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Full Length Research Paper

Evaluation of citrus, butternut and sprouting potato as mass rearing substrates for the oleander mealybug, *Paracoccus burnerae* (Brain) (Hemiptera: Pseudococcidae)

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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 which is above lower developmental thresholds of 7.6 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.

Key words: *Paracoccus burnerae*, temperature, degree-days, mass rearing, nymphal, fecundity, rate of development, lower developmental threshold, thermal constant.

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 (www.sel.barc.usda.gov/scalenet/scalenet.htm). 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 and 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 and Legner, 1999; Wheeler and 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 sprout-

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Abbreviations: DD, Degree-days; **LDT,** lower developmental threshold.

Table 1. Developmental times in days (mean ± SEM) for the biological cycle (egg to adult) of *P. burnerae* (Brain) (Hemiptera: Pseudococcidae) on citrus, sprouting potato and butternut at three constant temperatures.

Temperature (°C)	Food plant		
	Citrus	Sprouting potatoes	Butternut
22	48.6 (0.494)	36.9 (0.221)	*
25	40.4 (0.47)	30.1 (0.07)	*
27	35.9 (0.557)	25.8 (0.721)	*

*Cycle not completed.

ting potatoes have been found to be suitable substrates for the mass rearing of various Coccoidea (Blumberg and Swirski, 1977; Elder and Smith, 1995), the superfamily to which mealybugs belong. They have also been used to rear mealybugs other than *P. burnerae* (Saggara and Vincent, 1999; Serrano and Lapointe, 2002; Walton and Pringle, 2002; Wakgari and Giliomee, 2003; Kontodimas et al., 2004; Daane et al., 2004; Chong and Oetting, 2007).

The goal of this study is 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*.

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. Developmental 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 into citrus seedlings (X639), small-sized butternuts (Waltham) and potato sprouts (Mondial). 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 were allowed for at least 24 h 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. Three potatoes were used at each experimental temperature. As for citrus, an initial five seedlings with 10 eggs were used at each of the three temperatures. In cases were the mortality was high, the experiment was repeated on that particular citrus seedling. 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.

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 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. The equality of variances was tested using the Levene's (ANOVA) and Cochran C, Hartley, Bartlett homogeneity tests. The Tukey honestly significant difference (HSD) post-hoc test was used to compare the difference in treatments, in this particular case, the diet.

RESULTS AND DISCUSSION

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.

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 (Figure 1), but the regression slope was much steeper on sprouting potato than on citrus.

The theoretical lower developmental thresholds for the entire development of *P. burnerae* from egg to adult were estimated as 7.6 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

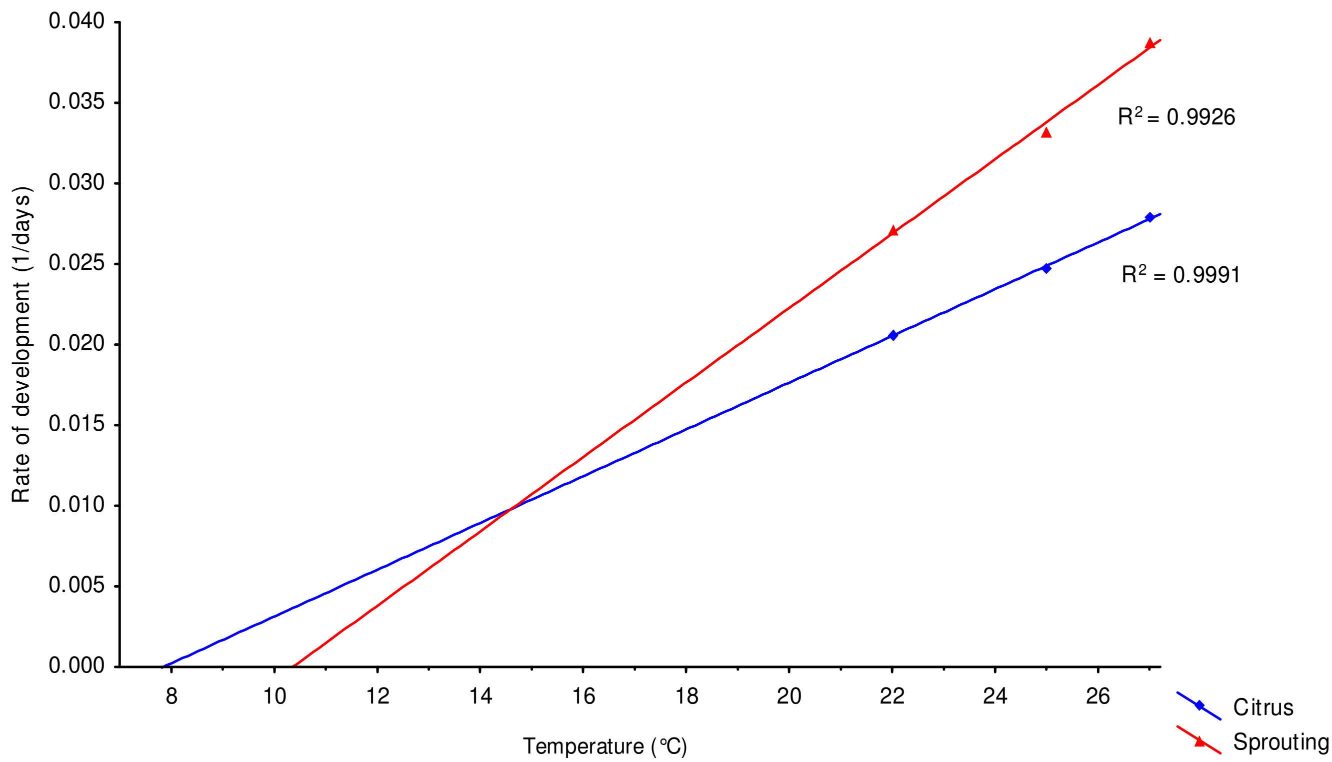

Figure 1. Relationship between rate of development (1/days), from egg to adult, and temperature (˚C) for *P. burnerae* (Brain) (Hemiptera: Pseudococcidae) on citrus and sprouting potato.

Table 2. Thermal constant values and regression coefficients for the biological cycle of *P. burnerae* (Brain) (Hemiptera: Pseudococcidae) 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
Potato	-0.0239 + 0.0023x	10.4	434.8	0.9926	0.05

LDT, Lower developmental threshold; DD, degree-days.

666.7 DD on citrus to complete development from egg to adult.

Fecundity

There were major differences between the mean fecund-dity 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 (Figure 2). A two way analysis of variance (ANOVA) showed that the interaction between temperature and substrate (diet) was not significant ($F = 0.076$, $P = 0.928$). However, diet was the most significant ($F = 29.479$, $P < 0.001$), followed by temperature ($F = 5.118$, $P = 0.025$). There were between-diet differences on the mean fecundity of *P. burnerae* (Tukey HSD test, $P < 0.001$).

Total development of *P. burnerae* was only realized on citrus (X639) and sprouting potato (Mondial) but not on butternut (Waltham). 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 and Beck, 1966; Saggara and Vincent, 1999; Serrano and 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

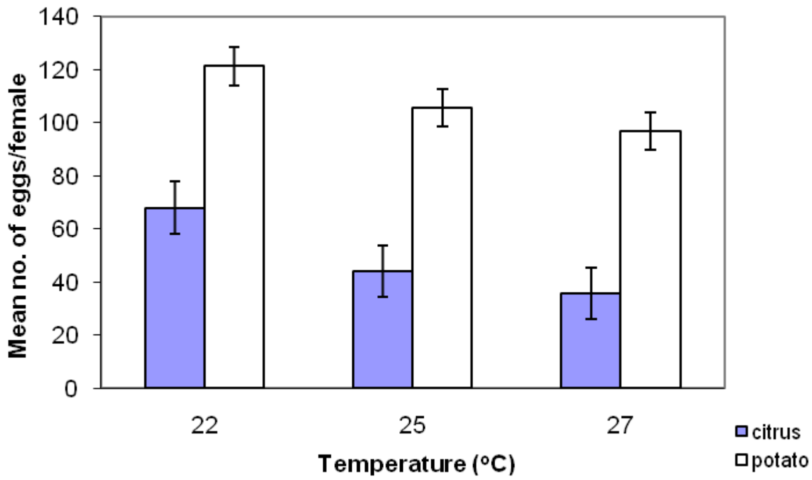

Figure 2. Comparison of female fecundity (mean number of eggs/female) (mean ± SEM) of *P. burnerae* (Brain) (Hemiptera: Pseudococcidae) on citrus and sprouting potato at three temperatures (22, 25 and 27°C).

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 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 and 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 to 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 when 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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