

2.4 RESULTS

2.4.1 Developmental times

Developmental time increased with a decrease in temperature (Table 1) and was significantly different between stages of development ($F = 5.759$, $df = 16$, $P = <0.001$). Females developed somewhat faster than males at all temperatures except 27°C. Developmental time was longer in the 1st nymphal instar than the 2nd and 3rd nymphal instars at all five temperatures except at 27°C for the 3rd nymphal instar. A positive linear relationship was obtained when the rate of development was regressed on temperature for the egg, 1st nymphal and pupal stages as well as the biological cycle. The rate of development was significantly influenced by temperature ($F = 25.953$, $df = 1, 3$, $P = 0.015$) (Fig. 1). In the 2nd and 3rd nymphal instars, the relationship was linear below 22°C, but became nonlinear above this temperature (Fig. 2). From the nonlinear model predictions, it was observed that the 2nd and 3rd nymphal instars could only develop above lower developmental thresholds of 10 and 14.8°C respectively. For the egg stage, the estimated lower developmental threshold (T_0) was 13.3°C and the thermal constant (K) 85.5 degree-days (Table 2).

Table 1. Developmental times in days (mean±SD) for each stage of *Paracoccus burnerae* on citrus at five constant temperatures

Developmental stage	Temperature (°C)				
	18	20	22	25	27
Egg	15.0 (1.37)	12.7 (1.1)	11.2 (1.3)	8.2 (1.3)	5.6 (0.21)
First instar	20.2 (2.54)	16.6 (3.5)	16.3 (2.1)	12.7 (2.6)	10.7 (2.3)
Second instar	16.9 (2.91)	12.2 (2.4)	10.3 (2.3)	9.9 (2.12)	9.5 (2.3)
Third instar (♀)	19.3 (2.08)	14.8 (2.6)	10.5 (0.6)	8.4 (1.7)	11.5 (3.0)
Male pupa	21.3 (0.52)	17.0 (1.4)	11.3 (1.2)	11.0 (0.6)	8.8 (1.2)
Adult male	4.8 (1.1)	*	*	4.3 (0.82)	2.4 (0.92)
Adult female	51.0 (14.1)	48.0 (6.73)	44.0 (5.66)	39.0 (9.86)	33.5 (3.32)
Egg to adult (♀)	71.4 (2.23)	56.3 (2.02)	48.2 (2.86)	39.1 (2.09)	37.7 (2.64)
Egg to adult (♂)	73.4 (1.82)	58.5 (2.52)	49.0 (2.74)	41.7 (1.91)	34.9 (2.24)
Egg to adult (♂+♀)	72.4 (1.41)	57.4 (1.6)	48.6 (0.6)	40.4 (1.9)	35.9 (1.9)

Note: * undetermined

Table 2. Thermal constant values and regression coefficients for immature stages and the biological cycle of *Paracoccus burnerae*

Stage	Regression equation	LDT (°C)	DD	R ²	P
Egg	-0.1556 + 0.0117x	13.3	85.5	0.8964	<0.02
1 st instar	-0.0361 + 0.0047x	7.7	212.8	0.9527	<0.005
Pupa	-0.0798 + 0.0071x	11.2	140.8	0.9283	<0.009
Egg-adult	-0.0130 + 0.0015x	8.7	666.7	0.9970	<0.001

LDT = Lower developmental threshold; DD = degree-days

The linear regression estimate of the lower developmental threshold for the 1st nymphal stage was lower than that for the egg and pupal stages. *P. burnerae* would require 666.7 degree-days above a lower developmental threshold of 8.7°C to complete one generation (egg to adult) (Table

2). Nonlinear regression estimates of the lower developmental threshold, optimum temperature and upper lethal temperature at 95% confidence interval are given in Table 3.

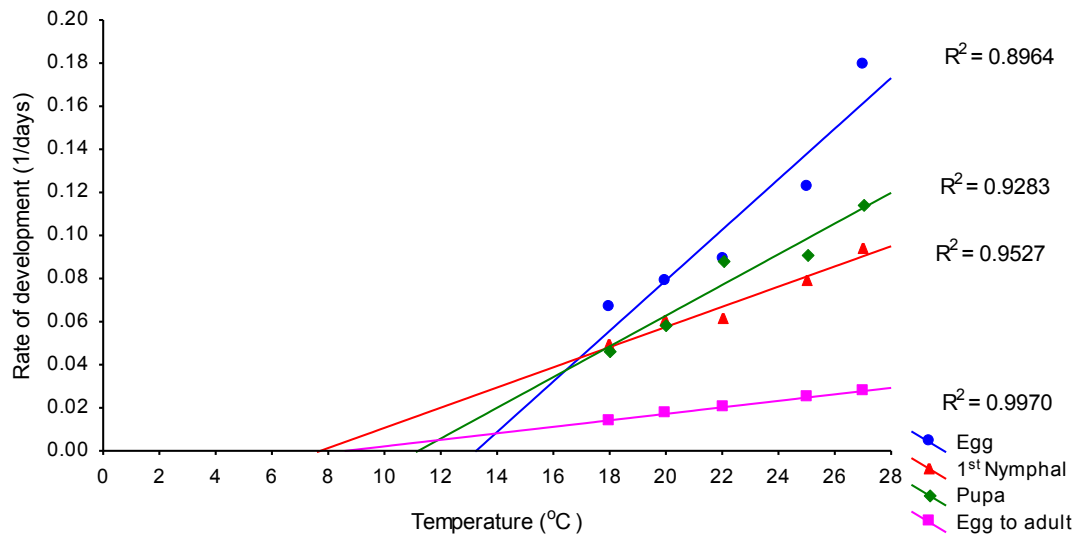


Fig. 1. Developmental rate of *Paracoccus burnerae* for the egg, 1st nymphal and pupal stages as well as biological cycle (egg – adult) at five constant temperatures (18, 20, 22, 25 and 27°C)

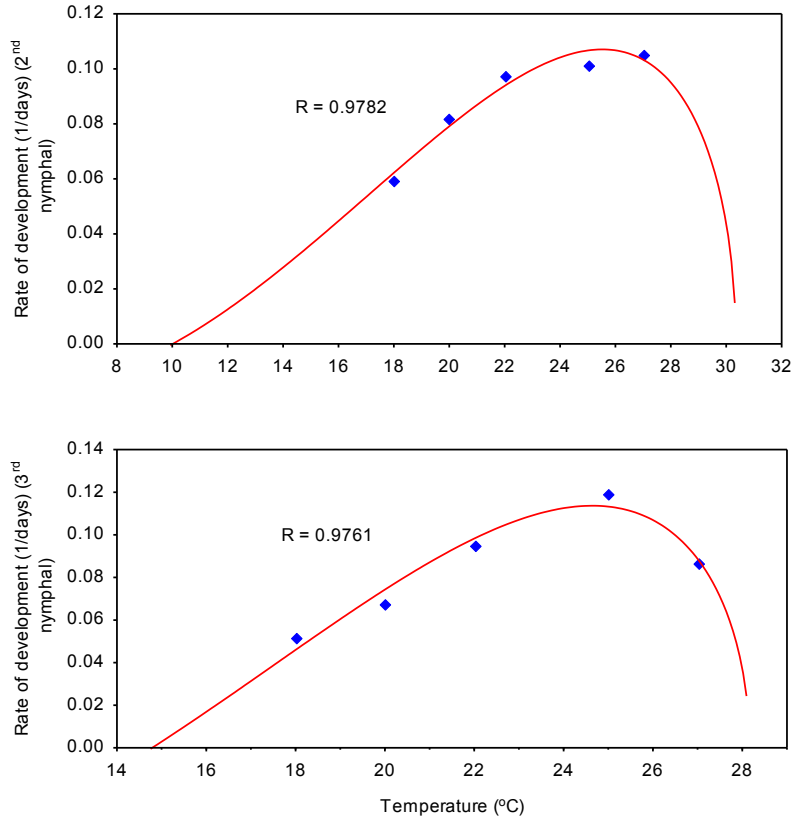


Fig. 2. Developmental rate of *Paracoccus burnerae* for the 2nd and 3rd nymphal instars at five constant temperatures (18, 20, 22, 25 and 27°C)

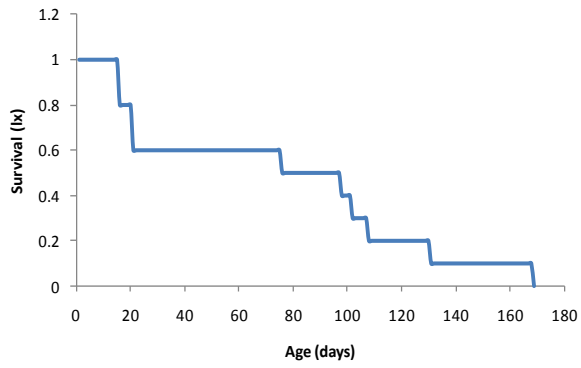
2.4.2 Survival, fecundity, population growth and life table parameters

Survival was much longer with lower mortality at 18°C than at the other temperatures (Fig. 3). It decreased with an increase in temperature from 18 to 27°C. Adults experienced heavy mortality at higher temperatures. Female longevity was longer than that of males at all the five temperatures (Table 4). The mean number of eggs/female (fecundity) was highest at 22°C (Fig. 4). Analysis of variance (ANOVA) showed no significant differences in the mean number of eggs per female ($F = 0.974$, $df = 4, 10$, $P = 0.464$) at the different temperatures.

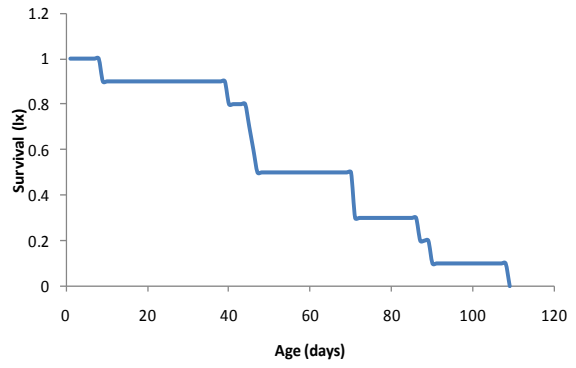
A life table was constructed to show the number of *P. burnerae* immatures entering adulthood as well as their survival and mortality rates (Table 5). According to the life table data, 18% of

females survived to adulthood. The 1st and 2nd nymphal stages were the most vulnerable. The net reproductive rate (R_0) and intrinsic rate of natural increase (r_m) were both highest at 22 and 25°C respectively. The mean generation time (T) decreased with an increase in temperature (Table 4). An adult sex ratio of 0.52:0.48 (male: female) was obtained from the life table (Table 5).

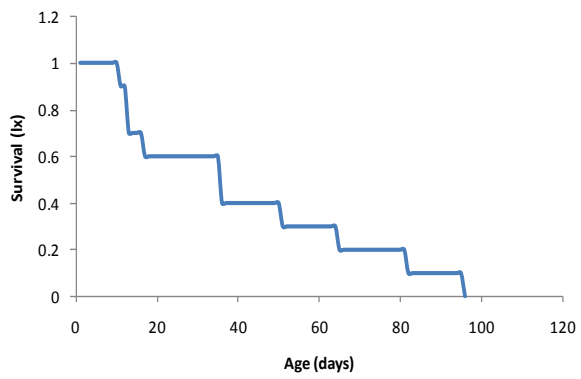
18°C



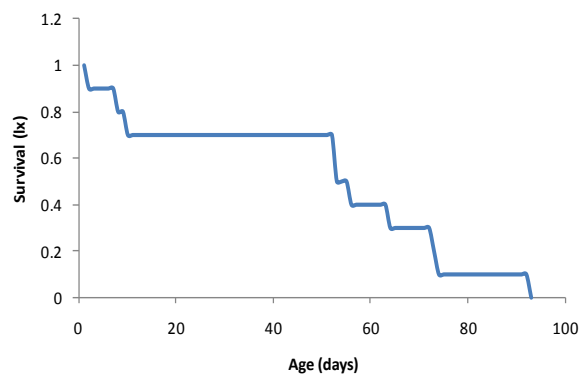
20°C



22°C



25°C



27°C

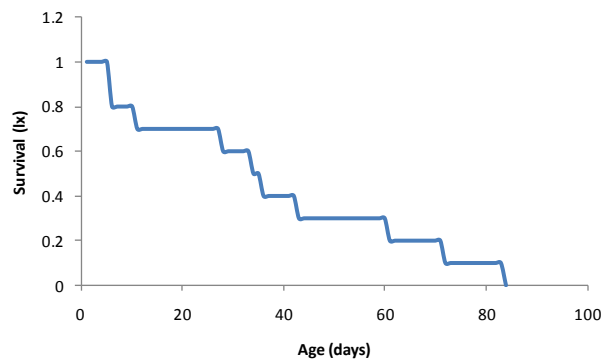


Fig. 3. Age specific survival (l_x) of *Paracoccus burnerae* at five constant temperatures

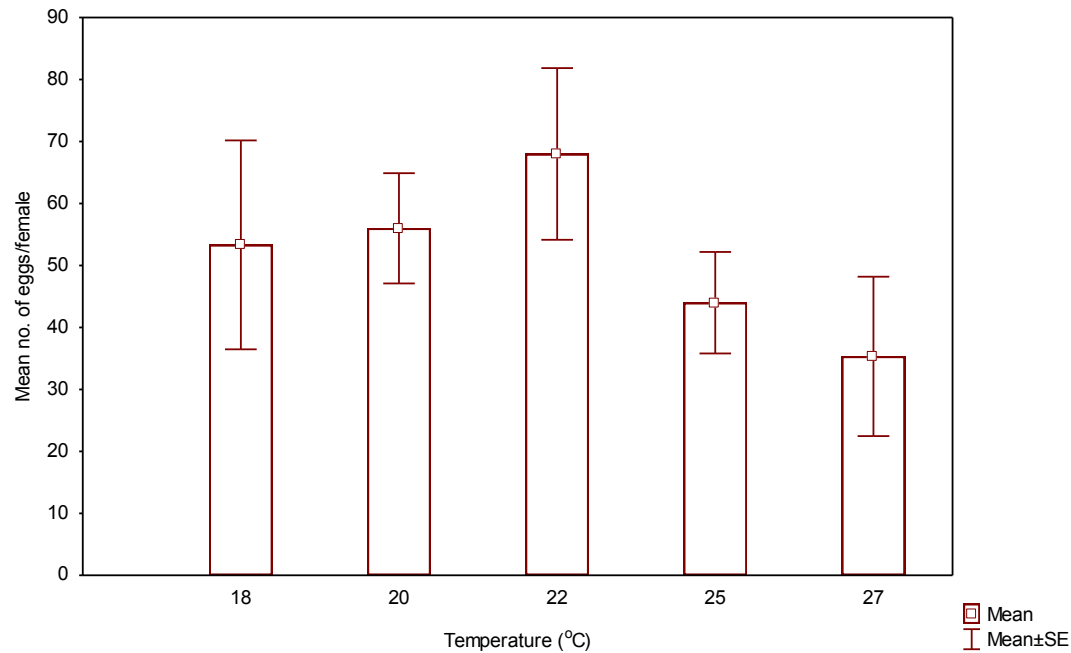


Fig. 4. Mean no. of eggs per female of *P. burnerae* at five constant temperatures

Table 3. Nonlinear regression estimates of developmental rates of 2nd and 3rd nymphal instars and the biological cycle of *P. burnerae* on temperature

Stage	T_0 (95% conf. int.) (°C)	T_L (95% conf. int.) (°C)	Optimum (°C)
2 nd instar	10.01	30.35	25.53
3 rd instar	14.79	28.16	24.64
Biological cycle	6.33	37.9	31.02

Table 4. Mean generation time (T), intrinsic rate of increase (r_m), net reproductive rate (R_o) and longevity of *P. burnerae* at 5 different constant temperatures

Temperature (°C)	R_o	r_m	T	Longevity (days)	
				Male	Female
18	8.7	0.024	90.6	4.8	51
20	14.1	0.032	82	*	48
22	15.1	0.039	69	*	44
25	13	0.045	56.9	4.3	39
27	8.37	0.037	57.1	2.4	33.5

* Longevity undetermined

Table 5. Life table for *P. burnerae* at five different constant temperatures. Data based on a cohort of 165 individuals

Stage	Initial no. of insects (nx)	No. dying	Mortality (dx)	Survival (lx)
Egg	165	32	0.194	0.806
Instar I-II	133	53	0.398	0.602
Pupa + Instar III	80	18	0.225	0.775
Adults	62	32	0.516	0.484
Sex ratio: (♂:♀)				
0.52:0.48				
Adult females	30			
Total		135	81.82	0.1818

2.5 DISCUSSION

Temperature had a marked effect on the development, survival and fecundity of *P. burnerae*. The egg, 1st nymphal, male pupal stages and egg to adult revealed a linear relationship between rate of development and temperature. No optimum and upper lethal temperatures (T_L) were obtained in the linear regression, as there was no turning point in the graphs for these stages. Of the four developmental stages, the egg stage had the highest rate of development and the highest lower developmental threshold (Fig. 1), which is typical of warm adapted species. Tropical species have been found to have higher lower developmental thresholds (Honek 1996). The crawlers (1st instar), on the otherhand, behaved more like a cold adapted species because they displayed the lowest rate of development and a very low lower developmental threshold. Having a very low lower developmental threshold in the most critical stage of mealybug development i.e., the dispersal phase, is an advantage for the survival of *P. burnerae* as it is able to withstand the cold climatic conditions associated with winter in some citrus growing regions.

The relationship between the rate of development and temperature for the 2nd and 3rd instars was linear within the lower experimental temperatures, but became nonlinear as the temperatures approached the optimum around 24 - 26°C (Fig. 2). These temperatures lie in the range of 22–28°C reported as optimal for insects of tropical origin by Nava *et al.* (2007). The optimum temperature of 31.02°C (Fig. 5) for the biological cycle and estimated from the nonlinear model was, however, outside the range of 22–28°C earlier observed as being optimal for insects from tropical conditions (Nava *et al.* 2007). Since the rate of development for *P. burnerae* was low and its thermal constant high in the entire biological cycle, a development very similar to cold adapted (temperate) species, there is a likelihood that it has broadened its thermal window over the years as a result of being exposed to a cooler climate.

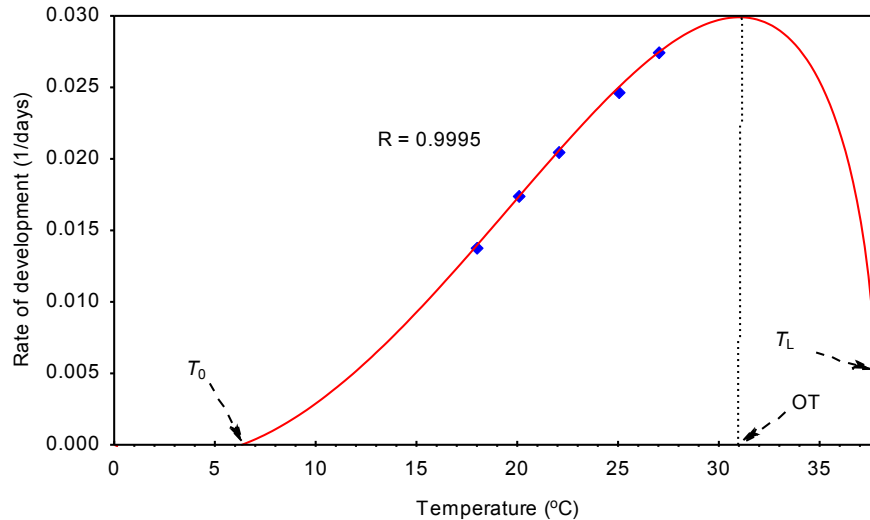


Fig. 5. Developmental rate of *Paracoccus burnerae* for the entire biological cycle (egg – adult) at five constant temperatures OT = Optimum temperature; T_L = Upper lethal temperature and T_0 = Lower developmental threshold

Survival increased at lower temperatures compared to higher temperatures in the immature stages (Fig. 3). The development time also decreased with an increase in temperature from 18 to 27°C. At lower temperatures this is expected primarily because the immature stages convert less food into body tissue resulting in low growth and thus longer developmental times (Atkinson & Sibly 1997). At higher temperatures, crawlers were observed to migrate from the leaves to the stems where they hid in cracks for a few days until the mealy, waxy layer thickened before migrating back to the leaves to begin feeding. This behaviour could have been one way of avoiding the effects of desiccation soon after hatching.

In similar studies on the developmental times of *P. burnerae* on citrus at 27°C, Cilliers & Bedford (1978) found slightly longer developmental times than those obtained in this study for the 1st, 2nd and 3rd nymphal instars, but shorter developmental times for the egg stage. The latter could be attributed to incubation of slightly older eggs by these authors. In this study, freshly laid eggs were used for all experiments.

The results show that temperature can affect the fecundity of *P. burnerae*. Fecundity was highest at 22°C, slightly lower at 18°C and much lower at 27°C. The fairly low fecundity experienced by *P. burnerae* at both low and high temperature could have been due to a decreased intake of food, resulting in increased maintenance costs. At temperatures between 20 and 24°C, there was probably a rise in food levels after maximum consumption was attained, resulting in decreased feeding activity and more energy being available for growth and reproduction. A reason for the generally low fecundity could have been the disturbances experienced during the daily counting of eggs as the females were irritated and had to form new ovisacs. In this process some energy was directed at producing the mealy wax instead of eggs. The movement of the seedlings caused by the constant air flowing into the growth chambers also made it difficult for the females to settle down after egg counting.

In the life table constructed from developmental time, survival and fecundity data, the net reproductive rate (R_0) of *P. burnerae* was found to be >1 at all five temperatures, an indication that it is capable of increasing its population numbers despite encountering high mortality levels in the 1st and 2nd nymphal instars.

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CHAPTER 3

IS THERE A LINK BETWEEN DEVELOPMENTAL RATE AND OCCASIONAL DOMINANCE OF THE OLEANDER MEALYBUG, *PARACOCCUS BURNERAE* (BRAIN) (HEMIPTERA: PSEUDOCOCCIDAE) ON CITRUS IN SOUTH AFRICA? †

3.1 ABSTRACT

The oleander mealybug, *Paracoccus burnerae* (Brain) is a pest of citrus in South Africa. This study was carried out to determine the effect of temperature on development rate of *P. burnerae* and to investigate whether development rate is the reason why *P. burnerae* is outcompeting the citrus mealybug, *Planococcus citri* (Risso), in the Eastern and Western Cape Provinces of South Africa. The influence of temperature on life history traits of *P. burnerae* was determined at 20, 22, 25 and 27°C and compared with corresponding data for *P. citri*. The rate of development increased linearly with an increase in rearing temperature in the embryonic, first nymphal and pupal stages but reached a climax at 26.13 and 28.6°C in the second nymphal stage of both species, respectively. *P. citri* exhibited lower developmental thresholds except in first instar, shorter degree-days and higher developmental rates than *P. burnerae*. Results of the current study indicated that the dominance of oleander mealybug over the citrus mealybug is neither linked to developmental rates nor sum of effective temperatures.

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3.2 INTRODUCTION

The oleander mealybug, *Paracoccus burnerae* (Brain) (Hemiptera: Pseudococcidae) is an afro-tropical species endemic to both southern and eastern Africa but has also been recorded in India (Ben-Dov *et al.* 2001). Since its discovery in the early 1900s in the (then) Transvaal Province of South Africa (Brain 1915), *P. burnerae* has managed to spread and establish in some parts of the country where temperate climatic conditions prevail.

P. burnerae is a pest of economic importance on citrus, deciduous fruit crops and a number of plant species due to its sap sucking activity on leaves, stems and fruits, as well as the large quantities of honeydew that it produces. The honeydew attracts a sooty mould fungus which reduces photosynthesis and blemishes fruit. Due to its endemic status, *P. burnerae* is also a pest of quarantine importance to the fruit export sector (Wakgari & Giliomee 2003).

The status of *P. burnerae* as a serious pest only became evident in the early 1990s in Swaziland and Natal (Hattingh *et al.* 1998). Subsequently, it became the most dominant mealybug species on citrus in some parts of the Eastern and Western Cape Provinces of South Africa (Moore & Kirkman 2005) during certain years. Previously, the citrus mealybug, *Planococcus citri* (Risso) was the most widespread and economically destructive mealybug species on citrus (Hattingh *et al.* 1998; Wakgari & Giliomee 2003). Reasons for the increase in prevalence of *P. burnerae* remains unknown (Hattingh *et al.* 1998) but may be due to a differential response to prevailing climatic conditions.

Temperature is considered to be the major external factor affecting the ability of an insect pest to establish and proliferate in a new area (Pilkington & Hoddle 2006). It is known to determine the rate of development in insects (Jarosik *et al.* 2004) and computations of the thermal constant (K) therefore allow for life stage duration estimates (Pilkington & Hoddle 2006). Thermal constants

are often used to generate predictive models of insect pest development in diverse environments, including stored products, greenhouses and orchards (Malina & Praslicka 2008).

The objectives of this study were to compare the effects of temperature on developmental rates of *P. burnerae* and *P. citri* as well as to investigate whether differences in developmental rates are the reason why the oleander mealybug has been occasionally outcompeting the citrus mealybug in the Eastern and Western Cape.

3.3 MATERIAL AND METHODS

Laboratory cultures of oleander mealybug, *P. burnerae*, were obtained from a colony reared on citrus in the greenhouse at the Department of Botany and Zoology, University of Stellenbosch.

Development time and rate of *P. burnerae* were determined at 20, 22, 25, and 27°C using growth chambers with relative humidity (r.h.) varying between 60 and 90% and a photoperiod of L16:D8.

Pre-ovipositing females obtained from the greenhouse were introduced onto potted citrus seedlings and were allowed to lay eggs for at least 24 hours before being withdrawn. The seedlings together with the eggs were then incubated in growth chambers at the temperatures and r.h. indicated above. A maximum of 10 eggs from each female ovisac was retained on each of the five experimental seedlings used as replicates at each of the four temperatures. The development of each individual was observed under a stereomicroscope and recorded daily.

The lower developmental threshold temperature (T_0) was determined by regressing $1/t$ on temperature, where t = time in days, for both *P. burnerae* and *P. citri* using Statistica 8.0

statistical package and then solving the regression equation $1/t = 1$, for both biological cycles and all the immature stages except the 2nd and 3rd nymphal stages. The nonlinear model described in Briere *et al.* (1999) was used to estimate the minimum, optimum and upper lethal temperatures at $\pm 95\%$ confidence interval for both the 2nd and 3rd nymphal stages of *P. burnerae*. The rate of development of the immature stages and total development time from egg to adult on citrus for both *P. burnerae* and *P. citri* were regressed on temperature to compare their rates of development at the four temperatures i.e., 20, 22, 25 and 27°C. Mean developmental time means were used in both linear and nonlinear regression models. This approach has also been adapted by Infante (2000); Walton & Pringle (2004); Aysal & Kivan (2008) and Blomefield & Giliomee (2009). The number of degree-days (DD) needed for development by both species was calculated using $DD=1/b$, where b is the slope of the regression equation. The data for *P. citri* were adapted from Arai (1996) who determined the effect of temperature on its developmental time on citrus. Comparing data for *P. burnerae* and *P. citri* from different countries is considered justified since both sets of data were obtained under controlled conditions. These experiments were carried out from February to December 2009.

3.4 RESULTS

Mean developmental times decreased with an increase in temperature from 20 to 27°C for both species (Tables 1 and 2) except for the third nymphal instar of *P. burnerae* where the developmental time increased at 27°C, showing a negative effect of higher temperature on development. The developmental time at 27°C (11.5 d) was longer than at 25°C (8.38 d) (Table 1). *P. citri* had the shortest developmental times in the embryonic stage at all four temperatures (Table 2). The decrease in developmental time with an increase in temperature was more pronounced with *P. burnerae* than *P. citri*.

Table 1. Mean (\pm SD) duration (days) of the development of *Paracoccus burnerae* incubated at four constant temperatures

Developmental stage	Temperature ($^{\circ}$ C)			
	20	22	25	27
Egg	12.67 (1.1)	11.16 (1.3)	8.17 (1.3)	5.58 (0.7)
1 st nymphal	16.6 (3.5)	16.3 (2.1)	12.7 (2.6)	10.65 (2.3)
2 nd nymphal	12.23 (2.4)	10.25 (2.3)	9.86 (2.12)	9.5 (2.3)
3 rd nymphal ($\text{\textcircled{f}}$)	14.8 (2.6)	10.5 (0.6)	8.38 (1.7)	11.5 (3.0)
Pupa ($\text{\textcircled{m}}$)	17.0 (1.4)	11.3 (1.2)	11.0 (0.6)	8.75 (1.2)
Egg to adult	57.4 (1.6)	48.61 (0.6)	40.42 (1.9)	35.86 (1.9)

Table 2. Mean (\pm SD) duration (days) of the development of *Planococcus citri* incubated at four constant temperatures

Developmental stage	Temperature ($^{\circ}$ C)			
	20	22	25	27
Egg	5.6 (1.5)	3.9 (1.8)	4.0 (2.1)	3.2 (1.3)
1 st nymphal	13.65 (0.5)	9.25 (0.1)	7.8 (0.3)	7.35 (0.2)
2 nd nymphal	9.35 (2.1)	7.15 (1.6)	6.95 (1.2)	6.55 (1.8)
3 rd nymphal ($\text{\textcircled{f}}$)	11.1 (1.7)	10.6 (2.7)	9.7 (3.6)	8.1 (1.8)
Pupa ($\text{\textcircled{m}}$)	12.3 (0.8)	9.2 (0.6)	8.7 (0.9)	7.0 (0.7)
Egg to adult	41.2 (2.1)	30.2 (1.0)	27.95 (0.7)	24.65 (0.8)

Data adapted from Arai (1996)

The development rates for both *P. burnerae* and *P. citri* increased with an increase in rearing temperature from 20 to 27°C with the third instar in *P. burnerae* being an exception to this observed trend. The relationship between temperature and developmental rate was positively linear in the embryonic, 1st instar and pupal stages (Fig. 1) but statistically weaker for *P. burnerae* in the pupal stage (Tables 3 and 4). The developmental rate was also positively correlated with temperature for the entire duration of the biological cycle (egg – adult) in both species (Fig. 1).

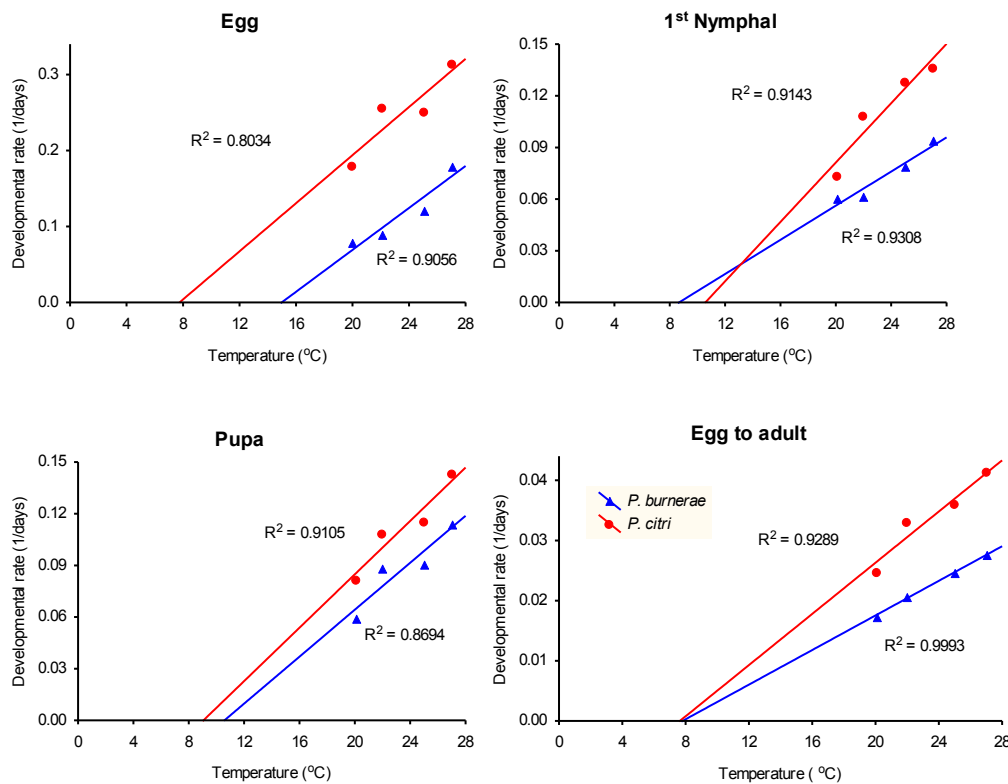


Fig. 1. Relationship between developmental rate and temperature for the egg, 1st nymphal and pupal stages and biological cycle (egg-adult) of *P. burnerae* and *P. citri*. Data for *P. citri* adapted from Arai (1996)

Table 3. Regression of developmental rates of immature stages and biological cycle (egg-adult) of *P. burnerae* on temperature, lower developmental thresholds (LDT) and degree-days (DD)

Stage	Regression equation	LDT (°C)	DD	R ²	P
Egg	-0.2068 + 0.0138x	15	72.5	0.9056	<0.05
1 st instar	-0.043 + 0.005x	8.6	200	0.9308	<0.05
Pupa	-0.0721 + 0.0068x	10.6	147.1	0.8694	>0.05
Egg-adult	-0.0112 + 0.0014x	8	714.3	0.9993	<0.01

Table 4. Regression of developmental rates of immature stages and biological cycle (egg-adult) of *P. citri* on temperature, lower developmental thresholds (LDT) and degree-days (DD)

Stage	Regression equation	LDT (°C)	DD	R ²	P
Egg	-0.1227 + 0.0158x	7.8	63.3	0.8034	>0.05
1 st instar	-0.0911 + 0.0086x	10.6	116.3	0.9143	<0.05
Pupa	-0.0702 + 0.0078x	9.0	128.2	0.9105	<0.05
Egg-adult	-0.0162 + 0.0021x	7.7	476.2	0.9289	<0.05

Data adapted from Arai (1996)

The relationship between temperature and developmental rate of the second nymphal instar of both species was nonlinear (Fig. 2) but did conform to a linear model within a certain range of temperatures before the graphs leveled off with the estimated optimum and upper lethal temperatures for development of *P. burnerae* and *P. citri* as shown in Table 5. In the third nymphal instar, the relationship between temperature and developmental rate was nonlinear for *P. burnerae* (Fig. 3) while that for *P. citri* was exponential. The estimated optimum and upper lethal temperatures for third instar of *P. burnerae* were 24.74 and 27.9 °C respectively (Table 5).

Since *P. citri* exhibited some kind of exponential growth, no estimates of the optimum and upper lethal temperatures were obtained. A linear model obtained from the *P. citri* dataset was unable to give a minimum temperature for development. Heat units estimated from the linear regressions revealed that *P. burnerae* required 714.3 degree-days (DD) and *P. citri*, 476.2 degree-days (DD) from egg to adult (Tables 3 and 4).

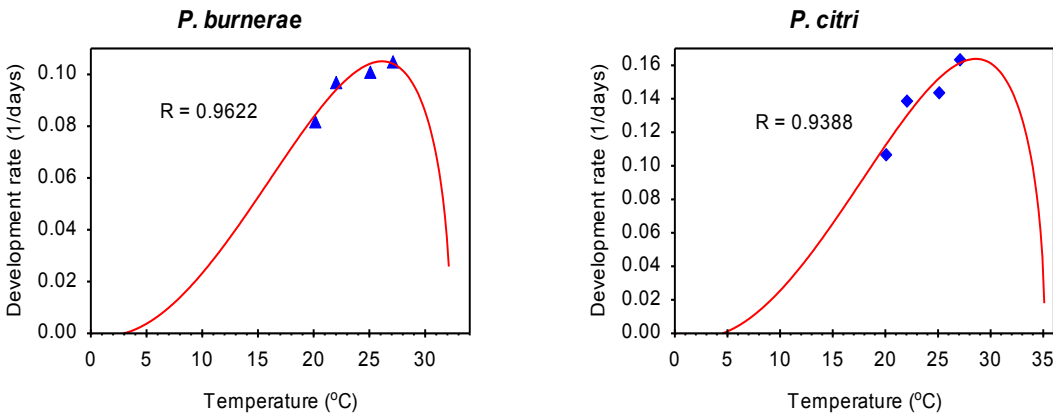


Fig. 2. Relationship between developmental rate and temperature for the 2nd nymphal instar of *P. burnerae* and *P. citri*. Data for *P. citri* adapted from Arai (1996)

Table 5. Non-linear regression estimates of developmental rates of immature stages of *P. burnerae* and *P. citri* on temperature

Stage	T_0 (\pm 95% conf. int.) ($^{\circ}$ C)	T_L (\pm 95% conf. int.) ($^{\circ}$ C)	Optimum ($^{\circ}$ C)
<i>P. burnerae</i>			
2 nd nymphal	2.96	32.28	26.13
3 rd nymphal	16.29	27.9	24.74
<i>P. citri</i>			
2 nd nymphal	4.54	35.13	28.6

T_0 = Lower developmental threshold and T_L = Upper lethal temperature

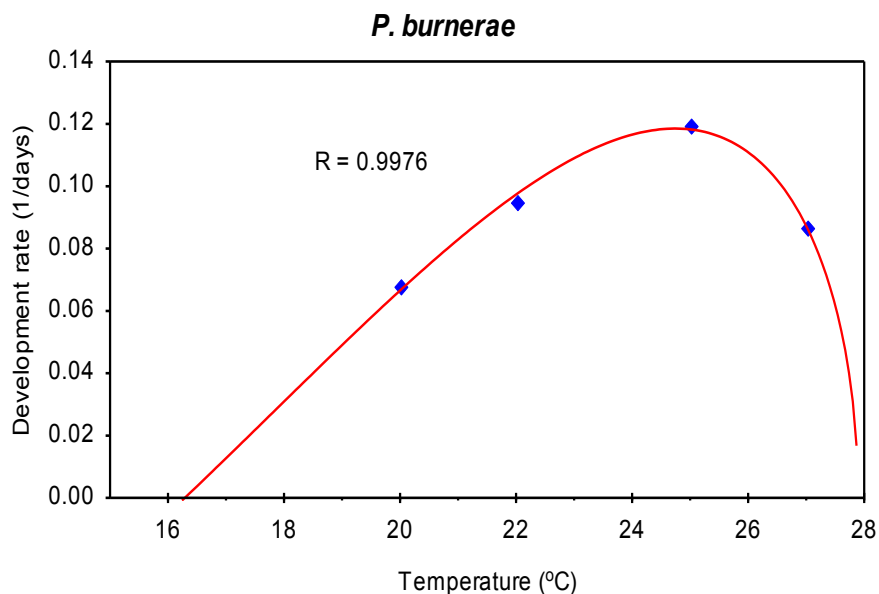


Fig. 3. Relationship between developmental rate and temperature for the 3rd nymphal instar of *P. burnerae*

3.5 DISCUSSION

Although it is difficult to know how well parameter estimates obtained at constant temperatures could be applied in the field, laboratory studies still remain helpful in our quest to understand the influence of temperature on development, survival and reproduction in insects (Omer *et al.* 1996; Wang *et al.* 1997). Thus in the current experiments we were able to confirm that the field observations of Hattingh *et al.* (1998), namely that the developmental rate of *P. burnerae* was slower than that of *P. citri*, were correct.

As with many insect life history studies conducted at constant temperatures on different species, developmental rates in this study increased linearly with an increase in temperature in all immature stages except the 2nd nymphal stage for both species. Since *P. burnerae*'s development rate reached a peak around 24-26°C in the second and third nymphal stages, it is reasonable to state that this was its optimum temperature in these stages. This temperature lies within the range

of 22 and 28°C reported to be optimal for insects from tropical conditions (Nava *et al.* 2007). In contrast, *P. citri*, a tropical to subtropical species with a worldwide distribution (Ben-Dov 1994), was the more resilient to high temperatures with an optimum temperature above 28°C at the second nymphal instar.

Regression analyses performed on the effects of temperature on developmental rate revealed that each developmental stage (embryo, nymph and pupa) required different amounts of heat (degree-days) to develop. Lower developmental thresholds (LDT) for each stage in both species were also very distinct. *P. citri* exhibited lower LDTs with increasing sum of effective temperatures (SET) in the all developmental stages except for the crawler (first nymphal) stage. This trend conforms to the predictions of Trudgill & Perry (1994) and Trudgill (1995) in Honek (1996) that cold adapted species of higher geographical latitudes (species S1 in Fig. 4) with a low LDT develop faster at low temperatures (below the point of intersection of both regression lines), while warm adapted species of lower geographical latitudes such as *P. burnerae* and *P. citri* (species S2) with a high LDT develop faster at high temperatures. Unlike *P. burnerae*, *P. citri* behaved like a cold adapted species (S1) and conformed to the prediction of a low LDT in the embryonic and pupal stages as well as the biological cycle, but retained its status as a warm adapted species by having a lower sum of effective temperatures. For complete development from egg to adult, there is little difference in the lower developmental thresholds of the two species but that of *P. citri* is slightly lower. However, in the crawler stage an exception to the predictions in Fig. 4. was observed for *P. burnerae*. Despite being a warm adapted species, *P. burnerae* behaved more like a cold adapted species with a lower developmental threshold than *P. citri* at the first instar stage. The SET and developmental rate were equally lower than those exhibited by *P. citri*. The fact that *P. burnerae* is able to develop at such low temperatures in the crawler stage, can only be ascribed to adaptive developmental plasticity attained over many years of exposure to lower temperatures. It is also possible that this developmental plasticity acquired from its increased range from the tropics into temperate climates such as those exhibited in the Eastern and Western Cape could have led to the observed broader thermal window.

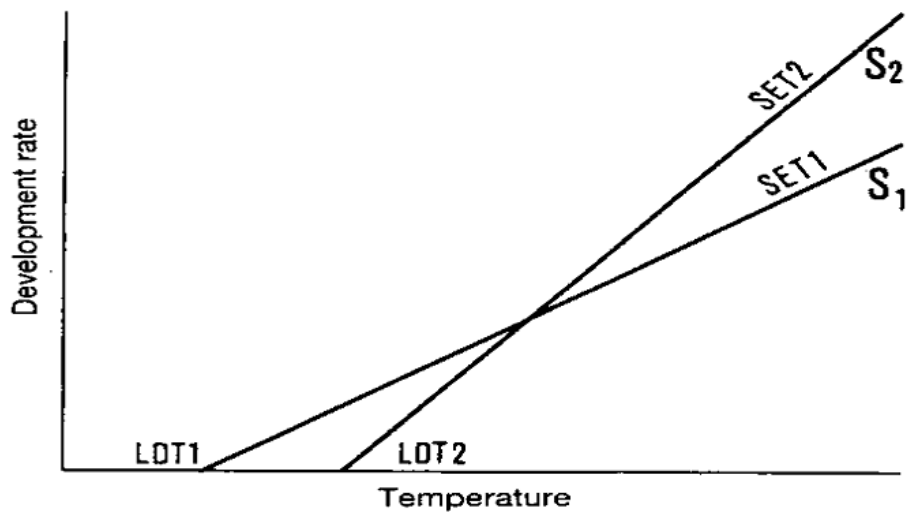


Fig. 4. Schematic presentation of the relationship between development rate and temperature in a cold adapted species S1, and a warm adapted species S2. Species S1 has lower LDT and higher SET (the slope of regression line is inversely proportional to the SET) than the species S2. Adapted from Honek (1996)

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CHAPTER 4

SEASONAL PHENOLOGY AND NATURAL ENEMIES OF THE OLEANDER MEALYBUG, *PARACOCCUS BURNERAE* (BRAIN) (HEMIPTERA: PSEUDOCOCCIDAE) IN SOUTH AFRICA

4.1 ABSTRACT

The importance of *Paracoccus burnerae* has risen over the years to an extent where it is now regarded as a quarantine pest for citrus fruit from South Africa. The field biology of *P. burnerae* on citrus in the Western Cape Province of South Africa was studied through periodic sampling of leaves from twigs enclosed in sleeve cages. The species composition and abundance of natural enemies was investigated. Both adult and immature stages attained maximum population peaks in March and *P. burnerae* had four generations. The highest level of mortality was experienced in the immature stages. Climate and an unidentified fungus were the key mortality factors. The level of abundance of the two observed predators, the harlequin beetle, *Harmonia axyridis* and the green lacewing, *Chrysoperla* sp. was relatively low. Although parasitism occurred in some cages, the level was low ranging between 1.62 to 9.43%. If biocontrol is the preferred method of controlling *P. burnerae*, suitable candidate parasitoids for inoculative biocontrol are *Acerophagus* sp., *Leptomastix* sp. and *Microterys nietneri*. The oleander mealybug does not share the same parasitoids with *Planococcus citri*, *Pseudococcus calceolariae* and *Pseudococcus longispinus* except the parasitoid *Coccophagus* sp. The most popular species of parasitoids used in the biological control of mealybugs, *Anagyrus* sp. and *Coccixenoides* sp. were insignificant in the case of *P. burnerae*.

4.2 INTRODUCTION

The oleander mealybug, *Paracoccus burnerae* is an important pest of citrus in South Africa (Pieterse *et al.* 2010). It was not reported in South Africa until March 1914 when it was first discovered on *Viburnum* sp. in Lydenburg (Brain 1915) in what was then Transvaal Province. Since then, it spread to Swaziland and Natal in the early 1990s where it was reportedly causing havoc (Hattingh *et al.* 1998). *P. burnerae* was until recently the most dominant mealybug species on citrus in certain parts of the Eastern and Western Cape Provinces of South Africa (Moore & Kirkman 2003, 2005). According to Hattingh *et al.* (1998), *P. burnerae* is found in all citrus growing zones of southern Africa. Its distribution range goes beyond southern Africa into east Africa and as far as India (Ben-Dov *et al.* 2001).

Paracoccus burnerae is of great concern to quarantine authorities in countries such as the USA, South Korea and China where it is regarded as a phytosanitary pest (Moore & Kirkman 2003; Wakgari & Giliomee 2003a and Pieterse *et al.* 2010). Fruit shipments originating from South Africa have been rejected in the past because they were found harbouring mealybugs. For example, almost 30% of apples and 9% of pears bound for export to the USA from South Africa in 2002 were rejected because of the presence of mealybug eggs and immature stages (Wakgari & Giliomee 2004). Rejection of this fruit by phytosanitary authorities was because they suspected the mealybugs to be endemic to South Africa (Wakgari & Giliomee 2004).

In view of the importance of this species on citrus there was need to supplement the laboratory studies on the biology of this species (Chapter 2) by investigating seasonal trends (phenology) and mortality factors of field populations under South African climatic conditions. In addition, the species composition and abundance of its natural enemies in two citrus growing regions of the Western Cape Province were investigated. The information would be useful in developing management strategies for this species which presently includes only chemical measures.

4.3 MATERIALS AND METHODS

4.3.1 Field orchards

Two abandoned commercial orchards, one planted with lemon and the other with oranges, were sampled from September 2008 to August 2009 at Blaauwklippen Farms, situated 6km South of Stellenbosch in the Western Cape Province of South Africa. An inspection of 100 fruits from each orchard was conducted prior to sampling to ensure that there was no naturally occurring mealybug population.

4.3.2 Phenology and sampling units

Ten citrus trees with healthy looking twigs from each of the two study orchards were selected at random and a tree at the beginning of the row with a selected tree marked for easy location. A healthy looking twig situated at mid-height of the canopy was chosen from each of the ten trees. The twigs were marked, infested with ovipositing females of *P. burnerae* and covered with gauze cages to keep out natural enemies. Each selected twig was at least 20cm long and bearing more than 20 leaves. When the eggs had hatched and the population had established itself, the sample population eventually included all developmental stages: three nymphal stages (designated as L1, L2 and L3), preovipositing females (POF), ovipositing females (OF) and eggs (E). Infestation of the twigs took place conducted approximately two months prior to sampling.

Sampling units on each tree comprised two leaves sampled twice monthly and taken to the laboratory for population census. From each twig, two leaves were collected on each sampling occasion. Each individual from the sampling unit was carefully examined under a stereomicroscope to establish whether it was alive and its developmental stage.

4.3.3 Parasitoid rearing

Three methods were used to rear parasitoids from field-sampled material. Firstly, young citrus trees, about 1m tall in bags, were infested with mealybugs in the greenhouse and then exposed to parasitoids in the orchards for two weeks every month for one year at Blaauwklippen, Stellenbosch and Boontjiesrivier, Citrusdal. After exposure they were enclosed in emergence boxes in the laboratory. The boxes were made of cardboard and each had two holes covered with an inverted funnel closed off with a vial facing a light source. In the second method, mealybug infested leaves were collected and placed in glass bottles with lids bearing holes covered by inverted glass funnels and vials attached to the narrow end of the funnel. In the third method, mummified mealybugs from infested material were placed in glass vials. The vials were plugged with cotton wool and kept in an incubator at a temperature of 25°C and 60-90% relative humidity. The emerging adult parasitoids were recorded daily and stored in 70% ethanol until identification. The proportion of each of the total parasitoid species reared was also determined. Predators were collected directly from the field and from the emergence boxes.

4.4 RESULTS

4.4.1 Phenology and life table

P. burnerae occurred on all parts of the citrus twigs (branches, leaves and fruit) throughout the year. During the dry summer months when the leaves were probably less palatable, most of the mealybugs would migrate towards the fruits. In winter, majority of the surviving individuals would cluster around the base of branches and leaves, and in fruit bracts. Others would seek shelter in rolled up leaves and under dry remains of the sooty mould fungus. A large proportion of second instar males were observed pupating on fruits, especially in the caged twigs that had fruit on them.

Although individuals were observed to move between leaves and fruits, only adults were observed migrating to and clustering around the base of the branches. Immature stages were present during each sampling period but their numbers varied throughout the year. The adult stages were a common occurrence at each sampling occasion as from February 2009. The population peaks for each group sampled (LI-III, POF and OF) varied greatly but all attained maximum peaks in March (Fig. 1).

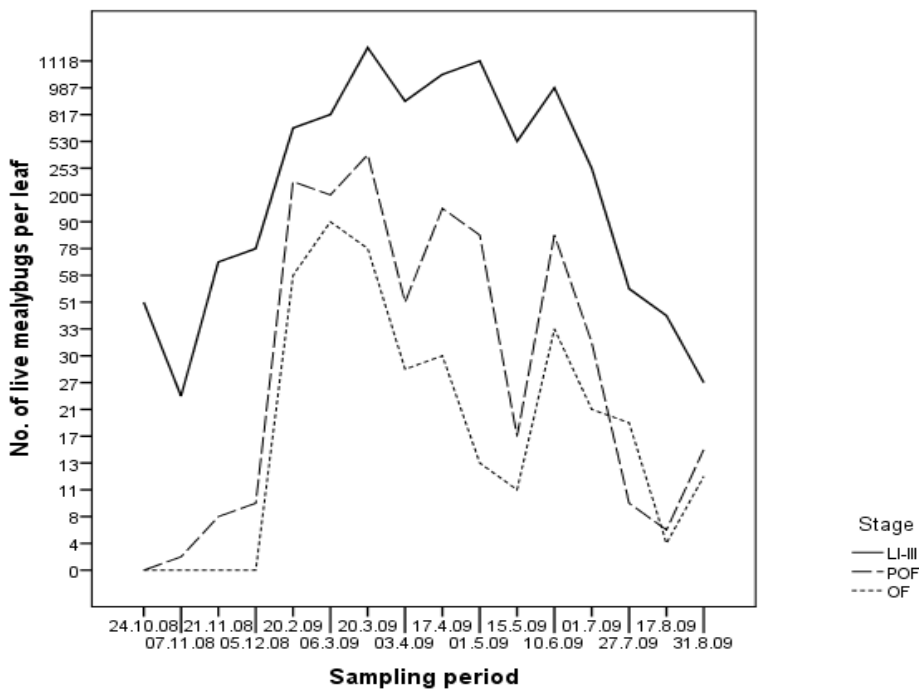


Fig. 1. Seasonal population curves for *P. burnerae* on citrus in Stellenbosch (October 2008 – August 2009)

In the 2008 to 2009 sampling season, population numbers of all stages of *P. burnerae* showed four peaks, indicating four generations in the field (Fig. 1). An estimate of the number of generations *P. burnerae* would undergo in the field in Stellenbosch based on thermal requirements obtained in the laboratory was five (Chapter 1).

The life table constructed shows that, only 397 ovipositing adult females survived from 9753 eggs during the 2008 to 2009 sampling season, giving 95.9% mortality. The egg, instar I-III and pre-ovipositing females experienced mortalities of 15.8, 86.3 and 64.7% respectively (Table 1). A wide range of factors some of which are unknown were responsible for the observed mortality. The known factors include infertility, malformation, entomopathogenic fungus, climate and parasitism.

Table 1. Life table of a field population of *P. burnerae* on citrus at Blaauwklippen, Stellenbosch

x	l_x^b	$d_x F^c$	d_x	q_x	$100d_x l_x^{-1}$	r_x^f	S_x
Egg	9753	Infertility Miscellaneous	1538	0.1577	15.77	15.77	0.8423
Instar I - III	8215	Malformation Climate Entomopathogenic fungus Parasitism Miscellaneous	7090	0.8631	86.31	72.70	0.1369
POF	1125	Entomopathogenic fungus Climate Miscellaneous	728	0.6471	64.71	64.71	0.3529
OF	397	Entomopathogenic fungus Climate Miscellaneous					
TOTAL			9356		95.93		0.0407

l_x^b , number of live insects at the beginning of each life stage x

$d_x F^c$, mortality factor

d_x , number of individuals dying in each stage

q_x , mortality rate

$100d_x l_x^{-1}$, apparent mortality (%) ($[d_x/l_x] \times 100$)

r_x^f , overall or real mortality (%) ($[d_x/9753] \times 100$)

S_x , survival rate

4.4.2 Parasitoids and predators

Nine species of primary hymenopteran parasitoids were obtained from adult and immature stages of *P. burnerae* from Citrusdal (Table 2), and eight from Stellenbosch (Table 3).

Table 2. Identity and number of parasitoids and predators attacking *P. burnerae* on Citrus from Citrusdal, Western Cape, from July 2008 to June 2009

Parasitoid/predator species	Family	Period when active	N (% of total)
Primary parasitoids			
<i>Acerophagus</i> sp.	Encyrtidae	May - Aug	23 (22.3)
<i>Leptomastix</i> sp.	Encyrtidae	March - Aug	54 (52.4)
<i>Microterys nietneri</i>	Encyrtidae	July & Aug; Oct - Jan	14 (13.6)
<i>Anagyrus</i> sp.	Encyrtidae	March & May	2 (1.9)
<i>Anagyrus</i> sp.1	Encyrtidae	March	1 (1.0)
<i>Anagyrus</i> sp.2	Encyrtidae	May & June	1 (1.0)
Unidentified sp.1	Encyrtidae	Aug	6 (5.8)
Unidentified sp.2	Encyrtidae	Nov	1 (1.0)
<i>Metaphycus</i> sp.	Encyrtidae	Aug	1 (1.0)
Total			103 (100)
Primary/secondary parasitoid			
<i>Coccophagus</i> sp.	Aphelinidae	Nov & April	6
Predators			
<i>Harmonia axyridis</i>	Coccinellidae		Low*
<i>Chrysoperla</i> sp.	Chrysopidae		Low*

*Relative abundance

Leptomastix sp., *Acerophagus* sp. and *Microterys nietneri* (Motschulsky) were the most dominant parasitoids recovered from *P. burnerae* in Citrusdal accounting for 52.4%, 22.3% and 13.6% respectively of the total number of parasitoids reared. At Stellenbosch, *Acerophagus* sp. and *Syrphophagus* sp. were the most dominant parasitoids, accounting for 70.6% and 10.1 % respectively. Two predators, the harlequin beetle, *Harmonia axyridis* (Pallas) (Coleoptera) and Green lacewing, *Chrysoperla* sp. (Neuroptera) were obtained from Citrusdal but their relative abundance was low. This also applies to Stellenbosch where the relative abundance of *Harmonia axyridis* was low.

Table 3. Identity and number of parasitoids and predators attacking *P. burnerae* on Citrus from Stellenbosch, Western Cape, from July 2008 to June 2009

Parasitoid/predator species	Family	Period when active	N (% of total)
Primary parasitoids			
<i>Acerophagus</i> sp.	Encyrtidae	May - Aug	77 (70.6)
<i>Leptomastix</i> sp.	Encyrtidae	Oct-Dec; May	5 (4.6)
<i>Microterys nietneri</i>	Encyrtidae	Oct & Nov	7 (6.4)
<i>Syrphophagus</i> sp.	Encyrtidae	Nov	11 (10.1)
<i>Anagyrus</i> sp.2	Encyrtidae	May & June	1 (0.9)
Unidentified sp.1	Encyrtidae	July & Aug	6 (5.5)
Unidentified sp.2	Encyrtidae	Nov	1 (0.9)
<i>Encyrtus aurantii</i>	Encyrtidae	Nov	1 (0.9)
Total			109 (100)
Primary/secondary parasitoid			
<i>Coccophagus</i> sp.	Aphelinidae	March - May	4
Predators			
<i>Harmonia axyridis</i>	Coccinellidae		Low*

*Relative abundance

4.5 DISCUSSION

A sharp decline in the number of immature stages was observed between October 2008 and part of November 2008 (Fig. 1). The low temperatures and rainy weather conditions during this time of year might have been the likely cause of the observed phenomenon. However, as the temperatures rose between November 2008 and March 2009, a corresponding increase in the population numbers of all individual stages occurred. This was followed by a gradual decline in the population numbers of all mealybug stages until winter.

In the life table (Table 1) it is suggested that climate and an unidentified fungus might have been the major causes of mortality in the field population of *P. burnerae*, affecting all post-embryonic stages. The effects of the entomopathogenic fungus on the mealybug population were very visible between end of May and Mid-August 2009. At this time of the year the humidity was high and perhaps favourable for growth of the fungus. There are several species of entomopathogenic fungi known to attack scale insects, for example, *Beauveria bassiana*, *B. brongniartii*, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus* and *Verticillium lecanii* (Shah & Pell 2003). It is also evident from the life table of *P. burnerae* that the critical and most vulnerable stage of development was the immature stage. Climate was considered to be the cause of population declines when no other mortality factors were evident. Similar studies by Browning (1959) on the long-tailed mealybug, *Pseudococcus adonidum* in South Australia found climate to be the key mortality factor on all stages of mealybug development. Furness (1976) also found climate to be responsible for most of the mortality of young crawlers (instar I) of the long-tailed mealybug, *Pseudococcus longispinus* in the dispersal stage.

Parasitism was one of the identified causes of mortality in the field population of *P. burnerae*. As a few gauze cages were unable to keep parasitoids out, the encyrtid wasp, *Acerophagus* sp. gained access and was able to parasitize *P. burnerae*. The parasitoid was observed attacking immature stages. However, the level of parasitism was low, ranging from 1.62 to 9.43 percent.

The level of parasitism was possibly low because the parasitoids did not have complete access to the mealybugs due to the gauze caging.

Pre-ovipositing and ovipositing female populations recovering from winter gave rise to the fourth generation in the spring of 2009. Since the adult females experienced the last population peak before the immature stages towards spring, chemical sprays should be applied at this time of year to prevent them from producing offspring and thus keep population numbers low. If biocontrol is the preferred method of controlling *P. burnerae* at this time of the year, *Leptomastix* sp., *Acerophagus* sp. and *Microterys nietneri* would be suitable candidates for inoculative biocontrol as they were active towards the end of winter in the two regions.

It is interesting to note from the work of Wakgari & Giliomee (2003b) that *P. burnerae* and the three other citrus mealybugs, *Planococcus citri*, *Pseudococcus calceolariae* and *Pseudococcus longispinus* do not share the same parasitoids, except for *Coccophagus* sp. Females of *Coccophagus* sp. are known to be primary parasitoids of mealybugs while the males can either be primary parasitoids of mealybugs or hyperparasitoids of other parasitoids (Pitkin 2003). *Anagyrus* sp., which is together with *Coccixenoides* sp. the most popular species used in the biological control of mealybugs, was an insignificant parasitoid in the case of *P. burnerae*.

Although predators are regarded as an important agent of mealybug mortality in South African orchards (Wakgari & Giliomee 2003b), the number encountered during this study was rather low. *Harmonia axyridis*, which has established recently in South Africa, is a voracious feeder on a wide variety of insects (Stals & Prinsloo 2007). In similar studies in Japan by McClure (1986), *H. axyridis* comprised 84% of the total number of predators captured and accounted for 97% of the mortality observed on the mealybug, *Matsucoccus matsumurae* (Kuwana) in less than four weeks. The detection of *Harmonia axyridis* actively searching for prey on uncovered infested twigs requires further investigation on whether it is really a predator of *P. burnerae* in South Africa. Lacewings have been found to be useful in the biological control of mealybugs in other

parts of the world. For example, in the USA, DeBach *et al.* (1947) found *Chrysopa californica* an active predator against the long-tailed mealybug, *Pseudococcus longispinus*. The same applies to Australia where Browning (1959) and Furness (1976) found *Chrysopa* sp. an active predator of this species. However, the numbers found in this study appeared to be too low to have a significant affect on the mealybug population.

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CHAPTER 5

EVALUATION OF CITRUS, BUTTERNUT AND SPROUTING POTATO AS MASS REARING SUBSTRATES FOR THE OLEANDER MEALYBUG, *PARACOCCUS BURNERAE* (BRAIN) (HEMIPTERA: PSEUDOCOCCIDAE)

5.1 ABSTRACT

Biological control programs of mealybug species have relied on sprouting potatoes, pumpkins and butternut for rearing of both mealybugs and their natural enemies. In this study, the suitability of sprouting potatoes, butternuts and citrus as mass rearing substrates for the oleander mealybug, *Paracoccus burnerae* was investigated. Developmental times, rate and fecundity on each substrate were determined and compared at three different temperatures. The developmental time on sprouting potatoes was shorter than on citrus. *P. burnerae* was unable to complete its life cycle on butternut. The rate of development increased linearly with an increase in temperature on both sprouting potatoes and citrus. *P. burnerae* required 666.7 degree-days on citrus and 434.8 degree-days on sprouting potatoes above lower developmental thresholds of 7.6°C and 10.4°C respectively to complete one generation. The mean number of eggs per female was higher on sprouting potatoes (121.3) than on citrus (68), but declined with an increase in temperature from 22 to 27°C. Despite the shorter shelf life, sprouting potatoes are the preferred host for mass rearing of the oleander mealybug.

5.2 INTRODUCTION

The oleander mealybug, *Paracoccus burnerae* (Brain), occurs from Kenya to South Africa and attacks approximately 35 species of plants, including important food and beverage crops such as potatoes, olives and coffee (Ben-Dov *et al.* 2001). In South Africa, however, *P. burnerae*, is now known to attack citrus and is one of three mealybug species with a quarantine status on citrus fruit bound for export to overseas markets (Wakgari & Giliomee 2003). As the demand for organic and pesticide free fruits increases, there is need for environmentally friendly, viable and risk free approaches to mealybug control such as using biological control measures.

The ability to mass rear *P. burnerae* is a vital step towards the culturing and colonization of its natural enemies, especially parasitoids. Mass rearing of these biocontrol agents on mealybugs facilitates their establishment, periodic colonization and inundative releases (Etzel & Legner 1999; Wheeler & Zahniser 2001). Therefore, if these agents are to be used against *P. burnerae*, an inexpensive mass rearing technique and a nutritionally efficient but simple diet has to be developed for *P. burnerae*. The nutritional regime should be capable of producing and supporting great numbers of insects at a low cost. Although numerous artificial diets for the mass production of various arthropods (Singh 1977) have been developed, no suitable mass rearing substrate for the oleander mealybug has been investigated.

An important requirement for a mass rearing substrate is a long shelf life, which obviates the regular provision of fresh food. In this regard butternut, pumpkins and sprouting potatoes have been found to be suitable substrates for the mass rearing of various Coccoidea (Blumberg & Swirski 1977; Elder & Smith 1995), the superfamily to which mealybugs belong. They have also been used to rear mealybugs other than *P. burnerae* (Saggara & Vincent 1999; Serrano & Lapointe 2002; Walton & Pringle 2002; Wakgari & Giliomee 2003; Kontodimas *et al.* 2004; Daane *et al.* 2004; Chong & Oetting 2007).

The goal of this study was to compare the development rate and fecundity of *P. burnerae* on citrus, butternut and potato sprouts for mass rearing purposes in order to assess the suitability of the three diets as mass rearing substrates for *P. burnerae*.

5.3 MATERIALS AND METHODS

Laboratory cultures of the oleander mealybug were obtained from a colony reared on citrus in the greenhouse at the Botany and Zoology Department of the University of Stellenbosch.

Development time and rate of *P. burnerae* on various hosts were determined at 22, 25 and 27°C using growth chambers with humidity varying between 60 and 90% and a light:darkness phase of L16:D8.

Pre-ovipositing females were obtained from the greenhouse and introduced onto citrus seedlings, small-sized butternuts and potato sprouts. Citrus seedlings were transplanted into plastic bottles while the butternuts and sprouting potatoes were placed in rectangular and transparent plastic containers whilst suspended on both ends by pieces of wire so that they could be turned around easily for observation. A fungicide was then applied to the points where the wire entered the rearing substrate to prevent fungal contamination. The substrates, together with the eggs, were then incubated and the females allowed at least 24 hours to lay eggs before being removed. A maximum of 30 eggs from each female ovisac was retained on every individual substrate used at the three temperatures. The development of each individual until maturity was observed under a stereomicroscope and recorded daily.

The emerging adult females were allowed to mate with males from within the cohort. When there were no males available, they were provided with males from the greenhouse. If mating

was successful and an ovisac was formed, eggs were then counted on a weekly basis until no further egg laying took place.

5.3.1 Data Analysis

The lower developmental threshold temperatures (T_0) for development to occur on both substrates were determined by regressing $1/t$ on temperature for *P. burnerae* using Statistica 9.0 statistical package and then solving the regression equation $1/t = 1$, where t = time in days, for both biological cycles (on citrus and potato). The rate of development from egg to adult on both citrus and sprouting potato was regressed on temperature and then compared at 22, 25 and 27°C. The number of degree-days (DD) needed for development by both species was calculated using $DD=1/b$, where b is the slope of the regression equation.

5.4 RESULTS

5.4.1 Developmental times

Total developmental time of *P. burnerae* for the entire biological cycle (egg to adult) was shorter on potato with a range from 35.9 days at 27°C to 48.6 days at 22°C on citrus and 25.8 days at 27°C to 36.9 days at 22°C on sprouting potato (Table 1). *P. burnerae* was unable to develop beyond the 3rd nymphal instar on butternut, with most of the mortality being experienced in the 1st and 2nd nymphal stages.

Table 1. Developmental times in days (mean±SD) for the biological cycle (egg – adult) of *Paracoccus burnerae* on citrus, sprouting potato and butternut at three constant temperatures

Temperature (°C)	Food plant		
	<i>Citrus</i>	<i>Sprouting potato</i>	<i>Butternut</i>
22	48.6 (0.6)	36.9 (1.13)	*
25	40.4 (1.9)	30.1 (0.36)	*
27	35.9 (1.9)	25.8 (5.68)	*

Note: * cycle not completed

5.4.2 Developmental rates

The relationship between temperature and rate of development on both citrus and sprouting potato was positively linear. The rate of development on both citrus and sprouting potato increased with an increase in temperature (Fig. 1), but the regression slope was much steeper on sprouting potato than on citrus.

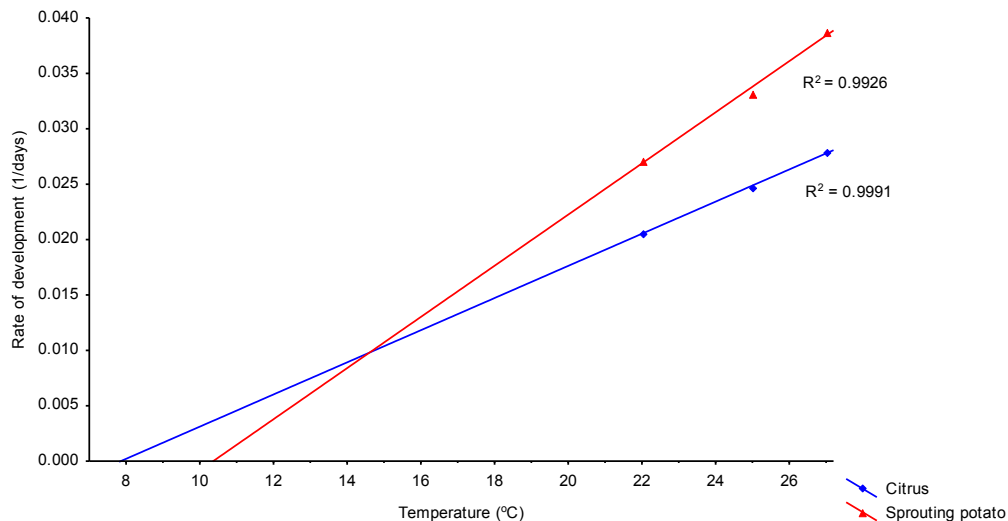


Fig. 1. Relationship between rate of development (egg to adult) and temperature for *P. burnerae* on citrus and sprouting potato

The theoretical lower developmental thresholds for the entire development of *P. burnerae* from egg to adult were estimated as 7.6°C and 10.4°C on citrus and sprouting potato respectively (Table 2). Thermal constant estimates from the regression equations revealed that *P. burnerae* required 434.8 DD on sprouting potato and 666.7 DD on citrus to complete development from egg to adult.

Table 2. Thermal constant values and regression coefficients for the biological cycle of *Paracoccus burnerae* on citrus and sprouting potato

Food plant	Regression equation	LDT (°C)	DD	R ²	P
Citrus	-0.0114 + 0.0015x	7.6	666.7	0.9991	0.02
Sprouting potato	-0.0239 + 0.0023x	10.4	434.8	0.9926	0.05

LDT = Lower developmental threshold and DD = degree-days

5.4.3 Fecundity

There were major differences between the mean fecundity per female of females raised on citrus and sprouting potato. Females raised on sprouting potato had the highest mean number of eggs. The mean fecundity per female on both substrates decreased with increasing temperature from 22 to 27°C (Fig. 2). Analysis of variance (ANOVA) showed significant differences in the mean fecundity per female on each substrate (*Citrus*: $F = 2.024$, $df = 2, 6$, $P = 0.213$; *Potato*: $F = 11.14$, $df = 2, 11$, $P = 0.0002$).

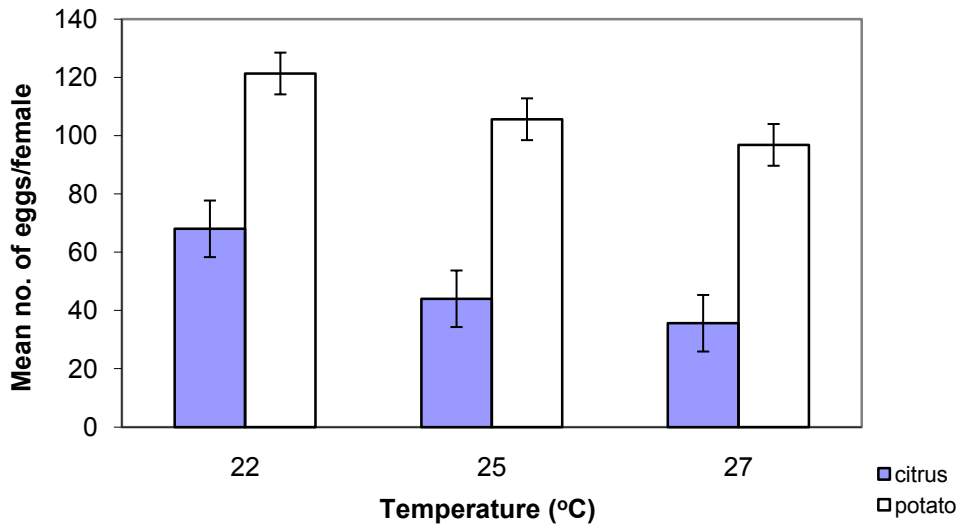


Fig. 2. Comparison of mean (\pm SE) female fecundity of *P. burnerae* on citrus and sprouting potato at three temperatures

5.5 DISCUSSION

Total development of *P. burnerae* was only realized on citrus and sprouting potato but not on butternut. However, in the laboratory, a complete life cycle from egg to egg on butternuts at a constant temperature of 22°C was found to vary from 37 to 50 days (V. Hattingh, unpublished data in Hattingh *et al.* (1998). A few other mealybug species such as the Comstock mealybug *Pseudococcus comstocki* (Kuwana), citrus mealybug *Planococcus citri* (Risso) and the spherical mealybug *Nipaecoccus viridis* (Newstead) have also been successfully reared on sprouting potatoes (Gothilf & Beck 1966; Saggara & Vincent 1999; Serrano & Lapointe 2002). Its inability to survive beyond the 3rd nymphal instar on butternut can possibly be due to the exterior coat which hardened with time resulting in reduced food uptake and allocation. As a result of this food limitation, the interaction between nymphs and host plant was decoupled, leading to their shortened survivorship and eventual death. The decreased developmental time observed on

sprouting potato compared to citrus might be a clear indication of the costs associated with diet quality as was found by Stockhoff (1993) for the gypsy moth, *Lymantria dispar*.

The results are consistent with observations of Wagner *et al.* (1984); Leddy *et al.* (1995) and Honek *et al.* (2002) on the effect of temperature on insect development. The rate of development of *P. burnerae* increased with increasing temperature on both citrus and sprouting potato. The effect of variation in diet quality between the two substrates was very distinct. Potatoes generated a much higher rate of development than on citrus seedlings, resulting in high levels of mean fecundity per female and shorter generation times. Even though there was a decrease in the mean number of eggs laid with increasing temperature on both substrates, the number was much lower on citrus. Therefore, there is a probability that as the energy levels surged on sprouting potatoes due to continuous shoot elongation, *P. burnerae* was able to reach a level of maximum consumption, resulting in a decrease in feeding costs, and therefore directing more energy towards growth and reproduction. The developmental stage of the foliage, nutrient and chemical composition of leaves/seedlings are some of the many factors that could have caused a reduction in fecundity on citrus.

The different influence of sprouting potatoes and citrus on the development of *P. burnerae* was also evident on the lower developmental threshold (LDT) and thermal constant (K). As the rate of development increased, a corresponding decrease in the thermal constant occurred on sprouting potatoes, but not on citrus. In contrast, the LDT on citrus was 2.8°C lower than on potato. This value, however, falls within the 1° and 3°C range of standard error biases in estimating developmental rate as observed by Janacek & Honek, unpublished data in Jarosik *et al.* (2002). Honek *et al.* (2002) also found a variation in the lower developmental threshold of *Autographa gamma* (Lepidoptera: Noctuidae) larvae reared on 9 different diets at 3 experimental temperatures. Campbell *et al.* (1974) found similar differences of 1 – 2°C in the LDTs of some aphids on 11 different host plants.

The lack of precision in lower developmental threshold and thermal constant has been found to be of no great importance for forecasting developmental duration. The error in temperature sum compensates for the inaccurate estimate of the lower developmental threshold that occurs when calculating the developmental time of a stage (Honek *et al.* 2003). The differences in diet also caused a 1.5 fold variation in the thermal constant. This variation in nutritional regimes between citrus and sprouting potatoes caused the plasticity reflected by the variation in the thermal constant. Variation in food quality has also been reported to be the most important cause of differences in the thermal constant of noctuid larva development (Honek *et al.* 2002).

As there was incomplete development on butternut, and lower reproduction and prolonged development on citrus compared to sprouting potatoes, the latter are the preferred host for mass rearing of *P. burnerae* for mass production and release of biocontrol agents.

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CHAPTER 6

GENERAL CONCLUSIONS

A comprehensive study on the biology of the oleander mealybug, *Paracoccus burnerae* (Brain), an important pest of citrus in South Africa (Pieterse et al. 2010) became accessory when *P. burnerae* reportedly became the most dominant mealybug species on citrus in some parts of the Eastern and Western Cape Provinces of South Africa (Moore & Kirkman 2005). Field based studies on its biology were supplemented by its developmental biology at constant temperature in the laboratory. The rate of development of *P. burnerae* on citrus was used to explain its suspected dominance over the citrus mealybug, *Planococcus citri* (Risso). A survey of the species composition and abundance of its natural enemies was carried out and an evaluation of three different diets was also conducted in order to identify the best host plant for its mass rearing. An understanding of the biology of *P. burnerae* and finding its natural enemies is an important milestone towards developing and consolidating existing control programs.

Developmental duration of the embryonic and immature stages of the oleander mealybug were affected by temperature. The rate of development was much slower at low temperature than at high temperature as reported by Jarosik *et al.* (2004) but increased with temperature in the embryonic stage, first instar, pupal stage and the biological cycle (egg to adult). The time spent in each developmental stage was longer at low temperatures. These results are consistent with those of other researchers who reported a similar trend with different species of mealybugs (Arai 1996; Walton & Pringle 2005). Survivorship of individuals was longer at low temperatures. Females of *P. burnerae* were observed to develop faster than males at temperatures of between 18 to 25°C (Table 1 in Chapter 2). Fecundity rose with increasing temperature but reached a maximum at 22°C and then dropped. This temperature might have been optimal for maximum food utilisation and resource allocation towards reproduction. Temperature has been found to

affect the amount of energy reserved for reproductive effort, while linked to food intake. It also has an influence on maintenance costs and affects ingestion rates (Butler & Burns 1991).

The embryonic stage of *P. burnerae* had the highest lower developmental threshold (T_0), fastest rate of development and required the least amount of heat to develop. The second and third instar rates of development reached an optimum between 24-26°C predicted as being optimal for insects from tropical conditions by Nava *et al.* (2007). The rate of development in the biological cycle (egg to adult) reached a peak at 31.02°C contrary to the assertion made by Nava *et al.* (2007). *Paracoccus burnerae* exhibited a broad thermal window of 24.7°C using the modal of Briere *et al.* (1999) and 22.3°C using the linear regression model. This thermal window exceeded the theoretical prediction of 20°C for insects based on Dixon *et al.* (2008) by between 2.3° and 4.7°C. This clearly suggests that *P. burnerae*, a tropical species, has broadened its thermal window as a result of long-term exposure to cooler climates. These phenotypic responses may be a result of adaptation (genetic) to different thermal environments or an unavoidable consequence of the effect of temperature on the insect's physiology during development (developmental plasticity or nongenetic) (Stillwell & Fox 2005).

In the life table constructed from developmental time, survival and fecundity data (Chapter 2), the net reproductive rate (R_0) of *P. burnerae* was found to be >1 at all five temperatures, an indication that it is capable of increasing its population numbers despite encountering high mortality levels in the 1st and 2nd nymphal instars.

The results of this study (Chapter 3) showed that *P. burnerae* has the ability to outcompete *P. citri* when temperatures are low in the most important stage of mealybug establishment, namely the crawler stage when the insect moves around before settling. This advantage would only exist in winter when temperatures are low. However, at later stages as well as during the entire biological cycle, *P. citri* responds better to temperature than *P. burnerae* because it develops faster. Thus, the oleander mealybug's suspected dominance over the citrus mealybug is neither linked to its sum of effective temperatures nor developmental rate. Since differences in

developmental rates and SET (sum of effective temperatures) are not responsible for the oleander mealybug's dominance, other factors such as differences in the ability to encapsulate parasitoids, the presence and effectiveness of biocontrol agents, thermal tolerance ranges and susceptibility to insecticides should be investigated to explain this phenomenon.

Despite experiencing heavy mortality in the post-embryonic stages in the field just like in the laboratory (Chapter 2), the population of *P. burnerae* increased resulting in four generations being observed in the 2008 to 2009 sampling season at Blaauwklippen, Stellenbosch. From the life table constructed in Chapter 4, the key mortality factors were climate and the entomopathogenic fungus. An unnoticeable fungus has reportedly been observed attacking mealybugs in the USA and when the rainy season began it completely destroyed the mealybug infestations (Chafin 1921). The entomopathogenic fungus observed at Blaauwklippen was also active during winter which is the rainy season in this part of the Western Cape Province. Mortality due to parasitism was very low and *P. burnerae* does not share the same parasitoids with the three other citrus mealybugs, *Planococcus citri*, *Pseudococcus calceolariae* and *Pseudococcus longispinus* except for the parasitoid *Coccophagus* sp. which is either a primary or secondary parasitoid. Although predators are regarded as an important agent of mealybug mortality in South African orchards (Wakgari & Giliomee 2003b), the level of predator abundance observed (Chapter 4) was negligible. If *P. burnerae* is to be controlled by use of biocontrol agents in both Citrusdal and Stellenbosch, the suitable parasitoids are *Acerophagus* sp., *Leptomastix* sp. and *Microterys nietneri* (Motschulsky).

As there was incomplete development on butternut, and lower reproduction and prolonged development on citrus compared to sprouting potatoes, the latter are the preferred host for mass rearing of *P. burnerae* for mass production and release of biocontrol agents. It is also clear from the results in Chapter 5 that host plant quality is an important factor in the fecundity of herbivorous insects. Constituents of host plant quality such as carbon, nitrogen and defensive metabolites have been cited as factors that directly affect potential and achieved fecundity in herbivores (Awmack & Leather 2002).

Further research is required to investigate the host stage preferences of the parasitoids of *P. burnerae*, parasitism rates, encapsulation of parasitoids, efficacy of biocontrol agents (including entomopathogenic fungi) and chemical sprays on *P. burnerae*. Physical and chemical differences between citrus and potato sprouts need further study.

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CHAPTER 7: ADDENDUM

PRACTICAL PROBLEMS AND SOLUTIONS IN STUDYING THE BIOLOGY OF A MEALYBUG SPECIES†

7.1 ABSTRACT

Researchers often present impressive results of their studies on the biology of the Coccoidea without mentioning the problems they came across and had to solve. In this paper the practical problems encountered during a study of the biology of the oleander mealybug, *Paracoccus burnerae* (Brain), an endemic pest of citrus in South Africa, are discussed.

7.2 INTRODUCTION

The oleander mealybug, *Paracoccus burnerae* (Brain) is a species mostly found in the southern part of the Afrotropical Region, while it has also been recorded from India (Ben-Dov *et al.* 2001). It feeds on a wide variety of plants, but in South Africa it is mainly known as a pest of citrus. South Africa exports citrus fruits to many countries of the world and some of them, notably the USA, China and South Korea, regard this species as a quarantine pest (Wakgari & Giliomee 2003; Pieterse *et al.* 2010). Since very little is known about the biology of this species, we were commissioned to do laboratory and field studies on its development and to determine the natural enemies associated with this species.

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7.3 DISCUSSION

The first problem was to obtain specimens of this species in citrus orchards. Despite it being a pest in some commercial orchards, we could not find any when we started the study. Thorough searching of the trees (leaves, bark, stems, young fruit) yielded nothing and we were told by orchardists that they only become conspicuous when the fruits are ripe. Since we had to start our studies in the summer at the beginning of the academic year, we could not wait for this to occur (in winter). Fortunately, we found a colony that someone was keeping for identification purposes, amongst other mealybug species, at our local quarantine station.

We brought the colony on young citrus seedlings to the greenhouse of the Department of Botany and Zoology at the University of Stellenbosch, South Africa. This is really a greenhouse for botany projects and it was not easy to persuade the professor of botany that we would not infest the other plants in the greenhouse with our mealybugs. We purchased young, one meter tall, citrus plants in black bags from a nursery, making sure they had lush young growth and no infestation of any kind, and replanted them into pots before infesting them with our colony by placing infested leaves on the new plants. To prevent the mealybugs from spreading, we placed the pots on brick islands in big plastic buckets with water (later it was considered unnecessary to replant them from the black bags, as we continuously replaced them). The buckets with the plants were placed on a dedicated and separate shelf in the middle of the greenhouse. One morning we found our nicely infested plants outside the greenhouse where a technician had put them with a note saying: “plants infested”.

Towards the end of the study, plants of other projects did get infested, and we had to act speedily in spraying them and the areas around our plants with an appropriate insecticide (chlorpyrifos). The plants in the greenhouse were well isolated from potential infectious insects outside, but towards the end of the study we experienced infestations with the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), the woolly whitefly, *Aleurothrixus floccosus* (Maskell), the citrus psylla, *Trioza erythrae* (Del Guercio), and an unidentified coccinellid. The larvae of

the latter could be killed by hand, while the others were controlled by treating infested leaves with an insecticide from a hand held sprayer.

One of our aims was to study the development of *P. burnerae* on citrus at different constant temperatures in incubators. Fortunately, several incubators were available so studies at different temperatures could run concurrently. In order to keep the plants in the limited space of the incubators, we had to obtain (from a nursery) very young seedlings, about 10cm tall, and plant them in small plastic bottles (12cm high, 6cm wide) with holes in the bottom for drainage. They did very well under a light:darkness regime of 16L:8D. We infested them by placing mature females on the leaves for oviposition, removing them later together with excess eggs.

The incubators had no humidity control and we soon found that it was too low for the mealybugs to thrive. We tried various measures to increase the humidity, and finally we placed our bottles on three soaked, brick shaped, so-called oases used by florists on a plastic tray in the incubator. This increased the humidity to between 60 and 90%, which was adequate for normal development of the mealybugs.

We also wanted to study the development of *P. burnerae* under field conditions. That is impossible in commercial orchards where numbers of insects are kept low with insecticides or biological control measures. We searched and found an abandoned citrus orchard on the outskirts of our town, Stellenbosch. We gained permission from the owner to infest the trees with *P. burnerae*. Merely placing infested seedlings in the trees did not result in a visible infestation, for reasons that were not clear. It may have been due to the effect of natural enemies, the condition of the neglected trees or climatic conditions. In order to facilitate establishment of the mealybugs we placed infested leaves on some 20 young branches from different trees and covered them with sleeve cages to exclude natural enemies. This proved successful, but unfortunately the sleeve cages were conspicuous and many of them were vandalized by inhabitants of nearby cottages, farm workers or passersby. The solution to this problem was to increase the number

and scatter them widely in the orchard in the hope that some would survive. Which is in fact what happened.

Finally, we wanted to obtain the natural enemies of *P. burnerae*. Again, the chances of finding them in a commercial orchard were remote as a result of the application of insecticides. So we searched for and found a situation where the farmer's house and garden was only some 30m from his commercial orchards that were known to have had problems with *P. burnerae* infestations. We then placed two or three plants from the greenhouse, infested with various stages of the mealybug, in the farmer's garden where they were looked after by his gardener. This site was some 200 km away from Stellenbosch in a different citrus region. The mealybugs were exposed for two weeks every month over a period of one year (except mid-winter) and brought back to the laboratory for possible parasitoid emergence in standard emergence boxes with a glass funnel and glass vials exposed to a light source. We also hid mealybug infested plants in the local abandoned orchard and treated them similarly. They had to be watered during the hot and dry summer months.

Solving the practical problems we experienced, enabled us to gather data on the development of *P. burnerae* at constant temperatures in the laboratory and at varying temperatures in the field, as well as its parasitoids. Life tables were also produced.

7.4 REFERENCES

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