THE ECOLOGY OF *PAPILIO DEMODOCUS* ESPER (LEPIDOPTERA: PAPILIONIDAE) ON CITRUS TREE PLANTATIONS IN SOUTHERN MOZAMBIQUE

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THE ECOLOGY OF *Papilio demodocus* Esper (LEPIDOPTERA: PAPILIONIDAE) ON CITRUS TREE PLANTATIONS IN SOUTHERN MOZAMBIQUE

By

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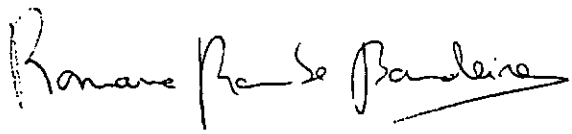
Declaration

I hereby declare that the work presented in this thesis was undertaken by me. It has not been accepted in any previous application for a degree. All quotations have been distinguished by quotation marks and all sources of information specifically acknowledged.



Romana Rombe Bandeira

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DEDICATED TO MY PARENTS MÁRIO ROMBE AND JOSEFA CHANDAMELA

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List of Abbreviations

EAU – Umbelúzi Agrarian Station

INIA – National Institute for Agronomic Research

M.A.P – Ministry of Agriculture and Fisheries

UEM – Eduardo Mondlane University

US – United States of America

g. - grams

mg - milligrams

cm - centimetres

mm - millimetres

m – metres

s - standard deviation

ha - hectares

l - litres

c. = circa

i.e. = that is

e.g. = for example

k = dispersion parameter of the negative binomial

h = hours

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THE ECOLOGY OF *Papilio demodocus* Esper (LEPIDOPTERA: PAPILIONIDAE) ON CITRUS TREE PLANTATIONS IN SOUTHERN MOZAMBIQUE

ABSTRACT

Following outbreaks of *Papilio demodocus* populations on citrus plantations and the consequent use of expensive control methods in southern Africa the project aim was to evaluate the natural regulating factors regulating the populations thus contributing to a more efficient pest control strategy in the Umbelúzi region of Moçambique. Field studies were carried out during the period March 1998 to November 1999 in the sites INIA, University Campus and LOMACO citrus orchards < 2 years old in Umbelúzi while laboratory research was conducted at the Faculty of Agronomy and Forest Engineering of the Eduardo Mondlane University at Maputo. The population distribution studies carried out in treated citrus plantations showed that *P. demodocus* egg population distribution was aggregated and that the coefficients a and b of Taylor's Power Law were respectively 0.28 and 1.12. Peak *P. demodocus* activity occurred between October and March, the rainy season. Generation time was 52 days, evolutive cycle lasted 41 days with a sex ratio of 1:1. During the rainy season the population net reproductive rate and capacity for increase were respectively 0.855 and -0.031. During the dry season these values were 1.467 and 0.005. Fecundity differed between seasons. 30 cohort life tables were produced from data collected in pesticide free orchards using trials established at INIA, University Campus and LOMACO 1° Maio field. The citrus plants utilised were *C. sinensis* cv. Valencia, *C. paradisi* cv. Marsh and cv. Star Ruby. The survival curves only differed between the dry and rainy seasons ($P<0.05$) but not between cultivars or sites. Larval disappearance at the second and fourth larval instars was the most important mortality factor but larval disappearance was not density-dependent. In general, mortality factors were not related to environmental factors. The effect of the initial population size on its changes between generations indicated density dependent processes between generations. Predators and parasitism under natural field conditions were seldom observed.

CHAPTER ONE

GENERAL INTRODUCTION

Fruit production is one of the most important income generating activities for rural households in Mozambique and helps to alleviate poverty while securing food in periods of food scarcity (appendix 1.1). Citrus fruit production also plays an increasingly important role in local traditional Agroforestry practices. Citriculture substantially contributes to the country's exports and economy (Oliveira e Castro, 1974). The Mozambican citrus plantation areas have expanded in the last few years, mainly as a result of overseas demand for citrus fruit but a part of citrus production is also absorbed in local markets.

Although the southern Africa sector of citrus production is relatively small compared to other citrus producing countries, southern Africa can supply off-season citrus products for northern markets (Hinton, 1994).

The region of Umbelúzi (southern Mozambique) is regarded as one of the most promising areas suitable for citrus production (map in appendix 1.2) (Gazeta do Agricultor, 1959); these reasons include climatic conditions, soil type and quality, water supply and roads. Since every single cultivated *Citrus* tree can bring a high net revenue from its yield when mature, any plant loss represents reduction in profits (Oliveira e Castro, 1974).

The major sweet orange cultivar in the Umbelúzi region is *Citrus sinensis* (L.) Osbeck cv. Valencia, which ripens from June to October. The most common grapefruit cultivars are *Citrus paradisi* Macf. cv. Marsh seedless and cv. Star Ruby. These ripen from April to June.

Orange fruit production can yield 173 kg/tree/year in managed orchards with grapefruit yielding 565 kg/tree/year in Florida, United States (Childers *et al.*, 1987). In Mozambique the yield seldom exceeds 100 kg/tree/year. Limitations to citrus fruit increased productivity include pests and diseases. In the region of Umbelúzi the most important diseases and insect pests include the aphids *Toxoptera aurantii* (B. de F.) (Homoptera:Aphididae) and *T. citricida* (Kirkaldy) (Homoptera: Aphididae) both agents of the Citrus Tristeza Virus, scale insects *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae), moths *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) , the leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracilariidae) , dipterous larvae *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) and *Ceratitis rosa* Karsch (Diptera: Tephritidae), mealybugs *Planococcus citri* (Risso) (Homoptera: Coccidae) and *Pseudococcus adonidum* (L.) (Homoptera: Pseudococcidae), mites *Tetranychus cinnabarinus* (Boisd.) (Acari: Tetranychidae), *Panonychus citri* (McG.) (Acari: Tetranychidae) and *Aceria sheldoni* (Ew.) (Acari: Eriophyidae) and the butterfly *Papilio demodocus* Esper (Lepidoptera: Papilionidae) (Valle Y March, 1968; Hill, 1983; Olmi, 1985) (table 1.1). Example of a disease is the Citrus Tristeza Virus (CTV). *P. demodocus* larvae feed on the leaves of the host plants. The damage is serious in seedlings and in young trees up to 2 years old (Honiball & Bedford, 1978; Netterville, 1994). Complete defoliation may occur when the infestation level is high. Damage can also result from larval feeding on the terminal twigs when the plant does not produce leaders and lateral growth dominates; consequently the tree becomes small and shrubby and one year's growth may easily be lost (Howard, 1906).

Table 1.1 Major *Citrus* pests in southern Mozambique (Hill, 1983)

Pest Species Scientific Name and Family Name	Common Name
Trioza erytreae (Del G.) (Hemiptera: Triozidae)	citrus psyllid
Aleurocanthus woglumi Ashby (Homoptera: Aleyrodidae)	citrus blackfly
Toxoptera aurantii (B. de F.) (Homoptera: Aphididae)	black citrus aphid
Toxoptera citricida (Kirk.) (Homoptera: Aphididae)	brown citrus aphid
Planococcus citri (Risso) (Homoptera: Coccidae)	root mealybug
Pseudococcus adonidum (L.) (Homoptera: Pseudococcidae)	long-tailed mealybug
Lepidosaphes beckii (Newman) (Homoptera: Diaspididae)	mussel scale
Aonidiella aurantii (Maskell) (Homoptera: Diaspididae)	california red scale
Chrysomphalus aonidum (L.) (Homoptera: Diaspididae)	purple scale
Saissetia oleae (Olivier) (Homoptera: Coccidae)	black scale
Ceroplastes rubens Mask (Homoptera: Coccidae)	pink waxy scale
Gascardia desctructor (Newst.) (Hemiptera: Coccidae)	white waxy scale
Icerya purchasi Mask (Homoptera: Margarodidae)	cotton cushion scale
Scirtothrips citrii (Moulton) (Thysanoptera: Thripidae)	citrus thrips
Ceratitis capitata (Wied.) (Diptera: Tephritidae)	medfly
Ceratitis rosa Karsch (Diptera: Tephritidae)	natal fruit fly
Tetranychus cinnabarinus (Boisd.) (Acari: Tetranychidae)	tropical red spider mite
Panonychus citri (McG.) (Acari: Tetranychidae)	citrus red spider mite
Aceria sheldoni (Ew.) (Acari: Eriophyidae)	citrus bud mite

This pest is most abundant from September to April in South Africa but in warmer climates it may occur throughout the year (Williams, 1969). Variations in *P. demodocus*

population activity was unrecorded under the climatic conditions of the Maputo region although large numbers of *P. demodocus* had been observed during the rainy season (Annecke & Moran, 1982). Little attention has been given to investigating the effects of environmental factors on *P. demodocus* population development, population structure, population dynamics, natural enemies and their impact on the pest population development, mortality key-factors and host preferences. This information is of vital importance in understanding *P. demodocus* biology and factors affecting population growth. An understanding of these factors would identify the key factor(s) regulating the pest populations. This would enable the environment to be managed in order to make it more favourable for the key factor and thus minimise the use of chemicals.

A wide range of chemical products have been recommended to control this pest when necessary (table 1.2).

Indiscriminate or repeated use of chemicals may lead to adverse effects on the environment including contamination of the ecosystem from pesticide residue build up, elimination of pest natural enemies and other beneficial organisms. The greater the use of pesticides, the more expense is involved in crop protection and production. Chemicals can be expensive. For example the biopesticide *Bacillus thuringiensis* Berliner. (C.A.) used in the region of Maputo costs approximately 35 US $ per litre which makes it more expensive than pesticides, at approximately 10-20 US $ litre. In addition, excessive pesticide usage may result in the development of resistance in pest populations.

A knowledge of pest biology provides an understanding of the factors affecting pest population numbers. The identification of the key factor would assist in the determination of the most appropriate pest developmental stages for control using pesticides thus reducing unnecessary preventive pesticide applications.

Table 1.2 List of pesticides recommended for the control of *P. demodocus* (a.i = active ingredient)

Pesticide	Formulation	Dosage	Information source
Endosulfan	sprays EC) (SC) (WP) or dusts	40 ml/100 l 100ml/100l water 100gr/100 l water	Honiball & Bedford (1978); Brink & Steyn (1993)
Methomyl	spray or dusts		Honiball & Bedford (1978); Brink & Steyn (1993)
Arsenite of soda	spray	1 pound of a.i. to 200 gallons water and 2-4 pounds of slaked stone lime	Howard (1906)
Paris Green Chemical	spray	1 pound of a. i. to 200 gallons water and 2-4 pounds of slaked stone lime	Howard (1906)
Malathion	aqueous spray to run-off point		Hill (1983)
Fenthion	aqueous spray to run-off point		Hill (1983)
Fenitrothion	aqueous spray to run-off point		Hill (1983)
DDT		0.2 % of a.i.	Bohlem (1973)
Chlorpyrifos			Brink & Steyn (1993)
Prothiofos			Brink & Steyn (1993)
Lebaycid		0.2 % of a.i.	Bohlem (1973)
Parathion		0.2 % of a.i.	Hepburn & Bishop (1954); Bohlem (1973)
Thiodan		0.2 % of a.i.	Bohlem (1973)
Bacillus thuringiensis	wettable powder	25 gr/100 litres of water	Netterville (1994)

Several studies in Asia (Chua, 1979; Badawi, 1981; Nagalingam, 1983) indicated that natural enemies could be the most important mortality factors of *Papilio demoleus* L. (Lepidoptera: Papilionidae), a major pest of citrus plants of that region. Tsubaki (1973), Watanabe (1976, 1979) demonstrated that *Papilio xuthus* L. (Lepidoptera: Papilionidae) populations were also regulated by their natural enemies. In Mozambique relatively little is known of any comparable type of regulation for *P. demodocus*.

The aims and objectives of the study are:

General Objective

Evaluation of the natural regulating factors of *P. demodocus* populations in order rationally to utilize or replace the use of pesticides in their control

Specific Objectives .

- Determination of *P. demodocus* population distribution parameters
- Determination of the *P. demodocus* life cycle under Umbelúzi conditions
- Development of *P. demodocus* life-tables to evaluate the relative role of natural enemies and other mortality factors in regulating *P. demodocus* population levels
- Evaluation of *P. demodocus* host range and preferences in the region of Umbelúzi
- Development of *P. demodocus* curves of population growth

CHAPTER TWO

LITERATURE REVIEW

2.1 BIOLOGY of *Papilio demodocus*

Papilio demodocus Esper was previously named *Princeps demodocus* until 1758. Following this the species was described as *Papilio demoleus* var. *demodocus* Bryk until 1798 (Van Son, 1949). *Papilio demodocus* is confined to Africa, where the distribution is continuous, (Hill, 1983; Olmi, 1985) and parts of Asia (Commonwealth Agricultural Bureaux International Institute of Entomology, 1987), (see figure 2.1.1). It is the most abundant African swallowtail butterfly (Williams, 1969; Scholtz & Holm, 1985).

Howard (1906), Van Son (1949), Williams (1969), Hepburn & Bishop (1954) and Scholtz & Holm (1985) all confirm that *P. demodocus* is oligophagous. It is common in cultivated areas, gardens and forest margins, feeding on citrus plants and other members of the families Rutaceae and Umbelliferae (table 2.1.1). Some of these plant species occur in forests and are common in the region around Maputo (voucher specimens at LMU Herbarium; Palgrave, 1977).

Smit (1964) cited by Honiball & Bedford (1978), stated that *P. demodocus* produces three generations per year in the Sundays River Valley, in south east South Africa.

Females lay their eggs in clusters or singly on the flush leaves (Honiball & Bedford, 1978; Hill, 1983). All caterpillar stages can feed on both flush and mature leaves resulting in complete defoliation of the plant when it is severely attacked.

Figure 2.1.1 *Papilio demodocus* distribution in the world (CAB International Institute of Entomology, 1987)

There are five larval instars. Larvae of the last three instars possess the osmeterium, an organ which expells a pungent secretion for defensive purposes following disturbance or threat (Honiball & Bedford, 1978; Hill 1983). The production of such a substance by the osmeteria in butterflies at the larval stages, when disturbed, is, probably to act as a deterrent to parasites and predators (Feltwell, 1986).

The *P. demodocus* life cycle in South Africa is given in detail by Van Son (1949); Hill (1983); Olmi (1985) and Annecke & Moran (1982). This consists of 4 days in the embryonic stage, 30 days for larval development with 14 days in the pupal stage.

Hepburn & Bishop (1954) and Honiball & Bedford (1978) indicated that six days passed from egg lay until egg hatch, the larval stages took 21 days and between 2 and 4 weeks for the pupae to eclode. Some pupae may overwinter. The complete life cycle in summer may be around one month while in winter it is considerably longer (Hepburn & Bishop (1954). In the Transvaal region (now Mpumalanga), pupae take only 14 days for adult emergence and the whole period from egg laying to adult emergence takes 33 days in summer (Howard, 1906). In the Cape region this

Table 2.1.1 *Papilio demodocus* preferred native host plants (Howard, 1906 = 1; Van Son, 1949 = 2; Hepburn & Bishop, 1954 = 3; Williams, 1969 = 4; Migdoll, 1994 = 6)

Species or genus	Family	Author
Calodendron capense (L. f.) Thunb.	Rutaceae	1, 2, 3, 4
Clausena	Rutaceae	2, 3, 4
Teclea	Rutaceae	2, 3, 4
Bubon	Umbelliferae	2, 4
Bubon	Umbelliferae	1, 2, 4
Vepris undata (Thunb.) Verdoorn	Rutaceae	3, 4
Toddalia asiatica (L.) Lam. (*Toddalia aculeata* Pers.)	Rutaceae	1, 2, 3, 4
Toddalia	Rutaceae	2, 3
Toddalia	Rutaceae	2, 3, 4
Hippobromus	Sapindaceae	1, 2, 4
Antoxylum capense (Thunb.) Harv. (*Fagara capensis*)	Rutaceae	3
Deverra (*Pituranthos*)	Umbelliferae	3
Ptaeroxylon obliquum (Thunb.) Radlk.	Ptaeroxylaceae	3
Peucedanum	Umbelliferae	1, 3
Foeniculum officinale All.	Umbelliferae	1, 3
Foeniculum vulgare Mill.	Umbelliferae	2, 3
Zanthoxylum	Rutaceae	6

period is longer as the winter and spring generations take longer to mature. In Pretoria, adults are already found in midle August while larvae are seen late in July and October. Last generations may mature about the middle or end of July, the pupae pass the dry season and the adults emerge from middle August and September. The adults oviposit early in October or in late September (Howard, 1906). In South Africa, Annecke & Moran (1982) noted that pupal diapause may occasionally occur in cooler southern regions. Hepburn & Bishop (1954) also observed that at times the pest overwinters in South Africa as pupae. Van Son (1949) and Williams (1969) noted that in South Africa *P. demodocus* is commonest from September to April but in tropical areas it occurs throughout the year. However, Hill (1983) indicated a need further to investigate the adult life span in relation to seasonal and other factors. At the adult stage, females are usually larger and paler than males (Williams, 1969). Scholtz & Holm (1985) indicated that the males have deeper yellow spots than the females.

Papilio demodocus has been observed to be parasitised by *Distatrix pallidicinctus* (Gahan) ((*Apanteles pallidicinctus* Gahan) Hymenoptera: Braconidae); *Pales coerulea* Jaen (Diptera: Tachinidae); *Sturmia dilabida* Villen (Diptera: Tachinidae) and an *Anastatus* sp. (Hymenoptera: Eupelmidae) in the larval stage. *Pteromalus puparum* L. (Hymenoptera: Pteromalidae) was identified as a pupal parasite while *Ooencyrtus* sp. (Hymenoptera: Encyrtidae) attacked the eggs (Searle, 1964; Annecke & Moran, 1982; Brink & Steyn, 1993). The bug *Afrius figuratus* (Germar) (Heteroptera: Pentatomidae) has been reported as a larval predator while adults have been observed to be predated by birds and mantids (Howard, 1906; Hepburn & Bishop, 1954; Honiball & Bedford, 1978). Although observations have been made of larvae being attacked by parasites, Hepburn & Bishop (1954) suggested that *P. demodocus* larvae are not heavily attacked by natural enemies.

Papilio demodocus shows little variation throughout Southern Africa. No subspecies have been described and only one aberration fully described by Van Son (1949) from Bechuanaland in South Africa is known (Van Son, 1949). This is explained by its continuous distribution throughout the continent (Van Son, 1949).

The fact that butterflies mostly rely on visual signals to find mates (Tinbergen *et al.*, 1942 cited by Paterson, 1991), together with the strongly limited host range of *P. demodocus* in the region of Umbelúzi, gives a clear indication that it is a species in which taxonomic and genetic characteristics coincide. Consequently, the existence of cryptic species or races is not suspected.

Other *Papilio* species that occur in the region of study include *P. dardanus cenea* Stol.; *P. antheus* Gr.; *P. colonna* Ward.; *P. pylades corrineus* Bert.; *P. leonidas* F. and *P. norania* Angas (specimens at the INIA entomological collections). These have not been extensively studied.

Migdoll (1994) indicated that fertile eggs are easily recognised by having a dark spot or band around the centre of the egg shell. The colour of fertile eggs varies from pale yellow to pale green when first laid turning pink or pale brown before developing a dark colour. Unfertile eggs remain a pure white.

2.2 INSECT POPULATION DISTRIBUTIONS

2.2.1 Introduction

Spatial distribution of insects has been studied and quantified in order to develop sequential sampling plans (Kuno, 1969). Kuno (1991) added that the analysis of spatial distribution patterns has been recognized as an indispensable part of the process of studying insect populations, providing useful information for interpreting pest spatial structures. These studies are necessary to understand a pest's ecology and behaviour for rapid assessment of pest density thus enabling forecasting in order to develop relevant sampling programmes. A reliable sampling programme contributes to effective integrated pest management programmes (Karandinos, 1976; Kuno, 1991).

The function ratio of insect sample variance to its mean is an important tool in insect population spatial pattern studies because it allows the prediction of variance for a given mean population size so that sequential sampling procedures can be developed. The function ratio between the variance and mean is more valuable if it remains stable in time and space or has a wide range of densities of natural populations (Ali *et al.*, 1998).

Environmental factors affecting insect population abundance and distribution can be classified into two groups: the abiotic and the biotic. Examples of the first group are the physical factors of the environment, particularly climate. Physical factors normally determine the zones of abundance of a given species in an area, in response to the frequency and intensity of the variation of these factors.

Examples of the second group include the relationships between organisms and biological products resulting from their activity, such as insect frass, corpses and exuviae. Ali *et al.*,

(1998) pointed out that the distribution pattern of an insect species may be a result of a combination of various factors, e.g. spatial differences in the habitats, different oviposition rates or movement behaviour of adults and larvae, respectively. Paterson & Fernando (1971) and Ali *et al*, (1998) concluded that *Chironomus attenatus* Walker and *Glyptotendipes barbipes* (Staeger) (Diptera: Chironomidae) tended to aggregate at low densities but exhibited a random distribution at higher densities, suggesting a behavioural dispersal related to high density levels.

Biotic factors also include interspecific interactions. Interspecific interactions may or may not act in favour of a certain animal species affecting, thus, its population sizes. Several types of interspecific interactions are recognised. Odum (1967) cited by Lara (1992) and Price (1984) included the following interspecific interactions:

- parasitism, where one or more individuals of a species can feed on one individual of another species and possibly use the same individual as a habitat.
- predation, in cases where one species uses individuals of other species as food.
- amensalism, interactions where one species is not affected but inhibits the other
- comensalism, interactions where only one species is favoured in the association, the other species neither benefits nor is negatively affected
- mutualism, an association between two species that benefits both species
- competition, the populations of two or more species compete for resources such as space, food, etc.
- neutralism, when populations of two or more species live in the same habitat without interference.

Intermediate stages between these categories exist (Price, 1984). All such factors have to be taken into consideration and evaluated in relation to estimating the population sizes of a specific organism.

The analysis of a population dispersion is also important in studies of predator-prey and host-parasite relationships (Anderson, 1974; Hassell & May, 1974; Southwood, 1978).

2.2.2 Population estimation

An accurate assessment of the number of samples needed at a particular time and place should be obtained after obtaining a preliminary set of samples (Room *et al.*, 1991).

Karandinos (1976) and Southwood (1978) stated that, within a homogeneous habitat, the number of samples needed is expressed by the equation

$$N=(s/E\bar{x})^2$$

where s is the standard deviation, $\bar{x}$ is the mean and E the predetermined standard error as a decimal of the mean (normally 0.05). For many purposes a standard error of around 5% of the mean is acceptable.

Absolute densities of insect populations can be estimated using direct observations or other methods such as removal sampling and mark-recapture techniques. However, mark-release-recapture methods for the purpose of population studies have been questioned if the demographic units are not clearly delimited (Ehrlich, 1984).

Absolute estimates or conversion of relative estimates to absolute estimates are essential for life table construction and analysis (Southwood, 1978).

2.2.3 Data transformation

Beall (1942) and Harcourt (1963) indicated that in entomological surveys the number of insects may vary in such a way that the standard deviations of the number of insects per sampling unit varies with the mean. In such cases, the results on insect counts can not be subjected to an analysis of variance without a suitable transformation. This is because the data obtained would not be normally distributed and may not be independent.

Forsythe & Gyrisco (1961) noted the four assumptions that the entomologist must observe before considering statistical inferences. These are:

- the treatment effects and the environmental effects must be additive
- the experimental errors must have a common variance
- the experimental errors must be independent
- the experimental errors must be randomly distributed

Forsythe & Gyrisco (1961) and Underwood (1997) indicated that one of the ways to avoid the need for data transformation due to data which is not normally distributed would be to select samples randomly. The assumption of independence of experimental errors of field data is usually satisfied by randomization of the samples in the field.

Forsythe & Gyrisco (1961) observed that it is possible to determine the best transformation required for use of insect count data in an analysis of variance by plotting the variances against the means. Data transformation can be carried out following an estimation of the value of the parameters *a* and *b* of the Taylor's Power Law. Taylor (1961) pointed out that in Taylor's Power Law, the variance (s^2) of samples of a population increases as a functional power of the mean density , that is, $s^2 = a * \bar{x}^b$ or $\log (s^2) = \log a + b \log \bar{x}$. The coefficients *a* and *b* are calculated by regression, plotting log values of variance against log values of mean of several samples. The coefficient *a* is the

intercept of the regression and b the gradient of the log transformed power law. The constant parameter a can be estimated reading off the axis y when $\bar{x} = 1$. The constant b can be calculated from the equation

$$\log s^2 = \log a + b \log \bar{x}$$

where s^2 is the variance and $\bar{x}$ the mean (Southwood, 1978).

Bartlett (1947) and Healy & Taylor (1962) observed that where data have to be transformed before further statistical analysis in order to satisfy the assumption of the statistical techniques such as additivity, homogeneity of variance and normality of distribution, if the quantity measured, usually a count, is designated x, the quantity analysed (the transformed value) is z, which can be either expressed as

$$z = \log x$$

$$z = x^{½}$$

$$z = \log (x + c)$$

or

$$z = (x + c)^{½}$$

The general form (Tukey, 1957) is

$$z = (x + c)^{p}$$

and the variances of a set of samples are related to the means by a power law so that

$$s^2 = a * \bar{x}^b$$

where, as indicated above, s^2 is the variance, a and b fitted coefficients of Taylor's Power Law, $\bar{x}$ the mean density and c a constant. Consequently, an appropriate variance-stabilizing transformation will be of the form

$$p = 1 - 1/2\ b$$

Values of z increase with increasing x for negative and positive values of p (Healy & Taylor, 1962).

The value of p can be estimated by solving the equation

$$p = 1 - 1/2\ b$$

Southwood (1978) pointed out that for populations in which the p values are -1, reciprocals should be used instead of original figures in data transformation. For p values of 0.5 and -0.5 square root transformations and reciprocal square root transformations are recommended whereas logarithmic transformations are best for p values of 0.

Healy & Taylor (1962) proposed tables for data transformation for p values equalling to 0.2, 0.4, 0.6, 0.8 and negative powers.

Beauchamp & Olson (1973) noted that in biological studies, transformations should precede the testing of hypotheses about regression relations. The variability around a fitted line increases in proportion to the mean size in most cases. This variability may be

stabilized by using a log transformation of the data so that the transformed data better satisfy the assumptions for parametric statistical methods.

2.2.4 Binomial family - a mathematical model of population distribution

Anscombe (1950) and Abrahamsen & Strand (1970) indicated that several theoretical distributions of overdispersed population distribution patterns have been studied. These theoretical distributions arranged as the skewness increases are namely: Thomas, Fisher Hh, Neyman, Polya-Aeppli, negative binomial and discrete lognormal. Waters (1959) indicated that the distributions most commonly fitting insect counts are the normal, Poisson, positive binomial, logarithmic, lognormal, Neyman type A and negative binomial. The first three distributions are random while the others are nonrandom. Nonrandom series are termed contagious or aggregated. In this work, the term "aggregated" has been adopted in this regard. Kuno (1991) indicated that among the aggregated distributions, two-parameter mathematical models of insect distribution include Neyman's, Thomas', Polya's, Poisson-binomial and the negative binomial. Nowadays, ecologists use only one, the negative binomial distribution (Kuno, 1991). The negative binomial distribution is a positive skew and has been indicated as the most applicable for describing zoological and microbiological population data (Anscombe, 1950; Abrahamsen & Strand 1970). This distribution can be derived from at least five mathematical and biological models (Anscombe, 1950). It has proved to be applicable for a wide diversity of biological data, particularly for insect counts, with k values indicating the degree of aggregation of a particular population (Waters, 1959).

The negative binomial probability model family is one that describes the manner in which a population is aggregated and, therefore, its distribution. The model is characterized by its large plasticity and this makes it the most commonly used statistical distribution for

investigating insect populations (Kuno, 1991). Kuno also noted that although the original negative binomial was defined only for k>0, the same model could be extended to include k<0, the positive binomial model. Hence, the negative binomial can describe distributions of all categories, that is, random (1/k = 0), aggregated (1/k > 0) and uniform (1/k < 0) distributions by varying the parameter k.

This model recognizes either the active aggregation in insect populations or aggregations due to environmental factors such as microclimate, soil type, resources and natural enemies (Southwood, 1978). The model has been found to be particularly useful in studies describing the pattern of attacks by predators on their prey (Hassell, 1978; Southwood, 1978).

Anscombe (1949), Bliss & Fisher (1953a), Waters (1959) and Abrahamsen & Strand (1970) showed that in a negative binomial distribution, as the variance approaches the mean, the over-dispersion decreases and k tends towards infinity and p to zero. The distribution is converging in a Poisson distribution. Otherwise, as the over-dispersion increases and k tends to zero, the negative binomial tends to Fisher's logarithmic series. Bliss & Fisher (1953a), Abrahamsen (1969) and Southwood (1978) observed that distributions in which values of k are larger than 2 approach the Poisson distribution.

When insects are distributed in such a way that the mean equals the variance, the population distribution is completely random (Beall, 1942). The ratio between the variance and mean of a population will equal to 1 when the distribution of individuals follows a Poisson series (Beall, 1942; Morisita, 1962).

The Poisson series occupies a central place in the binomial family. The Poisson series describes a random population distribution. The curve is described by one parameter as the variance is equal to the mean (Southwood, 1978). The uniform distribution indicates

that the presence of one individual in the population does not affect the distribution of other organisms of the same species. Sometimes, the variance is less than the mean implying a more regular or even distribution than that described by a Poisson series.

Distributions more skewed than the Poisson indicate that the population is overdispersed and that the variance is in excess of the mean. In ecological terminology it is designated aggregated, patchy or contagious (Abrahamsen & Strand, 1970).

Beall (1942) and Underwood (1997) noted that in most ecological studies, variance has been found to be larger than the mean and as observed above, in these cases, the population distribution is regarded as aggregated. Anscombe (1949) and Abrahamsen & Strand (1970) pointed out that many of the aggregated insect populations studied were well described by the negative binomial distribution, which is expressed by two parameters, namely the mean and the exponent k. k is then, the measure of the amount of aggregation and is referred to as the dispersion parameter (Anscombe, 1949). Bliss & Fisher (1953b) stated that the analysis and interpretation of most biological data are facilitated by stability in the variance so that only means need to be compared. A similar stability in the coefficient k would increase the utility of the negative binomial and increase confidence in its suitability for a given situation.

The values of k may not be constant for a population, varying with the mean (Anscombe, 1949; Bliss & Fisher, 1953b; Abrahamsen & Strand, 1970; Southwood, 1978). Bliss (1958) and Waters (1959) pointed out that frequency distributions like the negative binomial should be used only as descriptions of what has been found instead of being used as an analytical tool in ecological studies. The negative binomial parameter k as a measure of aggregation is a relative, not an absolute, measure. Consequently, it depends on the sampling unit chosen. The usefulness of k as a dispersion measure is improved by segregating and analysing the data for population levels or other strata. Studies on forest

insects recommend the use of the smallest natural unit for sampling, e.g. a leaf, and complement this data with counts on one or more larger units (Waters, 1959; Harcourt, 1963). Harcourt (1963) observed that for *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), the estimate of *k* for individual eggs laid singly approximated that for first instar larvae at the same densities.

There are three ways to estimate *k*, which is a non-negative characteristic exponent (Elliott, 1971). Approximate *k* estimation includes the moment method given by the equation

$$k = \bar{x}^2/(s^2-\bar{x})$$

where s^2 is variance and $\bar{x}$ the mean. This method is not adequate for populations exhibiting a degree of aggregation where *k* is 3 or less (Anscombe, 1950). Other approximate methods to estimate the value of *k* are mentioned, for example iterative solutions of the equations

$$\log (N/n_0) = k \log (1+(\bar{x}/k))$$

where N is the total number of samples
n_0 the number of samples with no animals
k the dispersion parameter
$\bar{x}$ the mean

This method gives an efficient estimate of *k* when the mean and *k* are small and the proportion of empty sample units is higher than 33% (Anscombe, 1950), that is, for populations with very small means and also for those with large means when the population shows a high degree of aggregation (Southwood, 1978).

Anscombe (1950), Bliss & Fisher (1953b), Abrahamsen & Strand (1970) and Southwood (1978) indicating a third and more precise method of k estimation, formulated the maximum likelihood method, by solving the two sides of the equations:

$$N\log_e(1+(\bar{x}/k))=\sum(A_x/(k+x))$$

where $\log_e$ are natural logs

A_x the sum of all frequencies of sampling units with more than x individuals

the other variables being as above

The efficiency of the first two methods vary with the mean and k but the maximum likelihood method of k estimation is efficient for all values of mean and k, although they are laborious to calculate (Abrahamsen & Strand, 1970). However, Southwood (1978) indicated that the maximum likelihood method of k estimation may still have a bias if the population mean is small and k large (Southwood, 1978).

Anscombe (1949, 1950) recommended a graphic method for testing the efficiency of the estimation of k.

In order to test the fit of the negative binomial as a model of the actual distribution of a pest population, several ways can be used to check its applicability (Anscombe, 1948, 1950 and Southwood, 1978):

- calculating the expected frequencies of each value and comparing with the actual values using a χ^2 test. Expected values are estimated as follows:

$$P_x = (\ \Gamma(k+x))/(x!\ \Gamma(k)) * ((\bar{x}/\bar{x}+k)^x\ * ((k/(k+\bar{x}))^k$$

where P_x is the probability of a sample with x individuals

values of x! and Γ (k) are respectively found in tables of factorials and log gamma functions taking into consideration that the χ^2 value has (n-3) degrees of freedom of comparisons (numbers) made between the expected and the real figures.

Low frequencies of expected values are pooled for the χ^2 test.

- using computer programmes e.g. Davies (1971) Number 41, for estimating *k* by the maximum likelihood method and testing its fit by calculating P_x.

Southwood (1978) stated that comparisons between actual and expected frequencies can be distorted by chance irregularities. In order to verify this, two tests based on the difference between the actual and expected moments (mean, variance or skewness) are compared with their standard errors (Anscombe, 1950; Bliss & Fischer, 1953b; Southwood, 1978). They indicated that the efficiency of these tests is dependent on mean values and the dispersion parameter *k*. For low means and *k* values, the most efficient test is the test based on the variance. In other cases, where values of the mean are high and large *k* values exist, the test based on the skewness should be used.

- for the test based on the variance, the statistic U (the difference between the actual variance and the expected variance) is calculated as

$$U = s^2 - ((\bar{x} + \bar{x}^{\,2})/k)$$

The value of *k* is estimated by the method

$$\log (N/n_0) = k \log (1 + (\bar{x} / k))$$

shown above (Anscombe, 1950). If U is significantly less than its standard error S.E.(U), the negative binomial may be taken as a satisfactory model. Southwood (1978) provided graphs for the calculation of the standard error of U.

- in the test based on skewness, the difference between the skewness of the data and its estimated value (T) is calculated from the mean and variance of the same samples as

$$T = (\Sigma f x^3 - 3 \bar{x} \Sigma f x^2 + 2 \bar{x}^2 \Sigma f x) / N - s^2 ((2 s^2 / \bar{x}) - 1)$$

T is compared with its respective standard error and if the negative binomial is an adequate model, T is smaller than the standard error S.E. (T). Southwood (1978) recommended a graphical method for the estimation of the standard error of T.

The sensitivity of the methods involving T or U may be low for small samples as these methods are intended to be applied to large samples (Abrahamsen & Strand, 1970).

Estimation of a common *k*

Because of the bias involved with the moment estimate of *k* and the maximum likelihood estimate, an alternative largely unbiased method was proposed by Bliss & Owen (1958). This is important when transforming data for the analysis of variance and sequential sampling. This method is based on the statistics x^1 and y^1. Estimation of the common k (k_c) can be carried out as described below. Samples are taken from several fields and analysed to determine the population mean, variance and respective *k* values (Bliss, 1958; Bliss & Owen, 1958; Peng & Brewer, 1994). It can be carried out by the moment method, involving the statistics x^1 and y^1 as indicated below

$x^1 = \bar{x}^2 - (s^2/N)$

and

$y^1 = s^2 - \bar{x}$

where $\bar{x}$ is the mean

s^2 is the variance

N = the number of individual counts for respective $\bar{x}$

Values of y^1 are plotted against x^1 and the regression fit through the origin has the slope 1/k. The inverse value of *k* is calculated and the approximate estimate of common *k* (k_c) will then be

$1/k_c = y^1 / x^1$

Bliss & Owen (1958) observed that the estimates of *k* based on the moment method can be corrected using the expression 1/k calculated as above.

Alternative graphical test of sample homogeneity can be obtained by plotting (1/k) against the mean $\bar{x}$ for every field. If a trend is not apparent, then the fitting of a common *k* is justified, otherwise the calculation may not be relevant (Southwood, 1978; Peng & Brewer, 1994).

2.2.5 Description of dispersion

Simultaneous changes in the dispersion pattern of a population and population size are of importance in interpreting population dynamics (Anderson, 1974; Hassel & May, 1974). The extent of aggregation or its change in a population provide indications of population characteristics (Southwood, 1978), including direct effects on insect population reproduction rates and survival thus affecting their population dynamics (Waters, 1959). Therefore, the study of the type of distribution is worthwhile.

Population distribution can be,

- regular, when the individuals tend to keep apart from each other
- random, when the presence of one individual does not affect others
- aggregated distribution, when individuals tend to attract others.

The degree of aggregation may be due to environmental factors, for example, habitat patchiness, differential predation or laying eggs in masses or individually. For low density populations, changes in the number of individuals in sampling units are often very low and their distribution is usually random (Southwood, 1978). Waters (1959) observed that populations of several forest insects (for example the Nantucket pine (Pinaceae) tip moth *Rhyacionia frustrana* (Comst.) (Lepidoptera: Tortricidae) and the spruce budworm (*Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) on balsam fir (*Abies balsamea* (L.) Mill. (Pinaceae)) showed a tendency to approach random distribution with an increase in population density. According to these authors, distribution when large numbers were present could go beyond the Poisson and become regular. This is, however, very infrequently observed. Some animal species such as leaf hoppers (Homoptera: Auchenorrhyncha) were observed to have different dispersion patterns within the same habitats, probably due to biological causes such as a greater level of

aggregation following increased reproduction levels in some parts of the habitat or owing to a reduced dispersion caused by different habitat dispersion suitability (Kuno, 1963). An example of an index suitable for description of animal populations is given by the expression:

$$s^2/(\bar{x}^{1/2})$$

which is 1 for a Poisson (random) distribution (Morisita, 1962; Southwood, 1978).

According to Southwood (1978) and Taylor *et al.* (1998) a more general approach to the variance-mean relationship is given by Taylor's Power Law:

$$s^2 = a * \bar{x}^{b}$$

where s^2 is variance
a and *b* are the constant parameters of Taylor's Power Law
$\bar{x}$ the mean

According to Taylor (1984) the coefficient *b* is a parameter which has been shown to be stable for a species and reflects a characteristic behaviour of individuals in the species, remaining constant regardless of the environment or habitats. In their studies, Taylor *et al.*, (1998) confirmed that the coefficient *b* shows a high degree of isotropy, making it an important tool in basic and applied ecology as well as in conservation and agriculture. The goodness-of-fit is judged by the significance of the correlation coefficient (Taylor *et. al.*, 1998).

According to Taylor *et al.* (1998) more robust *a* and *b* coefficients may be obtained with an increased data base. Ali *et al.*, (1998) pointed out that Taylor's Power Law

coefficient *b* did not vary with the season, suggesting a constant spatial pattern in the species *Glyptotendipes paripes* (Edward) (Diptera: Chironomidae). They observed that Taylor's Power Law model gave the best description of aggregated populations of *G. paripes* larvae compared to Iwao's (1968) patchiness regression and the negative binomial distribution, when describing the relationship between mean and variance. Ali *et al.*, (1998) observed that Taylor's Power Law model was less affected by variation in densities, space and time. For this reason, they found it more robust than Iwao's regression for describing the relationship between variance and mean. Peng & Brewer (1994) compared Taylor's Power Law, Iwao's patchiness regression and the negative binomial probability model as methods to analyze an insect's distribution by means of insect counts and concluded that Taylor's Power Law provided a better description of individual spatial aggregation for data from different locations and dates, with higher correlation coefficient (r^2) values between log variance and log mean density. In their studies, Meikle *et al.*, (1998) concluded that Taylor's Power Law model was more appropriate for analysis of insect distributions when compared to Lloyd's Mean Crowding Index.

Southwood (1978), Ali *et al.* (1998) and Meikle *et al.* (1998) stated that many workers considered Taylor's Power Law coefficient *b* as an appropriate measure of dispersion which is constant for a species. A coefficient *b* larger than 1 indicates that the population is aggregated, if *b* is equal to 1, the population distribution is random, while values of *b* smaller than 1 imply that the population is uniformly distributed. Southwood (1978) added that the coefficient *a* is a sampling factor.

Aggregation indices

In ecological studies, particularly on population dynamics, aggregation quantification is as important as the population density (Waters, 1959 and Harcourt, 1963).

Kuno (1991) observed that upon determining the general mathematical frequency distribution pattern of a population, the next step would be to quantify the properties of ecological parameters, classified into the indices of crowding and patchiness.

The departure from the random distribution of a population can be measured by the index of dispersion (Taylor *et al.*, 1978). Several indices have been proposed for measuring dispersion of individuals in a population (Morisita, 1964). Indices used to describe animal populations include (Southwood, 1978):

1) the simplest approach

$$s^2 * \bar{x}^{1/2}$$

where s^2 is variance and $\bar{x}$ the mean

2) index of clumping (David & Moore, 1954)

$$I_{DM} = (s^2/\bar{x}) - 1$$

where s^2 and $\bar{x}$ are as above

3) the coefficient *b* from the relationship variance and mean of a given species developed for the Taylor's Power Law

$$s^2 = a * \bar{x}^b$$

In most cases the dispersal is described by the Poisson distribution, the mean $\bar{x}$ being regarded as equalling the variance and, consequently, departures of the coefficient of dispersion (variation) $s^2/\bar{x}$ from 1 measures the departure from the Poisson related to randomness (Southwood, 1978). This is tested by the index

$$I_D = (s^2(n-1))/\bar{x}$$

where n is the number of samples and I_D is distributed as χ^2 with n-1 degrees of freedom (Southwood, 1978). In a Poisson distribution, values of I_D do not fall outside the limits given by 0.95 and 0.05 intervals of χ^2 presented in tables. Kuno (1991) showed that values of the coefficient of variation close to 0 indicate that the pattern of the population distribution is regular.

Kuno (1991) noted that for measuring the degree of crowding that characterizes individual frequency distributions, Lloyd's Mean Crowding is a suitable index.

Lloyd (1967) advanced the Index of Mean Crowding for analysing dispersions of mobile animals described by the negative binomial as a model. The mean crowding represents the mean number per individual of other individuals coexisting in the same quadrat. The Index of the Mean Crowding $\bar{m}$ would be expressed as

$$\bar{m} = m + (\sigma^2/m) - 1$$

where m and σ^2 are, respectively, the population real mean and variance and $\overline{m}$ is the amount by which the ratio between variance and mean exceeds 1 added to the mean. When the distribution follows a Poisson distribution pattern, $\sigma^2 / m = 1$ and the Index of Mean Crowding $\overline{m} = m$. Most estimates are based on samples and then, the Index of Mean Crowding $\overset{\bullet}{x}$ is given by

$$\overset{\bullet}{x} \cong \bar{x} + ((s^2/\bar{x}) - 1)$$

For dispersions described by the negative binomial model, variance can be calculated as

$$s^2 = \bar{x} + (\bar{x}^2 / k)$$

and substituting in the Index of Mean Crowding equation above, the $\overset{\bullet}{x}$ equation can be written

$$\overset{\bullet}{x} = \bar{x} + (\bar{x}/k)$$

Comparing mean crowding $\overset{\bullet}{x}$ to mean density $\bar{x}$, the ratio

$$\overset{\bullet}{x} / \bar{x}$$

would be simplified to

$$(\overset{\bullet}{x}/\bar{x}) = 1 + (1/k)$$

which gives 1/k as the proportion by which mean crowding exceeds mean density. This proportion is termed the patchiness for the species (Lloyd, 1967).

Iwao (1968, 1970) concluded that the relationship between mean crowding $\overset{\bullet}{x}$ and mean density $\bar{x}$ for a given species could be expressed for several densities using a linear regression

$$\overset{\bullet}{x} = \alpha + \beta\,\bar{x}$$

The coefficients α and β are characteristic for a species and its particular habitat (Southwood, 1978).

Kuno (1991) indicated that for measuring the patchiness, which corresponds to the relative magnitude of spatial variations in population density between quadrats, three indices could be used, namely the Morisita's I_δ , Kuno's C_A and Lloyd's $\overset{\bullet}{x}/\bar{x}$. Morisita's I_δ index is based on the probability that two randomly selected individuals are in the same quadrat (Morisita, 1971). Kuno's C_A is based on the concept of relative variance and Lloyd's $\overset{\bullet}{x}/\bar{x}$ on the crowding index described above. The use of these indices have been criticized (Taylor *et al.*, 1978 and Taylor, 1984), as field data values usually show significant correlations with mean densities.

The exponent k of the negative binomial model is also an index of aggregation in a population. Waters (1959) stated that k is a valid measure of aggregation and that there

were a number of examples from natural populations demonstrating its utility. The smaller the value of k the greater the extent of aggregation and consequently, the larger the value of k the more the distribution approaches the Poisson pattern of distribution. K values are often affected by the sampling unit size and consequently, comparisons have to be made between equal sample sizes (Waters, 1959; Harcourt, 1963; Southwood, 1978).

2.3 LIFE TABLES

Herbivorous insects such as *P. demodocus* are subjected to a high level of mortality from the embryonic up to the adult stage. This mortality is frequently as high as 98% or above and can occur while good quality food resources are still available. The identification of these mortality key-factors (Price, 1984) can open the way to their manipulation, thus improving crop protection against pests. This will contribute to the development of more environmentally friendly means of regulating insect pest populations (Richards & Davies, 1978). This leads to the necessity of clarifying the role of the regulatory mechanisms affecting insect population sizes within a particular natural or managed environment and is essential for the development of an integrated pest management strategy.

Apart from population reaction to abiotic factors, there may be interactions with other organisms. Two basic concepts in population regulation are recognised (Price, 1984):

- external factors that influence population numbers
- factor change within the population affecting population numbers and regulation

Density dependent factors regulate insect populations by increasing their impact when populations increase while their effect decreases as population levels fall. This is

manifested as proportions of the population being affected changes in response to a population increase in density. Factors that show no relationship in their influence related to population density will be density independent (Price, 1984). Most abiotic factors are unrelated to density (density independent). Others (e.g. some cases of predation by birds) may be inversely density dependent (Holling, 1965; Price, 1984; Hassell, 1987; Price, 1988). The mortality factors which regulate population size are related to population density changes caused by parasites, parasitoids, predators and pathogenic agents. The key mortality factor (or factors) is that which can be correlated with the total mortality of the insect population over a period of time (Roland, 1990).

Although no study has been carried out to investigate this possibility, Annecke & Moran (1982) suggested that *P. demodocus* populations could be regulated by natural enemies. Its populations are usually at equilibrium with occasional high infestation levels.

Ehrlich (1984) considered that the role of predation and parasitism in the dynamics of butterfly populations is still largely speculative. Apart from a few studies which showed that butterflies were regulated, for example *Papilio xuthus* (Tsubaki, 1973; Watanabe, 1976; Watanabe, 1979), neither predation nor parasitism have been shown to be regulatory factors in changes in butterfly population sizes.

Damage caused by pests depend on their abundance. This fact leads to the importance of continuing studies to clarify the role of biotic factors. In particular the manner in which biotic and abiotic factors influence population sizes (Carvalho, 1986). Therefore, knowledge of how populations fluctuate during periods of time around the equilibrium would make it possible to identify the appropriate timing for crop protection measures if the regulatory mechanism is no longer effective.

An assessment of the number of *P. demodocus* eggs, larvae and pupae killed by predators and parasites in conjunction with life table studies can be used to evaluate the impact of natural enemies. Similarly, the effects of inter and intraspecific competition should be evaluated although competition is not usually a factor regulating populations.

Southwood (1978) and Kuno (1991) showed that life tables could be used to assess field population dynamics in ecological studies. These provide the basic data for an understanding of the population dynamics of a given species. The information gathered should include birth rate, death rate, immigration and emigration at all stages of an insect generation over a period of time. Life tables can separate out the relative importance of physical and biotic factors and their relationship with population dynamics and assign numerical values in order to explain the differences in the number of individuals encountered at the various ages or life stages in the population while providing information on the causes of mortality of each stage. It would also help to establish why insect populations fluctuate temporally.

Several alternative techniques have been suggested to establish density dependence as a regulating mechanism in attempts to avoid possibly spurious positive correlations (Varley & Gradwell, 1960; Varley & Gradwell, 1968; Kuno, 1973; Royama, 1977; Elkinton *et al.*, 1990). Some limitations in the use and analysis of life tables include (Elkinton *et al.*, 1990):

- selection of the correct spatial scale on which to carry out research
- the fact that correlation does not prove causation
- most of the analytical techniques require collection of samples from the same

population over a period of many generations, at least 15.

Using key-factor analysis it is possible to determine mortality factors responsible for population changes between generations (Bellows Jr. *et al.*, 1992). Elkinton *et al.* (1990) observed that temporal density dependence is essential for a mortality agent to express its regulatory potential on a population. Hassell (1986) indicated that, at times, spatially density dependent mortality is reflected in temporal density dependence, even if no inter-generation numerical responses are present.

Three main types of life tables have been identified, namely static life tables, cohort life tables and rolling life tables (Southwood, 1978; Room *et al.*, 1991 and Bellows Jr. *et al.*, 1992). Cohort life tables (also age-specific or horizontal) are based on information of death rates collected from individuals of the same group as they get older. The disadvantage of cohort life tables can be that individuals are counted repeatedly; a process that can mask the true death rate and, thus, affect the eventual conclusions. Static life tables (time-specific or vertical) are constructed with information obtained on the population age structure on a single occasion. This sampling technique is valuable where individuals from a multi-stage population have generation overlap. In addition, for construction of static life tables, mortality factors must have constant effects on particular age classes in time. Rolling life tables combine information from cohort and static life tables, involving repeated censuses of populations to derive continuous estimates of age-specific mortality. It is of most value when there is no generation overlap. It is expensive as it is labour intensive (Southwood, 1978).

Room *et al* (1991) indicated that the main components appearing in life tables should include *ax:* the number of individuals that enter a given stage; *dxF:* the cause of a specific

mortality; *dx*: number of individuals that die from *dxF*; *lx*: population density transformed to a base of 1,000 to allow comparisons between sets of life tables; *100qx:* apparent mortality; *100rx*: real mortality; *Sx*: survival rate; *100ix*: indispensable mortality and *kx*: the mortality factor. Indispensable mortalities are dependent on the quantitative level of other mortalities in a life table. These may vary in different systems and so, indispensable mortality may not be included in life table studies (Bellows Jr. *et al.*, 1992).

In cohort studies, the values of *ax* are estimated by summing the numbers of living and dead individuals entered at a given stage *x*. The *kx* and *kxF* values are also standardised to a base of 1,000 individuals so that comparisons can be made between separate studies. Total mortality K is obtained by summing all the *kx* values. Podoler & Rogers (1975) recommended the regression of the several *kx* values against total K as an analysis method designed to assess which factor contributes the most input on total K in a generation. Price (1984) indicated that each k value and total K can be plotted against time so that the key factor is identified by graphical and regression analysis. In order to assess the density dependence of a given mortality factor, the specific k values are plotted against population density of the developmental stage on a log scale using regression analysis.

Life table budgets have been widely used in insect population studies (Southwood, 1978). A life budget provides details of absolute populations at different stages in their life cycle and also the effects of mortality factors, when known. These tables have the advantage of preserving information when data of age-specific life tables are corrected in order to start with a fixed number of individuals during comparisons of life tables. By correcting the population size to a base level the variations in population size of different generations, which might provide indications of the role of the various factors being analysed, can be lost (Southwood, 1978).

Because data for life tables correspond to population performance in particular circumstances (Room *et al.*, 1991), they may not be of use for managing a pest in, for example, species or cultivars other than the ones the data refer to. However, they do provide pointers regarding the most likely factors affecting mortality rates. Survival patterns could be different in each situation and at least three levels per factor are necessary for valid statistical modelling of population growth (Underwood, 1997).

Price (1990) suggested an alternative hypothesis with regard to the population dynamics of a latent, non-eruptive insect pest. This differed from the approach of Howard (1897), Lotka (1925) (both cited by Price, 1990) and Morris & Miller (1954) who claimed that natural enemies were usually responsible for regulation of the herbivore population.

Price's (1990) alternative is that

a) plant resources are the most important limiting factors for latent, non-eruptive insect species; the carrying capacity, low and stable, is defined by plant resources

b) the insects evolved adaptations so that for example, females do not lay eggs on plant resources of low quality; this female response to plant quality could be the most important factor in defining population size and its fluctuations

c) competition among females when oviposition resources are scarce result in stable populations close to the carrying capacity. In these populations, females tend to select young and vigorous plants or nutrient sinks in perennials. Insect populations that remain low and stabilised are a result of a relationship between plant-insect adaptive responses and phylogenetic constraints.

The net reproductive rate R_0 (number of times a population multiplies per generation) and capacity for increase r_c are adequate measures for population growth rate description in

most insects (Southwood, 1978). Southwood (1978), Price (1984) and Lara (1992) proposed the following equations for the calculation of R_0 and r_c:

$$R_0 = (N_{t+z}) / N_t$$

where z is the generation time and N_t the initial egg population size to provide the ratio of individuals in a population at the start of one generation to the numbers at the beginning of the previous generation or

$$R_0 = \sum lxmx$$

where x is the age class, lx is the survival rate during the age class x given by the probability of a newly hatched insect to be alive at age x and mx the number of progeny produced per female during age class x (specific mortality) that will produce females. The capacity for increase r_c was calculated as

$$r_c = (\log_e R_0) / T_c$$

where T_c is the cohort generation time (Price, 1984).

Room *et al.* (1991) considered that values of net reproductive rate (R_0) smaller than unity and of r_m (intrinsic rate of natural increase) less than zero indicate that the population is in decline while increasing populations exhibit values of R_o and r_m bigger than one and zero, respectively.

2.4 INSECT POPULATION DYNAMICS

Heliovaara & Vaisanen (1990) noted that the frequency, extent and severity of insect outbreaks have increased during the last 200 years. In addition, few insects had acquired pest status in the 1950s while insect pests now occur in high densities in areas where they did not previously occur. Explanations for these changes include the effects of annual changes in climate and intensive silvicultural or agricultural practices as the major causes affecting insect pests abundance (Heliovaara & Vaisanen, 1990).

Population abundance is dependent essentially on the various parameters affecting the population dynamics of the species. This is related to the fecundity and characteristics of the species in terms of development duration, behaviour and adaptative ability of various stages within a spectrum of environmental conditions (Wardle,1929; Carvalho, 1986).

Concepts such as life cycle (period between egg deposition to the cessation of adult reproduction), evolutive cycle (period between egg deposition to adult emergence), epidemiologic cycle (period between the commencement of egg deposition to the time when half of the eggs have been laid), biological cycle (number of generations that start and finish in a period of time) and similar terms are related to the generation concept. This concept links time to fecundity, becoming one of the fundamental elements for the estimation of multiplication rate. The embryonic developmental period may not correspond to the incubation period of eggs so, in studies of bioecology, knowledge of the incubation period frequently has a high priority because of its practical use in estimating the length of embryonic development in relation to environmental conditions (Carvalho, 1986).

Southwood (1978) defined natality as the number of births or the number of living offspring per female and fertility as the number of viable eggs laid by a female. Fecundity measures the total number of eggs produced by a female and is easier to measure.

Natality rate (population increase ignoring mortality and migration) depends on fecundity, egg fertility, sex ratio, percentage of sterile females and percentage of females that die before laying eggs. It is expressed as the number of eggs that hatch to produce larvae. Mortality rate (population reduction ignoring births and migration) is affected by the percentage of individuals in different age or stage groups and reduction of the population vigour due to environmental_factors e.g. poor nutritional food quality. Multiplication rate, the number of progeny from each female, is usually quite variable due to environmental conditions (Henson, 1968; Southwood, 1978; Ehrlich, 1984; Beck, 1986). Female age, size and weight are correlated to their fecundity. The last two can be affected by factors such as nutritional quality of food, temperature and moisture during their development (Henson, 1968; Fujii, 1975; Ehrlich, 1984; Beck, 1986). Fujii (1975) and Southwood (1978) citing Prebble (1941), Richards & Waloff (1954) and Taylor (1975) observed that the fecundity of a female was proportional to female weight and that female weight was directly related to size. Richards & Waloff (1954), cited by Southwood (1978), stated that the rate of oviposition is influenced by temperature and the realization of the potential fecundity is influenced by the longevity of the females. Nutrition and crowding have been reported as affecting female fecundity directly (Clark, 1963 cited by Southwood, 1978; Beck, 1986).

For this reason, mean multiplication rate can be estimated for a population in order to achieve better representation of its influence on population abundance. The mean number of offspring that individuals can produce (mean specific fecundity) can be highly variable (Carvalho, 1986; Lara, 1992).

An improvement in environmental conditions such as food nutrition quality (Schultz *et al.* 1990) can cause sudden and unexpected population outbreaks in insect populations. Information on the reproductive potential of a pest and the factors influencing that and causing a pest outbreak is essential.

Reproductive potential indicates the potential fecundity under ideal conditions. A practical way of precisely estimating the fecundity value is to rear insects under ideal conditions or by capturing females in situations of rapid population increase (Southwood, 1978). Dead females should be dissected to estimate their potential egg production. The number of eggs (fertile and infertile) gives a measure of the potential fecundity value of the insect. Using values for sex ratio, development duration and potential fecundity it is possible to estimate the reproductive potential value, using the expression:

$$X = pz^n$$

where X is the number of progeny

n the number of generations

p the initial population size

z the fecundity value using sex ratios (Carvalho, 1986).

If, for example, the number of eggs laid by a species was 100, the sex ratio is 1:1 and the initial population size 2 (1 female and 1 male), after one generation, there will be a total of 100 individuals.

Engelmann (1970) noted that in many insect populations, the sex ratio tends to be 1:1 because the major sex-determining mechanisms, balance between male and female sex determiners and epistatic sex determination establish this ratio. Consequently, individuals of both sexes are usually produced in equal numbers; however, some deviations have been observed, e.g. in *Lymantria dispar* L. (Lepidoptera: Lymantriidae) and *Hypolimnas bolina* (L.) (Lepidoptera: Nymphalidae) (Clarke, 1984). Engelmann (1970) defining espistatis as the masking of the expression of nonallelic genes stated that in a number of lepidopterous insects (e.g. *Bombyx mori* L. (Lepidoptera: Bombycidae) epistatic sex determination may be more common than the determination of sex by the balance between female and male determiners.

2.4.1 Pest movements

Information on insects with respect to a mobility capacity in relation to population density, age structure and genetic composition is essential in order to evaluate the population dynamics of a given species (Hughes, 1979). Drake (1991) pointed out the importance of incorporating information on both pest spatial heterogeneity and movement for the development of a population dynamics model.

Mark-recapture studies have been extensively used in the study of insect populations (Zhang & Schlyter, 1996; Sawada *et al.*, 1997; Bloem *et al.*, 1998; Mullen *et al.*, 1998). They enable the estimation of absolute insect population density, which is essential in the population dynamics studies. In addition, the method allows the determination of population natality and mortality rates as well as the immigration and emigration rates. Consequently, insect population longevity, dispersal and growth can be evaluated. The method has been extensively developed and is regarded as an appropriate alternative to the methods based on the counts of animals in quadrats. An advantage is that the

accuracy of the method does not depend on the assessment of the number of sampling units in the habitat (Southwood, 1978).

One of the techniques suitable for mark-recapture studies is Bailey's Triple Catch Method (Southwood, 1978; Begon, 1979). For this purpose a series of triple-catch estimates are carried out. Estimates are based on the formula

$N_2 = a^{\wedge}{}_1 \, n_2 / r_{21}$

where $a^{\wedge}{}_1$ is the estimate of the number of individuals marked on day 1 that could be recaptured on day 2

Using this logic, the population size on the second day can be estimated as N_2

$N_2 = a_2 \, n_2 \, r_{31} / r_{21} \, r_{32}$

where a_2 is the number of newly marked insects released on day 2
n_2 is the total number of animals captured on the day 2
r refers to the recaptures with the first marking representing the day of capture and the second marking representing the day of marking
r_{21} the number of insects captured on day 2 that had been marked on the first day
r_{31} the number of insects captured on day 3 exhibiting marks of the first day

2.4.2 Insect population growth curves

Model building assists in the generation of hypotheses concerning processes, populations or communities which can be tested by experimentation which can be rejected or

accepted (Kitching, 1983). Another function is the synthesis of knowledge concerning a phenomenon so that problems associated with that phenomenon can be investigated and eventually resolved. Models are built to answer specific questions, for example, to model the whole life-system of a pest species in a region (e.g. *Lymantria dispar* L. (Lepidoptera: Lymantriidae) in Europe (Znamenski & Liamcvev, 1983 cited by Montgomery, 1990 and *Cinara pinea* (Mordvilko) (Homoptera: Aphididae) (Kidd, 1990) or to evaluate integrated management strategies e.g. for the winter moth *Operophtera brumata* (L.) (Lepidoptera: Geometridae) in Canada (Roland, 1990). Models have proved to be extremely valuable in insect ecology and management (Kitching, 1983).

Models fall into four main classes (Kitching, 1983):

1- simple algebraic models of ideal populations

2- complex simulations of ecological processes such as predation, competition and movement

3- life-system models of single species, their hosts and natural enemies and

4- decision-making and tactical models at the crop or regional level

In model construction, after defining the system to answer the question being asked, it is necessary to identify measurable variables e.g. number of larvae, eggs or pupae in a population, define sources and sinks of material and connect these by material and information flows. These flows imply the existence of rate processes, e.g. egg hatch, larval development, death, adult emergence, etc. in order to approximate the dynamic changes observed in the various variables.

Following the identification of variables and constants the system can be defined. Following system definition, equations can be derived to encompass the changes that

occur through time. These will contribute to the basic model. The next step is to ascertain the rate at which the processes operate within a particular time period. The mathematical formulation of this relationship involves the estimation of parameter values within the statistical relationship chosen (e.g. the *a* and *b* axes in a regression model):

$y = a+bx$

Parameter values for a pest model are preferably values based on statistical analysis of data collected on the subject species at the location of interest over one or more seasons. Ideally, data collection and analysis should be done in parallel with the model-building process. Once built, the model must be verified (accurate reproduction of values and relationships built) and its predictive powers tested. An adequate correspondence between prediction and suitable test data (data not used in the parameter estimation stage of the work) may be an approximate reproduction of the major peaks and troughs in the test data and a difference of more than 10% between predictive and test data is generally unacceptable (Kitching, 1983).

Growth curves - simple models of ideal populations

Carvalho (1986) stated that population censuses for quantitative studies during a given time are needed to describe the population growth curves. Growth rate of a population expresses the difference between the birth and mortality rate. Birth rate (numerical growth of the population during a short time interval in the absence of mortality and migration) depends on several factors: fecundity, fertility of the eggs, proportion of sexes, percentage of sterile females in the population, female mortality before laying eggs, variation in the duration of pre-oviposition of eggs and the influence of environmental factors on each developmental stadium of the individuals within the population (evaluated

under field conditions). Mortality rate (numerical decrease of a population during a short time interval in the absence of births and migratory movements) is affected by the relative percentage of individuals of different groups of age (percentage of old individuals), poor population vitality due to environmental factors (evaluated in the field).

In growth curves of a population, focus should be directed at the curve structure (population composition in its various developmental stadia) because of the diverse influences that the different stadia of development have in the expression of birth and death rates (Carvalho, 1986 and Lara, 1992). Differences in susceptibility of the various developmental stadia of a pest population to the control measures have been observed. According to Price (1984) the equations

$$N_t = N_0 e^{(b-d)t} \text{ or } N_t = N_0 e^{(b-d)t} - E_t + I_t$$

where t = the time interval (short, for example 5 days)

N_0= number of existing individuals at the beginning of time interval (t)

b= number of births

d= number of deaths during time (t)

e= constant, logarithmic basis

E_t = number of individuals which leave the area

I_t = number of individuals joining the area during the time interval (t)

are used for population growth description when migration is not observed or when it does occur, respectively. Reproductive potential can be determined using the expression

$$x = p^{zn}$$

where x = number of progeny from a given population

n = number of generations

p = initial population size

z = the product of fecundity using sex ratios

In a case of embryonic multiplication, the factor *y* is introduced into the expression to represent the number of progeny resulting from the division of a unique egg. The expression takes the form

$$x = p^{(yz)n}.$$

Morris (1959) observed that as changes in animal populations (N) are mostly geometric, it is necessary to express N as a logarithm. Price (1984) noted that the relationship between the population size in a succeding generation (N_{n+1}) and the initial population size N_n is curvilinear and the variance increases with N. Variance is not independent of the mean population density. For these reasons, the data are transformed to log scales resulting in a linear relationship between the variables N_{n+1} and N_n. Variance becomes independent of the mean (Morris, 1955; Price, 1984). Price (1984) observed that the equation predicting population size of a pest in the generation N_{n+1} can be expressed as

$$\log N_{n+1} = \log F + bd \log N_{npq}$$

where *q* is the proportion of N_{np} that survives predation

N_{npq} the proportion that survives predation and parasitism

bd the slope of the line the contribution of the density-dependent factors

F, the intercept, is the effective rate of increase of the insect between generations when the population portion is not affected by a key factor

(the slope of the line *bd* refers to the slope resulting from density dependent factors)

Price (1984) pointed out that an acceptable result relates to 70% of the variation being explained by density-dependent factors.

When natural enemies do not have a large role in regulating the abundance of aphids, and there is no evidence of differences in natural enemy efficiency of the various species, then differences of a realized rate of increase for the different aphid species are the most important cause of changes in abundance between aphid species, their population dynamics between years can be expressed by the following equation (Dixon, 1990):

$$\text{Log } X_{t+1} = \log X_t + \log R - M \log X_t$$

where X_t and X_{t+1} are the peak numbers in a given season of year *t* and *t+1*

R = the realised rate of increased

M = the density-dependent factor

Leslie matrices

Matrices containing fecundity and survivorship terms are called Leslie Matrices (Kitching, 1983). These models have been extensively used in insect population studies (Lewis & Leslie, 1940 cited by Southwood, 1978; Cheke, 1995) to describe the transition of a population during a given period of time. Leslie Matrices examine the additive and subtractive process that operate on a population in any time period as follows

$$\Delta N = B-D+I-E$$

where ΔN is the change in the population

B is births

D = deaths

I = immigrants

E = emigrants

over the time period being studied

More complex models can be constructed using linear algebra to incorporate aspects of fecundity and survivorship. The matrix model represents a dynamic version of the standard age-specific life table with an added fecundity schedule. Having constructed the equation and decided on the size and structure of the initial population, it is possible to predict the size and structure of the population at intervals into the future by carrying out matrix multiplications (Rose, 1987).

The basic form of a Leslie Matrix is

$$\begin{pmatrix} f_0 & f_1 & f_2 & \cdots & f_{k-1} & f_k \\ p_{0,1} & 0 & 0 & \cdots & 0 & 0 \\ 0 & p_{1,2} & 0 & \cdots & 0 & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ 0 & 0 & 0 & \cdots & p_{k-1,k} & p_k \end{pmatrix} \times \begin{pmatrix} n_0 \\ n_1 \\ n_2 \\ \cdots \\ n_k \end{pmatrix}_t = \begin{pmatrix} n_{0t} & \Delta_{0t} \\ n_{1t} & \Delta_{1t} \\ n_{2t} + & \Delta_{2t} \\ \cdots & \cdots \\ n_{kt} & \Delta_{kt} \end{pmatrix}$$

where n is a vector representing the number of individuals in each of k age-classes

the f_i represent age-specific birth rates, that is, the fecundity of individuals in each age-class (the number of offspring born to individuals of each age within a fixed time period

p_{ij}'s are the age-specific survivorship terms

2.5 TEMPERATURE, RELATIVE HUMIDITY AND PHOTOPERIODS – INSECT DEVELOPMENT RELATIONSHIPS

Climate affects insect populations both by the individual or the combined effects of its various components. Varley (1963) and Clark *et al.* (1967) pointed out that weather plays an important role by its direct or indirect effects on insect populations sizes. Two main types of climatic effects are known to affect insect population sizes (Henson, 1968). Firstly the impact of an isolated and catastrophic change in ambient conditions leading to immediate mortality. Secondly, effects expressed more gradually on individual development, fecundity, behaviour or genetic selective change in the constitution of the population.

Several examples of weather as a controlling factor on population densities of some insect species are provided by Davidson & Andrewartha (1948a, 1948b). Examples include species in the genus *Thrips* (Thysanoptera: Thripidae) in Adelaide, Great Victoria Desert, Australia, where the geographical limit of the pest range could be attributed to climatic stress. Whittaker (1971) Greenbank (1956, 1963) observed similar effect of climatic stress when respectively, studying the insect *Neophilaenus lineatus* (L.) (Homoptera: Cercopidae), close to its range edge and southern sites of England and *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) in New Brunswick, Canada.

The degree to which weather can directly produce mortality in butterfly populations, other than influencing resource availability or insect fecundity, is unknown (Ehrlich, 1984).

Temperature is one of the most important factors which influence insect development as it affects its duration, life cycle and mortality. The effect of temperature depends on its

values, duration of the periods of exposure and also on relative humidity. The effect of temperature on the duration of pest population development can be expressed by the equation (Carvalho, 1986)

$$Y=k\ (t-c)$$

where y = development speed

t= temperature

k= thermic constant

c= zero constant of development

By knowing the duration of a stadial development of a given species for 2 temperatures, it is possible to construct a hyperbole establishing a relationship between stadium duration and values of temperature in the interval of zero development and upper limit of temperature for the insect species (Carvalho, 1986).

At least 10 temperatures separated by intervals of up to 2.5 °C are required for temperature-development relationship studies (Allsopp *et al.* 1991). More temperatures are needed to make the correct decision between alternative non linear models for the analyses, usually a range of temperatures at approximately 1°C intervals around the optimum. Allsopp *et al.* 1991 stated that for statistical description of the development time both the mean and, preferably the median, being less affected by abnormal values, are acceptable parameters to describe development times.

Wagner *et al.* (1984) pointed out that models of insect development rate or time versus temperature are mostly used to describe the temperature related curve of insect development. They indicated that models of the relationship between development rates

and temperature are widely used to predict insect development times under fluctuating temperatures.

Brier & Rogers (1981) cited by Allsopp *et al.*, (1991), concluded that field populations have lower developmental variability than laboratory populations and Twine & Evans (1991), in their studies, discovered that a population of an insect species could develop different characteristic temperature-development relationships after several generations.

Although differences between sexes have been found (Reed, 1965; Foley, 1981, cited by Allsopp *et al.*, 1991), the authors concluded that the differences were small and only significant in highly skewed sex ratios. A highly skewed sex ratio where there is a high dominance of females under given temperatures could, if that condition is perpetuated, result in a large increase in population numbers after a number of generations. Henson (1968), Gordon (1984), Carvalho (1986), White (1987) and Ayres & Scriber (1994) described in detail methods for the evaluation of temperature on insect populations.

Carvalho (1986) indicated that although " optimum ranges" of relative humidity can be found for each insect species, the effect of extreme values of relative humidity are not, in general, as critical as those of temperature for insects. Bateman (1968), however, pointed out that in some insect species, moisture can be the primary determinant of insect population numbers. He observed that summer rainfall favoured survival of pupae and newly emerged adults of *Dacus tryoni* (Froggatt) (Diptera: Tephritidae) in Australia. It also increased the fecundity of mature females as well as the rate of immigration.

Diapause synchronizes insect developmental stages with periods of the year to which they have become adapted. Diapause, as a seasonal adaptation in insects, is greater in importance in temperate zones and northern latitudes where climatic variations during the year are more marked (Danilevskii, 1965). Bean & Beck (1983) and Beck (1986)

reported that photoperiod effects may be observed on larval developmental rates, inducing diapause in some insect species e.g. *Ostrinia nubilalis* Hb. (Lepidoptera: Pyralidae), when the larvae are subjected to relatively low temperatures at the same time, as a mechanism to face adverse conditions in the life cycle. However, for *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) no form of diapause (embryonic, larval or pupal) was induced by changes in daylength.

2.6 EFFECT OF DIET ON LARVAL DEVELOPMENT

Ehrlich & Raven (1967) noted that plant herbivore relationships may be the most important interaction responsible for geographical organism diversity. Every species must obtain sufficient energy for successful reproduction. Plants and their parts vary in nutritional values for insects. This fact has important consequences for insect pests of plants and their selection of food (Bernays & Chapman, 1994).

The need for food varies with the stage of butterfly development. A species in its larval stage usually feeds on plant leaves and, in its adult stage, on nectar of flowers. Therefore, there is a synchronisation between insect development stage and the phenology of plants, i.e, between the insect life cycle and the availability and quality of food necessary for each development stage as well as between the insect life cycle and those of their predators, especially parasites (Carvalho, 1986). Only if synchronisation occurs can the agents have a significant role in the pest population control.

Larval growth of herbivores insects can be affected by factors such as water content, toughness of plant material, nutritional quality of plants, for example the free aminoacids, proteins and mineral contents of the food material (Matsuki & MacLean, 1994).

Host quality can be one of the most important factors regulating numbers of insect pests (Parry, 1974; McClure, 1988; Leather, 1990; Matsuki & MacLean Jr., 1994). The quality of food influences egg production in many phytophagous insects (Johansson, 1964). Egg output may vary with the species of plant, even if these all belong to the same genus. Within the same species, different varieties of food plant influence insect reproductive capacity, apart from the physiological conditions of the host plant. Feeny & Bostock (1968) and Price (1984) observed that tannins in plant leaves reduced larval weight and consequently the fecundity and population growth of *Operophtera brumata* (L.) (Lepidoptera: Geometridae). Johansson (1964) indicated that components important for insect reproduction include: water, carbohydrates, proteins and aminoacids, lipids, vitamins and minerals. White (1984), Mattson & Scriber (1986) and Watt (1990) indicated that chemicals such as nitrogen, phosphorus, water and tannins have been shown to be important in several insect-plant relationship studies.

Studies have indicated that some insect pests tend to develop faster on artificial diet than on host plants (Allsopp *et al.*, 1991). Howard (1906) stated that the more suitable the food is, the longer the larval stage for *Papilio demodocus*. Hagen *et al.*, (1984) recommended estimation of nutritional indices as follows:

Relative Consumption Rate (RCR)

$$RCR = \frac{\text{amount of food ingested (fresh wt of food eaten) (mg)}}{\text{duration of feeding period (days) x mean wt of insect during feeding period (mg)}}$$

Relative Growth Rate (RGR)

$$RGR = \frac{\text{wt. Gain of insect during feeding period (fresh) (mg)}}{\text{duration of feeding period (days) x mean wt. Of insect during feeding period (mg)}}$$

Assimilation Efficiency (AD)

$$AD = \frac{\text{amount ingested (mg)} - \text{faeces (mg)}}{\text{amount ingested (mg)}} * 100$$

Efficiency of Conversion of Assimilated or Digested Food (ECD)

$$ECD = \frac{\text{weight gained (mg)}}{\text{amount ingested (mg)} - \text{faeces (mg)}}$$

Efficiency of Conversion of Ingested Food (ECI)

$$ECI = \frac{\text{weight gained (mg)}}{\text{Amount ingested (mg)}} * 100$$

2.7 EFFECT OF LARVAL DENSITY ON DEVELOPMENT

Richards & Southwood (1968) observed that an increase in population density might alter the genetic or phenotypic characteristics of an insect population and thus lead to a reduction in numbers in the succeding generation. Pimentel (1961) suggested a possible action of a genetic feed-back mechanism in modifying relationships between different populations of a species. Wellington (1964) suggested that high density levels may induce intrinsic mechanisms of qualitative changes in a population as at high densities, high levels of group activity at an initial period would ensure the survival of many less active

members in a population of *Malacosoma pluviale* (Dyar) (Lepidoptera: Lasiocampidae). These less active members have reduced flight capability. Consequently, the second generation will include their progeny, amongst which, only some individuals have stronger flight capability. This process may continue and therefore, the deterioration preocess accelerates in the population. In the end, the inactive members may die.

Hassell (1987) stressed the importance of studies aimed at, not only assessing variability in processes between generations of insect populations, but also examining within-generation density dependent heterogeneity. This could be investigated through, for example, experimentally manipulated populations. Dempster (1968) observed that mortality in insect populations can be caused by overcrowding of individuals. He also reported a reduction in the mean adult weight of_insects specimens produced from crowded nymphs of the species *Arytaina spartii* (Guerin-Meneville) and *Arytaina genistae* (Latreille) (Homoptera: Psyllidae). Citty (1965), cited by_Richards & Southwood (1968), suggested that population quality may vary at different population densities since individuals developing at high densities appeared to be particularly vulnerable to diseases and other causes of mortality. This was attributed to be the case for the self regulation of the tent caterpillar *M. pluviale* (Wellington, 1960; Wellington, 1965).

Dempster & Pollard (1985) stressed the need to be cautious when interpreting data for density dependent regulation as spatial heterogeneity (within-generation) can be overlooked while testing temporal density dependence (between-generations).

Dempster (1968) working with crowded adults of the psyllid *Arytaina spartii* (Guerin-Meneville) and *Arytaina genistae* (Latreille) (Homoptera: Psyllidae) and Fujii (1975) observed that density in adults may affect their fecundity. The insects had reduced fecundity rates when compared to less dense populations. In his studies, Oloya (1964)

concluded that the number of eggs laid per psyllid female at a density of 60 adults per cage was only c. 33% of that laid at a density of 10 adults per cage. Fecundity, however, was not altered when the adults, previously crowded during their preoviposition stage, were isolated for egg deposition.

Cannibalism of eggs and small larvae by larger ones have been observed in many phytophagous insects and in butterflies, most species which lay single eggs are to some extent canniballistic (Gilbert, 1984). Increased mortality at high larval densities can also be due to starvation (Dempster, 1968). This phenomenon requires investigation on the effect of the pest larval density on development and survival.

2.8 EFFECT OF NATURAL ENEMIES ON PEST POPULATIONS

Established interdependencies between populations of the pests and their natural enemies can determine the population abundance of the pests, such that a given species may or may not express itself as a pest.

Huffaker *et al.* (1968); Kiritani & Dempster (1973) indicated several techniques to assess natural enemy impact on a pest population. Kiritani & Dempster (1973) classified the methods of evaluating natural enemy action into two categories; indirect methods by correlation between number or rate of increase of the host and numbers of the natural enemy and the direct methods. Direct methods can be divided into three types:

1) exclusion of the predator or parasite using several techniques, for example mechanical barriers and chemicals.
2) measuring the action of the natural enemy by the presence of the parasite or pathogen in the body of the host or by the presence of prey material in the gut or faeces of the predator

3) direct observation of the action of a natural enemy in the field. This can be time consuming.

Seymour & Jones (1991) stated that studies to evaluate the effectiveness of a predator should be undertaken in conditions as realistic as possible and preferably in the field. Laboratory studies of consumption rates of particular predators usually can tell us only how many prey the predator will consume with no alternative. Cage experiments, in which known numbers or densities of prey are exposed to known numbers of a particular natural enemy, are appropriate methods for evaluating natural enemy impact. The similarity of conditions in the cage to conditions found in the field are important in relation to the relevance of the results.

Hassell & Rogers (1972) developed models to describe host-parasite interactions; their model showed that the searching capacity of a parasitoid could be predicted taking into consideration three basic parasitoid responses, namely:

1) that the functional response to host density following the act of attacking a host or prey occupies a finite time. This could result in a reduction of parasitoid searching efficiency at high host densities.
2) a response to the host distribution
3) the response to other parasites, because a given parasitoid action can be modified by the presence of other parasitoids.

Seymour & Jones (1991) stated that when evaluating a parasitoid's effect on its host, if the parasitoid extends the length of time of host instar duration then raw parasitism rates overestimate net parasitism rates and should be corrected:

corrected parasitism rate = $1 - (1+k)\ S/2$

where k is the ratio (development time of parasitized instar)/(development time of unparasitized instar)

S = proportion of unparasitized larvae in the sample

This formula provides also the appropriate correction in the less common case of parasitism - shortening development time of an instar.

Richards & Southwood (1968) indicated that a predator with low reproductive rate may fluctuate with the numbers of its prey without regulating it.

Roland (1990) stressed the importance of the determination of the impact of the natural enemy relative to other mortality factors that act on the host insect population to understand pest population dynamics.

The roles of predation and parasitism in the dynamics of butterfly populations is still largely speculative. Apart from a few studies on this issue (Tsubaki, 1973; Brown & Vasconcellos Neto, 1976; Watanabe, 1976; Pough & Brower, 1977; Watanabe, 1979; Ehrlich & Ehrlich, 1982); neither predation nor parasitism have been shown to be regulatory factors in changes in butterfly population size (Ehrlich, 1984).

CHAPTER THREE

MATERIALS AND METHODS

(i) STUDY AREA

The study area is located in the southern Mozambique district of Boane and Namaacha, Maputo Province which are traversed by the river Umbelúzi (appendix 1.2). The main source of income of the Umbelúzi population comes from agriculture. The main crops include maize (*Zea mays* L. : Poaceae), beans (*Phaseolus vulgaris* L. : Fabaceae), cassava (*Manihot esculenta* Crantz : Euphorbiaceae) and fruits such as banana (*Musa* spp.: Musaceae) and pawpaw (*Carica papaya* L. : Caricaceae). Prior to independence peasants were not allowed to grow citrus plants owing to the possibility of spreading diseases and pests from the rural farms to the citrus company orchards in Umbelúzi. *Citrus* trees in local rural farms have all been recently planted.

The climate is semi-arid humid, the available mean period of effective rains varies from 5 to 8 weeks (Reddy, 1986). Average annual precipitation is 729 mm; mean air relative humidity 70% with higher values in February and March and lower values from May to August.

High temperatures occur from October to March, the rainy season, with January being the hottest month (maximum and minimum temperatures of 39° C and 20° C respectively). Lower temperatures occur from April to September (dry season), with maximum and minimum temperatures of 28° C and 11° C (appendix 3.1).

Engelen (1981) described the soils as alluvial with high fertility; the texture varies from sandy to clayey with regular drainage (appendix 3.2).

Natural vegetation in Umbelúzi includes trees and shrubs such as *Combretum* spp. (Combretaceae), *Acacia* spp. (Fabaceae), *Aloe* spp. (Aloaceae), *Euphorbia* spp. (Euphorbiaceae), *Sclerocarya birrea* (A. Rich) Hochst (Anacardiaceae) and grasses and herbs including *Panicum* spp. (Gramineae), *Amaranthus* spp. (Amaranthaceae) and *Tridax procumbens* (L.) (Asteraceae).

The sites where fieldwork was conducted include orchards that belong to three main owners, the private enterprises CITRUS SARL, LOMACO and those of the National Institute for Agronomy Research/ Ministry of Agriculture - INIA (appendix 1.2). At CITRUS SARL, citrus orchards occupy 125 hectares (ha), consisting of 73 ha of grapefruit, 46 ha of *Citrus paradisi* Macf. cv. Marsh seedless with the remaining 27 ha including grapefruit plantations with cultivars cv. Star Ruby and to a lesser extent cv. Rose. The remaining 52 ha consist of oranges, specifically *Citrus sinensis* (L.) Osbeck cv. Valencia late. The orchard is composed of small fields averaging 2.5 ha in size (field sizes, *Citrus* species, plant age and spacing in appendix 3.3).

The nursery, with the capacity to produce 50,000 plants per year, is located in Maputo, coordinates 25° 27' S and 32° 36' E while the orchards are situated at 26 ° 03' S and 32 ° 20' E. Altitude varies from 30 to 40 m above sea level. The nursery wind breaks are dominated by *Casuarina equisetifolia* L. (Casuarinaceae). By comparison with the nursery, the orchards at the CITRUS SARL have not been irrigated or received pesticide treatments since October 1997.

The orchards of the LOMACO enterprise are based at three main sites: Olsa Citrus D (coordinates 26° 11' S and 32° 12' E), Olsa Citrus A and B at the coordinates 26° 10' S

and 32° 11' E and 1° de Maio at 26° 05' S and 32° 15' E; altitude varies from 0 to 300 m. The sites occupy an area of, respectively, 72.90 ha; 103.00 ha and 163.07 ha. The nursery is located at the site 1° de Maio and can produce 50,000 plants per year. Live windbreaks dominated by *Casuarina equisetifolia* and occasionally *Grevillea robusta* A. Cunn. Ex R. Br. (Proteaceae) protect the orchards and nursery. The nursery capacity is aimed at 50,000 seedlings a year. Pesticides regularly applied are usually Thuricide, a bacterial insecticide (*Bacillus thuringiensis* Berliner. (C.A.)) var. *kurstaki* 16000 wettable powder at the rate of 25 g/100 l of water every seven or 10 days against *P. demodocus* and the organophosphorous insecticide Methidathion (Ultracide) and the carbamoxyloxime Aldicarb (Temik) to control aphids, thrips, mites and nematodes. The glyphosate herbicide (Round-Up) was used to control weeds.

The orchards of INIA are located at the coordinates 26° 03' S and 32° 22' E at c.100 m in altitude. This is the remains of an INIA trial that occupied an area of approximately 12 ha with a nursery with the capacity to produce 10,000 seedlings per year. *Citrus* species of this orchard include *C. sinensis* cv. Valencia; cv. Washington Navel; *C. paradisi* cv. Star Ruby; cv. Marsh and cv. Rose. This orchard was not treated with pesticide during the 4 years prior to 1998.

In the nurseries, fertilizer (NPK 12:24:12) is applied at the rate of 250 g/plant at the sowing period and subsequently every three months (Glória[1], personal communication).

In terms of rootstock behaviour in the Umbelúzi region, *C. lemon* (L.) Burm cv. Troyer citrange appears to be the most suitably adapted to local conditions although it takes longer than most cultivars to recover after grafting. The LOMACO company adopted the *C. lemon* cv. Carrizo and cv. Troyer citrange. The most common rootstock used in the Umbelúzi region are *C. lemon* cv. Troyer citrange, cv. Swingle citrumelo, cv. Carrizo,

[1] Head of the Nursery Section at LOMACO SARL

cv. *639* and cv. Miniola. The cultivars that are grafted on to the above rootstocks are *C. sinensis* cv. Valencia and *C. paradisi* cv Marsh, cv. Star Ruby, cv. Rose and cv. Nartia. Grafting takes place when the seedling is approximately 6 months old. Seedlings are produced throughout the year and usually transplanted before reaching two years of age. Pruning takes place only when the plants are already established in the definitive planting areas. Watering is carried out daily by aspersion. The rotation length for commercial *Citrus* trees in Umbelúzi is around 25 years.

Soil sampling was carried out with a screw auger in all study sites. The auger was inserted into the soil and withdrawn at 15-20 cm depth intervals down to 1 m. Soil analysis was carried out at the Soil Science Department, Eduardo Mondlane University. Standard procedures (Allen *et al.*, 1986) were followed for soil analysis (appendix 3.4).

Three field study areas of approximately 1.4 ha, 1.3 ha and 0.5 ha were established respectively at INIA (coordinates 26 °03.160'S; 32 °22.131' E and 100 m above sea level), University Campus (coordinates 25 °57.362' S; 32 °36.169' E and 80 m above sea level) and 1° de Maio LOMACO (coordinates 26° 05.509' S; 32° 15.083' E and 80 m above sea level) for life table data collection. At INIA and University Campus, 0.25 ha were planted with *C. paradisi* cv. Marsh, 0.25 ha with *C. paradisi* cv. Star Ruby while the remaining 0.9 ha at INIA and 0.8 ha at the University Campus trials were planted with *C. sinensis* cv. Valencia. At 1 °de Maio only the latter cultivar was planted. Plant spacing was 3 m with 7 m between rows. *C. jambhiri* cv. Rough lemon provided the rootstock.

NPK (12:24:12) fertilizer had been applied until April 1999 at 250 g/hole or plant before planting the seedlings and at three months intervals after planting. Watering at approximately five litres per plant/week was manual. The experimental orchards were managed as a LOMACO commercial orchard other than spraying with chemicals was

avoided. The only chemical applied in the experimental orchards was the herbicide glyphosate (Round-Up) for weed control. Plants were six months old and approximately 80 cm high when sampling commenced.

3.1 BIOLOGY OF *Papilio demodocus*

In order to ensure that a constant 50:50 proportion of females and males was placed in each cage for mating and egg deposition live 5th instar larvae were sexed (Underwood, 1994). Male larvae have one central sex pit on the ventral surface of the 9th abdominal segment whereas females have 2 pairs of pits, two on the 8th and two on the 9th abdominal segments. Dissection of adults confirmed that the pitting technique was accurate. Prior to examination, the larvae were immobilised by a short exposure to cotton wool containing 1% ether in hexane.

A female and male 5th instar larva were placed in each cage in March 1998 for a preliminary study of *P. demodocus* embryonic period, larval and pupal development times, adult life span, fecundity rate and sex ratio under field conditions. Five cages 30 cm in diameter and 60 cm high were placed in citrus plants aged approximately one year at INIA orchards and free of pesticides. Field air temperatures ranged from 16 to 27° C and air relative humidity from 45 to 78%.

Culturing *P. demodocus*

The larvae used in the experiments were fed on natural substrate (leaves of *Citrus sinensis* cv. Valencia) during the larval stage. In the laboratory and field cages different marking patterns using the commercial white fluid for document correction Tipp – Ex

containing thinner 1.1.1 Trichlorathan were made on the individuals at the start of the trials and after every moulting for recognition during the subsequent observations.

In all field and laboratory experiments, except the experiment testing the effect of temperature on insect development, prior to pupation, the specimens were transferred to 2mm mesh nylon cages (2 in total) over a metal frame the dimensions being 4 m x 6 m x 3 m in order to oviposit. The period before pupation was easily recognised as larvae stopped feeding, immobilised and started producing a whitish silky material.

The mating cages were kept at the University Campus and were exposed to natural daylight with ambient temperatures ranging from 11°C to 26°C during the dry season and 18°C to 30°C during the rainy season. Average air relative humidity varied from 60 to 80% during the rainy season and from 50 to 60% during the dry season.

Upon adult emergence, a 10% sucrose solution soaked on cotton wool was provided as well as the herb *Tridax procumbens* which was regularly visited by *P. demodocus* butterflies in the field. Larval and oviposition substrates were always plants or shoots of *C. sinensis* cv. Valencia separately for each group treatment except for the groups of specimens used to assess the effect of diet on *P. demodocus* performance where citrus cultivars were the same as those of the experiment for every treatment. Only the cultivar Valencia was used in the experiments because in previous studies, pest development time, survival and fecundity seemed to be unaffected by diet. Feeding materials were replaced every second day.

All adults emerging from pupae caged in the rainy season were used for mating and oviposition purposes but the proportion of female and male adults from the dry season was always kept constant; that is, for the emerging adults the sex ratio was always kept in

the proportion one female to one male and the excess butterflies removed from the mating cage.

3.2 *Papilio demodocus* POPULATION DISTRIBUTION

Sample size determination

A preliminary survey aimed at estimating the required number of samples in *P. demodocus* population distribution studies was carried out at 25 randomly selected orchards between 10th and 20th March 1998.

The orchards covered the range of plantations of different ages, species, cultivars and management practices in the region (tables 3.1.1 and 3.1.2).

The survey was conducted between 08.30 h and 11.30 h using randomly selected 30m x 30 m quadrangular plots containing approximately 40 plants per field.

The number of individuals in each stage, except the adult, were counted on each plant. Details on plant age, species, cultivar, height, pest position, natural enemies and meteorological data were also collected on each sampling occasion (appendix 3.5 and 3.6). Records of daily maximum and minimum air temperatures, relative humidity, radiation, wind speed and evaporation were provided by the INIA Meteorological Station in Umbelúzi. Plant height was measured with a measuring tape (to the nearest cm) for young trees and a hypsometer (1 dm precision) for trees $\geq$ 2.5 m. Data for distribution studies, sampled using same procedues as above described were not taken during the month of January 1999 due to high rainfall.

Table 3.1.1 Orchard age, species, cultivar and management practices in orchards > 2 years old (sprinkling = ground sprinkling)

Site	Orchard	Age (years)	Species	Cultivar	Irrigation system	Pesticides use
Olsa C. A&B	B7	35	*C. sinensis*	Valencia	sprinkling	treated
Olsa C. D	D24	35	*C. paradisi*	Marsh	sprinkling	treated
1°Maio	02	11	*C. paradisi*	Rose	sprinkling	treated
1°Maio	05	11	*C. paradisi*	Rose	sprinkling	treated
1°Maio	06	35	*C. sinensis*	Valencia	sprinkling	treated
INIA	EAU	3	*C. sinensis*	Valencia	manual	free
Citrus	3	3	*C. sinensis*	Joppa	sprinkling	free
Citrus	26	3	*C. paradisi*	Marsh	sprinkling	free
Citrus	7	3	*C. paradisi*	Marsh	sprinkling	free
Citrus	31	3	*C. paradisi*	Marsh	sprinkling	free
Citrus	20	5	*C. paradisi*	Star Ruby	sprinkling	free
Olsa C. D	D6	12	*C. sinensis*	Valencia	sprinkling	treated
1°Maio	10	35	*C. sinensis*	Valencia	sprinkling	treated

Table 3.1.2 Orchard age, species, cultivar and management practices in orchards < 2 years old (sprinkling = ground sprinkling)

Site	Orchard	Age (years)	Species	Cultivar	Irrigation system	Pesticides use
Olsa C. A&B	A4	2	*C. paradisi*	Marsh	sprinkling	treated
Olsa C. D	D15	2	*C. paradisi*	Marsh	sprinkling	treated
Olsa C. D	D20A	2	*C. paradisi*	Star Ruby	sprinkling	treated
Olsa C. D	D19	2	*C. paradisi*	Star Ruby	sprinkling	treated
Olsa C. D	D20B	2	*C. paradisi*	Star Ruby	sprinkling	treated
Olsa CD	D17	2	*C. paradisi*	Star Ruby	sprinkling	treated
1°Maio	11	2	*C. paradisi*	Rose	sprinkling	treated
1°Maio	10A	1	*C. sinensis*	Valencia	sprinkling	treated
1°Maio	1	1	*C. paradisi*	Marsh	sprinkling	treated
1°Maio	9	2	*C. paradisi*	Marsh	sprinkling	treated
INIA	EAU	2	*C. sinensis*	Valencia	manual	free
INIA	EAU	2B	*C. paradisi*	Marsh	manual	free

Host range of *P. demodocus*

In order to evaluate the host range of *P. demodocus* in the region, ten (30 x 10 m) rectangular plots were established in natural vegetation at two different sites in the District of Boane (site 1 at 26 ° 09'S and 32° 14'E and site 2 at 26° 05'S and 32° 15'E) from March 20th to 30th 1998. *P. demodocus* pre-reproductive satges were counted on every plant within the marked plot.

The sample plot size was determined as described by Hutchings (1986) by plotting the number of plant species against the sampling area; the curve stabilized when the area reached 300 m^2 (appendix 3.7) which establishes the minimum sampling area required.

Data analysis

Insect count means, variance and the number of samples necessary for pest population censuses for distribution studies were determined using procedures recommended by Bliss & Fisher (1953a), Southwood (1978) and Taylor *et al.* (1978).

The coefficients *a* and *b* of the Taylor' Power Law, the constant *p* and the estimation of the relationship between variance and mean were carried out as recommended by Southwood (1978) and Taylor *et al.* (1978) see 2.2.3. The dispersion parameter k of the negative binomial distribution was calculated using the methods described by Anscombe (1950), Elliott (1971) and Southwood (1978). Verification of the existence of a common k was carried out as recommended by Bliss & Owen (1958), Elliott (1971), Southwood (1978) and Peng & Brewer (1994) see 2.2.4.

For dispersion description, the departure of population distribution from ramdomness was calculated using the dispersion index as indicated by Taylor *et al.* (1978). The index of clumping was estimated using the techniques recommended by David & Moore (1954). The Lloyd's mean crowding index of the degree of crowding was used to characterize individual frequency distributions (Lloyd, 1967). In order to assess the proportion by which mean crowding exceeded mean density, the population patchiness was verified by means of a linear regression between the mean crowding and mean density for several densities (Iwao, 1968; 1970), see 2.2.5.

3.3 LIFE TABLES

Papilio demodocus feeds on citrus plants almost throughout the year. With a pest life cycle duration of less than two months, several cohorts were monitored. Three at 1 °de Maio, two at INIA and University Campus during both the rainy and dry season.

Owing to low egg numbers it was decided to breed a population for field release. This was carried out in the laboratory with one release during the dry season and another in the rainy season. Pairs of 5^{th} instar larvae collected from the field were caged at ambient air relative humidity and temperature. These ranged between 60 and 80% and 20 to 30 °C. Photoperiod cycles were set at 14:10 light: dark in climatic growth chambers. The growth chambers were of the type Angelantoni UC 26-8, 9/5-45D, with a useful volume of approximately 9 m^3. Temperature ranges can be set from + 5° C to + 45° C with a precision of ± 1° C. Relative humidity from 30% to 98% with a precision of ± 3 %. Each chamber was lit by 39 x 215 watts fluorescent lamps giving a total lighting of 30,000 lux.

The precise larval moulting day was not always identified but the larval stage was determined using developmental stages allowing, consequently, studies on particular mortality factors acting on a given developmental stage. For practical reasons related to size variability in larvae, individuals in the population were stratified into size classes and not ages for larval development stage identification in the field:

Very Small Larvae : < 7 mm
Small Larvae: 7≤11.9 mm
Medium Larvae: 12≤19.9 mm
Large Larvae (Brown): ≥20 mm

Data collection

Plants were sampled during the rainy season and in the dry season at INIA and UEM Campus while at 1°Maio site, the plants were sampled for six consecutive generations (three generations per season).

All plants in the 0.25 ha plots were sampled and recently laid eggs located. *Papilio demodocus* shows strong territorial behaviour and this made it easy to follow the female while laying eggs. The plants and leaves bearing the eggs were labelled for recognition and data collection. At the embryonic stage, eggs were sampled daily; at the larval and pupal stages at two day intervals. Because of the low number of larvae per plant together with their sedentary habits individuals were easily located and identified as they developed.

Data analysis

Life table data were constructed and analysed as indicated by Podoler & Rogers (1975), Price (1984), Room *et al.* (1991) and Bellows Jr. *et al.* (1992), see 2.3.

Net reproductive rate and capacity for increase were estimated for the characterization of population growth rate as indicated by Southwood (1978) and Lara (1992).

3.4 POPULATION DYNAMICS

3.4.1 Adult *P. demodocus* movements

Capture-recapture samples using Bailey's Triple Catch Method (Southwood, 1978) involved a series of triple-catch estimates at three-day intervals.

Papilio demodocus movement data were obtained in a study carried out between August and November 1998 at the three locations. Sampling was carried out in the early hours of the day (05.30 to 07.00 h) while butterflies were less active. Adult *P. demodocus* were caught with an entomological net and nail polish applied with a fine brush to the butterfly wing using different marking patterns to identify the sampling dates and locations.

The choice of the marker was decided after testing three different stains to evaluate newly emerged adult longevity after being marked on the wing in the second day of emergency. Two adults per stain (the commercial fluid Tipp-Ex, nail polish and permanent water proof marker) as well as a blank control treatment were subjected to each treatment.

3.4.2 Changes within generations

Population changes within generations were assessed using k-factor analysis with data obtained during the cohort life table studies as recommended by Varley *et al.* (1973), Southwood (1978), Kuno (1991) and Room *et al.* (1991) section 2.3.

3.4.3 Changes between generations

The importance of *P. demodocus* population changes in numbers in order to establish the density dependent regulation processes between generations, techniques recommended by Price (1984) were followed (section 2.4.1).

3.4.4 *Papilio demodocus* growth curves

Leslie matrices were used to describe the population dynamics in the rainy and dry season. Data collected from the life tables and other biological studies, namely the survival rate per age class, the age-specific number of offspring produced and the number of individuals in each age class per generation, provided the information necessary for the construction of the matrix as described by Kitching (1983) and Rose (1987) in section 2.4.

Only female populations were included in the matrix. The data from six consecutive generations from 1° Maio site and four generations from INIA and UEM sites were used in the matrix and thereafter the model for *P. demodocus* population dynamics.

Data analysis

Similarities of the growth curves between the three sites were tested by comparing data of four consecutive generations from INIA, University Campus and data from 1° Maio, using a χ^2 test.

3.5 EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON *P. demodocus* DEVELOPMENT

In order to assess development time and rates in relation to temperature and relative humidity a series of observations were carried out in the growth chambers.

In order to reduce intraspecific variability, insect specimens for a particular experiment were selected from the same batch. Batches of approximately 30 fertile eggs as well as 30 newly emerged larvae were subjected to temperatures of 20, 25, 30 and 35° C and relative humidity of 70% and a photoperiod regime of 14:10 light/dark. A relative humidity of 70 % was concluded after studying *P. demodocus* behaviour in 50, 60, 70, 80 and 90±5% in relative humidity regimes at 30° C (table 4.5.2).

Eggs were always rinsed in distilled water before being placed on *C. sinensis* cv. Valencia leaves also previously washed in distilled water to avoid contamination of eggs e.g. by fungal pathogens. Eggs were selected from those originating from parents previously subjected to similar treatments. This material was then placed on absorbent paper inside Petri dishes. Replicates were placed in the same growth chamber because of the limited rearing faculties available. Mortality, development times and egg production by the emerging females were recorded for each treatment.

This was a single factorial experiment set up in a randomized design.

The adults were transferred to oviposition cages immediately after adult emergence and temperature treatments kept separate.

Data analysis

Median and degree-days for the pest evolutive cycle data were the parameters used to describe development time.

Data on pest development time (degree-days) were investigated using analysis of variance (one-way ANOVA); survival probability, mean egg viability and egg production were subjected to regression and correlation analyses against the various thermal treatments. Because little data were available on pest development time (around only 30 specimens per treatment), the degree-days °C measurement was only obtained and analysed for the pest evolutive cycle periods.

3.6 EFFECT OF DIET ON *P. demodocus* DEVELOPMENT

The field trial

The investigation of the combined effects of various mortality factors on *P. demodocus* performance under field conditions was carried in a field experiment. The experiment was set up to include effects of the diet, predators, larval density and season on *P. demodocus* performance. The trial previously used for life table data collection at INIA was used for the assessment of the different field factors on *P. demodocus* population performance. The experiments were carried out in the months of August to November 1999. Because of a progressive decline in pest numbers to very low levels after the first generation (August to September), corresponding to the dry season, it was necessary to replicate the experiments in the following generation (October to November, wet season).

The effect of diet combined with factors like predator activity and season was assessed by observing, in total, 3 treatments for the 3 *Citrus* cultivars, namely *C. sinensis* cv. Valencia; *C. paradisi* cv. Star Ruby and cv. Marsh were studied. The factor regarding predator activity provided four treatments and exposed the larvae to 4 different levels of predator activity as indicated below:

- Control treatment, where no predator had access to the larvae inside the cages - treatment 0
- One individual of the mantid *Miomantis* sp. in a closed cage - treatment 1
- Open cages to allow access of birds and one mantid *Miomantis* sp. - treatment 2
- Open cages to allow access of birds, no mantids inside - treatment 3.

The seasonal factor consisted of dry and wet season observations. The experiment was designed as a factorial arrangement with a total of 3 factors (season, diet and predator activity). The experiment was arranged in a randomized complete block design with 3 replications. Ten larvae were established per plant/cage.

Metal cages supporting nylon mesh sized 1.0m x 1.0m x 1.2m were placed around each plant which were around 0.90 m tall. The caged plants, randomly selected from those not included in the previous life table data samples, were approximately 1 year old and were infested with recently hatched *P. demodocus* larvae.

Data analysis of field experiment

Larval survival probability had to be transformed by the square root transformation due to variance heterogeneity. Mean values were estimated for female fecundity. Data on larval longevity, larval headcapsule size and survival were analysed using General Linear Model ANOVA to assess the effect of *Citrus* cultivar, season and exposure to predator

species. Significance of differences between treatment means was assessed by Tukey's test.

The effect of cage type on the response variables was assessed by means of a T-test, regardless of the treatment cultivar, season or predator type.

Effect of citrus cultivar on larval development in the laboratory

Fifteen newly hatched larvae per treatment were placed in the growth chambers in order to assess the effects of three different citrus cultivars (treatments) on larval weight, survival rates and egg laying. This was a completely randomized single factor experiment.

Treatments consisted of fresh leaves on shoots of *C. sinensis* cv. Valencia, *C. paradisi* cv. Marsh and *C. paradisi* cv. Star Ruby placed in jars with water. The glass jars were 15 cm high with a volume of 1 l.

Fifth instar larvae were weighed on the 3rd day after moulting in order to obtain comparable values.

In a parallel study, groups of 10 newly moulted 5th instar larvae previously fed on fresh leaves of *Citrus jambhiri* cv. Rough lemon were exposed to one of three treatments: leaves of *Citrus sinensis* cv. Valencia, leaves of *C. paradisi* cv. Marsh and *C. paradisi* cv. Star Ruby. This was set up as a single factor, completely randomized design with the feeding period being five days from moulting. Larvae were marked with different marking patterns for identification. *Citrus* leaves were weighed prior to being placed into the respective compartment in the same growth chamber and the remains of the leaves

were weighed for use as an index of food consumption. Larvae were weighed at the beginning and end of the feeding periods.

Data analysis

Larval weight was subjected to General Linear Model ANOVA to assess differences between larval weight when fed on different cultivars. The combined effects of *Citrus* cultivar, season and rearing condition (laboratory, field dry season and field wet season populations) and mean egg production of all populations was assessed using General Linear Model ANOVA.

Nutritional indices (relative growth rate – RGR; relative consumption rate – RCR; assimilation efficiency – AD; efficiency of conversion of assimilated or digested food – ECD and efficiency of conversion of ingested food – ECI) were calculated as recommended by Hagen *et al.*, (1984), see 2.7. These indices were used to allow comparisons of these results with other studies (Scriber & Slansky, 1981; Slansky & Scriber, 1985; Stamp, 1994). Data were subjected to one-way ANOVA to assess the effect of cultivar on larval nutritional indices.

Leaf nutrient studies

Ten current year leaves of five plants of *C. sinensis* cv. Valencia (at 1° de Maio, INIA and University Campus), five plants of *C. paradisi* cv. Marsh and five of cv. Star Ruby (INIA and UEM sites) were collected for leaf nutrient analyses of nitrogen and tannins. This was carried out in October 1999 and in February 2000 using the same plants and aspect in order to reduce variation (Ibrikci, 1994). Leaves were chosen to be longer than 2.5 cm in order to obtain sufficient material per plant for the analysis. Standard procedures adopted by Hamze *et al.* (1984), Allen *et al.* (1986), Allen (1989), Zhang &

Dotson (1994) were followed. The leaves were collected between 10.00 and 11.00 h, washed in 1% HCL solution rinsed with distilled water. They were dried in an oven at 70 °C for approximately 48 hours in order to attain a constant moisture content. The dried leaf material was transported to Aberdeen where the analysis was carried out. Standard procedures described by Allen *et al.* (1986) were used for the analysis (appendix 3.8).

As the plants were immature a distinction between fruiting and non-fruiting or flowering and non-flowering shoots was not a possibility.

Data analysis

Nitrogen content of leaves was calculated as a percentage of oven dry weight. Data regarding nitrogen and tannin contents per *Citrus* cultivar and month were analysed by site, separately, because of the heterogeneity of variances between sites, cultivars and months. The data were submitted to the non parametric Kruskal-Wallis rank test and Mann-Whitney test for comparison of the values of nitrogen and water soluble tannins between cultivars, sites and time.

3.7 EFFECT OF *P. demodocus* DENSITY ON ITS DEVELOPMENT

The effect of larval density in the field was assessed using a single factor experiment including five treatments: 2, 4, 6, 8 and 10 insects per plant/cage. Cages were built as described in section 3.6. Plant species and cultivars included *C. sinensis* cv. Valencia, *C. paradisi* cv. Marsh and cv. Star Ruby.

The variables quantified were the 5th instar headcapsule size (width from the left to the right side of the head) just prior to pupation, larval development period, proportion surviving and female fecundity.

In the laboratory groups of 15 newly hatched larvae were caged and placed per treatment for assessment of the effect of density on larval weight, survival rates and egg laying. The five density levels were ensured by placing the desired larval numbers in the required numbers of quadrangular wooden 50 x 50 x 70 cm cages. For example, for the level 2, 2 newly hatched larvae were separately placed in 8 cages and 15 of these larvae were randomly selected and daily observed. In total there were 8 cages for density level 2, 4 cages for level 4, 3 cages for level 6, 2 for level 8 and 10. Larvae were handled as indicated in section 3.3.

Treatments compared larval densities of 2, 4, 6, 8 and 10 in the form of a single factor experiment in a completely randomized complete block design. 5th instar larvae were weighed on the 3rd day after moulting in order to quantify the effects of density.

Data analysis

Larval weight was analysed by regression against larval density levels. Multiple regression analysis was performed for the evaluation of the combined effects of larval density and female proportion on the mean number of eggs laid in all populations (laboratory, wet and dry seasons).

The mean values of larval headcapsule size, larval development periods and survival were regressed against larval density level.

3.8 EFFECT OF NATURAL ENEMIES ON *P. demodocus* DEVELOPMENT

3.8.1 Diagnosis of diseases and pathogens of samples of field populations

The specimens collected from the field were transported to the laboratory in containers. The transparent plastic containers were 0.75 l in volume and covered with a porous lid. In the laboratory, specimens were handled with forceps regularly flamed in a Bunsen burner. Eggs were placed on sterilized absorbent paper tissues. The specimens were retained for periods of over one week in order to determine pathogen infection.

Eggs

In total, 227 and 209 eggs were collected during the rainy (October 1998 to March 1999) and dry (May to July 1999) seasons, respectively. These were retained in a sterile cabinet in the laboratory under ambient temperature and relative humidity for development of possible diseases and pathogens.

Larvae and pupae

During the rainy season (October 1998 to March 1999), 91, 98, 82, 59, 45 larvae were collected; during the dry season (May to July) 102, 67, 78, 79 and 38 larvae of the five instars were collected and assessed in the laboratory for identification of parasites and pathogens. 32 pupae were collected during the rainy (October 1998 to February 1999) and 24 in the dry season (May to July 1999). Specimens were retained in the same conditions as for eggs.

3.8.2 Effect of predators on *P. demodocus* development

The effect of natural enemies on *P. demodocus* development in the field was assessed in an experiment combining this factor to other factors like diet and season, as described in

section 3.6. The experiment was designed as a factorial arrangement with a total of 3 factors (season, diet and predator activity). The experiment was arranged in a randomized complete block design with 3 replications. Ten larvae were established per plant/cage. The four treatments for the investigation of the exposure of *P. demodocus* to its natural enemies were:

- Control treatment, where no predator had access to the larvae inside the cages - treatment 0
- One individual of the mantid *Miomantis* sp. in a closed cage - treatment 1
- Open cages to allow access of birds and one mantid *Miomantis* sp. - treatment 2
- Open cages to allow access of birds, no mantids inside - treatment 3.

The experiment was conducted only in one location because of low mantid numbers, one of the predators. The mantid species found in citrus orchards was of the genus *Miomantis* (Dictyoptera: Mantidae) and birds were mostly *Lybius* sp. (Lybiidae), *Chrysococcyx* sp. (Culidae) and *Larus* sp. (Laridae).

Data analysis

Data on larval longevity, survival and headcapsule size were analysed using General Linear Model ANOVA.

Software for data analyses

All the data were analysed using the statistical package MINITAB for Windows release 12.1 except for the test of the model on population dynamics and all χ^2 tests which were carried out using MICROSOFT EXCEL 97.

CHAPTER FOUR

RESULTS

4.1 BIOLOGY OF *Papilio demodocus*

Observations on *P. demodocus* habits were carried out in order to obtain detailed knowledge about the insect's biology. In total, 1953 eggs were laid in the two oviposition cages by the emerging adults (table 4.1.1) from life table studies (section 3.3). The studies indicated that the eggs are usually laid singly on the underside of terminal leaves (90.7%) or otherwise on the upperside of older leaves and other parts of the plants such as twigs (9.3%). The differences were tested using the Mann-Whitney test for the proportion of eggs laid per plant part (the underside of leaves or upperside and plant branches) by caged females from the life table studies (table 4.1.2). The test W revealed significant differences between plant parts as a substrate for oviposition, i.e. most eggs deposited on the underside ($P < 0.001$); $W = 495.0$, 95% confidence interval = (82.6, 90.0) around the mean 86.2 % egg laid on the underside of leaves during the two seasons (rainy season occurs from October to March and the dry season from April to September).

Table 4.1.1 Mean fecundity per season, generation and cultivar (dry season = April to September and rainy = October to March; ad.= adults; inc.= included; gen. = generation)

Site	season	cultivar	eggs	ad. ♂	ad. ♀	pairs inc.	mean eggs/♀	gen.
UEM	dry	Valencia	132	2	3	2	66.0	4
UEM	dry	Marsh	147	4	2	2	73.5	4
UEM	dry	Valencia	0	0	1	0	0.0	5
UEM	dry	Star Ruby	0	0	0	0	0.0	4
UEM	dry	Star Ruby	0	0	0	0	0.0	5
UEM	dry	Marsh	42	1	1	1	42.0	5
INIA	dry	Valencia	0	1	0	0	0.0	5
INIA	dry	Marsh	0	0	0	0	0.0	5
INIA	dry	Marsh	0.0	0	2	0	0.0	4
INIA	dry	Valencia	51	1	1	1	51.0	4
INIA	dry	Star Ruby	0	0	1	0	0.0	5
INIA	dry	Star Ruby	39	1	1	1	39.0	4
1°MAIO	dry	Valencia	109	2	2	2	54.5	5
1°MAIO	dry	Valencia	0.0	1	0	0	0	4
1°MAIO	dry	Valencia	93	1	1	1	93.0	6
1°MAIO	rainy	Valencia	93	3	3	all	31.0	1
1°MAIO	rainy	Valencia	66	3	3	all	22.0	2
1°MAIO	rainy	Valencia	146	10	8	all	18.25	3
UEM	rainy	Valencia	127	3	4	all	31.75	1
UEM	rainy	Marsh	138	1	4	all	34.5	1
UEM	rainy	Valencia	49	3	2	all	24.5	2
UEM	rainy	Marsh	86	2	3	all	28.67	2
UEM	rainy	Star Ruby	0	1	0	all	0	1
UEM	rainy	Star Ruby	57	3	2	all	28.5	2
INIA	rainy	Valencia	116	3	5	all	23.2	1
INIA	rainy	Marsh	0	0	1	0	0.0	1
INIA	rainy	Star Ruby	63	0	1	all	63.0	1
INIA	rainy	Valencia	0	2	0	all	0.0	2
INIA	rainy	Marsh	307	4	4	all	76.75	2
INIA	rainy	Star Ruby	92	4	2	all	46.0	2

Table 4.1.2 Total egg numbers deposited by all females (10 during the dry and 42 during the rainy season) per plant part and season in the oviposition cages (Sept. = September; Oct. = October)

Season	Total egg numbers	Underside of leaves	Other plant parts
Rainy (Oct. to March)	1340	1223	117
Dry (April to Sept.)	613	549	64
Total	1953	1772	181

The eggs are spherical, white, yellow or greenish when first laid and darken to grey or pinkish brown as they age. Fertile eggs are bright and a dark spot (the embryo) develops in the interior. A dark stripe or spots appear and encircle the egg when it is about to hatch. Unfertile eggs remain white. Desiccated eggs have an opaque appearance and fail to hatch, showing, at the start, the appearance of being fertile but fail to develop owing to the absence of an embryo. Eggs take approximately 4 days to hatch. After hatching, the remains of the egg shell are eaten by the newly hatched larvae. A high proportion of the laid eggs are not viable due to desiccation and infertility (section 4.3, table 4.3.6 and 4.3.7).

The larvae are fleshy, with a swollen thoracic region bearing several tubercles when young. As the larvae age they become glabrous. In the first thoracic segment there is an orange coloured organ in the shape of a V, the osmeterium. Five larval instars occur and two distinct stages were observed in the larval period, which are: the initial 4 instars are dark brown with a yellow anterior region and posterior region and a white mark in the middle of the dorsum (See plate 4.2.1). These four stages last approximately two weeks before the production of the final instar with a green ground colour and dark brown markings on the sides. This stage lasts about 1 week. All larval stages preferably feed on

immature leaves but when these are no longer available they attack the mature leaves of citrus plants at all larval development stage (personal observations) and will consume terminal twigs as well as the bark of young plants (plate 4.2.2).

The pupae are vertically suspended and attached by a silk filament around the thorax to a plant branch in a protected area in the interior of the canopy (plate 4.2.3). After the larvae cease to feed and just prior to pupation the 5^{th} instar larvae immobilize and produce a silken whitish material. This stage takes approximately 48 hours. Pupae are mostly pale brown when attached to the citrus branch. This stage lasted 9 to 14 days (section 4.3).

The adults are large, up to 10 cm across the wings. They are blackish in colour, with small yellow markings (plate 4.2.4). A full description is given by Howard (1906).

Plate 4.2.1 *P. demodocus* third instar larva (enlarged 1 : 1.2)

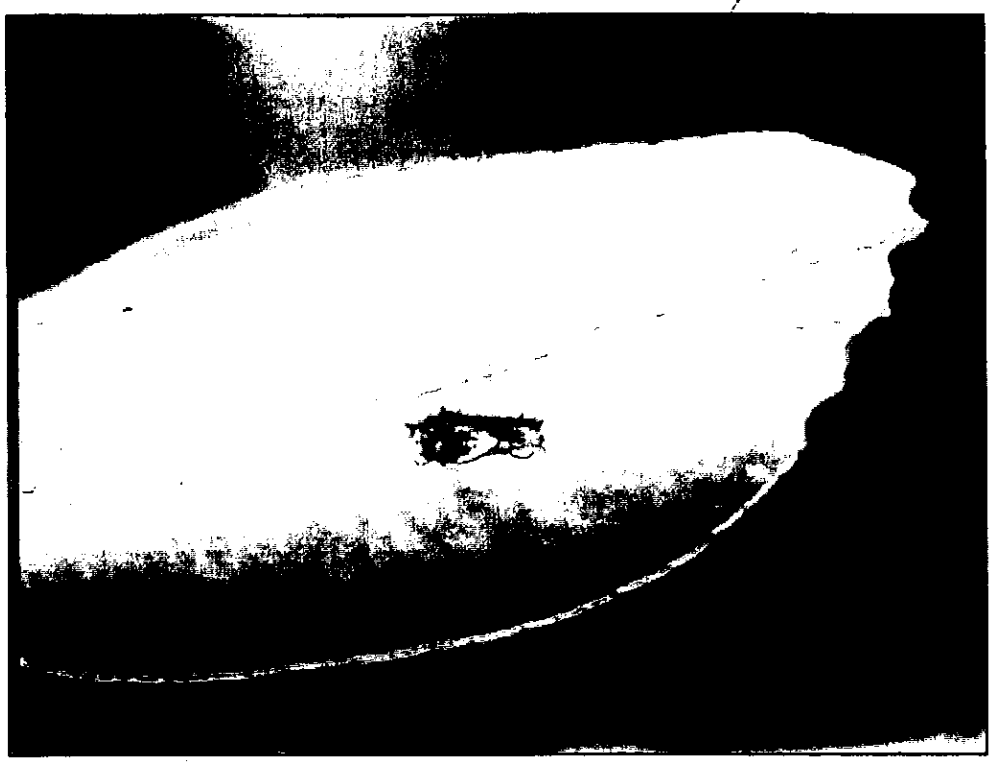


Plate 4.2.2 *P. demodocus* fifth instar larva (enlarged 1 : 1.2)

Plate 4.2.3 *P. demodocus* pupa (reduced 1 : 0.5)

Plate 4.2.4 *P. demodocus* butterfly (inside an oviposition cage, reduced 1 : 0.5)

4.2 *Papilio demodocus* POPULATION DISTRIBUTION

4.2.1 Preliminary survey

The preliminary survey was aimed at determining the required number of samples for insect distribution studies in natural vegetation. Plot size was determined as the sampling area where the number of plant species stabilized (section 3.2 and appendix 3.7). The survey showed that no *P. demodocus* larvae were found feeding on natural vegetation. The vegetation was dominated by trees and shrubs such as *Acacia* spp. (Fabaceae), *Aloe* spp. (Aloaceae), *Euphorbia* spp. (Euphorbiaceae), *Combretum* spp. (Combretaceae), *Sclerocarya* spp. (Anacardiaceae), *Tridax procumbens* (Asteraceae) and grasses (Gramineae) (appendix 3.7). None belonged to the Rutaceae or Umbelliferae, the preferred larval host plants (Van Son, 1949; Hepburn & Bishop, 1954).

Distribution of *P. demodocus* was restricted by the range of the larval host plants of the genus *Citrus* in the Umbelúzi region. Adults were frequently seen visiting flowers of the herb *Tridax procumbens* (L.) (Asteraceae) where they fed on the nectar.

Egg population censuses carried out in 911 plants in order to estimate the required number of samples in citrus orchards (sections 2.2.3 and 3.2) showed that for studies of pest distribution in trees < 2 years old and treated with insecticides, a minimum of 2,380 plants had to be sampled for data to be representative of population levels (table 4.2.1, appendix 4.1 and 4.2).

Owing to low insect densities in old orchards (> 2 years old) it was calculated that 43,499 trees should be sampled on each occasion in order to be representative; in untreated orchards < 2 years old the number of samples was calculated to be at least 6,165.

The insect density in older orchards was, therefore, too low to enable statistical analyses. The total area sampled was 47,550 m^2, of which 6,300 m^2 were in Olsa A&B, 18,150 m^2 in Olsa D and 23,100 m^2 in 1° Maio. Due to the insect preference for young plants later sampling was carried out on plants up to two years old.

Table 4.2.1 Insect counts in citrus orchards to determine number of samples (1 plant in the orchards occupies an approximate area of 21 m^2 with spacing of 7 m x 3 m)

Orchard age	Pesticides use	Insect Counts	Insect Density /21 m^2	Mean	Variance	Standard Deviation	Number of samples
<2	Yes	71	0.19	0.19	0.215	0.463	2,379.01
<2	No	33	0.09	0.09	0.125	0.353	6,164.66
>2	Both	9	0.02	0.02	0.043	0.209	43,498.94

During the preliminary study in all locations, three pupae were found to be parasitised (two in Olsa D and one in 1° Maio sites) by a parasitoid of the genus *Pteromalus* (Hymenoptera: Pteromalidae).

4.2.2 Population distribution parameters

Hypothesis

H_0: the population distribution of *P. demodocus* is random

H_1: the presence of an individual affects the behaviour of other individual in *P. demodocus* populations resulting in aggregation

The Index of Clumping (David & Moore's, 1954), Taylor's Power Law coefficients, Lloyd's Mean Crowding Index and the parameter k of the negative binomial distribution model were used to test the null hypothesis (section 3.2).

In total, 25,561 plants (< 2 years old) were sampled for distribution studies at LOMACO orchards; 13,549 during the dry season and 12,012 during the rainy season over a 12 month period (section 3.2). Egg numbers comprised 93.0% of the total *P. demodocus* specimens observed (6,103) while the percentage of larvae and pupae were respectively 6.4 and 0.6%. Mean densities were too low to carry out statistical analysis for each developmental stage. Therefore, only eggs were included in the distribution studies. The number of insects was low, with a mean of 0.222 eggs per plant (or 21 m^2), standard error 0.006, variance 1.014 (table 4.2.2).

The relationship between variance and mean for the total population using David & Moore's (1954) equation (section 3.2) was approximately 2.15, which indicates that the population distribution was not random. The relationship between the mean crowding per sample (the mean number per individual of other individuals coexisting in the same quadrat) and the mean density per sample showed that the two variables were correlated. The equation expressing the relationship between mean crowding and mean density for *P. demodocus* (section 3.2) at various density levels (Iwao, 1968, 1970) was $y = 0.454 + 1.515\ x$, $P < 0.001$, $s = 0.659$, $r^2 = 87.4\%$ and r^2 (adjusted) = 87.1% (figure 4.2.1). Therefore, *P. demodocus* population distribution is characterized, in this relationship (Southwood, 1978), by a coefficient $\alpha = 0.454$ and $\beta = 1.515$. The overall Index of Mean Crowding was 2.677 for this population and the proportion by which mean crowding exceeded mean density (the degree of patchiness for *P. demodocus*) was 12.11.

The dispersion parameter k calculated by the maximum likelihood method (section 3.2) varied between samples (from data in appendix 3.5) and the overall value was estimated as being 0.09 for the whole population. The value of p was estimated as 0.43, which indicates that the population could be described as a negative binomial distribution, approaching Fisher's logarithmic series. The population parameters a and b during the year were found to be 0.253 and larger than 1, respectively (table 4.2.2).

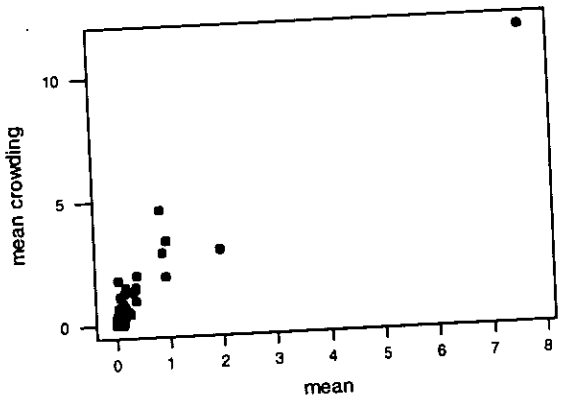
Table 4.2.2 Population mean, standard error, variance and coefficients a and b of Taylor's Power Law for the dry and rainy seasons

Characteristics	Total year	Rainy Season	Dry Season
mean insect density plant (or 21 m^2)	0.222	0.414	0.052
variance	1.014	2.020	0.088
standard error of mean	0.006	0.013	0.003
a	0.253	0.401	0.078
b	1.12	1.16	0.993
F	(1,51) = 1284.78	(1,35) = 854.02	(1,14) = 896.70
adjusted r^2	96.1%	98.4%	96.0%
s of regression	0.161	0.134	0.117
P of regression	< 0.001	< 0.001	< 0.001

The slope b was larger than 1 for the whole population during the year and during the rainy season, showing that the population had an aggregated distribution. During the dry season, the slope was approximately 1 (table 4.2.2), indicating that at lower densities the population distribution was random. In every season and for the population of this year,

the correlation coefficient r^2 accounted for more than 95% of the variance indicating a good fit of the Taylor Power Law model (table 4.2.2 and figures 4.2.2 and 4.2.3).

Figure 4.2.1 The relationship between mean crowding and insect mean density per sample (each point represents one observation)



Variance was found to be larger than the mean, indicating that the distribution was aggregated (Southwood, 1978). The population dispersion parameter k of the negative binomial model, estimated by iterative solution of the equations

$$\log (N/n_0) = k \log (1+(\bar{x}/k))$$

where N is the total number of samples

n_0 the number of samples with no animals

k the dispersion parameter

$\bar{x}$ the mean (Anscombe, 1953; Southwood, 1978)

showed also that this population was aggregated. The maximum likelihood method of estimating the parameter *k* of the negative binomial estimated by iterative solution of the equations

$$N\log_e(1+(\bar{x}/k))=\sum(A_x/(k+x))$$

where $\log_e$ are natural logs

A_x the sum of all frequencies of sampling units with more than *x* individuals

the other variables being as above (Southwood, 1978)

indicated that the population distribution was skewed towards the left, that is, higher insect frequencies were found on the left side of the insect mean per sampling area than would have been observed in a normally distributed population and that the estimation of *k* as being 0.09 for the whole population (data in appendix 3.5) may have been in excess (Southwood, 1978).

Figure 4.2.2 Log variance plotted against log $\bar{x}$ insect numbers for estimation of the parameter *a* of Taylor's Power Law (each point represents one observation).

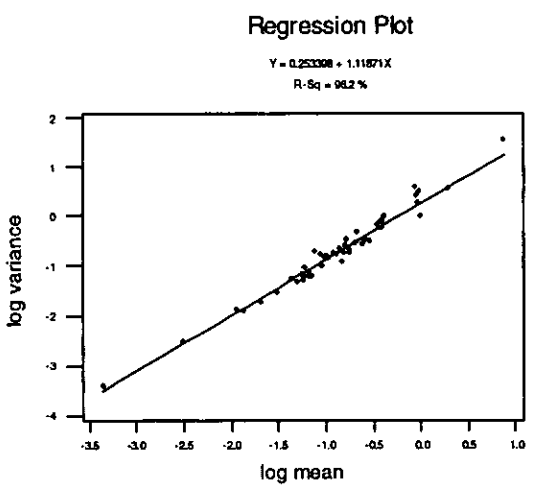
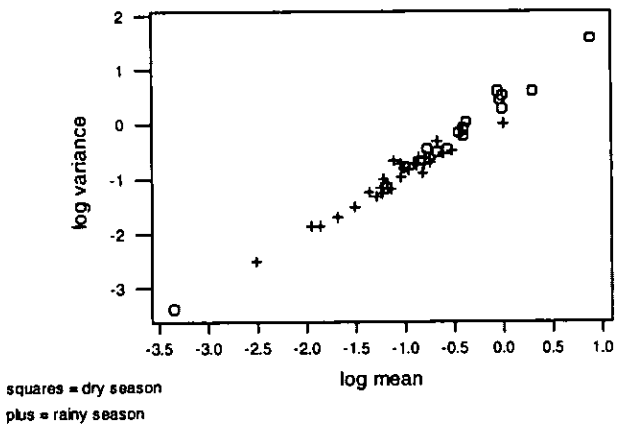


Figure 4.2.3 Relationship between the variance and mean on a log scale for the rainy and dry seasons (each point represents one observation)



The Anscombe (1950) graph to verify the efficiency of the k estimator indicates that for a mean density of 0.222 and dispersion parameter k = 0.09, the efficiency of k estimation lies between 50 and 75%.

Verification of the existence of a common k

Hypothesis

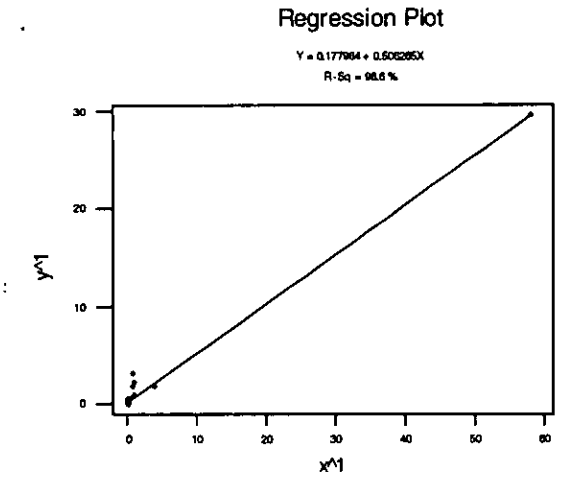
H_0: there is no association between the variables 1/k and mean density and consequently, there is a common k

H_1: the variables 1/k and mean density are correlated and consequently, there is not a common k

The dispersion parameter k varied between plots. Although the dispersion parameter k appeared to be unstable in the population, the analysis by regression of the relationship between 1/k and the mean (Elliott, 1971 and Southwood, 1978) showed that there was not a common k_c. The graphical test of the homogeneity of the samples plotted (1/k) ($=y^1/x^1$) against the mean ($\bar{x}$), as indicated in the section 2.1, for every field showed that there was a trend for aggregation, so, the fitting of a common k was not justified (figure 4.2.5 and table 4.2.4).

In verification of the need to estimate a common k for all sets of field plot data by the moment method given by the equation $k = \bar{x}^2/(s^2-\bar{x})$ where s^2 is variance and $\bar{x}$ the mean, a regression line was observed (figure 4.2.4 and figure 4.2.5).

Figure 4.2.4 Regression line between y^1 and x^1 for common k (k_c) verification (each point represents one observation)



For the relationship between the statistics y^1 and x^1 for the estimation of a common k ($x^1 = \bar{x}^2 - (s^2 - N)$; N = number of individual counts for respective $\bar{x}$ and $y^1 = s^2 - \bar{x}$), the

standard deviation (s) was 0.491; r^2 = 98.6% and adjusted r^2 (adjusted) was 98.5% with $F_{(1,51)}$=3,524.94 and P<0.001 (table 4.2.3).

Figure 4.2.5 The relationship between 1/k against log $\bar{x}$ (each point represents one observation)

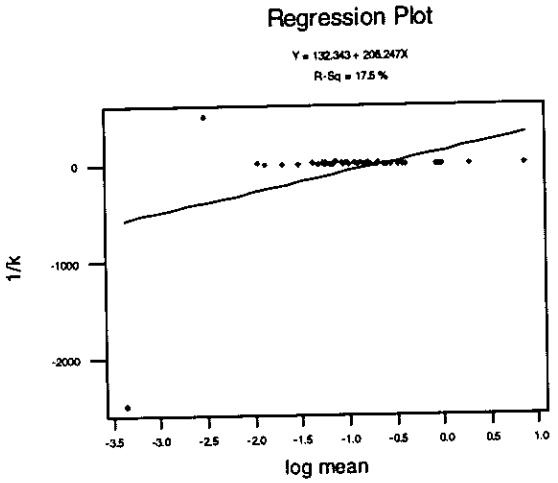


Table 4.2.3 ANOVA for the regression equation of y^1 against x^1 (** significant at P = 0.01)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F	P
Regression	1	847.93	847.93	3,524.94	0.000**
Residual Error	51	12.27	0.24		
Total	52	860.19			

In the relationship between 1/k and log $\bar{x}$ to assess the degree to which these two variables are related (section 2.2.5) the standard deviation (s) was 338.9; r^2 (adjusted) = 17.9%; $F_{(1,45)} = 11.01$ and P = 0.002. Tables 4.2.3 and 4.2.4 give the analysis of variance for these regression relationships. The coefficient of variation approached values of 0, with most of them lying between 0 and 1, confirming that the population showed an aggregated distribution such as the coefficient *b* of the Taylor Power Law which was > 1 for this population (table 4.2.2).

Table 4.2.4 ANOVA for the regression equation of 1/k against log $\bar{x}$ (** significant at P = 0.01)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F	P
Regression	1	1264108	1264108	11.01	0.002**
Residual Error	45	5166999	114822		
Total	46	6431107			

These data (tables 4.2.3 and 4.2.4 and figures 4.2.4, 4.2.5) show that the dispersion parameter k varies as the mean density increases.

Temperature, relative humidity and wind speed effect on insect abundance

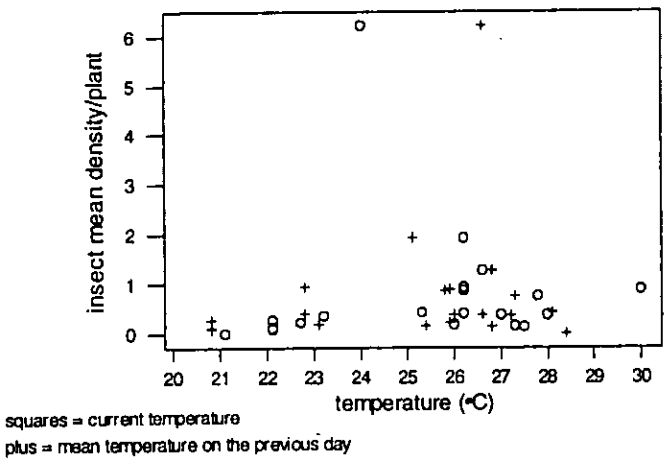
Hypotheses

H_0: *P. demodocus* density in the field is not affected by temperature, relative humidity and wind speed

H_1: *P. demodocus* density in the field is affected by temperature, relative humidity and wind speed

Multiple regression analysis was carried out to assess the effect of the explanatory variables (temperature, relative humidity and wind speed) on the response variable (insect density) per season, during the insect distribution studies (section 3.2). Temperature, air relative humidity and wind speed measured at every sampling occasion in 20 surveys during the rainy season and 38 during the dry season had no significant relationships with pest density. Multiple regression equation for the rainy season Y = -1.05 + 0.009 temperature - 0.004 air relative humidity + 0.017 wind speed, s = 1.482; R^2 = 6.1%, R^2 (adjusted) = 0.0%; $F_{(3,16)}$ = 0.03; P=0.991. During the dry season the equation was Y = -0.473 - 0.014 temperature - 0.001 air relative humidity - 0.002 wind speed, s = 0.073; R^2 = 13.2%; R^2 (adjusted) = 5.5%; $F_{(3, 34)}$ = 1.72 and P = 0.182. The stepwise regression analysis indicated that of all meteorological factors, mean temperature on the previous day of sampling was the factor that appeared to affect the most the number of insects found during the rainy season but this was not statistically significant, with y = 281.370 - 36.145 x + 1.536 x^2 - 0.022 x^3; s = 1.409; r^2 = 10.2%; r^2 (adjusted) = 3.9%; $F_{(3, 16)}$ = 2.4 and P = 0.09. Figure 4.2.6 shows the insect mean density observed at various field temperature levels.

Figure 4.2.6 Temperature (°C) on the current and the previous sampling day related to insect mean density per plant during the rainy season (each point represents one observation)



The correlation between insect mean density and temperature was not significant for the inclusion of temperature in the prediction model.

Assessment of the influence of the time of the year on *P. demodocus* abundance

Hypothesis

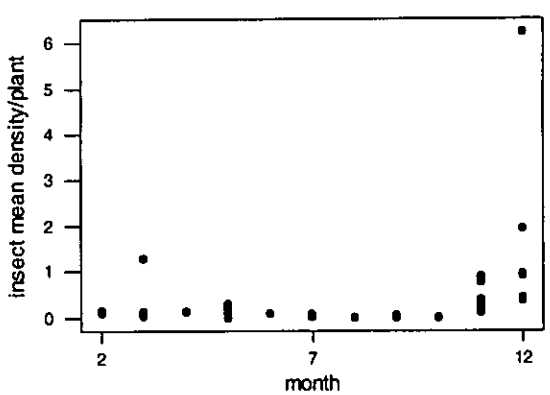
H_0: *P. demodocus* abundance does not vary with time of the year

H_1: *P. demodocus* abundance varies with the time of the year

Data of egg population sampled in the field during population distribution studies (section 3.2) were used to assess the influence of time on the mean number of eggs found per

plant in the fields during different. months of the year using one-way ANOVA. This analysis showed that there were differences on the mean number of eggs per plant between months with $F_{(10, 49)} = 2.06$; $P = 0.047$ and pooled standard deviation = 0.7805 (data in appendix 3.6). *Papilio demodocus* abundance reached its highest values in the months of the rainy season, that is, from October to March (figure 4.2.7).

Figure 4.2.7 Mean egg density per plant in different months of the year (each point represents one observation)



The graph shows that for *P. demodocus*, higher insect numbers could be expected in the field, during the months of October to March.

4.3 *Papilio demodocus* LIFE TABLES

Insect censuses for cohort life tables construction were carried out in three sites (INIA, University Campus and plot 23 at 1° Maio) (section 3.3). In the region of Umbelúzi, *P. demodocus* generations overlapped throughout the year. Observations did not support the conclusion that mortality factors affecting particular developmental stages had constant effects on the specific developmental stages and that this was constant as time passed, basic assumptions for the validation of static life-tables (Room *et al.*, 1991), data in appendix 4.3. Among the life table types, cohort life tables were the most practical for this study and also because rolling life tables would be excessively costly and time consuming. Variables measured were larval development periods, survival and total larval longevity, evolutive life cycle and female fecundity (section 3.3). Pupal weight was not taken in any experiment to avoid destruction of the specimens caused by removal of the pupae from the substrate to which they were attached.

Different numbers of life tables were produced (section 4.3.1) per cultivar and season. The trials at INIA and University Campus were set up late in the year and comprised the three cultivars being studied for which two life tables were constructed for the rainy and dry seasons. At the 1° Maio site, which belongs to the company LOMACO, only cultivar Valencia had been planted at this orchard but the plot had been established earlier allowing three life tables to be constructed for each season.

4.3.1 Development time

Hypothesis

H_0: there is no difference in larval development time between the three cultivars, the three sites and the two seasons

H_1: larval development time differs between the three cultivars, sites and seasons

Data on larval development time were collected by recording larval development periods from cohort life tables in the field (section 3.3). Tables 4.3.1-4.3.4 give the overall development periods on the three different cultivars during the rainy and dry season of 1998/1999. The period from the embryonic stage to pupation lasted 21.59 days in *C. sinensis* cv. Valencia, 21.54 days in *C. paradisi* cv. Marsh orchards and 22.12 for the cultivar Star Ruby.

The median length of time for the total life cycle was estimated as 52 days; 52 days during the rainy season and 53 days in the dry season. The median for the evolutive life cycle lasted 41 days in total, 41 days in cv. Valencia orchards, 41 days in cv. Marsh orchards and 42 days in cv. Star Ruby orchards. The data showed that there were no differences between the dry and rainy seasons in *P. demodocus* development time, $F_{(1, 108)} = 0.10$; $P = 0.750$ (table 4.3.5). Larval development time did not differ between cultivars, $F_{(2, 108)} = 1.04$; $P = 0.356$, or between sites, $F_{(2, 108)} = 1.44$, $P = 0.241$. Adult life span was not different between seasons, $F_{(1, 108)} = 0.01$; $P = 0.907$.

Table 4.3.1 Developmental periods at the three different *Citrus* cultivars and sites in the period 1998/1999 (lar. is larvae; data refer only to specimens that reached adulthood)

Stage	Total		Rainy Season		Dry Season	
	Mean±S.E	Median	Mean±S.E	Median	Mean±S.E	Median
Yellow eggs	1.68±.05	2.0	1.72±.05	2.0	1.59±.11	2.0
Pink Eggs	1.77±.04	2.0	1.78±.05	2.0	1.76±.10	2.0
Dark eggs	1.89±.04	2.0	1.86±.04	2.0	1.97±.11	2.0
Total eggs	5.33±.07	5.0	5.35±.08	5.0	5.28±.17	5.0
<7mm larvae	3.78±.06	4.0	3.79±.07	4.0	3.76±.15	4.0
7≤11.9mm larvae	3.63±.06	4.0	3.67±.06	4.0	3.52±.13	4.0
12≤19.9mm larvae	3.69±.06	4.0	3.71±.07	4.0	3.66±.11	4.0
≥20mm brown lar.	3.67±.05	4.0	3.68±.06	4.0	3.62±.14	4.0
5th instar larvae	6.89±.07	7.0	6.76±.08	7.0	7.28±.15	7.0
♀ 5th instar larvae	7.02±.10	7.0	6.88±.11	7.0	7.40±.19	8.0
♂ 5th instar larvae	6.77±.10	7.0	6.65±.11	7.0	7.14±.23	7.0
Total larvae	21.65±.16	22.0	21.65±.16	22.0	21.66±.42	22.0
Pupae	14.04±.12	14.0	14.00±.13	14.0	14.17±.25	14.0
♀ pupae	14.18±.18	14.0	14.17±.18	14.0	14.20±.44	15.0
♂ pupae	13.90±.15	14.0	13.81±.18	14.0	14.14±.37	14.0
Evolutive cycle	41.07±.22	41.0	40.94±.22	41.0	41.07±.59	41.0
Adults	11.04±.18	11.0	11.06±.22	11.0	10.97±.29	11.0
♀ adults	11.12±.26	11.0	11.10±.33	11.0	11.20±.37	11.0
♂ adults	10.95±.24	11.0	11.02±.29	11.0	10.71±.45	11.0
Total longevity	52.00±.28	52.0	52.00±.31	52.0	51.66±.60	53.0
♀ total longevity	52.18±.41	52.0	52.31±.46	52.0	51.80±.91	53.0
♂ total longevity	51.83±.37	52.0	51.70±.42	52.0	52.50±.80	52.5

Table 4.3.2 Developmental periods from the embryonic stage to adulthood for *P. demodocus* on *C. sinensis* cv. Valencia (lar. is larvae; data refer only to specimens that reached adulthood).

Stage	Total		Rainy Season		Dry Season	
	Mean±S.E	Median	Mean±S.E	Median	Mean±S.E	Median
Yellow eggs	1.61±.06	2.0	1.68±.07	2.0	1.38±.13	1.0
Pink Eggs	1.81±.05	2.0	1.81±.05	2.0	1.81±.10	2.0
Dark eggs	1.88±.05	2.0	1.89±.05	2.0	1.88±.16	2.0
Total eggs	5.29±.11	6.0	5.38±.12	6.0	5.00±.22	5.0
<7mm larvae	3.80±.08	4.0	3.81±.09	4.0	3.75±.17	4.0
7≤11.9mm larvae	3.58±.08	4.0	3.62±.08	4.0	3.44±.20	3.5
12≤19.9mm larvae	3.70±.08	4.0	3.72±.08	4.0	3.63±.18	4.0
≥20mm brown lar.	3.58±.07	4.0	3.66±.08	4.0	3.31±.18	3.0
5th instar larvae	6.88±.10	7.0	6.72±.10	7.0	7.44±.18	8.0
♀5th instar larvae	6.97±.14	7.0	6.76±.16	7.0	7.40±.19	8.0
♂5th instar larvae	6.79±.13	7.0	6.68±.14	7.0	7.14±.23	7.0
Total larvae	21.58±.21	22.0	21.59±.20	22.0	21.56±.60	22.0
Pupae	14.03±.16	14.0	14.04±.18	14.0	14.00±.35	14.0
♀pupae	14.06±.24	14.0	14.12±.27	14.0	14.20±.44	15.0
♂ pupae	14.00±.20	14.0	13.96±.23	14.0	14.14±.25	14.0
Evolutive cycle	40.86±.28	41.0	40.93±.28	41.0	40.63±.81	40.0
Adults	11.10±.24	12.0	11.25±.29	12.0	10.63±.43	11.0
♀ adults	11.20±.34	11.0	11.20±.43	11.0	11.20±.37	11.0
♂ adults	11.00±.36	11.0	11.29±.39	12.0	10.71±.45	11.0
Total longevity	51.94±.35	52.0	52.17±.38	52.0	51.19±.82	50.5
♀ total longevity	52.17±.51	52.0	52.32±.58	52.0	51.80±.91	53.0
♂ total longevity	51.71±.47	52.0	52.04±.50	52.0	52.21±.80	52.5

Table 4.3.3 Development time on *Citrus paradisi* cv. Marsh in the period 1998/1999 (lar. is larvae; data refer only to specimens that reached adulthood)

Stage	Total		Rainy Season		Dry Season	
	Mean±S.E	Median	Mean±S.E	Median	Mean±S.E	Median
Yellow eggs	1.75±.10	2.0	1.67±.11	2.0	1.89±.18	2.0
Pink Eggs	1.75±.10	2.0	1.78±.10	2.0	1.70±.21	2.0
Dark eggs	1.89±.09	2.0	1.78±.10	2.0	2.10±.18	2.0
Total eggs	5.39±.13	5.0	5.22±.10	5.0	5.70±.30	5.0
<7mm larvae	3.64±.15	4.0	3.67±.16	4.0	3.60±.31	3.5
7≤11.9mm larvae	3.71±.10	4.0	3.78±.13	4.0	3.60±.16	4.0
12≤19.9mm larvae	3.61±.12	4.0	3.61±.16	3.5	3.60±.16	4.0
≥20mm brown lar.	3.71±.10	4.0	3.61±.12	4.0	3.90±.18	4.0
5th instar larvae	7.00±.15	7.0	6.89±.16	7.0	7.20±.29	7.0
♀ 5th instar larvae	7.20±.18	7.0	7.08±.19	7.0	7.67± .33	8.0
♂ 5th instar larvae	6.77±.23	7.0	6.50±.22	6.5	7.00± .38	7.0
Total larvae	21.54±.34	21.0	21.61±.36	21.0	21.4±.72	21.5
Pupae	14.29±.21	14.0	14.17±.25	14.0	14.5±.37	15.0
♀ pupae	14.40±.27	14.0	14.17±.30	14.0	15.33±.33	15.0
♂ pupae	14.15±.32	14.0	14.17±.48	14.0	14.14±.46	14.0
Evolutive cycle	41.36±.40	41.0	40.94±.50	41.0	42.1±.64	43.0
Adults	11.14±.33	11.5	10.94±.48	11.5	11.5±.37	11.5
♀ adults	11.27±.49	12.0	11.17±.61	12.0	11.67±.33	12.0
♂ adults	11.00±.45	11.0	10.50±.76	10.5	11.43±.53	11.0
Total longevity	52.50±.57	53.0	51.89±.80	52.0	53.6±.56	54.0
♀ total longevity	52.67±.83	53.0	52.33±1.02	52.5	54.00±.58	54.0
♂ total longevity	52.31±.79	53.0	51.00±1.32	50.5	53.43±.78	54.0

Table 4.3.4 Development time on *Citrus paradisi* cv. Star Ruby in the period 1998/1999 (lar. is larvae; data refer only to specimens that reached adulthood)

Stage	Total		Rainy Season		Dry Season	
	Mean±S.E	Median	Mean±S.E	Median	Mean±S.E	Median
Yellow eggs	1.88±.08	2.0	1.93±.07	2.0	1.67±.33	2.0
Pink Eggs	1.65±.12	2.0	1.64±.13	2.0	1.67±.33	2.0
Dark eggs	1.88±.08	2.0	1.86±.10	2.0	2.00±.00	2.0
Total eggs	5.41±.12	5.0	5.43±.14	5.0	5.33±.33	5.0
<7mm larvae	3.94±.14	4.0	3.86±.14	4.0	4.33±.33	4.0
7≤11.9mm larvae	3.71±.17	4.0	3.79±.16	4.0	3.67±.33	4.0
12≤19.9mm larvae	3.82±.13	4.0	3.79±.16	4.0	4.00±.00	4.0
≥20mm brown lar.	3.94±.10	4.0	3.86±.10	4.0	4.33±.33	4.0
5th instar larvae	6.77±.16	7.0	6.79±.19	7.0	6.67±.33	7.0
♀ 5th instar larvae	6.86±.26	7.0	7.00±.32	7.0	6.50±.50	6.5
♂ 5th instar larvae	6.70±.21	7.0	6.67±.24	7.0	7.00±	7.0
Total larvae	22.12±.36	22.0	21.93±.39	22.0	23.00±1.00	22.0
Pupae	13.65±.30	13.0	13.57±.29	13.0	14.00±1.15	14.0
♀ pupae	14.29±.52	15.0	14.40±.40	15.0	14.00±.2.00	14.0
♂ pupae	13.20±.29	13.0	13.11±.31	13.0	14.00±	14.0
Evolutive cycle	40.82±.70	42.0	41.0±.57	42.0	40.0±3.51	43.0
Adults	10.59±.37	11.0	10.5±.42	10.5	11.0±1.00	12.0
♀ adults	10.43±.78	10.0	10.40±1.03	10.0	10.50±1.50	10.5
♂ adults	10.70±.37	11.0	10.56±.38	11.0	12.00±	12.0
Total longevity	51.41±.78	52.0	51.5±.75	52.0	51.0±3.21	52.0
♀ total longevity	51.14±1.28	52.0	52.20±1.11	52.0	48.50±3.50	48.5
♂ total longevity	51.60±1.02	52.0	51.11±1.01	52.0	56.00±	56.0

Table 4.3.5 Mean, standard error and median for larval development time at 3 different sites during the 1998/1999 period (N = number of larvae; S.E. is the standard error; wet and dry are seasons)

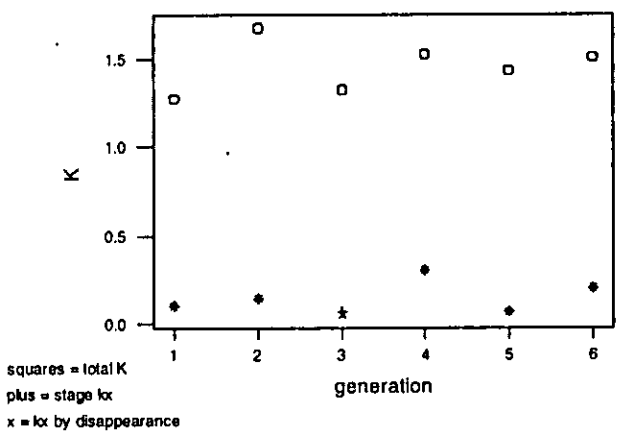
Site	N			Mean±S.E.			Median		
	Year	wet	dry	Year	wet	dry	Year	wet	dry
1° de Maio	37	30	7	21.84± 0.25	21.63±0.26	22.71±0.61	22	22	23.0
INIA	35	27	8	21.83±0.27	21.44±0.28	23.13±0.55	22	22	23.0
University Campus	42	28	14	21.33±0.29	21.86±0.29	20.29±0.58	21	22	19.5

The dry season occurs between April to September and the rainy season from October to March. During the months of the rainy season, mean temperatures were around 26.3°C while during the dry season mean temperatures were around 19.3°C. Mean daily maximum temperatures in the region of Maputo were situated between 33.6 and 27.2 °C during the rainy season and between 31.4 and 26.6°C in the dry season. Average minimum temperatures ranged between 22.5 and 16.5° C during the rainy season and between 18.9 and 11.4° C in the dry season. Values of relative humidity varied from 53% during the dry to 75% during the rainy season (appendix 3.1).

A total of 114 (57 males and 57 females) individuals reached adulthood, from the orchards INIA, University Campus and 1° Maio. Of these 85 were from generations from the rainy season (42 females and 43 males) and 29 from the dry season (15 females and 14 males) (tables 4.3.1-4). In total, 30 life tables were prepared for the 1998/1999 period (section 3.3). Of these, 14 life tables were built for *C. sinensis* cv. Valencia (six at 1° de Maio/LOMACO site, four at INIA and the remaining four at the University Campus sites). Eight life tables were prepared for *C. paradisi* cv. Marsh and for *C.*

paradisi cv. Star Ruby, four at INIA and four at the University Campus (tables 4.3.6 and 4.3.7). Kx values for every developmental stage were calculated and summed for the developmental stage and for each generation. Graphical key-factor analysis (figure 4.3.1) was carried out by plotting *k* from the set of life tables for each consecutive generation with each of the stage-specific k-values as recommended by Price (1984) and Bellows Jr. *et al.* (1992). Individual kx values were plotted against total mortality K per generation to assess by regression analysis the factors that contributed most to total mortality (Royama, 1981). Total K was also plotted against initial density (table 4.4.2) on a log scale to assess by regression analysis the occurrence of density dependent processes within generations. The highest rate of mortality (k-value) for the entire generation occurred during the larval stages (section 4.4, table 4.4.2).

Figure 4.3.1 Graphical analysis of the contribution of larval disappearance in small larvae (2^{nd} instar) to total K per generation (□ = total mortality of all developmental stages; + = total mortality for the second instar larval development stage; x = mortality caused by larval disappearance; each point represents one observation)



Stepwise regression was used to assess the most important meteorological factors on mean pest density per sampling occasion. This method allowed the selection of the variables that showed the greatest influence on the residuals for inclusion in the relationship model.

From the life tables built (cumulative data in tables 4.3.6 and 4.3.7), particular *kx* values for missing individuals were subjected to multiple regression and correlation analyses against environmental data (current, mean, minimum and maximum temperature of the sampling day, current and mean relative humidity of the sampling day, current and mean wind speed, rainfall and evaporation) as recommended by Kuno (1991), Room *et al.* (1991) and Bellows Jr. *et al.* (1992) (sections 2.3 and 3.3).

Egg desiccation contributed to 50.6% to the total egg mortality (tables 4.3.6 and 4.3.7). Egg infertility contributed to 26.6% to egg mortality. The life table data (tables 4.3.6 and 4.3.7) showed that in the rainy season some of the white or yellowish eggs (19.8%) were infertile with 30.3% not being viable owing to desiccation. Other mortality factors were either unknown or rare, such as egg displacement from the substrate where the egg had been laid, or due to the innapropriateness of the substrate after drying (table 4.3.6 and 4.3.7).

Table 4.3.6 Cumulative life table data for *P. demodocus* in the rainy season 1998/1999

Stage	ax	lx	dxF	dx	100qx	Sx	100rx	kx
Yellow eggs	2473	3.39	dislodged	2	0.08		0.08	
			desiccated	748	30.25		30.25	
			infertile	489	19.77		19.77	
			substrate dried	2	0.08		0.08	
			unknown	107	4.33		4.33	
			total	1348	54.51	0.45	54.51	0.342
Pink eggs	1125	3.05	dislodged	0	0.00		0.00	
			desiccated	239	21.24		9.66	
			infertile	77	6.84		3.11	
			substrate dried	1	0.09		0.04	
			unknown	98	8.71		3.96	
			total	415	36.88	0.63	16.78	0.200
Dark eggs	710	2.85	dislodged	2	0.28		0.08	
			desiccated	103	14.51		4.16	
			substrate dried	13	1.83		0.53	
			unknown	158	22.25		6.39	
			total	276	38.87	0.61	11.16	0.214
1st inst. larvae	434	2.64	disappeared	132	30.41		5.34	
			dead, desiccated	2	0.46		0.08	
			cannibalism	0	0.00		0.00	
			substrate dried	0	0.00		0.00	
			unknown	15	3.46		0.61	
			total	149	34.33	0.66	6.03	0.182
2nd inst larvae	285	2.46	disappeared	69	24.21		2.79	
			dead/unknown	4	1.40		0.16	
			total	73	25.61	0.74	2.95	0.129
3rd inst larvae	212	2.33	disappeared	72	33.96		2.91	
			parasitised	0	0.00		0.00	
			unknown	3	1.42		0.12	
			total	75	35.38	0.65	3.03	0.189
4th inst larvae	137	2.14	disappeared	20	14.60		0.81	
			parasitised	0	0.00		0.00	
			unknown	1	0.73		0.04	
			total	21	15.33	0.85	0.85	0.073
5th inst larvae	116	2.06	disappeared	9	7.76		0.36	
			parasitised	0	0.00		0.00	
			unknown	1	0.86		0.04	
			total	10	8.62	0.91	0.40	0.039
pupae	106	2.03	parasitised	13	12.26		0.53	
			unknown	8	7.55		0.00	
			total	21	19.81	0.80	0.32	0.096
adults	85	1.93						
total population						0.034		1.464

Table 4.3.7 Cumulative life table data for *P. demodocus* in the dry season 1999

Stage	ax	lx	dxF	dx	100qx	Sx	100rx	kx
Yellow eggs	1149	3.060	dislodged	0	0.00		0.00	
			desiccated	312	27.15		27.15	
			infertile	323	28.11		28.11	
			substrate dried	0	0.00		0.00	
			unknown	3	0.26		0.26	
			total	638	55.52	0.45	55.52	0.352
Pink eggs	511	2.708	dislodged	0	0.00		0.00	
			desiccated	65	12.72		5.66	
			infertile	73	14.29		6.35	
			substrate dried	0	0.00		0.00	
			unknown	4	0.78		0.35	
			total	142	27.79	0.72	12.36	0.141
Dark eggs	369	2.567	dislodged	2	0.54		0.17	
			desiccated	35	9.49		3.05	
			substrate dried	10	2.71		0.87	
			unknown	103	27.91		8.96	
			total	150	40.65	0.59	13.05	0.227
1st inst. larvae	219	2.340	disappeared	66	30.14		5.74	
			dead, desiccated	0	0.00		0.00	
			cannibalism	0	0.00		0.00	
			substrate dried	0	0.00		0.00	
			unknown	3	1.37		0.26	
			total	69	31.51	0.69	6.00	0.164
2nd inst larvae	150	2.176	disappeared	43	28.67		3.74	
			dead/unknown	1	0.67		0.09	
			total	44	29.34	0.71	3.83	0.127
3rd inst larvae	106	2.025	disappeared	20	18.87		1.74	
			parasitised	0	0.00		0.00	
			unknown	0	6.60		0.61	
			total	27	25.47	0.75	2.35	0.127
4th inst larvae	79	1.898	disappeared	19	24.05		1.65	
			parasitised	0	0.00		0.00	
			unknown	5	6.33		0.44	
			total	24	30.38	0.70	2.09	0.158
5th inst larvae	55	1.74	disappeared	12	21.82		1.04	
			parasitised	0	0.00		0.00	
			unknown	4	7.27		0.35	
			total	16	29.09	0.71	1.39	0.117
pupae	42	1.623	parasitised	9	21.43		0.78	
			unknown	1	2.38		0.09	
			total	10	23.81	0.76	0.87	0.118
adults	29	1.505						
total population						0.03		1.555

From a total population of 2473 eggs only 434 individuals survived to the very small larval stage. Most of the mortality occurred during the egg stage since very small larvae represented only 17.6 % of the egg population; small larvae comprised 65.7 % of the previous developmental stage; medium larvae as many as 74.4 % of the small larvae; large brownish larvae were 64.6 % of the previous stage while final larval instar 84.7 % of the last larval stage. Of these larvae, 91.4% achieved pupation (tables 4.3.6 and 4.3.7). Other decreases in population numbers occurred during the pupal stage with only 80.2% of pupae reaching adulthood. It was also observed that 12.3% of the pupae found in the field during life table studies were parasitised by the parasitoid *Pteromalus* sp.

4.3.2 Sex ratio

Of the 85 butterflies from the life tables studies (section 3.3) that emerged in the oviposition cages into which 5th instar larvae had been transferred prior to pupation, 42 were females and 43 males, giving a proportion of 49.4%:50.6% for the rainy season; during the dry season 15 females and 14 males made a proportion 51.7%:48.3%. In total this proportion consisted of 50% females (57) and 50% (57) males. Sex ratio was established at 1:1 for this population.

Tables 4.6.4, 4.6.5 and 4.6.6 (section 4.6) respectively show the mean and comparisons of means for egg production per cultivar and rearing condition (including season).

4.3.3 Survival curves

Hypothesis

H_0: there are no differences of *P. demodocus* survival curves between the three cultivars, seasons and sites

H_1: *P. demodocus* survival curves are different between cultivars, seasons and sites

Data regarding survivorship from the life table studies (section 3.3.), on a log scale to stabilize the variability of the data around the regression line (Bliss, 1970; Beauchamp & Olson, 1973), were subjected to regression analyses against life span in days. Polynomial regressions were carried out by plotting the survivorship data against time in days. These regressions provided better fits than linear regression to assess survival curve patterns (figures 4.3.3; 4.3.4 and 4.3.5). The parameters of the survival curves (intercept, linear regression slope, quadratic regression slope and cubic regression slope) were compared by using General Linear Model Analysis of Variance (ANOVA) in order to include missing values to enable the similarity of development time curves on the three different cultivars, two seasons and sites to be assessed.

Season had no significantly different effects on pest survival probability at the end of the larval stage and 5th instar larval headcapsule size with P = 0.159 and P=0.407 respectively (tables 4.8.8 and 4.8.1, section 4.8). The constants of the survival curves (intercept and slopes) were not statistically different (P>0.05) between cultivars and sites with $F_{(2,24)}$ = 1.00; P = 0.383 for cultivars and $F_{(2,24)}$ = 1.68; P = 0.208 for locations. However, the survival pattern showed significant differences between seasons with $F_{(1,24)}$ = 4.85; P = 0.038. Pooled standard deviation of 0.1843 for slopes and 0.323 for intercepts (figures 4.3.2-5 and appendix 4.4). The findings support the hypothesis that citrus cultivars and

sampling sites do not affect *P. demodocus* survival pattern. Population survival curves did not differ between cultivars and sites, P>0.05 but were different between seasons, P < 0.05 (table 4.3.8, 4.3.9 and figures 4.3.2-5).

Table 4.3.8 ANOVA for the survival curve parameters (intercept, linear slope, quadratic and cubic regression lines) between locations, seasons and cultivars; * significant at P = 0.05)

Source of variation	Degrees of freedom	Seq SS	Adj SS	Adj MS	F	P
Location	2	1.77×10^{-6}	1.04×10^{-6}	0.52×10^{-6}	1.68	0.208
Cultivars	2	0.62×10^{-6}	0.62×10^{-6}	0.31×10^{-6}	1.00	0.383
Season	1	1.50×10^{-6}	1.50×10^{-6}	1.50×10^{-6}	4.85	0.038*
Error	24	7.43×10^{-6}	7.43×10^{-6}	0.31×10^{-6}		
Total	29	11.32×10^{-6}				

Comparisons of the survival curve parameters between the two seasons are given in table 4.3.9.

Table 4.3.9 Survival curve parameters from the cohort life tables (intercept, linear slope, quadratic and cubic regression lines) for comparison of the effect of the two seasons on *P. demodocus* survival curves pattern (* significant at P = 0.05)

Parameter	Difference of means	Standard error of difference	T-value	Adjusted P-value
intercept	0.3169	0.114	2.779	0.0104*
linear slope	-0.01374	0.015	-0.9176	0.3679
quadratic slope	0.000309	0.001	0.4270	0.6732
cubic slope	-0.000014	0.000	-2.201	0.0376*

Figure 4.3.2 Cumulative survival curve of *P. demodocus* on *Citrus* sp. in the Maputo and Umbelúzi regions during the period 1998/1999 (on a log scale; each point represents one observation).

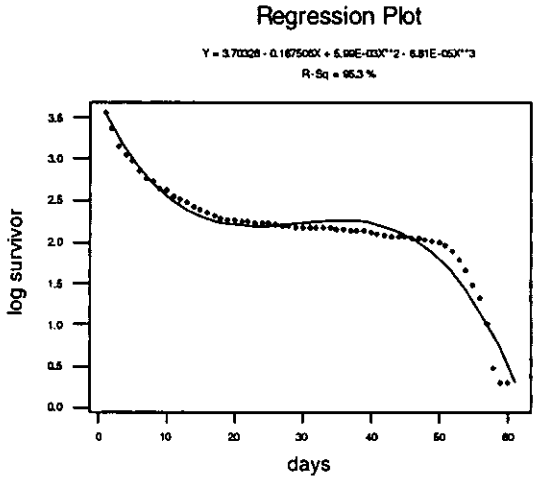


Figure 4.3.3 Cumulative survival curve of *P. demodocus* during the rainy season of 1998/1999 (on a log scale; each point represents one observation)

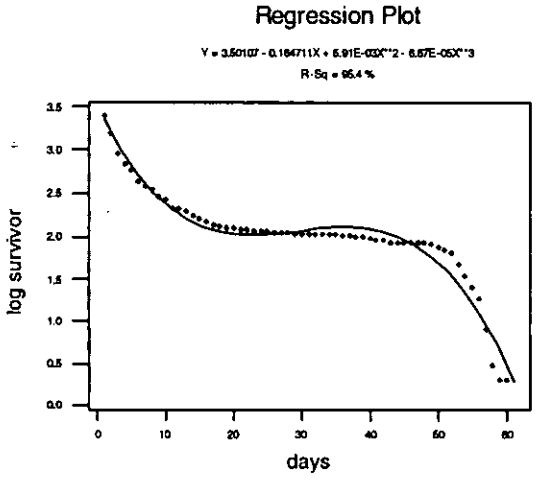


Figure 4.3.4 Cumulative survival curve of *P. demodocus* during the dry season of 1999 (on a log scale; each point represents one observation)

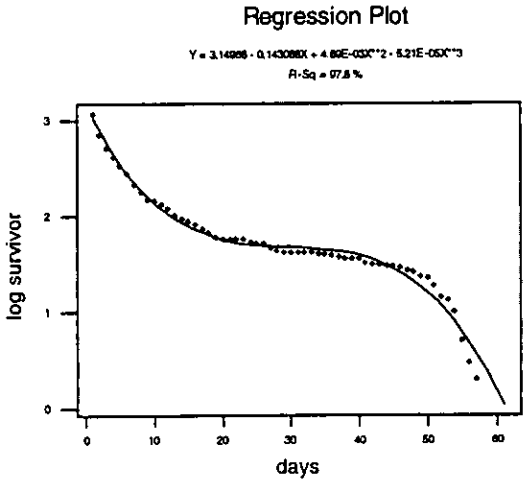
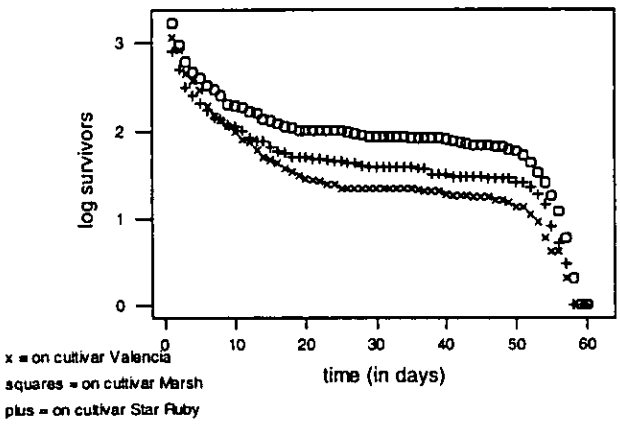


Figure 4.3.5 Cumulative survival of *P. demodocus* on the cultivars Valencia (x), Marsh (□) and Star Ruby (+) (on a log scale; each point represents one observation)



Survival curves indicate a high death rate at the younger developmental stages with survival stabilizing from approximately day 20 (last larval instars) until day 50 (adults) when mortality rates increased (figures 4.3.2-5).

Regarding survival terms, the null hypothesis being investigated that survival curve parameters were similar for the three cultivars was accepted but was rejected between the two seasons.

4.3.4 *Papilio demodocus* reproductive rate

Hypothesis

H_0: there are no differences in the net reproductive rate and capacity for increase between females of the generations from the two seasons

H_1: the reproductive rate and capacity for increase between the two seasons are different

For characterization of population growth rate, net reproductive rate R_0 and capacity for increase r_c were estimated as indicated by Southwood (1978) with the emerging adults collected from all fields during the life table studies and held on the oviposition cages (section 3.3) and table 4.1.1. Net reproductive rate R_0 and capacity for increase r_c were estimated using life table data for the description of population growth rate. The net reproductive rate was estimated as the ratio between the population size at the end of the generation and the population size at the start of the generation period. The capacity for increase was estimated as the ratio of log net reproductive rate and cohort generation time. Cohort generation time (Lara, 1992) was estimated as 52, the mean, from the life tables. Table 4.3.10 summarizes the values of the net reproductive rate and capacity for

increase for *P. demodocus*. The data used in this section were included in the statistical analysis in section 4.6.1.2 (tables 4.6.3, 4.6.5 and 4.6.6).

Table 4.3.10 Mean and standard error for survival rate, fecundity, R_o and r_c during the two seasons (S.E. = standard error; R_0 is the net reproductive rate; r_c is capacity for increase; * only the number of adults included in fecundity studies)

Population	Survival	Adults*	Eggs laid±S.E.	R_0±S.E.	r_c ±S.E.
Dry season	0.027	22	59.9±7.21	1.467±0.359	0.005±0.004
Rainy season	0.034	81	35.7±5.11	0.855±0.169	-0.031±0.023
Total	0.030	103	44.6±4.90	1.081±0.178	-0.017±0.015

One of the generations of the rainy season produced only one male adult (University Campus, cv. Star Ruby) and one population produced only one adult female (INIA site, cv. Marsh). Another produced only two male adults and no females (INIA site, cv. Valencia, see table 4. 1.1). These were excluded as atypical populations (table 4.1.1) and so, R_0 was set at 0.855. The capacity for increase r_c was estimated as being -0.031 (table 4.3.10). Taking into consideration the parameters given above and the global survival rate (table 4.3.6); adult population of 81 individuals as well as the sex ratio of approximately 1:1; the field population average eggs laid per female was established as 35.7. The population was in decline, probably owing to the approach of the dry season where numbers of the insect decrease. During the dry season the R_0 was estimated as being 1.467 with an r_c of 0.005 indicating that the population was increasing. From the life tables (table 4.3.7), survival rate during the dry season, average number of eggs laid per female (table 4.1.1), the adult population and the same sex ratio as during the rainy season (1:1) supported the conclusion that this population was increasing.

4.4 POPULATION DYNAMICS

4.4.1 Adult *P. demodocus* movements

Effect of markers

Hypothesis

H_0: adult longevity of *P. demodocus* is not affected by nail polish, the permanent water proof marker and the commercial fluid Tipp-Ex

H_1: the longevity of *P. demodocus* butterflies is affected when marked with nail polish, permanent water proof marker and the commercial fluid Tipp-Ex

Data regarding adult longevity to assess the effect of different markers on *P. demodocus* life span (section 3.4) were analysed using One Way ANOVA (details in appendix 4.5). The experiment pooled standard deviation was 1.369 and no significant differences between the use of the markers and control treatment on adult longevity was found ($F_{(3,4)} = 0.07$; $P = 0.975$). Nail polish was chosen for marking butterflies in the mark-recapture studies.

Movements

Hypothesis

H_0: there is no evidence that numbers of *P. demodocus* individuals entering or leaving the region affect pest population size

H_1: *P. demodocus* migration affects its population size

The types of movements studied included the within-field (100m), between-field (1 km) and within-region movements (20 kms) of adults as indicated by Drake (1991), see sections 2.4.1 and 3.4.1. The numbers of butterflies caught, marked and recaptured were however too low (table 4.4.1) for statistically meaningful conclusions to be drawn about immigration, birth and death rate processes in the population. The interval of three days was considered to be long enough to allow butterflies to become randomized between the marked and the unmarked individuals. This interval seemed also suitable since it enhanced the likelihood of finding live marked individuals as adult life span had a median of 11 days (table 4.3.1). The durability of the marker was appropriate for the period of study as it lasted more than the adult life span. Insufficient data were collected for meaningful analysis of data on adult pest movements.

Table 4.4.1 Data regarding mark-recapture studies (n1 = number of insects captured and marked on day 1; n2 = total number of insects captured on day 2; n3 = number of insects captured on day 3; r21 = insects captured on day 2 marked on day 1; r31 = insects captured on day 3 marked on day 1; r32 = insects captured on day 3 marked on day 2)

Site	n1	n2	n3	r21	r31	r32
INIA	9	11	8	1	0	2
1° Maio	7	13	9	1	2	0
UEM	7	10	10	0	1	0

4.4.2 Changes within generations

Hypothesis

1) H_0: there are no differences between mortality factors effect on *P. demodocus* numbers between developmental stages

H_1: mortality factor effects on *P. demodocus* developmental stages are different

2) H_0: there is no evidence of density dependent mortality factors within a generation of *P. demodocus*

H_1: mortality factors are density dependent within generations of *P. demodocus*

Spatial density dependent processes were analysed by regressing particular kx factors for specific development stage against insect density and kx factors against generation mortality observed during the life table studies per generation (section 3.4.2). The working hypothesis that there was no evidence of differences between mortality factors on insect numbers in each developmental stage was rejected since particular *kx* values of *P. demodocus* plotted for the various developmental stages of the pest against total mortality K showed different relationship patterns between these variables (figure 4.4.1, table 4.4.2 and appendix 4.6). Table 4.4.2 presents the regression statistics only for the relationships where kx contributed most to total K. The contribution of the larval disappearance to total K was only significant for the second, third and fourth larval instar, but none was related to larval density. Correlations in the relationship between *kx* and *lx* values were not significant for any developmental stage.

Figure 4.4.1 Relationship between kx by 4th instar larval disappearance against total K (each point represents one observation)

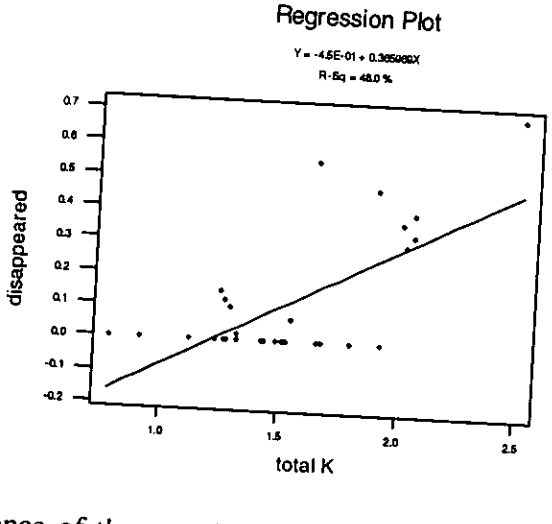


Table 4.4.2 Significance of the contribution of larval disappearance to total K and the relationship between larval disappearance and insect density (y = relationship equation; r^2 = correlation coefficient; s = standard deviation; ** significant at P = 0.01; kx = mortality factor at a given stage; kxd = mortality factor by disappearance; K = total mortality in the generation; lx = larval density; 2nd, 3rd, 4th = second, third and fourth larval instar)

Instar	y	r^2	r^2 (adjusted)	s	$F_{(1, 28)}$	P	Relationship
2nd	-0.236 + 0.267x	33.9%	31.6%	0.142	14.38	0.001**	kx vs K
2nd	-0.221 + 0.255x	31.4%	28.9%	0.144	12.79	0.001**	kxd vs K
2nd		0.00	0.00%	0.173	0.00	0.950	kxd vs lx
3rd	-0.062 + 0.141x	9.9%	6.7%	0.163	3.07	0.09	kxd vs K
4th			47.2%	0.155	26.93	<0.01**	kx vs K
4th	-0.480 + 0.399x	49.0%	46.1%	0.145	25.83	<0.01**	kxd vs K
4th		1.5%	0.0%	0.199	0.42	0.520	kxd vs lx

kx values per development stages plotted against environmental factors using multiple regression analysis did not show any consistency among the series of life tables and there was no evidence of any association between these two groups of variables, with P>0.05 (appendix 4.7) for all developmental stages except the dark egg, second larval instar and fifth larval instar (table 4.4.4). The number of observations on mortality factors influence per development stage varied according to the effect of these factors on particular sampling days. Information on the number of samples and plots from where the data were collected is summarised in appendix 4.8 and 4.9a-4.9i. At the dark egg, second and fifth larval stages significant relationships between meteorological data and *kx* values per development stage were found (table 4.4.3). In these relationships, R^2 was 88.7% for dark eggs, 25.5% for small larvae (2^{nd} instar) and for 5^{th} instar larvae R^2 was 95.1 % with P = 0.014, P= 0.036 and P<0.001 respectively. Standard deviation was 0.072 for dark eggs, 0.126 for 2^{nd} instar larvae and for 5^{th} instar larvae standard deviation was 0.032. Multiple regression analysis is presented in table 4.4.4.

Table 4.4.3 Equations for the relationship between *kx* values against environmental factors for the dark egg stage, 2nd and 5th instar larval stages using multiple regression analysis (E. = equation; $Y1_{(kx)}$ for the dark eggs; $Y2_{(kx)}$ is the equation for the 2nd instar larvae and $Y3_{(kx)}$ the equation for the 5th instar larvae; P = probability ; a = intercept; the other symbols are coefficients for the meteorological data: c.t for for current temperature; m.t. for for mean temperature; mi.t. for minimum temperature; ma.t. for maximum temperature; c.r.h. for current relative humidity; m.r.h. for mean relative humidity; m.w. for mean wind speed; sun for solar radiation; ev. for evaporation; rain for rainfall; * significant at P = 0.05 and ** significant at P = 0.01)

E. and P	a	c.t.	m.t.	mi.t.	ma.t.	c.r.h.	m.r.h.	m.w.	sun	ev.	rain
$Y1_{(kx)}$	-67.9	0.90	1.96	-0.7	0.43	0.337	-0.05	0.85	-0.50	0.124	0.523
P	0.004	0.002	0.007	0.002	0.003	0.002	0.119	0.009	0.017	0.046	0.024
	**	**	**	**	**	**		**	*	*	*
$Y2_{(kx)}$	0.09	-0.04	0.13	0.06	0.11	-0.01	0.01	-0.01	-0.02	-0.00	-0.00
P	0.86	0.09	0.86	0.87	0.76	0.07	0.07	0.14	0.05	0.90	0.97
									*		
$Y3_{(kx)}$	0.71	-0.04	-1.71	0.94	0.80	0.00	0.03	-0.09	0.00	0.07	-0.03
P	0.21	0.04	0.00	0.00	0.33	0.00	0.02	0.00	0.00	0.02	0.00
		*	**	**		**	*		**	*	**

Table 4.4.4 Details of the multiple regression analysis regarding *kx* values against meteorological factors for dark egg stage, small (2nd instar) and 5th larval instar (* significant at P = 0.05; ** significant at P = 0.01; a) for dark egg stage; b) for small larvae c) for 5th larval instar)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F	P
Regression a)	10	0.617960	0.061796	12.00	0.014*
Residual error	4	0.020600	0.005150		
Total	14	0.638560			
Regression b)	10	0.37011	0.03701	2.34	0.036*
Residual error	29	0.45943	0.01584		
Total	39	0.82954			
Regression c)	10	0.306463	0.030646	29.97	0.001**
Residual error	5	0.005112	0.001022		
Total	15	0.311575			

These results indicate that solar radiation is correlated with kx value for the 5th larval instar and negatively correlated with the kx value for the dark egg and 2nd instar larvae. Kx value for dark egg stage was positively correlated with current, mean and maximum temperature , current relative humidity, mean wind, evaporation and rain while negatively related to minimum temperature. The kx value for the 5th larval instar was also affected by the current temperature, and relative humidity, average temperature and relative humidity in the previous sampling day, minimum temperature, evaporation and rainfall in the sampling day.

4.4.3 Changes between generations

Hypothesis

H_0: population size changes are not affected by the initial population size

H_1: population changes are dependent on the initial population size

Mean values of egg counts taken for cohort life table studies (sections 2.4.2 and 3.4) per generation in all sites (table 4.4.5) were used to assess the effect of the initial population size on population size at the end of the generation (section 2.4.2). In figure 4.4.2 the effect of the egg population size in the second generation on population size of the third generation was assessed, in figure 4.4.3 the effect of egg population size in the fourth generation on the population size of the fifth generation was assessed while in figure 4.4.4 egg population size of the third generation effect on population size of the fourth generation was assesssed. The null hypothesis stating that there was no evidence that changes in population sizes between generations were density dependent was rejected in this analysis. The regression equations describing the relationship of population changes between generations suggested that there was evidence for the existence of density-dependent processes occurring between generations and between seasons (table 4.4.5 and figures 4.4.2-4).

Table 4.4.5 Cumulative egg population size in different generations and 0.25 ha plots (G1= October/November 1998 generation; G2 = December/January 1998/99 generation; G3 = February/March 1999; G4 = April/May; G5 = June/July; G6 = August/September)

Site	Cultivar	G1	G2	G3	G4	G5	G6
UEM	Valencia		132	93	98	63	
UEM	Marsh		184	68	102	12	
UEM	Star Ruby		324	161	107	31	
INIA	Valncia		64	88	55	101	
INIA	Marsh		85	152	92	114	
INIA	Star Ruby		107	231	66	102	
1 ° Maio	Valencia	112	288	384	34	108	64

Figure 4.4.2 Population changes related to the initial population size between consecutive generations of the rainy season (generation 2 and generation 3 in table 4.4.5)

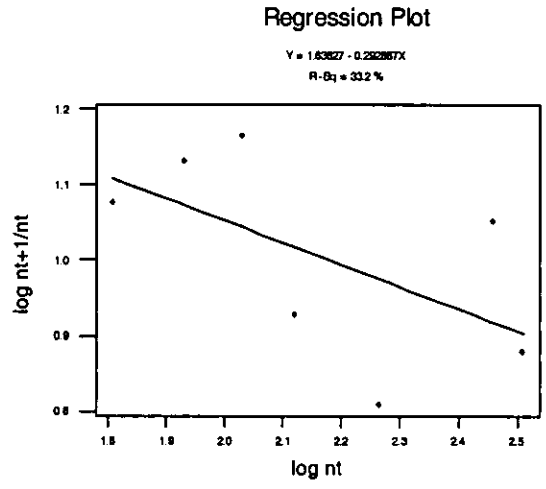


Table 4.4.6 Details of the regression analysis of the population changes between consecutive generations in the course of the year (DF is degrees of freedom; SS is sum of squares; MS mean squares; REG. is regression; RES. E is residual error; TOT. is total; A) is rainy season; B) is dry season and C) the period between rainy and dry season; P is probability; y is the relationship equation; S = standard deviation and r^2 the correlation coefficient; * significant at P = 0.05)

ANOVA A)								
SOURCE	DF	SS	MS	F	P	y	S	r^2 (ADJ.)
REG	1	0.0361	0.0361	2.48	1.76	1.64-0.293 X	0.120	19.8%
RES.E	5	0.0726	0.0145					
TOT	6	0.1087						
ANOVA B)								
SOURCE	DF	SS	MS	F	P	y	S	r^2 (ADJ.)
REG.	1	0.3084	0.3084	12.50	0.017*	3.28-1.24 X	0.157	65.7%
RES.E	5	0.1234	0.0247					
TOT.	6	0.4318						
ANOVA C)								
SOURCE	DF	SS	MS	F	P	y	S	r^2 (ADJ.)
REG.	1	0.1404	0.1404	34.25	0.02*	2.14-0.582 X	0.064	84.7%
RES.E	5	0.0205	0.0041					
TOT.	6	0.1609						

Figure 4.4.3 Population changes relating the initial population size and consecutive generations of the dry season (generation 4 and generation 5 in table 4.4.5)

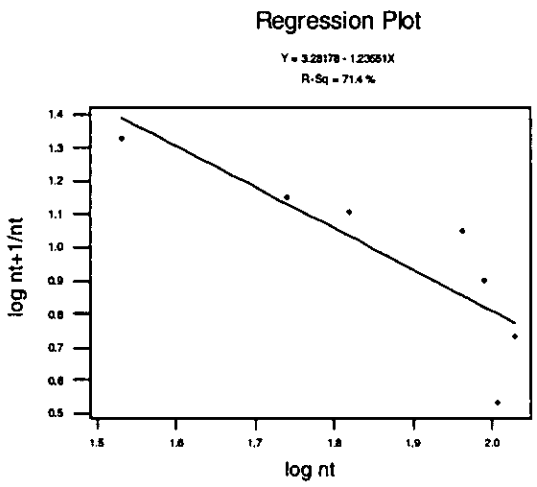
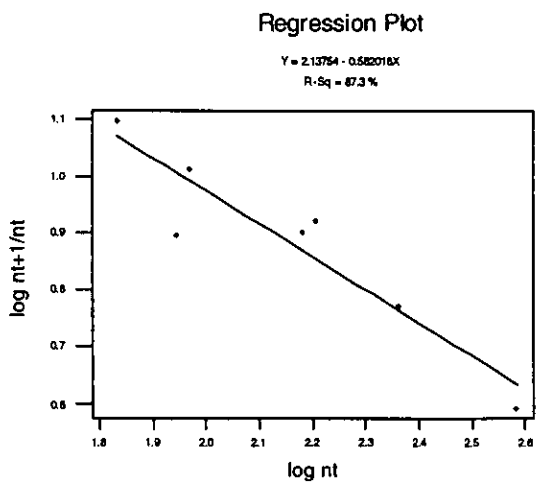


Figure 4.4.4 Population changes relating the initial population size and consecutive generations of different seasons (generation 3 and generation 4 in table 4.4.5)



4.4.4 *Papilio demodocus* population growth curves

Hypothesis

H_0: there were no differences between growth curves for *P. demodocus* population in different sites

H_1: there were differences between growth curves from different sites

Egg counts carried out in the field during the life table studies (section 3.3, tables 4.3.6, 4.3.7 and 4.4.5) showed that the egg population increased by 51.02% between the first (G1 in table 4.4.5) and the second generation (G2) and decreased 0.59% between the second and the third (G3) generation in the rainy season. Differences between consecutive generations of the rainy (G3) and dry season (G4) in terms of egg population reached 52.93%. During the first (G4) and second generation (G5) of the dry season the reduction in egg population reached 4.15% and 15.63% between the second and third (G6) generation (table 4.4.5). Leslie matrices built (as in figure 4.4.5) using information (summarized in tables 4.3.5, 4.3.7 and table 4.1.1) on survival rates, fecundity and population size collected during the life table studies were used to construct the population growth curves. Data from INIA, UEM and 1° Maio sites were used to construct the growth curves in order to assess similarities of the egg and larval population growth curve during the year between these sites. From the matrices (table 4.4.7), the other developmental stages (larval, pupal and adult) produced the highest increase in numbers in the last generation of the rainy season and the minimum levels during the dry season. Figures 4.4.6 and 4.4.7 represent the graphical population dynamics of the pest population from February to December of the same year, respectively, for the egg and larval age classes. A general decrease in pest numbers is observed in the generations of the dry season (figure 4.4.6 and 4.4.7).

Figure 4.4.5 Example of the diagram used to build Leslie matrices with life table data (E= egg stage; L1 = first larval instar; L2 = second larval instar; L3 = third larval instar; L4 = fourth larval instar; L5 = fifth larval instar; P = pupal stage; A = adult stage; a1, a2 = fertility terms for the adults respectively during the 4 initial and 4 final days of reproduction; same letters in lower case = survival rate terms specific to the developmental stage; I.A.C. = initial age composition; time interval between columns is 4 days giving a matrix of thirteen rows and thirteen columns for a 52 days generation)

E	L1	L2	L3	L4	L5	L5	P	P	P	P	A	A	= developmental stages
0	0	0	0	0	0	0	0	0	0	0	a1	a2	= age-specific fertility terms

e	0	0	0	0	0	0	0	0	0	0	0	0		E
0	l1	0	0	0	0	0	0	0	0	0	0	0		L1
0	0	l2	0	0	0	0	0	0	0	0	0	0		L2
0	0	0	l3	0	0	0	0	0	0	0	0	0		L3
0	0	0	0	l4	0	0	0	0	0	0	0	0		L4
0	0	0	0	0	l5	0	0	0	0	0	0	0		L5
0	0	0	0	0	0	l5	0	0	0	0	0	0	x	L5
0	0	0	0	0	0	0	p	0	0	0	0	0		P
0	0	0	0	0	0	0	0	p	0	0	0	0		P
0	0	0	0	0	0	0	0	0	p	0	0	0		P
0	0	0	0	0	0	0	0	0	0	p	0	0		P
0	0	0	0	0	0	0	0	0	0	0	a	0		A
0	0	0	0	0	0	0	0	0	0	0	0	a		A

I.A.C.

Table 4.4.7 Average number of insects per developmental stage in sites INIA, UEM and at the site 1° Maio (indicated respectively by letters I, U and M) for *P. demodocus* (estimated using Leslie Matrix; G = generation; Larv. = larvae; Pup. = pupae; Ad. = adults)

G	Eggs (I)	Eggs (U)	Eggs (M)	Larv (I)	Larv (U)	Larv (M)	Pup. (I)	Pup. (U)	Pup. (M)	Ad. (I)	Ad. (U)	Ad. (M)
1			93.0			16.0			0.9			0.3
2	89.5	132.5	66.0	30.0	26.0	23.9	1.6	1.8	0.8	1.0	0.4	0.2
3	92.0	64.0	146.0	27.9	15.2	78.0	1.8	1.0	4.3	0.4	0.3	1.0
4	45.5	139.5		9.0	21.5		0.4	1.5		0.1	0.3	
5	41.0	42.0	109.0	11.0	4.0	22.0	1.9	0.7	0.8	0.4	0.3	0.2
6			93.0			37.0			1.7			0.1

Figure 4.4.6 Numbers of eggs estimated at 52 day time interval during 1998-1999 in 0.25 ha plots using Leslie matrices for 1° Maio, INIA and University Campus sites

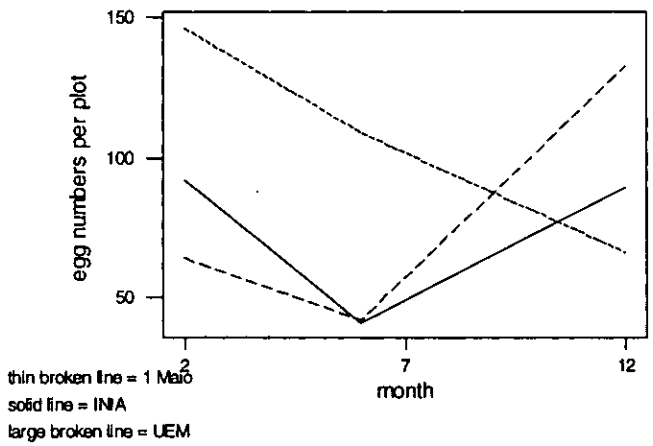
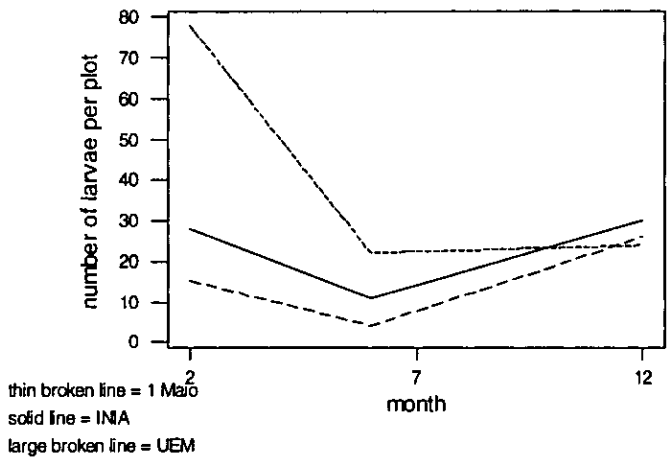


Figure 4.4.7 Numbers of larvae estimated at 52 day time interval during the year in 0.25 ha plots using Leslie matrices for 1° Maio, INIA and University Campus sites



Using the χ^2 test, the similarities of the growth curves between sites for egg and larval developmental stages were assessed. The critical value for the comparisons between the three growth curves by the χ^2 test at P = 0.05 with two degrees of freedom was 5.99. Calculated χ^2 values were 131.4, 70.3 and 494.4 for eggs respectively for comparisons of the curves between the sites INIA and 1° Maio, INIA and University Campus and 1° Maio and University Campus (figure 4.4.6). The calculated χ^2 values were 80.6, 36.2 and 29.3 for the larval stages respectively for the comparisons between INIA and 1° Maio, INIA and University Campus and 1° Maio and University Campus (figure 4.4.7). The χ^2 test showed that the Leslie model was not appropriate for these data. However, it can be regarded as a basis to test for the importance of other factors contribution to explain the variation of *P. demodocus* population sizes.

4.5 EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON *P. demodocus* DEVELOPMENT

4.5.1 Larval development time and development rate in the laboratory

Hypotheses

H_0: *P. demodocus* larval development time and rate are not affected by temperature and relative humidity

H_1: *P. demodocus* larval development time and rate are affected by temperature and relative humidity

Papilio demodocus development periods at selected temperatures and constant relative humidity were observed in the laboratory to assess the effect of temperature on *P. demodocus* performance (section 3.5). Development rates were determined as the reciprocals of the median number of days for development at a given temperature (table 4.5.3). They were plotted against temperature (Wagner *et al.*, 1984) in order to describe the relationship between temperature and *P. demodocus* development rates (figure 4.5.1). *Papilio demodocus* physiological time was calculated by summing up the accumulated degree-days from egg deposition to adult emergence (section 3.5). Regression analysis using the quadratic model was carried out to assess the effect of relative humidity on larval development rate. Temperature was not regressed against larval development rate because neither the regression quadratic model nor the cubic model gave satisfactory curves describing the relationship between the two variables.

Temperature had a significant effect on *P. demodocus* larval developmental time, with

$F_{(3, 82)} = 162.76$ and $P < 0.001$, (table 4.5.1 and 4.5.2).

Table 4.5.1 ANOVA for the effect of temperature on larval development time (relative humidity = 70%; ** significant at P = 0.001)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F	P
Temperature	3	4294826	1431609	162.76	<0.001**
Error	82	721246	8796		
total	85	5016072			

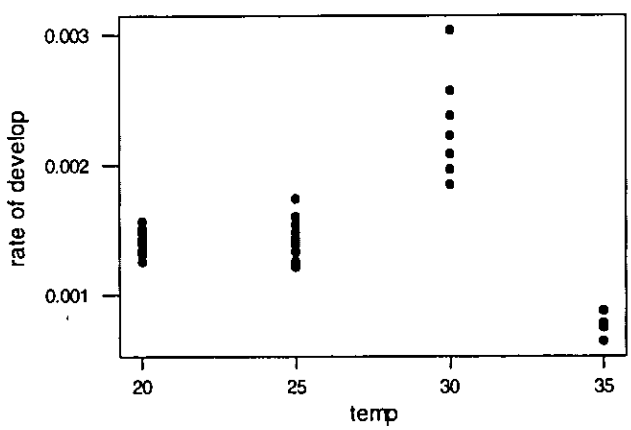
Table 4. 5.2 Larval development time mean and standard error of *P. demodocus* in degree-days at selected temperatures (N = number of insects; relative humidity = 70%)

Temperature level (°C)	N	Mean of degree-days	Standard Error of Mean
20	28	690.7	7.16
25	23	669.6	32.70
30	29	432.4	7.67
35	6	1341.7	55.5

Table 4.5.3 Development rate of *P. demodocus* at selected temperatures (N = number of insects; relative humidity = 70%)

Temperature (° C)	N	Median (days)	Development rate day $^{-1}$
20	28	690	0.00145
25	23	700	0.00143
30	29	420	0.00238
35	6	1330	0.00075

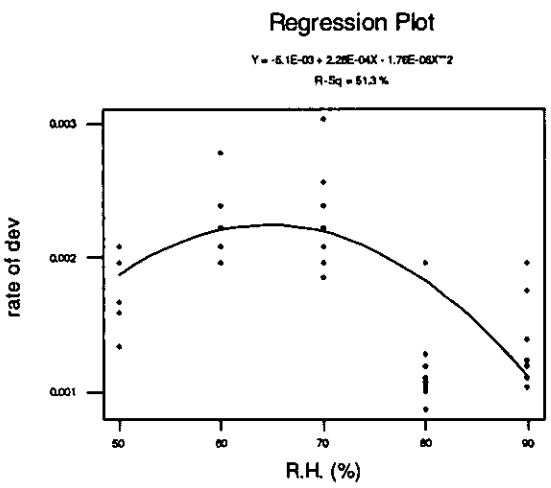
Figure 4.5.1 *Papilio demodocus* development rate (rate of deve) days $^{-1}$ as affected by temperature in °C at 70% relative humidity (each point represents one observation)



In the relationship between the insect development rate and temperature (figure 4.5.1), standard deviation was 0.0004. Figure 4.5.2 shows the relationship between development

rate and air relative humidity, where $F_{(2,92)} = 48.41$ and $P < 0.001$. 51.3% of variation in *P. demodocus* development rate was accounted for by relative humidity.

Figure 4.5.2 Effect of relative humidity on the development rate at 30° C (dev = development rate; each point represents one observation)



The graph (figure 4.5.1) and the regression line (figure 4.5.2) respectively showing the relationship between *P. demodocus* performance as affected by temperature and relative humidity indicated that both temperature and relative humidity have a positive effect on *P. demodocus* performance at lower levels, turning to negative effects at higher levels (figures 4.5.1 and 4.5.2).

4.5.2 Survival probability, fecundity and egg viability

Hypotheses

H_0: *P. demodocus* survival, egg deposition and viability rate are not affected by temperature and relative humidity

H_1: *P. demodocus* survival, egg deposition and fecundity are affected by temperature and relative humidity

Papilio demodocus egg viability, survival probability and fecundity at selected temperatures and relative air humidity levels were studied in the laboratory as indicated in section 3.5. Table 4.5.4 presents the proportion of egg viability, *P. demodocus* survival and egg production per temperature and relative humidity treatment. The variables egg viability, survival probability and fecundity were not significantly related to temperature and relative humidity (figures 4.5.3 and 4.5.4). Table 4.5.5 presents the statistical analysis for the relationship between these variables.

Table 4.5.4 Survival probability, average egg production and egg viability per temperature and relative humidity (Temp. = temperature)

Temp (°C)	Relative humidity (%)	Initial egg number	Egg dying (%)	Survival (%)	Total egg number	Average Egg Production	Adult ♀	Total number adults
20	70	54	48.1	51.9	256	17.1	15	28
25	70	38	39.5	57.9	247	22.5	11	23
30	50	30	55.8	44.2	466	42.4	11	19
30	60	39	43.6	56.4	472	42.9	11	22
30	70	45	35.6	64.4	511	34.9	16	29
30	80	80	82.5	15.0	54	9.0	6	11
30	90	52	50.0	26.9	68	9.7	7	14
35	70	42	66.7	14.3	19	6.3	3	6

Table 4.5.5 Regression analysis for the relationship between different treatments of temperature and relative humidity and *P. demodocus* survival, fecundity and egg viability

Relationship	Equation	R^2 (%)	F	P
Egg production vs temperature	$-192.65 + 16.590 x - 0.31 x^2$	77.8	(2,1)=1.754	0.4700
Egg dying vs temperature	$306.755 - 20.797 x + 0.397 x^2$	92.0	(2,1)=5.759	0.2830
Survival vs temperature	$-301.135 + 28.729 x - 0.561 x^2$	89.2	(2,1)=4.146	0.3280
Egg production vs humidity	$58.0829 + 0.157 x - 0.00821 x^2$	87.5	(2,2)=7.030	0.1245
Egg dying vs humidity	$82.3971 - 1.157 x + 0.0102 x^2$	7.0	(2,2)=0.075	0.9301
Survival vs humidity	$-100.134 + 5.04 x - 0.0414 x^2$	49.0	(2,2)=0.961	0.5099

Figure 4.5.3 Effect of temperature (°C) on egg viability (%) at 70% relative humidity (each point represents mean of 30 specimens or more)

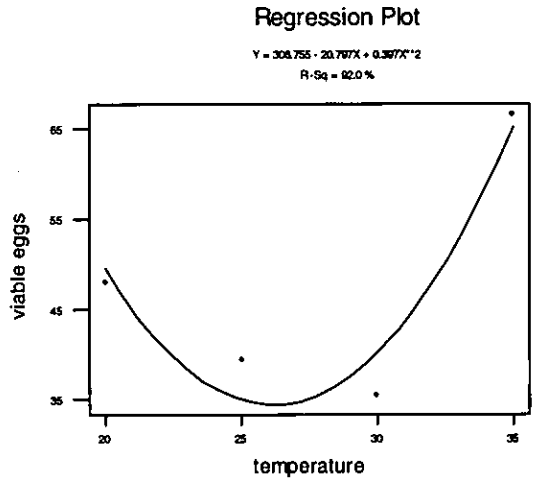
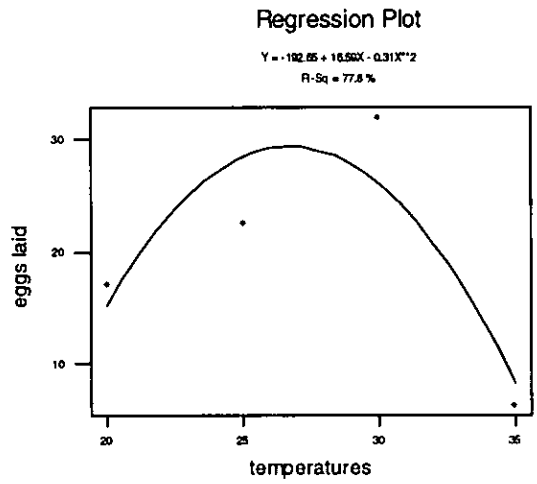


Figure 4.5.4 Effect of temperature (° C) on egg production at 70% relative humidity (each point represents mean of 3 females or more)



4.5.3 Larval headcapsule size

Hypothesis

H_0: there is no evidence that larval headcapsule size differs with temperature

H_1: there is evidence that larval headcapsule size differs between temperatures

Measurements on larval headcapsule size were taken from fifth instar larvae developed at selected temperatures in the laboratory (section 3.5). The analysis of variance for the headcapsule size in relation to temperature was not significant, $F_{(1,84)} = 3.10$; P=0.083; $r^2 = 3.6\%$; r^2 (adjusted) = 2.4%; standard deviation of 0.439. Table 4.5.6 shows the mean headcapsule size for every temperature treatment.

Table 4.5.6 Larval headcapsule size means (5^{th} instar) by temperature level (relative humidity = 70%; N = number of insects)

Temperature (° C)	N	Mean x 10^{-1} mm	Standard Error
20	28	32.607	0.094
25	23	32.870	0.072
30	29	32.897	0.058
35	6	32.667	0.333

4.6 EFFECT OF DIET ON *P. demodocus* DEVELOPMENT

The objectives of the experiments were to evaluate the suitability of three *Citrus* cultivars as food for the pest in the laboratory (section 3.6).

4.6.1 Experiment 1

4.6.1.1 Larval weight

Hypotheses

1) H_0: the *Citrus* cultivars Valencia, Marsh and Star Ruby do not differentially affect *P. demodocus* larval weight

H_1: the *Citrus* cultivars Valencia, Marsh and Star Ruby differentialy affect *P. demodocus* larval weight

2) H_0: *P. demodocus* larval weight is the same when reared in different growth chambers

H_1: *P. demodocus* larval weight is affected by the growth chamber

Larval weight was measured on fifth instar larvae reared in the laboratory on their third day after moulting (section 3.6). In the laboratory, diet did not differentially affect *P. demodocus* fifth instar larval weight; $F_{(2,137)} = 0.13$; P=0.880. The results showed that larval weight did not vary between cultivars or growth chambers (tables 4.6.1 and 4.6.2).

Table 4.6.1 ANOVA for 5th larval instar weight in different cultivars and growth chambers

Source of variation	Degrees of freedom	Seq. Sum of Squares	Adjusted Sum of Squares	Adjusted Mean Squares	F	P
Chamber	1	0.000767	0.000770	0.000770	0.34	0.561
Cultivar	2	0.000581	0.000581	0.000291	0.13	0.880
Error	137	0.310792	0.310792	0.002269		
Total	140	0.312140				

Table 4.6.2 Mean and standard error of larval weight (5th instar) per cultivar in the laboratory

Cultivar	N	Mean (g)	Standard error of mean
Valencia	48	2.4462	0.008
Marsh	47	2.4496	0.006
Star Ruby	46	2.4511	0.006

4.6.1.2 Fecundity

Hypotheses

1) H_0: the *Citrus* cultivars Valencia, Marsh and Star Ruby do not differentially affect *P. demodocus* fecundity

H_1: the *Citrus* cultivars Valencia, Marsh and Star Ruby differentialy affect *P. demodocus* fecundity

2) H_0: *P. demodocus* fecundity is similar for generations produced in different places (the dry season, rainy season and those reared in the laboratory)

H_1: fecundity varies between generations produced in different places (the dry season, rainy season and those reared in the laboratory)

Combining data from insects observed and developed in the field at the egg and larval stages during the life table studies and transferred, prior to pupation, to oviposition cages (section 3.3) with data from insects reared in the laboratory (section 3.6), the effect of the rearing condition on female fecundity was assessed. The cultivars Valencia, Marsh and Star Ruby showed no significant differences in egg deposition when compared to the effect of the place where the insects had developed (tables 4.6.3 and 4.6.4). Differences were found between rearing conditions (table 4.6.3). Higher values of egg deposition were observed in females in the dry season (table 4.6.5), with P = 0.0224 (table 4.6.6).

Table 4.6.3 ANOVA to assess the effect of cultivar and rearing condition on egg deposition (* significant at P = 0.05; place refers to the area where the larvae developed, that is, field rainy season, field dry season and laboratory)

Source of Variation	Degrees of Freedom	Seq. Sum of Squares	Adjusted Sum of Squares	Adjusted Mean Squares	F	P
Cultivar	2	418.0	334.6	167.3	0.55	0.587
Place	2	2641.1	2641.1	1320.5	4.33	0.028*
Error	19	5793.3	5793.3	304.9		
Total	23	8852.3				

Table 4.6.4 Mean egg deposition per cultivar (N = number of larvae; cultivar Valencia involved both insects reared in the laboratory and those from the field life table studies; Marsh and Star Ruby cultivars refer only to data observed during field life table studies)

Cultivar	N	Mean eggs laid	Standard Error	Range
Valencia	15	40.56	5.25	(18.25, 93.00)
Marsh	5	51.10	10.1	(28.70, 76.80)
Star Ruby	4	44.13	7.25	(28.50, 63.00)
Total populations	24	43.35	4.00	(18.25, 93.00)

Table 4.6.5 Mean egg deposition per rearing condition (N = number of larvae)

Rearing place	N	Mean eggs laid	S.E. of Mean	Range
Field dry season	7	59.86	7.21	(39.00, 93.00)
Field rainy season	12	35.68	5.11	(18.25, 76.75)
Laboratory	5	38.65	4.97	(21.00, 50.75)
Total populations	24	43.35	4.00	(18.25, 93.00)

Table 4.6.6 Comparisons between means of egg deposition per rearing condition (* significant at P = 0.05)

Comparisons between rearing places	Difference of means	Standard error of difference	T-value	Adjusted P-value
field dry season and field rainy season	-24.51	8.372	-2.928	0.0224*
field dry season and laboratory	-17.58	10.803	-1.627	0.2590
field rainy season and laboratory	6.935	10.13	0.6843	0.7753

The analysis of variance (table 4.6.3) and comparisons of means (table 4.6.6) showed that populations of the dry season and those of the wet season and laboratory were different in terms of egg deposition. Generations of the dry season laid more eggs than those of the wet season or under laboratory conditions.

4.6.2 Experiment 2

Nutritional indices

Hypothesis

H_0: nutritional indices of *P. demodocus* larvae are similar when the larvae are fed on the cultivars Valencia, Marsh or Star Ruby

H_1: nutritional indices of *P. demodocus* larvae are different between cultivars when fed on the cultivars Valencia, Marsh or Star Ruby

Larval nutritional indices from 10 specimens were estimated as indicated in section 3.6. One-way analysis of variance indicated that the three cultivars did not show significant differences for relative growth rates (RGR), relative consumption rate (RCR), assimilation efficiency (AD), efficiency of conversion of assimilated food (ECD) and efficiency of conversion of ingested food (ECI) ($P>0.05$) (tables 4.6.7, 4.6.8 and appendix 4.10)

Table 4.6.7 ANOVA data for the nutritional indices (data from ten 5th instar larvae fed on the cultivars Valencia, Marsh and Star Ruby)

Indice	$F_{(2,27)}$	P	Pooled Standard deviation
ECI	0.48	0.627	5.536
RCR	0.76	0.477	0.003
RGR	0.26	0.775	0.003
AD	1.99	0.156	4.046
ECD	0.45	0.643	11.840

Table 4.6.8 Mean values of nutritional indices of 10 specimens per *Citrus* cultivar

Citrus cultivar	RCR Mean	RGR Mean	AD Mean	ECD Mean	ECI Mean
Valencia	0.04614	0.01589	58.34	60.04	34.46
Marsh	0.04693	0.01650	61.39	55.85	35.18
Star Ruby	0.04542	0.01663	61.59	60.35	36.81
Total	0.04617	0.01634	60.45	58.75	35.48

4.6.3 Leaf nutrient analysis

Hypotheses

H_0: the level of water soluble tannins and nitrogen are similar between cultivar, site and time

H_1: the level of water soluble tannins and nitrogen are different between cultivar, site and time

To indirectly assess the influence of food quality of different citrus plants under different site condition and consequently, on the pest abundance and distribution, citrus leaves were collected for nutrient analyses in < 2 years old citrus plants in the months of October and February (section 3.6). The Kruskal-Wallis rank test was used to assess differences between two or more samples and the Mann-Whitney test was used to identify, when necessary, the samples that were different, comparing pairs of samples.

Using Kruskal-Wallis rank test, no significant differences were found in nitrogen and tannin contents between the two sampling months (October 1999 and February 2000), tables 4.6.9 and 4.6.10. The values of nitrogen and soluble tannins are given as percentage of leaf dry weight.

Table 4.6.9 Kruskal-Wallis test for nitrogen levels on citrus leaves (Median in % of dry weight) between February and October per site (Z = confidence interval, DF = degrees of freedom, P = probability)

Site	Month	N	Median (%)	Rank	Z	H	DF	P
1°Maio	February	10	2.93	8.3	-1.90	3.62	1	0.057
1°Maio	October	11	3.28	13.5	1.90			
	Overall	21	3.16	11.0				
UEM	February	15	1.79	18.9	0.47	0.22	1	0.641
UEM	October	20	1.72	17.3	- 0.47			
	Overall	35	1.73	18.0				
INIA	February	34	2.55	28.2	- 1.78	3.16	1	0.075
INIA	October	29	3.00	36.4	1.78			
	Overall	63	2.60	32.0				

Cultivar nitrogen levels were statistically different at the INIA ($P<0.01$) but not at the UEM site (table 4.6.11). In order to assess the nitrogen levels in the leaves of cultivars the Mann-Whitney test was carried out for the cultivars at the INIA location (table 4.6.12). Cultivar Valencia had higher levels of nitrogen followed by the cultivar Star Ruby (table 4.6.13). In this respect, at INIA, cultivar Valencia was different to cultivar Marsh ($P<0.01$) and to cultivar Star Ruby ($P<0.01$). The cultivars Marsh and Star Ruby had also different nitrogen contents ($P<0.01$) (table 4.6.12).

Table 4.6.10 Kruskal-Wallis test for tannin levels on citrus leaves (Median in % of dry weight) between February and October per site (Z = confidence interval, DF = degrees of freedom, P = probability)

Site	Month	N	Median (%)	Rank	Z	H	DF	P
1°Maio	February	11	57.35	9.7	- 0.99	0.97	1	0.324
1°Maio	October	10	58.11	12.4	0.99			
	Overall	21	57.45	11.0				
UEM	February	27	76.89	29.6	- 0.17	0.03	1	0.861
UEM	October	32	78.43	30.4	0.17			
	Overall	59	77.28	30.0				
INIA	February	44	59.22	41.5	0.90	0.80	1	0.370
INIA	October	34	58.66	36.9	- 0.90			
	Overall	78	58.74	39.5				

Table 4.6.11 Kruskal-Wallis analysis for nitrogen content on citrus leaves (Median in % of dry weight) between cultivars per site (Z = confidence interval, DF = degrees of freedom, P = probability; ** = significant at P = 0.01)

Site	Cultivar	N	Median (%)	Rank	Z	H	DF	P
UEM	Valencia	12	1.93	21.2	1.36	2.01	2	0.366
UEM	Marsh	12	1.72	15.5	- 1.06			
UEM	Star Ruby	11	1.71	17.2	- 0.30			
Overall		35	1.73	18.0				
INIA	Valencia	19	3.08	48.7	4.76	40.62	2	<0.001**
INIA	Marsh	22	2.02	13.1	- 6.00			
INIA	Star Ruby	22	2.65	36.5	1.42			
Overall		63	2.60	32.0				

Table 4.6.12 Mann-Whitney test for nitrogen levels (Median in % of dry weight) between cultivars at INIA site (N = sample size; V = Valencia, M = Marsh and SR = Star Ruby; C.I. = confidence interval; W = test; P = probability; ** = significant at P = 0.01)

Comparisons	N V M SR	Median (%) V M SR	C.I.	W	P
	19 22 22	3.08 2.02 2.65			
V vs M			95.2% (0.9101, 1.1901)	608.0	<0.0001**
V vs SR			95.2% (0.1300, 0.5599)	508.0	0.0046**
M vs SR			95.0% (-0.9399, -0.510)	287.5	<0.0001**

Foliar nitrogen levels were significantly different between sites for the three cultivars (tables 4.6.13 and 4.6.14). Site 1° Maio had higher nitrogen levels followed by INIA site (table 4.6.13). The INIA site had higher levels of nitrogens than UEM site both for Marsh and Star Ruby cultivars (table 4.6.14).

Table 4.6.13 Differences in nitrogen content (Median in % of dry weight) for the cultivar Valencia between sites (Z = confidence interval, DF = degrees of freedom, P = probability; ** = significant at P = 0.01)

Site	N	Median (%)	Rank	Z	H	DF	P
INIA	19	3.08	31.0	1.62	27.52	2	<0.0001**
UEM	12	1.93	6.5	- 5.21			
1 Maio	21	3.16	33.9	2.88			
Overall	52	3.03	26.5				

Table 4.6.14 Analysis of nitrogen levels of leaves (Median in % of dry weight) of the cultivars Marsh and Star Ruby between INIA and UEM sites (Z = confidence interval, DF = degrees of freedom, P = probability; ** = significant at P = 0.01)

Site	Cultivar	N	Median (%)	Rank	Z	H	DF	P
INIA	Marsh	22	2.02	22.4	3.89	15.15	1	<0.001**
UEM	Marsh	12	1.72	8.5	- 3.89			
Overall	Marsh	34	1.92	17.5				
INIA	Star Ruby	22	2.65	22.1	4.32	18.62	1	<0.001**
UEM	Star Ruby	11	1.71	6.7	- 4.32			
Overall	Star Ruby	33	2.52	17.0				

Assessment of differences in tannin levels between cultivars was carried out using a Mann-Whitney test for each site (table 4.6.15). At the UEM site, differences in tannin levels were observed between the cultivars Valencia and Star Ruby (P=0.0079) whereas at INIA, differences were observed between the cultivars Valencia and Star Ruby (P<0.01) as well as between the cultivars Marsh and Star Ruby (P<0.01) (table 4.6.16). Table 4.6.16 shows also that at the UEM site, cultivar Star Ruby had higher levels of tannins than the cultivar Valencia. At the INIA site the cultivars Valencia and Marsh had lower levels of tannins than the cultivar Star Ruby.

Table 4.6.15 Kruskal-Wallis analysis for tannin content on citrus leaves (Median in % of dry weight) between cultivars per site (Z = confidence interval, DF = degrees of freedom, P = probability; ** = significant at P = 0.01)

Site	Cultivar	N	Median (%)	Rank	Z	H	DF.	P
UEM	Valencia	20	73.81	22.9	- 2.26	7.97	2	0.019*
UEM	Marsh	20	76.93	29.1	- 0.30			
UEM	Star Ruby	19	80.00	38.4	2.59			
Overall		59	77.28	30.0				
INIA	Valencia	30	60.13	48.6	2.80	22.75	2	<0.001**
INIA	Marsh	26	60.31	45.4	1.63			
INIA	Star Ruby	22	53.54	20.1	- 4.74			
Overall		78	58.74	39.5				

Plants of the cultivar Valencia at the three sites had different tannin contents in the leaves (table 4.6.17). Valencia plants had higher tannin levels at the UEM site followed by INIA (table 4.6.17). Between sites, tannin contents between INIA and 1° Maio were different at P = 0.0018; plants from INIA and UEM were different at P<0.01 and plants from 1° Maio and UEM differed at P<0.01 in terms of tannin contents on Valencia plants (table 4.6.17). Similarly, the cultivars Marsh and Star Ruby differed in tannin contents between the sites INIA and UEM, with P<0.01 for both Marsh and Star Ruby (table 4.6.18). Higher tannin levels were observed for the site UEM for the cultivars Marsh and Star Ruby (table 4.6.18).

Table 4.6.16 Mann-Whitney test for tannin levels (Median in % of dry weight) between cultivars at INIA and UEM sites (N = sample size; V = Valencia, M = Marsh and SR = Star Ruby; C.I. = confidence interval; W = test; P = probability; ** = significant at P = 0.01)

Compari sons/site	N V M SR	Median (%) V M SR	C.I.	W	P
UEM	20 20 19	73.81 76.93 80.0			
V vs M			95.0% (-6.306, 1.301)	364.0	0.2184
V vs SR			95.2% (-15.87, -1.700)	305.0	0.0079**
M vs SR			95.2% (-11.78, 0.43)	335.5	0.0721
INIA	30 26 22	60.13 60.31 53.54			
V vs M			95.0% (-1.557, 4.863)	889.0	0.5821
V vs SR			95.1% (4.808, 12.434)	1034.0	<0.0001**
M vs SR			95.2% (3.691, 9.156)	825.0	0.0001**

Table 4.6.17 Mann-Whitney test for tannin levels (Median in % of dry weight) between sites on cultivar Valencia (I = INIA, U = UEM, M = 1° Maio; N = sample size; C.I. = confidence interval; W = test; P = probability; ** = significant at P = 0.0 1)

Comparisons/ cultivars	N I U M	Median (%) I U M	C.I.	W	P
Valencia	30 20 21	60.13 73.81 57.45			
I vs M			95.0% (1.216, 6.628)	944.0	0.0018**
I vs U			95.1% (-16.982, -8.154)	544.0	0.0000**
M vs U			95.1% (-21.205, -14.006)	231.0	<0.0001**

Table 4.6.18 Kruskal-Wallis rank test for tannin content (Median in % of dry weight) on the cultivars Marsh and Star Ruby between sites (** = significant at P = 0.0 1)

Cultivar	Site	N	Median (%)	Rank	Z	H	DF	P
Marsh	INIA	26	60.31	14.2	-5.36	28.75	1	<0.001**
Marsh	UEM	20	76.93	35.6	5.36			
Overall		46	63.79	23.5				
Star Ruby	INIA	22	53.54	11.5	-5.46	29.86	1	<0.001**
Star Ruby	UEM	19	80.00	32.0	5.46			
Overall		41	62.19	21.0				

4.7 EFFECT OF *P. demodocus* DENSITY ON ITS DEVELOPMENT

The objective of this study was to evaluate the effect of the density of larvae of *P. demodocus* on their survival, development and reproductive potential both in the laboratory and under field conditions (section 3.7).

4.7.1 Larval weight

Hypotheses

H_0: there is no difference between the weight of larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

H_1: there is a difference between larval weight for larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

Data on larval weight were collected from fifth instar larvae reared in the laboratory (section 3.7). The variable larval weight showed variance heterogeneity even after data transformation. Regression analysis showed that larval weight did not have a significant relationship with larval density Y = 2.29 + 0.0088 x; $F_{(1,3)}$= 0.47; P = 0.543, standard deviation of 0.080; r^2 = 13.5%; r^2 (adjusted) = 0.0%, (table 4.7.1).

Table 4.7.1 Mean of 5th larval instar weight per density level in the laboratory (N = number of larvae)

Density level	N	Mean (g)
2	15	2.311
4	10	2.264
6	11	2.404
8	11	2.446
10	8	2.308

4.7.2 Larval development time

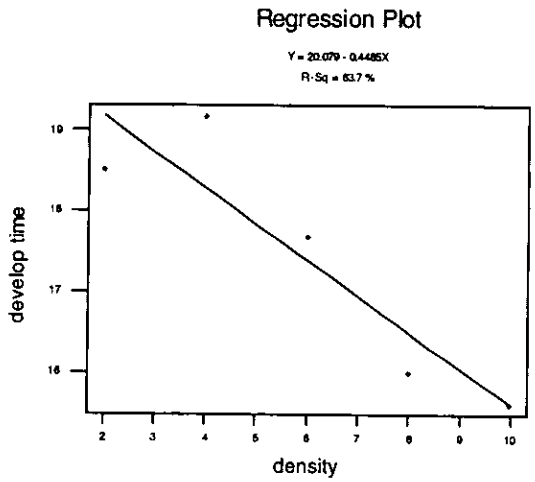
Hypotheses

H_0: larval development time is the same for larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

H_1: larval development time is different for larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

Observations on larval development time as affected by larval density were made analysing data from larvae developed at five density levels (2, 4, 6, 8 and 10 insects per treatment) both in the laboratory and in the field experiment set up for this purpose (section 3.7). The relationship between larval density on its developmental time in the field during the dry season was expressed by the equation y= 20.1-0.448 x with a standard deviation of 0.7232%; r^2 = 83.7%; r^2 (adjusted) = 78.2%; $F_{(1,3)}$ = 15.38; P = 0.029 (figure 4.7.1). In higher larval densities (10 larvae per cage), larval development time was reduced by as much as 78.2% in the field experiment.

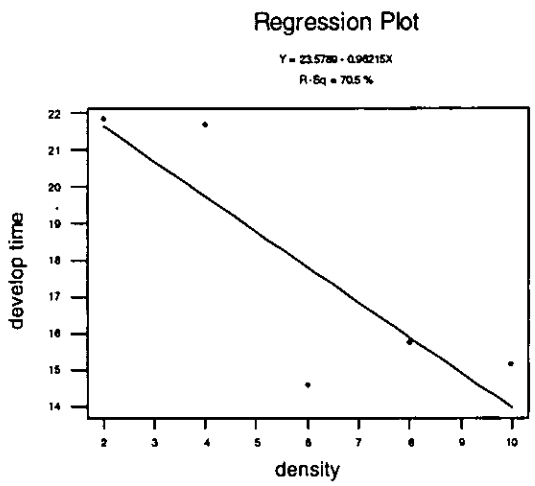
Figure 4.7.1 The relationship between larval development time and larval density in the field during the dry season (each point represents the mean of one serie of data)



This relationship is negative, which indicates that larval development time decreased as larval density increased. During the rainy season, however, this relationship was not

demonstrable. The equation describing the relationship was y = 23.6-0.962 x; standard deviation of 2.272; r^2 = 70.5%; r^2 (adjusted) = 60.7%; $F_{(1,3)}$ = 7.17; P = 0.075, significant at P < 0.10 (figure 4.7.2).

Figure 4.7.2 The relationship between larval development time (develop. time) and larval density in the field during the rainy season (each point represents the mean of one serie of data)



The models accounted for more than 70% (P<0.05 during the dry season and P<0.10 during the rainy season) of the variability in *P. demodocus* development time at different density levels.

4.7.3 Survival probability

Hypothesis

H_0: larval survival probability is the same for larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

H_1: larval survival probability is different for larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

Studies on the larval survival probability in relation to larval density levels were conducted analysing data from larvae reared in the laboratory and larvae held in field cage experiments (section 3.7). In the laboratory, the relationship between larval density and its survival was y = 99.3 - 4.33 x; s = 11.61; r^2 = 65.0%; r^2 (adjusted) = 53.4%; $F_{(1,3)}$ = 5.58; P = 0.099 (figures 4.7.3-5). In the filed, survival probability during the rainy season was not correlated with density levels, P=0.06. The equation for this relationship was y = 110-4.79 x; standard deviation = 10.29; r^2 = 74.3%; r^2 (adjusted) = 65.7%; $F_{(1,3)}$ = 8.68. During the dry season the two variables were negatively correlated with y = 91.2-2.29 x; standard deviation = 4.240; r^2 = 79.6%; r^2 (adjusted) = 72.8%; $F_{(1,3)}$ = 11.70 and P = 0.042 (figure 4.7.4). The relationship between larval density level and larval survival explained more than 65% of the variance of *P. demodocus* around the regression line. These relationships hold both for field and laboratory populations.

Figure 4.7.3 Survival probability against larval density in the laboratory (each point represents the mean of one serie of data)

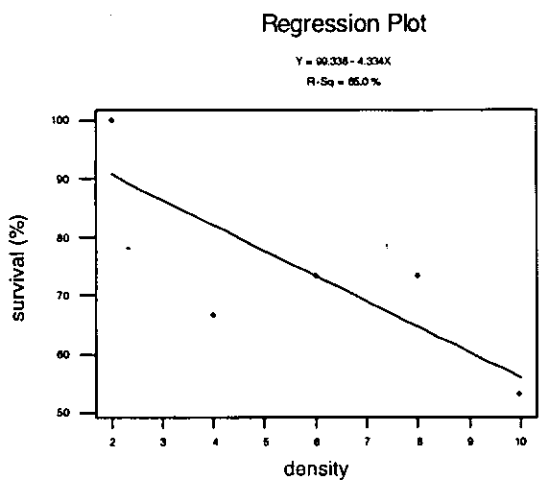


Figure 4.7.4 Survival probability of larvae against density in the field during the dry season (each point represents the mean of one serie of data)

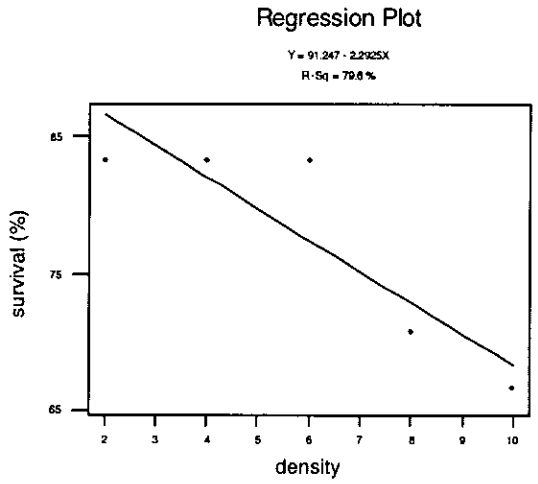
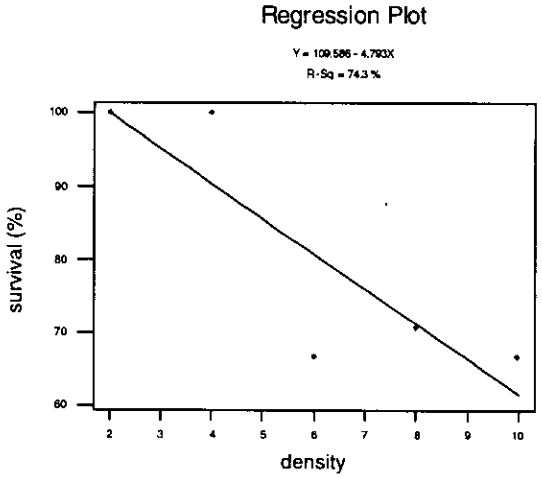


Figure 4.7.5 Survival probability versus larval density level in the field during the rainy season (each point represents the mean of one serie of data)



4.7.4 Larval headcapsule size

Hypothesis

H_0: larval headcapsule size is the same for larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

H_1: larval headcapsule size is different for larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

Larval headcapsule size data were recorded from fifth instar larvae that had developed in the field experiments and in the laboratory to assess the effect of larval density on larval headcapsule size (section 3.7). Regression analysis showed that the larval headcapsule size was not related to insect density. The relationship was not significant at 5% level with $y = 33.0 - 0.054\ x$; standard deviation = 0.130; $r^2 = 69.9\%$; r^2 (adjusted) = 59.9%; $F_{(1,3)} = 6.98$ and $P = 0.077$ but significant at $P < 0.10$. for the dry season. During the rainy season the relationship was described by the equation $y = 33.1 - 0.066\ x$; standard deviation = 0.136; $r^2 = 76.1\%$; r^2 (adjusted) = 68.1%; $F_{(1,3)} = 9.53$; $P = 0.054$.

4.7.5 Fecundity

Hypotheses

1) H_0: *P. demodocus* fecundity is the same for larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

H_1: *P. demodocus* fecundity is different for larvae reared at densities of 2, 4, 6, 8 and 10 individuals per cage

2) H_0: *P. demodocus* fecundity is the same for females at different proportions in the population

H_1: *P. demodocus* fecundity is different for females at different proportions in the population

Research on the effect of larval density and female proportion on female fecundity was carried out by observing egg production in the oviposition cages by the emerging adults from field life tables studies and by the insects reared in the laboratory (sections 3.3 and 3.7). Regression analysis of populations from dry, wet season and laboratory conditions, separately, showed that the number of eggs laid was not affected by insect density. The relationship between egg deposition and insect density level in laboratory was y = 56.7-3.01 x; r^2=73.3%, r^2 (adjusted) = 64.4%, s = 6.634, $F_{(1,3)}$ = 8.22 and P=0.064; for the population of the rainy season this relationship was described by the equation y = 45.4 - 1.44 x, r^2=9.9%, r^2(adjusted) = 0.9%, s=17.63; P=0.319.

Multiple regression analyses between insect density, female proportion in the population and egg deposition were carried out with data of all individuals in the density studies to assess the combined effects of the explanatory variables on *P. demodocus* fecundity. Female proportions varied in the populations of the dry and rainy seasons (table 4.3.11, section 4.3). Observing all populations, it was found that the regression relationship of progeny production as affected by female proportion and insect density expressed by the equation Y = 73.6 - 0.307 female proportion - 2.70 insect density with ANOVA of the regression equation detailed in tables 4.7.2 and 4.7.3. Insect density affected the number

of eggs laid (P=0.018) but female proportion did not differentialy affect egg deposition (P=0.446); s = 17.73; r^2 = 25.4% and r^2 (adjusted) = 18.3%. Effect of larval density and female proportion on egg deposition was expressed by the equation $Y_{(eggs\ laid)}$ = 73.6 – 2.7 insects – 0.307 female proportion.

Table 4.7.2 Regression details of the effect of female proportion and insect density on egg deposition (* significant at P = 0.05; ** significant at P = 0.01)

Predictor	Coefficient	Standard deviation	T	P	$F_{(2,21)}$	P
constant	73.61	21.10	3.49	0.002**		
♀%	-0.3066	0.395	-0.78	0.446		
density	-2.703	1.056	-2.56	0.018*		
ANOVA					3.58	0.046*

Table 4.7.3 ANOVA for the regression equation of egg deposition versus insect density and female proportion (*significant at P = 0.05)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Regression	2	2250.4	1125.2	3.58	0.046*
Residual error	21	6601.9	314.4		
Total	23	8852.3			

The proportion of females in the population did not affect egg laying. This means that the placing caged butterflies of the wet season in different proportions of females: males (table 4.1.1) did not affect the number of eggs laid assuming they were counted as eggs per female. Reproductive performance of these populations therefore, were similar to that of females of the dry season where caged butterflies were always kept in the same proportion (number of females: number of males per cage).

4.8 EFFECT OF NATURAL ENEMIES ON *P. demodocus* DEVELOPMENT

4.8.1 Diagnosis of diseases and pathogens of samples of field populations

In the diagnosis of diseases and pathogens of samples of field populations (section 3.8.1), no egg, larva or adult had been parasitised or diseased. From the life table studies (sections 3.3 and 4.3) only 13 pupae (12.6 %) presented signs of adult parasite emergence and these were dead. During the rainy season, 3 (9.4 %) of the pupae collected in the field were attacked and 2 (8.3 %) during the dry season were attacked by the parasite *Pteromalus* sp.

4.8.2 Effect of predators on *P. demodocus* development

The objective of the study in this section was to assess survival and development of 10 *P. demodocus* larvae on three different cultivars (Valencia, Marsh and Star Ruby) in the two seasons in order to establish the relative importance of 4 predator activity under field conditions (section 3.6 and 3.8.2). The experiment had a factorial design with 3 factors (2 seasons, 3 cultivars and 4 predator activity) all with 3 replications.

4.8.2.1 Larval headcapsule size

Hypotheses

1) H_0: there are no differences in larval headcapsule size of larvae exposed to different predator activity (no predator, one *Miomantis* sp. only, access of birds and one *Miomantis* sp and access of birds)

H_1: larval headcapsule size of larvae exposed to different predator activity are different (no predator, one *Miomantis* sp. only, access of birds and one *Miomantis* sp and access of birds)

2) H_0: there are no differences in larval headcapsule size of larvae fed on different cultivars (Valencia, Marsh and Star Ruby)

H_1: larval headcapsule size of larvae fed on different cultivars (Valencia, Marsh and Star Ruby) is different

3) H_0: there are no differences in larval headcapsule size of larvae developed in different seasons

H_1: larval headcapsule size of larvae developed in different seasons is different

General linear model ANOVA was carried out to assess any indirect effect of predator activity on larval headcapsule size of the fifth instar larvae (section 3.8.2). The influence of mantid activity on larval headcapsule size could result from an indirect effect of larval headcapsule size as larger *P. demodocus* larvae might attract their predators in a different way to the smaller larvae. This analysis showed that all treatments were significantly

different from each other regarding 5th larval instar headcapsule size and predatory activity level. Differences were found in terms of larval headcapsule size, when subjected to the four treatments (predator type) , with $F_{(3,82)} = 3.02$; P=0.034; pooled standard deviation was 0.271 (table 4.8.1). Table 4.8.1 shows that cultivar and season had no effect on larval headcapsule size.

Table 4.8.1 General Linear Model ANOVA for the effect of season, cultivar and predator type on 5th larval instar headcapsule size (* significant at 5% level of significance; ** significant at P = 0.01)

Source of variation	Degrees of Freedom	Seq. Sum of Squares	Adjusted Sum of Squares	Mean Squares	F	P
Season	1	0.3597	0.3069	0.3069	0.69	0.407
Cultivar	2	1.2140	1.7381	0.8690	1.96	0.147
Predator	3	4.0097	4.0097	1.3366	3.02	0.034*
Error	82	36.3042	36.3042	0.4427		
Total	88	41.8876				

Mean larval headcapsule size for each predator level is presented in table 4.8.2. The table shows an increase in larval headcapsule size for larvae subjected to predator level 2 (one *Miomantis* sp. in the cage).

The result of the T test showed that cage type (closed cages and open cages to allow access of birds) did not affect the variable responses of the experiment, P = 0.64 with t = -0.46 and 81 degrees of freedom for the larvae headcapsule size.

Table 4.8.2 Mean and standard error of 5th larval instar headcapsule size per predator type (S. E.= standard error)

Predator type	N	Mean (mm x 10^{-1})	S.E.
No predator (0)	40	32.550	0.124
1 mantid (1)	12	32.917	0.083
Birds and 1 mantid (2)	8	32.250	0.313
Birds (3)	29	32.828	0.100

Fifth instar larvae subjected to one mantid per treatment showed an increased headcapsule size when compared to treatment 2 (one mantid specimen and exposure to birds), at 6% level of significance (table 4.8.3), with a mean of 32.250 x 10^{-1} mm while the larvae subjected to one mantid specimen had a mean of 32.917 x 10^{-1} mm.

Table 4.8.3 Comparisons between means of 5th larval instar headcapsule size per predator treatment

Treatments	Difference of means	Standard error of difference	T-value	Adjusted P-value
0 and 1	0.4283	0.222	1.931	0.2234
0 and 2	-0.3685	0.263	-1.402	0.5015
0 and 3	0.2531	0.163	1.554	0.4106
1 and 2	-0.7968	0.313	-2.549	0.0598
1 and 3	-0.1752	0.233	-0.753	0.8749
2 and 3	0.6216	0.269	2.315	0.1030

Larvae that were not consumed by mantids had larger headcapsule sizes compared to larvae subjected to birds or both birds and mantids or even larvae not exposed to predators.

Under field conditions, differences were not found in terms of larval headcapsule size $F_{(2,82)} = 1.96$; $P=0.147$ nor for larval longevity $F_{(2,233)} = 1.01$; $P=0.367$ (tables 4.8.1 and 4.8.5) per cultivar. Mean values of 5th instar larval headcapsule size and longevity per cultivar are given in table 4.8.4.

Table 4.8.4 Mean values for 5th larval instar headcapsule size and larval longevity in the field experiment (N = number of insects)

Cultivar	N	Headcapsule size (10^{-1} mm) mean	development time (days) N (80)
Valencia	30	32.633	11.700
Marsh	27	32.519	10.712
Star Ruby	32	32.813	12.412
All cultivars	89	32.663	11.608

4.8.2.2 Larval longevity of original populations

Hypotheses

1) H_0: there are no differences in larval longevity of larvae exposed to different predator activity

H_1: larval longevity of larvae exposed to different predator activity are different

2) H_0: there are nó differences in larval longevity of larvae fed on different cultivars (Valencia, Marsh and Star Ruby)

H_1: larval longevity of larvae fed on different cultivars (Valencia, Marsh and Star Ruby) is different

3) H_0: there are no differences in larval longevity of larvae developed in different seasons

H_1: larval longevity of larvae developed in different seasons is different

General linear model ANOVA showed that differences were found for larval longevity when subjected to the four predatory levels, with $F_{(3, 233)} = 11.82$; $P<0.001$. Pooled standard deviation was 0.628 (table 4.8.5), indicating an acceptable experiment variance. Cultivars did not differentially affect larval longevity, $P > 0.05$ (table 4.8.5). Season differentially affected larval longevity. Comparing the mean longevity for each season using the Tukey test showed that the difference of means of the two seasons was -2.050; the standard error of the difference was 0.986; T-value = -2.079 and P = 0.0387 (tables 4.8.6).

Table 4.8.5 General Linear Model ANOVA for *P. demodocus* longevity for each season, cultivar and predator type (** significant at 1% level of significance; ** = significant at P = 0.0 1)

Source of variation	Degrees of Freedom	Seq. Sum of Squares	Adjusted Sum of Squares	Mean Squares	F	P
Season	1	252.15	252.15	252.15	4.32	0.039*
Cultivar	2	116.61	116.61	58.30	1.00	0.370
Predator	3	2053.08	2053.08	684.36	11.73	0.000**
Season x predator	3	78.62	78.62	26.21	0.45	0.718
Error	230	13416.72	13416.72	58.33		
Total	239	15917.18				

Larval longevity of the original population was significantly higher at the treatment with no predator and exposure only to birds compared to the treatments with one mantid specimen and one mantid and exposure to birds (table 4.8.6 and 4.8.7). Although birds have been reported to be predators of *P. demodocus* adults (Hepburn & Bishop, 1954), there was no evidence to make this conclusion in this study. The importance of birds in reducing larval numbers remains unclear. The reduction in larval longevity amounted to 38.67% for larvae subjected to one mantid in the cage compared to the control treatment with no predatory activity.

The result of the T test showed that the two types of cages (open cages to allow access of birds and closed cages) did not affect experiment results for larval longevity, P = 0.13; t = 1.52; 236 degrees of freedom.

Table 4.8.6 Mean and standard error (S.E.) of larval longevity (N = number of larvae; treatment 0 = no predator; 1 = 1 mantid; 2 = birds and 1 mantid; 3 = birds; data summarised in table 4.8.6)

Predator type	N	Mean Longevity (days)	S.E.	Median
No predator (0)	60	15.38	1.090	20.00
1 mantid (1)	60	9.43	0.959	7.00
Birds and 1 mantid (2)	60	8.17	0.886	6.00
Birds (3)	60	13.45	1.010	16.00

Table 4.8.7 Tukey Simultaneous Tests for comparisons between means of larval longevity subjected to different predator treatments (* significant at P = 0.05; ** significant at P = 0.01; predator treatment as above)

Treatments	Difference of means	Standard error of difference	T-value	Adjusted P-value
0 and 1	-5.950	1.394	-4.267	0.0002**
0 and 2	-7.217	1.394	-5.175	<0.0001**
0 and 3	-1.933	1.394	-1.386	0.5091
1 and 2	-1.267	1.394	-0.9084	0.8004
1 and 3	4.017	1.394	2.8805	0.0224*
2 and 3	5.283	1.394	3.789	0.0011**

4.8.2.3 Survival probability

Hypotheses

1) H_0: there are no differences in larval survival probability of larvae exposed to different predator activity

H_1: larval survival probability of larvae exposed to different predator activity is different

2) H_0: there are no differences in larval survival probability of larvae fed on different cultivars

H_1: larval survival probability between larvae fed on different cultivars is different

3) H_0: there are no differences in larval survival probability of larvae developed in different seasons

H_1: larval survival probability of larvae developed in different seasons is different

Survival probability was estimated as the proportion of individuals that reached the adult stage in relation to the initial number of insects subjected to the same treatment. Raw data were subjected to the square root transformation due to variance heterogeneity. Tables 4.8.8, 4.8.9 provide the analysis of variance, mean and standard error of mean for survival probability per predator type and season (table 4.8.10). There were no significant differences in survival probability between cultivars and seasons.

The reduced mortality of *P. demodocus* larvae not exposed to predators is marked and the magnitude of this reduction was 93.12%; 90.87% and 63.97% less than that of the

larvae on cages with treatments 1 (one mantid), 2 (one mantid and access to birds) and 3 (access to birds), respectively (table 4.8.9). Neither season or cultivar differentially affected *P. demodocus* survival probability $P > 0.05$ (table 4.8.8).

Table 4.8.8 General Linear Model ANOVA for *P. demodocus* 5th larval instar survival probability per season, cultivar and predator type (** significant at P = 0.01)

Source of variation	Degrees of Freedom	Seq. Sum f Squares	Adjusted Sum of Squares	Mean Squares	F	P
Season	1	8.84	8.84	8.84	2.01	0.159
Cultivar	2	6.50	6.50	3.25	0.74	0.481
Predator	3	1225.02	1225.02	408.34	92.65	<0.001**
Error	113	498.00	498.00	4.41		
Total	119	1738.36				

Table 4.8.9 *Papilio demodocus* survival probability mean and standard error (S.E.) per predator type (N = number of larvae)

Predator	N	Mean (%)	S.Error
No predator	30	85.56	2.82
1 mantid	30	5.89	1.75
Birds and 1 mantid	30	7.81	2.42
Birds	30	30.83	3.55

Table 4.8.10 Mean and standard error (S.E.) for 5th larval instar headcapsule size, larval longevity and survival probability per season (N = number of larvae)

Season	Headcapsule size			larval longevity				Survival probability		
	N	Mean ($mmx10^{-1}$)	S.E.	N	Mean (days)	S.E	Median	N	Mean (%)	S.E.
Dry	48	32.604	0.106	120	12.633	0.722	13.00	60	34.91	4.77
Rainy	41	32.732	0.099	120	10.583	0.759	8.00	60	30.13	4.40
The 2 seasons	89	32.663	0.073	240	11.608	0.527	11.00	120	32.52	3.24

The null hypotheses stating that the probability of larval survival, longevity and headcapsule size was the same for larvae exposed to different predatory levels were rejected as differences were found for larval survival, headcapsule size and larval longevity when subjected to different predatory levels.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

P. demodocus biology and life tables

Eggs of *P. demodocus* were usually laid on the underside of the leaves. Tiritilli & Thompson (1988) explain the consistent oviposition behaviour of *Papilio zelicaon* Lucas (Lepidoptera: Papilionidae) on the undersides of leaves by ovipositing females as being due to the relative ease of ovipositing on the undersides because they are usually larger than the host plant leaves and the extension of the abdomen easily enables egg deposition. They pointed out that the undersides of leaves offer protection against wind and rain which dislodge eggs particularly in coastal habitats. Desiccation could be another explanation for this phenomenon as humidity is higher on lower leaf surfaces (Vesala, 1998).

Egg desiccation during the rainy season (October to March) affected 44.1% of total egg production and 35.9% in the dry season (April to September) (tables 4.3.6 and 4.3.7). Besides the higher mean relative humidity observed during the rainy season compared with the dry season (appendix 3.1), more eggs were killed by desiccation than during the dry season (tables 4.3.6 and 4.3.7). This is probably explained by the short incubation

period, with a median of five days (table 4.3.1). Consequently, even if eggs are exposed to high temperature and low humidity levels for only a short period of time they may still be affected by desiccation.

Higher temperature levels were observed during the rainy season (appendix 3.1). During the months of the rainy season, mean daily temperatures reached 26.3°C while during the dry season mean daily temperatures were 19.3°C. Values of relative humidity averaged between 53% during the dry and 75% during the rainy season, respectively.

The *P. demodocus* survivorship curve combined characteristics of the type III (high mortality rates at early stages) and Type I (low mortality rates at late developmental stages) on a cubic regression model, during the life cycle. This has been recorded for other insect species such as *Oncopeltus fasciatus* Dallas (Heteroptera: Lygaeidae) (Price, 1984). The heavy mortality of *P. demodocus* at the early stages confirms Price's (1984) findings that most free living and exposed insects exibit such a mortality curve. In general, the curve fell into Price's (1984) type A category of larval survivorship where mortality reaches 70% or more by mid larval stages. Differences in survivorship curve shapes were observed between the seasons (table 4.3.8 and 4.3.9). This agrees with Watanabe (1976) for *Papilio xuthus* and Tiritilli & Thompson (1988) for *Papilio zelicaon*. Hayes (1981, 1985) suggested that fluctuations in butterfly populations could be explained by focusing on the factors that affect natality in cases where larval and pupal survivorship is relatively constant.

The net reproductive rate R_0 and the capacity for increase r_c of the generations from the rainy season indicated that the population was decreasing while there was an increase in the dry season (table 4.3.10). This fits in with the model of the population changes of this insect during the year as shown by population changes as affected by the initial population size (table 4.4.5) and the population growth curves using the Leslie Matrix

(figures 4.4.6 and 4.4.7). The models predicted a reduction in population size at the end of the rainy season.

Brink & Steyn (1993) noted that following dry conditions in 1992, the *P. demodocus* outbreak in 1992/1993 in South Africa may have been caused by drought related physiological stress followed by heavy rain and increased flushing of the citrus trees. They associated *P. demodocus* outbreaks with drought conditions in the dry season followed by high rainfall in the rainy season. However, some *P. demodocus* changes in abundance resulting from marked generation to generation variability in population growth rates (table 4.3.10 and 4.6.3) could be attributed to variation in the natality or even the fecundity rate. Fecundity was correlated with the season during the year (table 4.7.2). Adult life span did not differ between the two seasons (section 4.3.1), so, longevity may not have contributed to increased fecundity of the dry season females. Density-dependent processes between generations may have been responsible for the reduction of *P. demodocus* population levels in the dry season generations, the reproductive rate increase towards the end of the dry conditions and the beginning of the rainy season with favourable conditions being re-established (table 4.3.10 and 4.6.3). Williams & Liebhold (1995a) observed that climatic changes affected the extent of outbreaks of the lepidopterous insects *L. dispar* and *C. occidentalis* in Oregon and Pensylvannia, United States, since defoliated areas decreased when temperatures increased. However, they did not find evidence to support the Climatic Release Theory (Price, 1984; Pedigo, 1997) which postulates that outbreaks are triggered by climatic factors favourable for population growth. By contrast, Williams & Liebhold (1995b) observed that for *L. dispar*, in New England, United States, there was evidence that local population oscillations resulted from density-dependent mechanisms operating on the insect population, which may be synchronized over wide areas exposed to similar weather patterns.

The population fluctuations of *Colias alexandra* Edwards (Lepidoptera: Pieridae) between years was related to reduced natality where the population numbers were reduced when adult females failed to achieve their potential fecundity (Hayes, 1981). For that population, adult removal (by predation, incidental losses, etc) seemed to be an unimportant factor and, consequently, potential fecundity reduction may have been important through oviposition interference e.g. male interference and competition for oviposition sites.

In this study, the ways in which different components of mortality interacted were identified as follows:

- graphical key-factor analysis indicated larval disappearence as the key-factor for *P. demodocus* (figure 4.3.1). The mortality factor that was most highly correlated with generation change was that which occurred during the second, third and fourth larval stages (figure 4.4.1 and table 4.4.2).
- life table analysis showed that the main mortality factors are unrelated to population density (table 4.4.2 and appendix 4.6). There was no association between the key-factor on its population density as the regression analysis showed no significant relationship between larval disappearance and its population density ($P>0.05$). kx values plotted against larval density of the succeeding generation suggested that the mortality factor and larval density variables were also unrelated.
- spatial density-dependent processes accounted for only up to 50.7% of variation in population size for the second larval instar ($P = 0.068$), 3.1% in the third instar and 0% in the fourth instar larval population variation of *P. demodocus* population size ($P>0.05$). In this way, the key-factor determining the larval population change was contributing mostly to the total generation mortality but the key-factor (larval disappearance) did not determine the population density. The lack of evidence for density dependent processes within generations, however, does not necessarily imply

that the processes do not exist (Dempster & Pollard, 1985). The limited number of generations assessed during the life tables studies (section 4.3) may have contributted to the difficulties with detecting spatial density related processes. Hassell (1986, 1987) pointed out that density dependent processes have proved difficult to detect when sampling size is small.

The vulnerable stages of *P. demodocus* are, in conclusion, the second, third and fourth larval instars because the mortality that occurred in these developmental stages contributed most to the total mortality in the generations (table 4.4.2).

Varley & Gradwell (1960) and Varley *et al.* (1973) observed that winter disappearance was also the key factor for the winter moth *Operophtera brumata* (L.) (Lepidoptera: Geometridae) measured by the difference between larval density on a log scale prior to overwintering and during the following winter.

Population distribution

Papilio demodocus larvae were to be found on citrus plantations only. As the Umbelúzi region is a unique *Citrus* growing area in the southern Maputo Province, it was anticipated that this pest would be restricted to the area such that movement from other sites would not contribute to population size fluctuations.

Papilio demodocus poses a serious threat for young trees not only due to their vulnerability after defoliation but also young orchards seem to be the most vulnerable to *P. demodocus* attack (table 4.2.1 and appendices 4.1 and 4.2). These observations confirm those reported by Honiball & Bedford (1978), Olmi (1985), Annecke & Moran

(1982), and Netterville (1994). Annecke & Moran (1982) suggested that a young leaf possess less developed oil cells which could make them more attractive to *P. demodocus* larvae.

The numbers of *P. demodocus* immature stages other than eggs were too low to estimate meaningful distributions. This may be partly explained by the pesticide treatment of orchards in the LOMACO sites. The biopesticide *Bacillus thuringiensis* is specific to larval stages and consequently, these were the most affected, thus contributing also to the low pupal numbers. Few alternative sites were available and the owners were not willing to stop *B. thuringiensis* applications during the field studies.

The mean density for *P. demodocus* of approximately 0.22 eggs per plant suggests that if the population distribution was regular, then around one individual egg would be observed for every five plants. Consequently, one in five plants could be defoliated or even lost (personal observation) due to *P. demodocus* activity in the Umbelúzi region. Egg mean density during the preliminary survey (table 4.2.1) was less than 0.22 eggs per plant because orchards >2 years old were included in the sampling. The mean density of 0.22 eggs per plant and the population parameters estimated in this study are valid for orchards receiving chemical treatments. Pesticide free fields would be expected to have a much higher mean density and consequently more damage. Field cohort life table studies (tables 4.3.6 and 4.3.7) free from pesticides registered a mean of 1 egg per plant.

The value of $p = 0.43$ from the power law relating variance to mean in population distribution studies (section 2.2.3) indicates that variance on *P. demodocus* counts can be stabilized by data transformation using the tables developed by Healy & Taylor (1962) when carrying out analysis of variance of insect counts.

The results of Taylors' Power Law, Lloyd's Mean Crowding and the negative binomial probability model indicated an aggregated spatial distribution of *P. demodocus* populations. Therefore a sequential sampling plan could be developed based on any of these three models.

Papilio demodocus populations showed, however, a slight aggregated distribution (figure 4.2.2 and table 4.2.2). Natural populations on plants were very loosely aggregated, being widely dispersed throughout the stands. The mean/variance ratio (coefficient of variation) approached 0, confirming that the individuals in this population distribution were slightly aggregated (table 4.2.2). The pest lays eggs singly, although at times the butterfly may come back to a visited plant to lay additional eggs (Howard, 1906) which explains the slight aggregation. In addition plants in close vicinity of a butterfly laying eggs would have more chance of being chosen. The low level of aggregation in the field may also be caused by small microclimatic variations in the habitat. The insect itself demonstrated no preferences for specific citrus cultivars or variation in soil quality and natural enemies (table 4.3.8). *Papilio demodocus* behaviour itself does not appear to be actively aggregational. Variation in population aggregation for the developmental stages were dismissed. Harcourt (1963) observed similar distribution patterns of egg and first instar larval stages for *Leptinotarsa decemlineata* outbreaks in which the females lay eggs singly.

In this study, *P. demodocus* showed different values of Taylor's Power Law coefficient b during the dry and rainy season (table 4.2.2). Changes in the values of the coefficients *a* and *b* using the Taylor's Power Law under particular conditions have also been observed in other insect populations, e.g. the insects *Rhyacionia frustana* (Comst.) (Lepidoptera: Tortricidae) and *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) (Waters, 1959). Changes in the values of *b* can be attributed to different behaviour related to the pest density (Southwood, 1978; Taylor *et al.*, 1998) probably partly explained by

intraspecific competition in which, the generations of the dry season, characterized by a random distribution pattern and with low density levels where individuals do not affect the presence of each other would turn, in the rainy season, to a more aggregated population. Table 4.2.2 indicated that a higher percentage of variance around the regression line was explained by a higher variance around the mean density in the rainy season than during the dry season. The difference of the slope *b* values during the seasons for *P. demodocus* may also have occurred because the number of samples was higher during the rainy season, and consequently more adequate than data of the dry season for this calculation (Abrahamsen & Strand, 1970; Taylor *et al.*, 1998). In this case, the difference may not have any biological significance.

The negative binomial model for *P. demodocus* population distribution, with a dispersion parameter k value of 0.09, which is close to the logarithm series distribution, suggests a strongly aggregated distribution. There was, therefore, an apparent contradiction between the negative binomial model and other models describing the population distribution as a slightly aggregated pattern, such as Taylor's Power Law and the Lloyd's Mean Crowding regression. The maximum likelihood estimator of the dispersion parameter produced skewed equations, denoting that k estimation was in excess (Southwood, 1978). Consequently, the efficiency of k estimation could not be determined. The moment method to estimate the parameter k was considered to be an approximate one and inadequate for populations with k less than 3 (Southwood, 1978) which was the case with *P. demodocus*. The distribution parameter k varied in relation to mean density thus indicating that a common k was absent.

In summary, Taylor's Power Law described *P. demodocus* distribution more realistically than the negative binomial probability model. The inadequacy of the binomial distribution model may also have been related to the chosen plot size (0.09 ha), which may have not been the most appropriate. Waters (1959) and Harcourt (1963) recommended sampling

units as small as a plant leaf for studies with forest insect species. It is possible, therefore, that better fits of the model are obtained using different sampling unit sizes. Low *P. demodocus* population sizes could not be quantified owing to the large number of samples (table 4.2.1). Better fits of the Taylor's Power Law than other dispersion indices have also been observed in several studies, e.g. for *Anisota senatoria* (J.E. Smith) (Lepidoptera: Saturnidae) by Coffelt & Schultz (1994); Ali *et al.* (1998) and Taylor *et al.* (1998).

Population dynamics

Population densities were greater during the rainy season (figures 4.4.6 and 4.4.7). Towards the dry season, population numbers fell (tables 4.4.5 and 4.4.7). Egg population density declined from March to July and then began to increase from July until December (figure 4.2.7). The changes in average insect density per plant were associated with the time of the year (figure 4.2.7). The establishment that *P. demodocus* peak period of activity occurred during the rainy season agrees with those of Williams (1969) and Annecke & Moran (1982) in the warmer northern regions of South Africa. This may be partly due to differences in the reproductive rates as stated above. The dynamics of *P. demodocus* in Maputo is thus understood to be primarily determined by the degree to which the population of a given generation responds to intraspecific interference, i.e., to the initial population size. Although predator level had a significant effect on *P. demodocus* larval survival (table 4.8.7), natural enemy presence and activity under uncaged field conditions was negligible and consequently, predators were not included in the population dynamics model. Temperature (figure 4.2.6 and appendix 4.7) was not significantly correlated with insect mean density and was ommitted from the population dynamics model.

The population dynamics matrix (figures 4.4.6 and 4.4.7) indicated that in the generations following the rainy season, the egg and larval developmental stages of the population decreased as conditions became dryer. The later developmental stages (pupae and adults) maintained their numbers. These figures may not totally immitate what happens in reality as the mean of offspring laid by females of the dry season refer only to the females kept in pairs in the oviposition cages. Larger numbers of females than males (table 4.1.1) had to be released to keep the proportion of females and males constant throughout the experiment. Another feature that may explain differences between the curve describing *P. demodocus* population growth in different sites is that although natural conditions were immitated as much as possible in the oviposition cages, caged individuals may not behave in the same way as those on the wild, concerning reproductive biology, as pointed out by (Blossey & Hunt, 1999) for other insect species such as *Galerucella calmariensis* L. and *G. pusilla* Duftschmidt (Coleoptera: Chrysomelidae). In their studies, field rearing produced more offspring than greenhouse rearings.

The Leslie matrix model prediction (figures 4.4.6 and 4.4.7) for 1° Maio and other sites was different. A disparity between values of the three curves is seen in the rainy season generations for the egg stage (figure 4.4.6). Discrepancies between values of the three growth curves are more pronounced at four (end of the rainy season) out of 18 comparisons. This difference can also be accredited to difficulties in assessing the effect of the proximity of sprayed orchards at the LOMACO study plot, perhaps leading females to choose to oviposit at this unsprayed plot. Consequently, the values of eggs laid may have been overestimated. Survival curves proved similar in life table studies between the three sites. Consequently, the existence of differences in *P. demodocus* population dynamics at the three sites are not suspected.

The study on population dynamics was an intensive one, in localized areas. It did not however, provide information on detailed mechanisms of *P. demodocus* population dynamics. This can be explained as study areas (0.25 ha/plot) seemed to have been too small for adequate extensive work on the population dynamics encompassing simultaneous studies over a large portion of the population activity range in order to assign the fluctuations in *P. demodocus* populations. This was unavoidable owing to the need for a large number of man-hours to achieve the necessary sample intensity.

The period of study was also relatively short. *Papilio demodocus* is a widespread species, consequently, the conclusions made on the basis of the population in this study area alone must not be generalized. Consequently, the effect of season was not accurately represented by the Leslie matrix model but some generalizations at the level of basic trends in *P. demodocus* abundance might be carried out in the future as the model highlighted areas for developing information on *P. demodocus* population dynamics in the study area.

Intrinsic density-dependent processes have been shown to occur in *P. demodocus* (table 4.4.5 and figures 4.4.3 and 4.4.4) and can be regarded as being significant in determining population numbers under field conditions. These processes probably include reproductive arrest by male interference on female fecundity.

Although significant only to changes in the populations of the dry season and changes of population size between the two seasons, the predictive values of log (N_t) (figures 4.4.3 and 4.4.4) in the model were high since a large proportion of the variance in the rate of population change could be explained by population levels of the previous generation. 65.7% and 84.7 % of variation in population size in the dry season and between rainy and dry season are accounted for by population changes in previous generations (table 4.4.5). Regulation by density-dependent processes in *P. demodocus* suggested that the

population could be regarded as being in a stable equilibrium, due to the total population net reproductive rate R_0 of 1.081 and capacity for increase r_c of -0.0176 (table 4.3.10). A population reaches a stable equilibrium if no further disturbances are added to the system where the insect populations exist (Room *et al.* 1991). However, the use of pesticides, common in most citrus orchards, may interfere with the stable system, with density levels and, thus, lead to higher reproductive rates (table 4.6.3); therefore, permanent outbreaks may result in treated orchards. The insect status may then, be affected. AliNiazee, (1983) observed an excessive population build-up of *Melissopus latiferreanus* (Wals.) (Lepidoptera: Olethreutidae) resulting from pesticides use in the Willamette Valley of Oregon, United States.

Summarising, these studies revealed that egg population size through intraspecific interference is an important factor affecting the population dynamics of *P. demodocus* in the Maputo region.

Adult *P. demodocus* movements

The data on mark and recapture studies suggest that the adult population in the study areas was low and migration based on the data (table 4.4.1) would not provide meaningful conclusions.

Although not adequately measured due to high adult mobility, migration appeared to be so negligible (table 4.4.1) that emigration or immigration could be regarded as of little importance as determinants of numerical changes in populations of *P. demodocus*.

Papilio demodocus belongs to the group of butterfly species that do not migrate from their original habitat. Feltwell (1986) observed that some butterfly species exibit a territorial behaviour and remain confined to their place of origin, particularly species in the genus *Papilio*.

Several authors have indicated that migration in insects could be due to a reaction to unsuitable environmental conditions. Kennedy (1961) on aphids and locusts, Southwood (1962) and Dempster (1968) on *Anthocoris sarothamni* D. & S. (Coleoptera: Anthocoridae) both concluded that in most cases migration is density-induced. Clark *et al.*, (1967) observed that *Perga affinis* Kirby (Hymenoptera: Pergidae) migrates following food depletion as did the spruce budworm *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae) (Huffaker & Messenger, 1964). Adverse environments in relation to scarcity of food, resting places, space and other resources are unrelated in the case of *P. demodocus* in Maputo thus minimising the necessity for migration.

Abiotic factors

Meteorological factors such as air temperature, air relative humidity and wind speed did not have significant effects on *P. demodocus* numbers (appendix 4.7), probably because the range of variation was limited on the different sampling occasions within the area.

Meteorological factors had little importance on insect pest abundance data (appendix 4.7); this was observed in life tables findings by plotting kx values against environmental factors (appendix 4.7); it was observed again in the studies of temperature-insect pest development relationships (table 4.5.5 and figures 4.5.3 and 4.5.4) and in the distribution studies of insect density in the field plotted against current meteorological data (appendix

4.7). However, some observations indicated that temperature reduced larval developmental periods in the laboratory and also from the indirect effect of temperature from *P. demodocus* egg population size variations related to mean monthly meteorological conditions (figure 4.2.7). At 35°C a higher proportion of insects failed to reach full maturity. Development was most rapid at 30° C, followed by the temperatures of 20° and 25 °C (tables 4.5.1, 4.5.2, 4.5.3 and figure 4.5.1). Best indices of development rate were reached at 60 and 70% of relative humidity, reducing to the minimum development rate indice at 90% (figure 4.5.2). Briere & Pracros (1998) found similar patterns of survival, development time and egg viability for the moth *Lobesia botrana* Dennis & Schiffermuller (Lepidoptera: Tortricidae).

The effect of temperature and relative humidity on insect population characteristics such as egg viability, larval headcapsule size and egg production was not significant under laboratory conditions (table 4.5.5). Santos & Shields (1998) noted that temperature in ranges between 18 and 30°C did not affect larval development rates, headcapsule size and weight in *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae).

Apparently small differences in degree-days between the rainy and dry season in the field (appendix 3.1) are, probably, responsible for the uniformity in larval survival probability, headcapsule size, adult life span and fecundity for *P. demodocus* in the study area. The two seasons appeared to be at a similar thermal regime and *P. demodocus* development could continue throughout the year (appendix 3.1). In this region, pupal diapause did not occur, probably due to a narrow range of temperatures and day length. Howard (1906), Hepburn & Bishop (1954) and Annecke & Moran (1982) indicated that in southern regions of South Africa diapause could occur in pupae, probably after the larvae experience relatively cooler temperatures. Ford (1968) noted for tse-tse flies (*Glossina morsitans* Westwood (Diptera: Glossinidae), that climate and weather determined optimal zones for their activity. Similar effects may act on *P. demodocus* and may

explain the differences found between its occurrences in South Africa and in the Maputo region. Van Son (1949), Hepburn & Bishop (1954), Williams (1969) and Honiball & Bedford (1978) noted that in southern and cooler regions of South Africa *P. demodocus* activity is confined to the rainy season while in warmer parts of South Africa and northern regions of the continent, including Maputo, the insect is active throughout the year. Ford (1968) observed that climate and weather determined optimal zones for *G. morsitans* activity and *P. demodocus* is more active in the warmer regions of southern Africa compared to southern South Africa where temperatures can drop to freezing levels.

The lack of a diapause by *P. demodocus* in the Maputo region is explained by the slight changes of the climatic factors during the course of the year (appendix 3.1). The results of *P. demodocus* growth agree with findings by Danilevskii (1965) who reported that, depending on the insect species, the upper limit for insect survival is situated around 30° C and 35° C with a lower limit of 5° C to 10° C. The range of the biologically most suitable temperature lies between 25° C to 30° C. Beck (1986) researching the moth *Agrotis ipsilon*, Mays & Kok (1997) researching *Evergestis rimosalis* (Guenee) (Lepidoptera: Pyralidae) and Fantinou *et al.* (1998) studying *Sesamia nonagrioides* (Levebvre) (Lepidoptera: Noctuidae) concluded that day length was not involved in diapause. A similar phenomenon may occur in the case of *P. demodocus.*

Effect of diet

Papilio demodocus developed a strong oligophagy to citrus plants in the region of study Howard (1906) in the Transvaal region (Mpumalanga) of South Africa where it is found only on citrus orchards and never seen feeding on natural vegetation.

The experiments conducted in the laboratory (section 4.6.1 and 4.6.2) showed that diet of several cultivars did not affect *P. demodocus* 5th larval instar weight (table 4.6.1), larval nutritional indices (table 4.6.7) or egg deposition (table 4.6.3). Therefore, the insect could be regarded as a pest of equal importance for all of the cultivars included in this study.

Although food is regarded as the most obvious essential requisite for most insect species and could seem to have a controlling influence on insect numbers (McNeill & Southwood, 1973), the data obtained from this study suggested that this possibility was not relevant for *P. demodocus* in the Maputo region since even when larval food of different cultivars was available in large amounts (i.e. they were not nutritionally stressed), in separate experiments, differences on *P. demodocus* survival (table 4.3.8), larval nutritional indices (table 4.6.1) and population performance (table 4.6.3) were not demonstrable. In the cage experiment assessing the effect of diet and season on *P. demodocus* performance, neither citrus cultivar nor season influenced larval headcapsule size (table 4.8.1). Larval longevity varied between seasons but not between cultivars and sites (table 4.8.5) and 5th instar larval survival was not affected by cultivar or season (table 4.8.8).

Leaf nutrient analysis showed highly significant differences between cultivars and sites on citrus leaf nitrogen content (tables 4.6.11-4.6.14). Leaf analysis also confirmed that differences in soluble tannins were significant between cultivars and study sites (tables 4.6.15-4.6.19). Nitrogen and tannin contents did not significantly vary between seasons (tables 4.6.9 and 4.6.10). Cultivar Valencia (table 4.6.12) and site 1° Maio had higher levels of nitrogen (table 4.6.13) whereas the site UEM (table 4.6.15) and cultivar Star Ruby (table 4.6.16) registered higher levels of soluble tannins. The differences in nitrogen and tannin levels between cultivars and sites lead to the conclusion that *P.*

demodocus is not affected by nitrogen and tannin contents on plant leaves within the range observed in this study.

In the life table studies (survival curves parameters, table 4.3.8) and laboratory experiments (larval weight (table 4.6.1) and nutritional indices (table 4.6.7)) it was found that *P. demodocus* behaviour was similar in all the sites and for the three cultivars, even at different nitrogen content levels. Survival curves only varied between seasons (table 4.3.8). *Papilio demodocus* did not show any preferences in food requirements from among the three cultivars Valencia, Marsh and Star Ruby, feeding equally on any of the cultivars (tables 4.6.1, 4.6.3 and 4.6.7). Nitrogen has a major influence on the population dynamics of many herbivorous invertebrates (Hosking *et al.* 1990). White (1984) and Hain (1987) indicated that the highest concentrations of nitrogen, phosphorous and potassium occur in young rapidly expanding leaves and a decline has been observed with leaf age. McNeill & Southwood (1973) observed that nitrogen was a limiting factor in the diet of *Leptopterna* sp. (Heteroptera: Miridae). In their studies, nitrogen influenced fecundity and the choice for feeding sites. Larval weight and adult fecundity of *O. brumata* was also positively affected by increased nitrogen levels (Feeny & Bostock, 1968). Watt (1990), pointed out that although food sources rich in nitrogen result in better larval growth and survival in insects such as *Panolis flammea* (D. & S.) (Lepidoptera: Noctuidae) in Scotland, it must not be assumed that differences in plant nutritive condition and insect performance will necessarily result in differences in insect abundance.

It has been observed that although increased concentrations of soluble nitrogen in trees under stress may positively affect defoliatiors (White, 1984), secondary compounds often increase in stressed trees (Mattson & Haack, 1987) so that this negative effect acts in an antagonisitic way (Heliovaara & Vaisanen, 1990). In the end, plant chemistry may exert both a positive and negative influence on *P. demodocus*.

The low sensitivity of *P. demodocus* to different levels of tannins between cultivars and sites can be a consequence of evolved mechanisms to produce much of the microsomal mixed function oxidases in the midguts (Krieger *et al.*, 1971; Price, 1984) in order to be able to feed on the plant chemicals found in most plant species of the family Rutaceae, Umbelliferae and related families. Even at significantly different tannin levels in the diet, *P. demodocus* survival remained similar (table 4.3.8). Lepidopterous larvae contain enzymes in the midgut tissues (Krieger *et al.* 1971) which are microsomal mixed function oxidases that detoxify toxic compounds, catalyze several reactions and are often involved in the development of insecticide resistance in insect populations (Price, 1984). These enzymes promote the detoxification of toxic products. Plants with phenolic compounds such as tannins can be toxic to non specific insects but may be unable to deter insects with high microsomal oxidase production (Price, 1984). Neal & Berenbaum (1989) observed that *Papilio polyxenes* Fabr. (Lepidoptera: Papilionidae) evolved mixed function oxidases 10 and 46 times less sensitive to inhibition by myristicin and safrole (which are inhibitors of the mixed function oxidases) respectively than *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). Products such as safrole, myristicin and sesamin are inhibitors of mixed function oxidases to some insects but some others are less sensitive (Price, 1984; Neal & Berenbaum, 1989). Neal & Berenbaum (1989) found that higher mixed function oxidase activity and decreased sensitivity to mixed function oxidase inhibitors are important adaptations that allow the *P. polyxenes* larvae to feed on many umbelliferous plants that contain the toxic compounds furanocoumarins and methyleneidioxyphenyl such as myristicin and safrole. Feeding also on umbelliferous plants in the wild (Van Son, 1949), it is possible that *P. demodocus* has reduced its sensitivity to mixed function oxidase inhibitors.

When, by a physiological adjustment, the insect is able to use a plant species with high levels of a toxic compound for non specific insects, that insect gains a source of food,

usually not utilized by other herbivores and consequently, competition for food is minimized (Price, 1984). The ability of an insect to feed on plant tissues with high levels of toxic compounds may enable that insect to benefit from the antibiotic properties of many toxic compounds protecting the insect against pathogens as did *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) (Jones *et al.*, 1962; Reichstein *et al.*, 1968). The insect can also develop unpalatable characteristics so that predation pressure is reduced, e.g. *Caryedes* sp. (Coleoptera: Bruchidae) (Brower & Brower, 1964; Price, 1984). As *P. demodocus* larvae feed equally on plants with low and high tannin levels (tables 4.6.15-4.6.19), it has probably developed an unpalatable characteristic protecting them from predators and reducing the attack by pathogens.

Hunter & West (1990) showed that for free-living lepidopterous larvae, leaf structure was more important than leaf chemistry in determining population abundance. They found evidence that for *Diurnea fagella* (D. & S.) (Lepidoptera: Oecophoridae), distribution preference for the host tree *Quercus robur* L. (Fagaceae) spring leaves was related to habitat (including leaf structure) selection rather than nutritional quality of leaves on the same plant species. The apparent insensitivity of *P. demodocus* (tables 4.6.15-4.6.19) to soluble tannin levels in citrus leaves may also be related to the low effect of leaf nutritional quality on this insect performance.

Effect of density levels on population performance

The population performance of *P. demodocus* in terms of insect pest survival (figure 4.7.3) and larval weight (table 4.7.1) was not significantly related to any increase in insect pest density levels in the laboratory. There was, however, a reduction in insect survival probability in the field (figure 4.7.4) and egg deposition with an increase of insect pest

density (table 4.7.2) suggesting that density could be a regulating factor for *P. demodocus* populations. The results were not significantly different in the range between 2 to 10 insects per rearing space. There was however, a significant effect of insect pest density levels on egg deposition when 15 females in one cage laid their eggs (table 4.7.2). When individuals collected from the field reached 15 females per cage, the number of eggs deposited was significantly reduced compared to egg deposited by females at lower density levels (table 4.7.2). Oloya (1964) found similar results for psyllids. The density levels of 2 to 10 insects per cage studied in this experiment used to assess the effect of density on *P. demodocus* performance may have not been the most advisable range for density level studies of *P. demodocus* population performance. However, time was a constraint in repeating this experiment at higher density levels.

These data on *P. demodocus* fecundity in relation to density levels provide additional evidence that population density regulated *P. demodocus* population sizes.

Population density was negatively correlated with survival probability in the field (figure 4.7.4) and larval development time during the dry season (figure 4.7.1). Larval headcapsule size was not affected by density, (table 4.5.6).

The regression models of the *P. demodocus* population changes as affected by the initial size showed that variance in population (84.7% around the regression line, figure 4.4.4) was due to the initial population size and indirectly, fecundity.

As for many insects including *Rhizopertha dominica* Fabricius (Coleoptera: Bostrychidae), reproductive rate increases from low density levels and it ceases increasing at high insect numbers (Varley *et al.*, 1973). A similar phenomenon occurred with *P. demodocus* (table 4.2.2). The causes for the decrease of the multiplication rates may include decreased fecundity, for example by male interference as decreased longevity of

adults is not observed for *P. demodocus* during the rainy season (section 4.3.1). Klomp (1968) noted that for the insect *Bupalus piniarias* L. (Lepidoptera: Geometridae) at higher densities, larval interference resulted in a delayed density dependent effect on reproduction as pupae were lighter and the females emerging from them laid less eggs.

Natural enemies

Larval disappearance was the main cause of population decrease while pupae were attacked by a parasite, *Pteromalus* sp (table 4.4.2 and appendix 4.6). The key factor was identified as larval disappearance at the second, third and fourth instar stage (appendix 4.6). Extremely few predators were found in the orchards during the sampling work in all fields whether subjected to chemical treatment or not. Little is known about the effect of natural enemies on *P. demodocus* numbers in the field. Parasitism was negligible and was observed only for pupae in the study plots. The parasite *Pteromalus* sp. was responsible for a 12.6% reduction of pupal numbers in the field. However, this mortality did not contribute to the pupal population size changes (0.0%; P =0.361) and accounted for only 6.5% of total generation mortality (P = 0.093). Mantids *(Miomantis* sp.) were rarely found in the study plots (just three in all 25,561 sampling units at different sampling occasions) and birds were regarded as of very little impact on *P. demodocus* population density, probably due to a relatively low efficiency in finding the prey (section 4.8). These findings demonstrate a very low level of parasitism and predatory activity during the study period and consequently, parasitism and predation had little effect on population abundance. Although caged mantids were able to feed on *P. demodocus* larvae (tables 4.8.5 and 4.8.8), exclusion studies can, however, tell us only how many prey a generalist predator consumes if no alternative food is given (Seymour & Jones, 1991). Since the predators appeared at very low densities in the field, it is suggested that they probably

had low reproductive rates (Richards & Southwood (1968) and so, had little effect on *P. demodocus* population size.

Larval disappearance in the first instar may have not been due to walking or predatory effect, but probably was a result of difficulties with initial feeding. After death, the larvae, usually less than 7 mm in length may have been dislodged from the leaves by winds or rain. Dislodgement by winds and rain may also increase the number of disappeared live larvae in the younger instars.

Negligible parasitism has been observed in other insect species such as the aphid *Acyrthosiphon spartii* (Koch) (Homoptera: Aphididae) (Smith, 1966b cited by Waloff, 1968; Waloff, 1968). Total pupal parasitism at 4% has been reported by Humble (1985) in *Operophtera* spp. (Lepidoptera: Geometridae) on Vancouver Island, United States, and that was partly due to hyperparasites which had a negative impact on the parasites. By contrast, Watanabe (1976, 1981), Hayes (1985) and Lederhouse *et al.* (1987) have found that predators caused most of the mortality in populations of *Papilio xuthus* in Japan, *Colias alexandra* Edwards (Lepidoptera: Pieridae) in Colorado, United States and *Papilio polyxenes asterius* Stoll in the United States respectively. Lederhouse *et al.* (1987) observed that birds accounted for 65% of predation of *P. polyxenes.*

The exclusion experiment results (tables 4.8.8 and 4.8.9) indicated that predator activity affected *P. demodocus* survival. Expected survival of *P. demodocus* was longer when free from predators than when exposed to mantids and both mantids and birds.

Larval headcapsule size was smaller in the cages where only birds were excluded than in cages where one *Miomantis* sp. specimen had been placed. Larval headcapsule size was smallest in cages where the activities of both mantids and birds were low. The results (tables 4.8.1, 4.8.2 and 4.8.3) showed, conclusively that the highest increase in the size of

larval headcapsule size observed in larvae exposed to one mantid is probably due to a non preference by the mantid species to large *P. demodocus* larvae, contributing to a reduced efficiency of *Miomantis* sp. as a *P. demodocus* natural enemy.

While captive mantids have been shown to feed on *P. demodocus*, the predator may behave differently if given a choice. Mantids have been reported as generalist predators (Hurd & Eisenberg, 1989). Under natural conditions, *Miomantis* sp., may have other preferred food, which includes most insect pests, eggs and mites (Acosta, 1998). Hepburn & Bishop (1954) observed that a predator might be repelled by *P. demodocus* larvae, because of the defensive emission of an aggressive odour (terpenic compounds) by the osmeterium when disturbed. This indicates that larval disappearance may not in this case have been primarily caused by predators, but for example by difficulties with initial feeding, dislodgement of young larvae from the plant leaves by strong winds or rain. It may however be related to predatory activity as the cage experiments showed (tables 4.8.5 and 4.8.8) that the mantid *Miomantis* sp. and birds fed on larvae of all developmental stages.

The impact of predators on *P. demodocus* population growth remains, however, uncertain. Unlike other lepidopterous insects such as *Papilio xuthus* (Watanabe, 1976; 1979; 1981) and *Papilio polyxenes asterius* Stoll Lederhouse *et al.* (1987), natural enemies (mostly birds) did not exert a high impact on *P. demodocus* performance (table 4.8.7).

Morris (1963) stated that regulatory mechanisms by natural enemies usually vary at different population levels. As pointed out by Holling (1961, 1968), even after showing a response to their prey density in some occasions, predators may, however, exert quite different effects on their prey in relation to prey density or other factors. Waloff (1968) noted that some generalist predators may respond to an increase in their host numbers

with an increase in their own abundance but at low prey densities they move to other sites in search for more abundant food. An example of such a predator is *Anthocoris sarothamni* Douglas and Scott (Coleoptera: Anthocoridae) which increased in population numbers following host population increase but left the area at low prey densities. There is no evidence that *Miomantis* sp. acts in the same way as they did in the field experiment in relation to *P. demodocus* different levels. Their impact on *P. demodocus* populations is not clear. Losses on *P. demodocus* numbers on the caged and uncaged plants were not different, by the t-test (section 4.8.2.2). Consequently, dispersal of *P. demodocus* is considered a minor factor determining larval disappearance and *P. demodocus* larval abundance in the field.

In the study plots, parasites did not attack *P. demodocus* eggs *and* larvae. In South Africa, *P. demodocus* has been affected by parasitoids at all immature stages (Brink & Steyn, 1993). This may be explained by the fact that in Maputo, *P. demodocus* populations were not found in the wild. In natural vegetation, free of pesticides, more parasitoids may be found. However, in the study area, members of the Rutaceae and Umbelliferae plants were seldom observed. In South Africa, where the pest has been recorded from native plant species e.g. *Foeniculum officinale* All. (Umbelliferae), *F. vulgare* Mill. (Umbelliferae), *Vepris undata* (Thunb.) Verdoorn (Rutaceae), *Toddalia asiatica* (L.) Lam. (Rutaceae) (Van Son, 1949; Hepburn & Bishop, 1954; Honiball & Bedford, 1978), there may be a synchronization between the pest and its parasitoids. The ineffective parasitoid impact on *P. demodocus* in Maputo can also be an explanation for the relatively high pest numbers compared to those observed in South Africa, where the species does not appear to be of real economic importance (P. Joubert[2]). It can also be concluded that meteorological factors in the Maputo region (annual temperature of 21°C and relative humidity of 70%, Reddy, 1986) favour *P. demodocus* population growth and development when compared to cooler temperatures in South Africa, with daily minimum

[2] Institute of Tropical and Subtropical Crops in Nelspruit, South Africa.

mean of -3°C while summer temperatures rarely exceed 26°C (Calvin & Wettlaufer, 2000). Annual average temperature varies from 14° to 17° C (West Coast Investment Initiative, 1998). Van Son (1949), Hepburn & Bishop (1954), Williams (1969) and Honiball & Bedford (1978) confirmed that *P. demodocus* was more abundant in northern parts of southern Africa where it occurs throughout the year. In the southern cooler regions of South Africa (e.g. Cape Province) *P. demodocus* has been reported as experiencing winter diapause and its life cycle taking longer during the winter season (Hepburn & Bishop, 1954).

The constant application of *B. thuringiensis* may have made any density dependent processes invisible as the density never achieved levels uncomitant with the factors being measurable.

In conclusion, natural enemies seem not to play an important role in the population dynamics of *P. demodocus* providing further evidence in support of a density related process related to changes in the net reproductive rate R_0 and the intrinsic rate of increase r_c appearing to be the major factors regulating the insect numbers. Several factors may have contributed to larval disappearance, the *P. demodocus* key factor, like larval dispersal, predator activity, environmental factors. However, separately, these factors are of little importance in the reduction of *P. demodocus* survival under natural field conditions (appendix 4.6 and 4.7).

With the accummulated information, it can be concluded that *P. demodocus* showed evidence of population regulation (section 4.3 and 4.4) as insect numbers significantly differed between generations of different seasons but mortality key factors were not density dependent within generations and the insect was not affected by cultivars (section 4.6) or natural enemies in the field. Consequently, density induced mortality is rare and unprobable under field conditions.

As Klomp (1962) cited by Wilson (1968) pointed out, an insect species population density may not be maintained or controlled by chance and so, *P. demodocus* is more likely not to be an exception.

As observed above, density related processes seem to act through reduced fecundity rate (capacity for increase and net reproductive rate, section 4.3.4, 4.4.3 and 4.7.5) at higher density levels during the rainy season (table 4.6.3).

REFERENCES

Abrahamsen, G. 1969 Sampling design in studies of population densities in Enchytraeidae (Oligochaeta). *Oikos*. **20**, 54-66.

Abrahamsen, G. & Strand, L. 1970 Statistical analysis of population density data of soil animals, with particular reference to Enchytraeidae (Oligochata). *Oikos*. **21**, 276-284.

Acosta, E.W. 1998 The Poster Child of Beneficial Insects. In: *Biocontrol Network* http://www.biconet.com

Ali, A.; Gu, W-D.; Lobinske, R.J. 1998 Spatial distribution of chironomid larvae (Diptera: Chironomidae) in two central Florida lakes. *Environmental Entomology*. **27** (4), 941-948.

AliNiazee, M.T. 1983 Pest status of filbert (hazelnut) insects: A 10-year study. *Canadian Entomologist*. **115** (9), 1155-1162.

Allen, S.E. 1989 (ed) *Chemical analysis of Ecological Materials*. 2nd edition. Oxford: Blackwell Scientific Publications. 368 pp.

Allen, S.E.; Grimshaw, H.M.; Rowland, A.P. 1986 Chemical analysis. In: *Methods in Plant Ecology*. 285-344 pp. Moore, P.D. & Chapman, S.B. (eds.) 2nd edition. Oxford: Blackwell Scientific Publications.

Allsopp, P.G.; Daglish, G.J.; Taylor, M.F.J.; Gregg, P.C. 1991 Measuring development of *Heliothis* species. 90-101pp. In: Heliothis Research Methods and Prospects. (ed.) Zalucki, M.P. New York: Springer-Verlag New York INC

Anderson, R.M. 1974 Population dynamics of the cestode *Caryophyllalus laticeps* (Pallas, 1781) in the bream (*Abramis brama* L.). *Journal of Animal Ecology*. **43**, 305-321.

Annecke, D.P. & Moran, V.C. 1982 *Insects and mites of cultivated plants in South Africa*. Durban: Butterworths & CO(SA) (PTY) LTD. 383 p.

Anscombe, F.J. 1948 The transformation of Poisson, binomial and negative binomial data. *Biometrika*. **35**, 246-254.

Anscombe, F.J. 1949 The statistical analysis of insect counts based on the negative binomial distribution. *Biometrics*. **5** (1-4), 165-173.

Anscombe, F.J. 1950 Sampling theory of the negative binomial and logarithmic series distributions. *Biometrika*. **37**, 358-382.
Ayres, M.P. & Scriber, J.M. 1994 Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera: Papilionidae) *Ecological Monographs*. **64** (4), 465-482.

Badawi, A. 1981 Studies on some aspects of the biology and ecology of the citrus butterfly *Papilio demoleus* L. in Saudi Arabia (Papilionidae, Lepidoptera). *Journal of Applied Entomology*. **9** (3), 286-292.

Bartlett, M.S. 1947 The use of transformations. *Biometrics*. **3**, 39-52.

Bateman, M.A. 1968 Determinants of abundance in a population of the Queensland fruit fly. In: *Insect Abundance*. pp 119-131. (ed. T.R.E. Southwood). Royal Entomological Society. Symposium 4. Surrey: Adlard And Son Limited.

Beall, G. 1942 The transformation of data from entomological field experiments so that the analysis of variance becomes applicable. *Biometrika*, **31-32**, 243-262.

Bean, D.W. & Beck, S.D. 1983 Haemolymph ecolysteroid titres in diapause and nondiapause larvae of the European corn borer, *Ostrinia nubilalis*. *Journal of Insect Physiology*. **29** (9), 687-693.

Beauchamp, J.J. & Olson, J.S. 1973 Corrections for bias in regression estimates after logarithmic transformation. *Ecology*. **54**, 1403-1407.

Beck, S.D. 1986 Effects of photoperiod and thermoperiod on growth of *Agrotis ipsilon* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America*. **79** (5), 821-828.

Begon, M. 1979 *Investigating animal abundance: capture and recapture for biologists*. Great Britain, Wilmer Brothers Limited.

Bellows Jr., T.S.; Driesche, R.G. van; Elkinton, J.S. 1992 Life-table construction and analysis in the evaluation of natural enemies. *Annual Review of Entomology*. **37**, 587-614.

Bernays, E.A. & Chapman, R.F. 1994 Chemicals in plants. In: *Host-Plant Selection by Phytophagous Insects*. 14-60. USA: Chapman & Hall, INC.

Bliss, C.I. 1958 The analysis of insect counts as negative binomial distributions. In: *Genetics, Cytology, and Biometrics. Proceedings Tenth International Congress of Entomology*. pp 1015-1032. Vol 2. New Haven, Conn.

Bliss, C.I. & Fisher, R.A. 1953a Fitting the negative binomial distribution to biological data. *Biometrics*. **9**, 1-4.

Bliss, C.I. & Fisher, R.A. 1953b Fitting the negative binomial distribution to biological data and note on the efficienct fitting of the negative binomial. *Biometrics*. **9**, 177-200.

Bliss, C.J. & Owen, A.R.G. 1958 Negative binomial distribution with a common k. *Biometrika*. **45**, 37-58.

Bloem, S.; Bloem, K.A.; Knight, A.L. 1998 Assessing the quality of mass-reared codling moths (Lepidoptera: Tortricidae) by using field release-recapture tests. *J. Econ. Entomol.* **91** (5), 1122-1130.

Blossey, B. & Hunt, T.R. 1999 Mass rearing methods for *Galerucella calmariensis* and *G. pusilla* (Coleoptera: Chrysomelidae), biological control agents of *Lythrum salicaria* (Lythraceae). *J. Econ. Entomology*. **92** (2), 325-334.

Bohlem, E. 1973 *Crop Pests in Tanzania and their Control*. Germany: Verlag Paul Parey. 142 p.

Briere, J.F. & Pracros, P. 1998 Comparison of temperature-development growth models with the development of *Lobesia botrana* (Lepidoptera: Tortricidae). *Environ. Entomology*. **27** (1), 94-101.

Brink, T. & Steyn, W.P. 1993 A pest outbreak of orange dog: *Inligtings Bulletin Institut Vir Tropiese en Subtropiese Geuwasse*. **249**, 5-6.

Brower, L.P. & Brower, J.V.Z. 1964 Birds, butterflies and plant poisons: a study in ecological chemistry. *Zoologica*. **49**, 137-159.

Brown, K.S. & Vasconcellos Neto, J. 1976 Predation of aposematic ithomiinse butterflies by tanagers. (*Pipraeidea melanonota*). *Biotropica*. **8**, 136-141.

Calvin, M. & Wettlaufer, D. 2000 Fires in the Southern Cape Peninsula, Western Cape Province, South Africa. *IFFN* number 22.

Carruthers, V. 1997 *The Wildlife of Southern Africa. A Field Guide to the Animals and Plants of the Region*. Western Cape: Southern Book Publishers. 310 p.

Carvalho, J.P. 1986 *Introdução à Entomologia Agrícola*. Fundação Calouste Goulbenkian, Lisboa. 361 p.

Cheke, R. A. 1995 Potential rates of increase of solitarious and gregarious phases of the African armyworm *Spodoptera exempta* (Lepidoptera: Noctuidae). *Ecological Entomology*. **20**, 319-325

Childers, C.C.; Duncan, L.W.; Wheaton, T.A.; Timmer, L.W. 1987 Arthropod and nemathod control with Aldicarb on Florida citrus. *Journal of Economic Entomology*. **80**, 1064-1071.

Chua, T.H. 1979 Parasites of the citrus butterfly, *Papilio demoleus* L. *malayanus* Wall *Ooencyrtus malayensis*, *Pteromalus puparam*, *Scellionus* spp. *Malaysian Applied Bio*. **7** (2), 93-98.

Clark, L.R.; Geier, P.W.; Hugues, R.D.; Morris, R.F. 1967 The ecology of insect populations in theory and practice. London: Methuen. 232 p.

Clarke, C.A. 1984 Upsets in the sex-ratio of some Lepidoptera. In: *The Biology of Butterflies*. Symposium of the Royal Entomological Society of London. Number II. pp. 255-258. (ed. R.I. Vane-Wright & P.R. Ackery). London: Academic Press INC (London) LTD.

Coffelt, M.A. & Schultz, P.B. 1994 Within-tree distribution and a fixed-precision-level sampling plan for the orange striped oakworm (Lepidoptera: Saturnidae). *J. Econ. Entomology*. **87** (2), 382-388.

Commonwealth Agricultural Bureaux International Institute of Entomology 1987 *Distribution Maps of Pests*. UK: CABInternational Institute of Entomology.

Danilevskii, A.S. 1965 *Photoperiodism and Seasonal Development oflinsects*. Great Britain: Robert Cunningham & Sons Ltd. 283 p.

David, F.N. & Moore, P.G. 1954 Notes on contagious distributions in plant populations. ***Annals of Bot. Lond. N.S.*** **18,** 47-53.

Davidson, J. & Andrewartha, H. G. 1948a Annual trends in a natural population of *Thrips imaginis* (Thysanoptera). *Journal of Animal Ecology. 17*, 193-199.

Davidson, J. & Andrewartha 1948b The influence of rainfall, evaporation and atmospheric temperature on fluctuations in the size of a natural population of *Thrips imaginis* (Thysanoptera). *Journal of Animal Ecology*. **17**, 200-222.

Davies, R.G. 1971 Computer Programming in Quantitative Biology. London & New York: Academic Press. 492 pp.

Dempster, J.P. 1968 Intra-specific competition and dispersal: as exemplified by a psyllid and its anthocorid predator. In: *Insect Abundance.* pp 8-17. Symposium, 4. The Royal Entomological Society. (ed. T.R.E. Southwood). Surrey: Adlard And Son Limited.

Dempster, J.P. & Pollard, E. 1985 Spatial heterogeneity, stochasticity and the detection of density dependence in animal populations. *Oikos.* **46** (3), 413-416.

Dixon, A.F.G. 1990 Population dynamics and abundance of deciduous tree-dwelling aphids. In: *Population Dynamics of Forest Insects.* pp 11-23. (eds.) Watt, A.D.; Leather, S.R.; Hunter, M.D.; Kidd, N.A. Intercept Ltd. Andover.

Drake, V.A.1991 Methods for studying adult movements in *Heliothis.* In: *Heliothis Research Methods and Prospects.* (ed. M.P. Zalucki). 109-121 pp. New York: Springer-Verlag New York INC.

Ehrlich, P.R. 1984 The structure and dynamics of butterfly populations. In: *The Biology of Butterflies.* Symposium of the Royal Entomological Society of London. Number II. pp. 25-40. (ed. R.I. Vane-Wright & P.R. Ackery). London: Academic Press INC (London) LTD.

Ehrlich, P.R. & Ehrlich, A.H. 1982 Lizard predation on tropical butterflies. *Journal of Lepidopterists' Society.* **36**,148-152.

Ehrlich, P.R. & Raven, P.H. 1967 Butterflies and plants. *Sci. Amer.* **216** (6), 104-113.

Elkinton, J.S.; Gould, J.R.; Ferguson, C.S.; Liebhold, A.M.; Wallmer, W.E. 1990 Experimental manipulation of gypsy moth density to assess impact of natural enemies. In: *Population Dynamics of Forest Insects.* pp 275-287. (eds. A. D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Elliott, J. M. 1971 Some methods for the statistical analysis of samples of benthic invertebrates. 2nd edition. UK: Freshwater Biological Association Scient. Publ. 25, 148 p.

Engelen, J.V. 1981 *Umbeluzi. Estacao Agrária. Cartografia Hidro-Pedológica.* Faculdade de Agronomia. U.E.M. 21 p.

Engelmann, F. 1970 *The Physiology of Insect Reproduction.* Great Britain, Pergamon Press Inc. 307 p.

Fantinou, A.A.; Tsitsipsis, J.A.; Karandinos, M.G. 1998 Diapause termination in *Sesamia nonagrioides* (Lepidoptera: Noctuidae) under laboratory and field conditions. *Environ. Entomology.* **27** (1), 53-58.

Feeny, P. 1970 Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology.* **51**, 565-581.

Feeny, P.P. & Bostock, H. 1968 Seasonal changes in the tannin content of oak leaves. *Phytochemistry.* **7**, 871-880.

Feltwell, J. 1986 *The Natural History of Butterflies.* Kent: Groom Helm LTD. 133 p.

Ford, J. 1968 The control of populations through limitation of habitat distribution as exemplified by tse-tse flies. In: *Insect Abundance.* pp 109-118. Symposium 4. The Royal Entomological Society. (ed. T.R.E. Southwood). Surrey: Adlard And Son Limited.

Forsythe, H.Y. & Gyrisco, G.G. 1961 Determining the appropriate transformation of data from insect control experiments for use in the analysis of variance. *Journal of Economic Entomology.* **54**, 859-861.

Fujii, K. 1975 A general simulation model for laboratory insect populations I. From cohort of eggs to adult emergences. *Res. Popul. Ecol.* **17**, 85-133.

Gazeta do Agricultor 1959 *A Citricultura no Sul do Save.* Direccao de Agricultura e Florestas. L. Marques.

Gilbert, L.E. 1984 The biology of butterfly communities. In: *The Biology of Butterflies.* Symposium of the Royal Entomological Society of London. Number II. pp. 41-54. (eds. R.I. Vane-Wright & P. R. Ackery). London: Academic Press INC (London) LTD.

Gordon, H.T. 1984 Growth and development of insects. In: *Ecological Entomology.* pp. 53-77. (eds. C.B. Huffaker. & R.L. Rabb). USA: John Wiley & Sons.

Hagen, K.S.; Dadd, R.H.; Reese, J.1984 The food of insects. In: *Ecological Entomology.* pp. 79-112. (eds. C.B. Huffaker. & R.L. Rabb). USA: John Wiley & Sons.

Greenbank, D.O. 1956 The role of climate and dispersal in the initiation of outbreaks of the spruce budworm in New Brunswick. I. The role of climate. *Canadian Journal of Ecology.* **34**, 453-476.

Greenbank, D.O. 1963a Climate and the spruce budworm. In: *The Dynamics of Epidemic Spruce Budworm Populations.* pp 174-180. (ed. R.F. Morris). Mem. Entomol. Soc. Canada. 31.

Greenbank, D.O. 1963b The development of the outbreak. In: *The Dynamics of Epidemic Spruce Budworm Populations.* pp 19-23. (ed. R.F. Morris). Mem. Entomol. Soc. Canada. 31.

Hain, F.P. 1987 Interactions of insects, trees and air pollutants. *Tree Physiology.* **3**, 93-102.

Hamze, M.; Nimah, M.; Zaabout, M. 1984 Effectiveness of six digestion procedures to evaluate the status of major elements (Ca, K, Mg and Na) in Citrus leaves. *Communications in Soil Science and Plant Analysis.* **15** (9), 1135-1145.

Harcourt, D.G. 1963 Population dynamics of *Leptinotarsa decemlineata* (Say) in Eastern Ontario. I. Spatial pattern and transformation of field counts. *The Canadian Entomologist.* **95**, 813-820.

Hassell, M.P. 1978 *The Dynamics of Arthropod Predator-Prey Systems.* Princeton NJ: Princeton University Press.

Hassell, M.P. 1986 Detecting density dependence. *Trends in Ecology and Evolution.* **1**, 90-93.

Hassell, M.P. 1987 Detecting regulation in patchily distributed animal populations. *Journal of Animal Ecology.* **56**, 705-713.

Hassell, M.P. & May, R.M. 1974 Aggregation in predators and insect parasites and its effect on stability. *Journal of Animal Ecology.* **43**, 567-594.

Hassell, M.P. & Rogers, D.J. 1972 Insect parasite responses in the development of population models. *Journal of Animal Ecology.* **41**, 661-676.

Hayes, J.L. 1981 The population ecology of a natural population of the pierid butterfly *Colias alexandra. Oecologia (Berl.).* **49**, 188-200.

Hayes, J.L. 1985 Predator-prey interactions between the mite *Balaustium* sp. and the pierid butterfly *Colias alexandra. Ecology.* **66**, 300-303.

Healy, M.J.R. & Taylor, L.R. 1962 Tables for power-law transformations. *Biometrika.* **49**, 557-559.

Heliovaara, K & Vaisanen, R. 1990 Changes in population dynamics of pine insects induced by air pollution. In: *Population Dynamics of Forest Insects.* 209-218 pp. (eds. A.D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Henson, W.R. 1968 Some recent changes in the approach to studies of climatic effects on insect populations. In: *Insect Abundance.* pp 37-46. Symposium 4. The Royal Entomological Society. (ed. T.R.E. Southwood). Surrey: Adlard And Son Limited.

Hepburn, G.A. & Bishop, H.J. 1954 The insect Pests of Citrus in South Africa. *Entomology Series.* 41. Porth Elizabeth: Union of South Africa. Dept. of Agriculture. 31 p.

Hill, D.S. 1983 *Agricultural insect pests of the tropics and their control.* 2nd Edition. Cambridge: Cambridge University Press. 746 p.

Hinton, L. 1994 *The Southern Hemisphere Market for Fruit and Vegetables.* Monograph 27. Cambridge: Granta Editions Limited. 182 p.

Holling, C.S. 1961 Principles of insect predation. *Annual Review of Entomology.* **6,** 163-182

Holling, C.S. 1965 The functional response of predators to prey density and its role in mimicry and population regulation. *Mem. Entomolog. Soc. Can.* **45**, 1-60.

Holling, C.S. 1968 The tactics of a predator. In: *Insect Abundance.* pp 47-58 (ed. T.R.E. Southwood) Symposium, 4. The Royal Entomological Society. Surrey: Adlard And Son Limited.

Honiball, E. & Bedford, E.C.G. 1978 *Citrus* butterflies. In: Citrus Pests in The Republic of South Africa. (ed. E.C.G. Bedford). 243 p.

Hosking, G.P.; MacKenzie, R.M.J.; Zondag, R.; Hutcheson, J.A. 1990 Effect of *Neomycta pulicaris* Pascoe (Coleoptera: Curculionidae) on hard beech *Nothofagus truncata.* In: *Population Dynamics of Forest Insects.* pp 105-111. (eds. A. D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Howard, C.W. 1906 The orange-tree butterfly, *Papilio demoleus. Transvaal Agric. J.* **4** (16), 727-732.

Huffaker, C.B. & Messenger, P.S. 1964 Population ecology-historical development. In: *Biological Control of Insect Pests and Weeds*. pp 45-73. (eds. P. DeBach & E.I. Schlinger). London.

Huffaker, C.B.; Kennett, C.E.; Matsumoto, B.; White, E.G. 1968 Some parameters in the role of enemies in the natural control of insect abundance. In: *Insect Abundance*. pp. 59-75 (ed. T.R.E. Southwood) Symposium, 4. The Royal Entomological Society. Surrey: Adlard And Son Limited.

Hugues, R.D. 1979 Modelling bushfly populations. *J. Appl. Ecol.* **16**, 117-139.

Humble, L.M. 1985 Final-instar larvae of native pupal parasites and hyperparasites of *Operophtera* spp. (Lepidoptera, Geometridae) on southern Vancouver Island. Canadian Entomologist. **117** (5), 525-534.

Hunter, M.D. & West, C. 1990 Variation in the effects of spring defoliation on the late season phytophagous insects of *Quercus robur*. In: *Population Dynamics of Forest Insects*. pp 123-135. (eds. A. D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Hurd, L.E. & Eisenberg, R.M. 1989 Temporal distribution of hatching times in three sympatric mantids (Mantodea: Mantidae) with implications for niche separation coexistence. In: *Proceedings of the Entomological Society of Washington*. **91** (1), 55-58.

Hutchings, M.J. 1986 Plant population biology. In: *Methods in Plant Ecology*. 377-435 pp. 2nd edition. Moore, P.D. & Chapman, S.B. (eds.) Oxford: Blackwell Scientific Publications.

Ibrikci, H. 1994 Macro element status of mandarin orchards in Southern Turkey. *Communications in Soil Science and Plant Analysis*. **25** (17-18), 2971-2980.

Iwao, S. 1968 A new regression method for analysing the aggregation pattern of animal populations. *Res. Popul. Ecol.* **10**, 1-20.

Iwao, S. 1970 Analysis of contagiousness in the action of mortality factors on the western tent caterpillar population by using the $\overline{m}$-m relationship. *Res. Pop. Ecol.* **12**, 100-110

Johansson, A.S. 1964 Feeding and nutrition in reproductive processes in insects. In: *Insect* Reproduction. Symposium 2. pp. 43-55. The Royal Entomological Society of London. (ed. K.C. Highman). Surrey: Adlard And Son Limited.

Jones, D.A.; Parsons, J.; Rothschild, M. 1962 Release of hydrocyanic acid from crushed tissues of all stages in the life-cycle of species of the Zygaeninae (Lepidoptera). *Nature.* **193**, 52-53.

Karandinos, M.G. 1976 Optimum sample size and comments on some published formulae. *Bulletin of Entomological Society of America.* **22**, 417-421.

Kennedy, J.S. 1961 A turning point in the study of insect migration. *Nature.* **189**, 785-791.

Kidd, N.A.C. 1990 A synoptic model to explain long-term population changes in the large pine aphid. In: *Population Dynamics of Forest Insects.* pp 317-327. (eds. A. D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Kiritani, K. & Dempster, J.P. 1973 Different approaches to the quantitative evaluation of natural enemies. *Journal of Applied Ecology.* **10**, 323-330.

Kitching, R.L. 1983 *Systems Ecology: An Introduction to Ecological Modelling.* St. Lucia: University of Queensland Press, Australia.

Klomp, H. 1968 A seventeen-year study of the abundance of the pine looper, *Bupalus piniarius* L. (Lepidoptera: Geometridae). In: *Insect Abundance.* pp. 98-108 (ed. T.R.E. Southwood) Symposium, 4. The Royal Entomological Society. Surrey: Adlard And Son Limited.

Krieger, R.I.; Feeny, P.P.; Wilkinson, C.F. 1971 Detoxication enzymes in the guts of caterpillars: An evolutionary answer to plant defenses? *Science.* **172**, 579-581.

Kuno, E. 1963 A comparative analysis on the distribution of nymphal populations of some leaf- and planthoppers on rice plant. *Res. Popul. Ecol.* **5**, 31-43.

Kuno, E. 1969 A new method of sequential sampling to obtain the population estimates with a fixed level of precision. *Res. Pop. Ecol.* **11**, 127-136.

Kuno, E. 1973 Statistical characteristics of the density-independent population fluctuation and the evaluation of density-dependence and regulation in animal populations. *Res. Pop. Ecol.* **15**, 99-120.

Kuno, E. 1991 Sampling and analysis of insect populations. *Annual Review of Entomology.* **36**, 285-304.

Lara, F.M. 1992 *Principios de Entomologia.* 3rd edition. SP: Icone Editora. 331 pp.

Leather, S.R. 1990 The role of host quality, natural enemies, competition and weather in the regulation of Autumn and Winter populations of the Bird Cherry Aphid. In: *Population Dynamics of Forest Insects.* pp 35-44. (eds. A. D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Lederhouse, R.C.; Codella, S.G.; Cowell, P.J. 1987 Diurnal predation on roosting butterflies during inclement weather: a substancial source of mortality in the black swallowtail, *Papilio polyxenes* (Lepidoptera: Papilionidae). *J.N.Y. Entomol. Soc.* **95** (2), 310-319.

Lloyd, M. 1967 Mean crowding. *Journal of Animal Ecology.* **36**, 1-30.

Matsuki, M. & MacLean Jr., S.F. 1994 Effects of different leaf traits on growth rates of insect herbivores on willows. *Oecologia.* **100** (1-2), 141-152.

Mattson, W.J. & Haack, R.A. 1987 The role of drought stress in provoking outbreaks of phytophagous insects. In: *Insect Outbreaks.* pp. 365-407. (eds. P. Barbosa and J.C. Schultz). San Diego: Academic Press.

Mattson, W.J. & Scriber, J.M. 1986 Nutrition ecology of insects folivores of woody plants: nitrogen, water, fiber and mineral considerations. In: *The Nutritional Ecology of Insects, Mites and Spiders.* pp. 105-146. (eds. F. Slansky Jr. and J.G. Rodriguez). New York: John Wiley.

Mays, W.T. & Kok, L.T. 1997 Oviposition, development, and preference of the cross-striped cabbageworm (Lepidoptera: Pyralidae). *Environ. Entomol.* **87** (2), 382-388.

McClure, M.S. 1988 The armored scales of hemlock. In: *Dynamics of Forest Insect Population.* Patterns, Causes, Implications. (ed. A.A. Berryman). New York: Plenum Press.

Mckillop, A.R. 1986 *The Relationship Between Feeding Levels of Sitrophosomus melanogrammus (Forster) and the Foliar Chemistry of Betula pubescens (Ehrh).* Aberdeen: University of Aberdeen. Thesis for the B.Sc. Forestry (Hons.) degree.

McNeill, S. & Southwood, T.R.E. 1978 The role of nitrogen in the development of insect/plant relationships. In: Biochemical Aspects of Plant and Animal Coevolution. pp. 77-98 (ed.) J.B. Harborne. London: Academic Press.

Meikle, W.G.; Markham, R.H.; Holst, N.; Djomamou, B.; Schneider, H.; Vowotor, K.A. 1998 Distribution and sampling of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in Maize stores in benin. *Journal of Economic Entomology*. **91** (6), 1366-1374.

Migdoll, I. 1994 Field Guide. Butterflies of Southern Africa. Cape Town: Struik Publishers (Pty)Ltd). 256 p.

Ministério da Educação 1986 *Atlas Geografico*. 2nd Ed. vol 1. Sweden: Esselte Map Service AB.

Montgomery, M. E. 1990 Role of site and insect variables in forecasting defoliation by gypsy moth. In: *Population Dynamics of Forest Insects*. pp 73-84. (eds. A. D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Morisita, M. 1962 Iδ- Index, a measure of dispersion of individuals. *Research Population Ecology*. **4**, 1-7.

Morisita, M. 1964 Application of Iδ- Index to sampling techniques. *Research Population Ecology*. **6**, 43-53.

Morisita, M. 1971 Composition of the Iδ- Index. *Research Population Ecology*. **13**, 1-27.

Morris, R.F. 1955 The development of sampling techniques for forest insect defoliators, with particular reference to the spruce budworm. *Can. Journal of Zoology*. **33**, 225-294.

Morris, R.F. 1959 Single-factor analysis in population dynamics. *Ecology*. **40**, 580-588.

Morris, R.F. 1963 Predictive population equations based on key factors. *Mem. Ent. Soc. Can.* **32**, 16-21.

Morris, R.F. & Miller, C.A. 1954 The development of life tables for the spruce budworm. *Canadian Journal of Zoology*. **32**, 283-301.

Mullen, M.A.; Wileyto, E.P.; Arthur, F.H. 1998 Influence of trap design and location on the capture of *Plodia interpunctella* (Indian meal moth) (Lepidoptera: Pyralidae) in a release-recapture study. *J. Stored Prod. Res.* **34** (1), 33-36.

Nagalingam, B. 1983 Ocurrence of hymenopteran parasites on the larvae of lemon butterfly *Papilio demoleus* (L.) in India. *Madras Agricult. Journal*. **70**, (4) 274.

Neal, J.J. & Berenbaum, M. 1989 Decreased sensitivity of mixed-function oxidases from *Papilio polyxenes* to inhibitors in host plants. *Journal of Chemical Ecology.* **15** (1), 439-446.

Netterville, R.M. 1994 *Identification Manual for Citrus Pests.* South Africa: Outspan Citrus Centre. 83p.

Oliveira e Castro, F.R. 1974 Considerações sobre o fomento da fruticultura em Moçambique. Gazeta do Agricultor. **29** (297),135-140.

Oloya, M. 1964 *Some Observations on the Biology of Psyllids, Arytaina genistae and Arytaina spartii on Broom, Sarothammus scoparius, and the Effect of Crowding on their Fecundity.* London: D.I.C. Thesis, Imperial College.

Olmi, M. 1985 *Apontamentos de Entomologia Agrícola.* Parte I e II. Maputo: U.E.M.

Parry, W. H. 1974 The effects of nitrogen levels in Sitka Spruce needles on *Elatobium abietinum* populations in north-eastern Scotland. *Oecologia.* **15**, 305-320.

Palgrave, K. C. 1977 *Trees of Southern Africa.* 4th edition. Struik Publishers, Cape Town. 959 p.

Paterson, C.G. & Fernando, C.H. 1971 Studies on the spatial heterogeneiy of shallow water benthos with particular reference to the Chironomidae. *Canadian Journal of Zoology.* **49**, 1013-1019.

Paterson, H.E.H. 1991 The recognition of cryptic species among economically important insects. In: *Heliothis: Research Methods and Prospects.* 1-10pp. (ed.) M.P. Zalucki. Springer-Verlag New York Inc.

Pedigo, L.P. 1997 Insect Ecology. In: *ENT 671 Lecture Notes* http://www.ent.iastate.edu/dept/courses/ent671/syllabus.html

Peng, C.; Brewer, G.J. 1994 Spatial distribution of the red sunflower seed weevil (Coleoptera: Curculionidae) on sunflower. *Environmental Entomology.* **23** (5), 1101-1105.

Pimentel, D. 1961 Animal population regulation by the genetic feed-back mechanism. *Am. Nat.* **95**: 65-79.

Podoler, H. & Rogers, D. 1975 A new method for the identification of key factors from life-tables. *J. Animal Ecology.* **44**, 85-114.

Pough, F.H. & Brower, L.P. 1977 Predation by birds on great southern white butterflies as a function of palatability, sex and habitat. ***Am. Midl. Nat.*** **98**, 50-58.

Price, W.P. 1984 *Insect Ecology.* USA: John Wiley & Sons. 607 p.

Price, P.W. 1988 Inversely density-dependent parasitism: the role of plant refuges for hosts. *Journal of Animal Ecology.* **57**, 89-96.

Price, P.W. 1990 Evaluating the role of natural enemies in latent and eruptive species: new approaches in life table construction. In: *Population Dynamics of Forest Insects.* pp 221-232. (eds. A.D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept LTD.

Reddy, S.OJ. 1986 Agro-climate of Mozambique as Relevant to Dry-Land Agriculture. *Comunicação* 47, Série Terra e Água. Maputo: INIA.

Reichstein, T.; Von Euw, J.; Parsons, J.A.; Rothschield, M. 1968 Heart poisons in the monarch butterfly. *Science.* **161**, 861-866.

Richards, O.W.; Davies, R.G. 1978 *IMMS' Outlines of Entomology.* 6th ed. London: Chapman and Hall Ltd. 254 p.

Richards, O.W. & Southwood, T.R.E. 1968 The abundance of insects: introduction: In: *Insect Abundance.* pp 1-7. Symposium, 4. The Royal Entomological Society. (ed. T.R.E. Southwood). Surrey: Adlard And Son Limited.

Roland, J. 1990 Interaction of parasitism and predation in the decline of winter moth in Canada. In: *Population Dynamics of Forest Insects.* pp 289-302. (eds. A.D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Room, P.M.; Titmarsh, I.J.; Zalucki, M.P.; 1991 What are life tables? In: *Heliothis: Research Methods and Prospects.* pp 69-89. (ed.) M.P. Zalucki. Springer-Verlag New York Inc.

Rose, M.R. 1987 *Quantitative Ecological Theory: an Introduction to Basic Models.* U.K: Billing & Sons Limited. 203 p.

Royama, T. 1977 Population persistence and density-dependence. *Ecological Monographs.* **47**, 1-35.

Royama, T. 1981 Evaluation of mortality factors in insect life table analysis. *Ecological Monographs.* **51**, 495-505.

Santos, L. & Shields, E.J. 1998 Temperature and diet effect on black cutworms (Lepidoptera: Noctuidae) larval development. *Journal of Economic Entomology.* **91** (1), 267-273.

Sawada, K. ; Nomakuchi, S.; Masumoto, T.; Suzuki, N.; Shiotsu, Y.; Koyanagi, H.; Sugiura, N.; Okuda, N. 1997 Fluctuation of protandry in eclosion of *Anthocharis scolymus* (Lepidoptera: Pieridae): can males eclose optimally under evolutionary equilibrium. *Environ. Entomol.* **26** (3), 572-579.

Scholtz, C. H.; Holm, E. 1985 *Insects of Southern Africa.* Durban: Butterworth Publishers (PTY) LTD. 502 p.

Schultz, J.C.; Foster, M.A.; Montgomery, M.E. 1990 Host plant-mediated impacts of a baculovirus on gypsy moth populations. In: *Population Dynamics of Forest Insects.* pp 303-313. (eds. A.D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Scriber, J.M. & Slansky, F. Jr. 1981 The nutritional ecology of immature insects. *Annual Review of Entomology.* **26**, 183-211.

Searle, C.M. 1964 A list of the insect enemies of citrus pests in southern Africa. Nelspruit: Institute for Tropical and Subtropical Crops. *Tech. Comm. Dept. Agric. Serv. Repub. S. Africa.* vol. **30**, 1-18.

Seymour, J.E. & Jones, R.E. 1991 Evaluating Natural Enemy Impact on *Heliothis.* In: *Heliothis: Research Methods and Prospects.* (ed.) Zalucki, M.P 80-89pp. Springer-Verlag New York Inc.

Slansky, F.Jr. & Scriber, J.M. 1985 Food consumption and utilization. pp. 87-163. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology.* Vol. 4. (eds. G.A. Kerkut & L.I. Gilbert). Oxford: Pergamon.

Southwood, T.R.E. 1978 *Ecological Methods with Particular Reference to the Study of Insect Populations.* 2nd Ed. Cambridge: Cambridge University Press. 524 p.

Stamp, N.E. 1994 Simultaneous effects of potassium, rutin and temperature on performance of *Manduca sexta* caterpillars. *Entomologia Experimentalis et Applicata.* **72**, 135-143.

Taylor, L.R. 1961 Aggregation, variance and the mean. Nature (Lond.). **189**, 732-735.

Taylor, L.R. 1984 Assessing and interpreting the spatial distribution of insect populations. Annual Review of Entomology. **29**, 32-57.

Taylor, L.R.; Woiwod, I.P.; Perry, J.N. 1978 The density-dependence of spatial behaviour and the rarity of randomness. *Journal of Animal Ecology*. **47**, 383-406.

Taylor, L.R. 1984 Assessing and interpreting the spatial distribution of insect populations. *Annual Review of Entomology*. **29**, 321-357.

Taylor, R.A.J.; Lindquist, R.K.; Shipp, J.L. 1998 Variation and consistency in spatial distribution as measured by Taylor's Power Law. *Environmental Entomology*. **27** (2), 191-201.

Tiritilli, M.E. & Thompson, J.N. 1988 Variation in swallowtail/plant interactions: host selection and the shapes of survivorship curves. *Oikos*. **53**, 153-160.

Tukey, J.W. 1957 On the comparative anatomy of transformations. *Annual Math. Statist*. **28**, 602-632.

Turley, M.; Granett, J.; Omer, A.D.; De-Benedictis, J.A. 1996 Grape phylloxera (Homoptera: Phylloxeridae) temperature threshold for establishement of feeding sites and degree-day calculations. *Environ. Entomol*. **25** (4), 842-847.

Tsubaki, Y. 1973 The natural mortality and its factors of the immature stages in the population of the swallow-tail butterfly *Papilio xuthus*. *Japanese Journal of Ecology*. **23** (2, 10), 210-217.

Twine, P.H. & Evans, M.L. 1991 Collecting immature and adult *Heliothis* for distribution, host and parasite records. In: *Heliothis: Research Methods and Prospects*. 11-21 pp. (ed.) M.P. Zalucki. Springer-Verlag New York Inc.

Underwood, A. J. 1997 *Experiments in Ecology. Their logical design and interpretation using analysis of variance*. Cambridge: Cambridge University Press. 504 p.

Underwood, D.L.A. 1994 Methods for sexing Lepidoptera larvae using external morphology. *Journal of the Lepidopteralist' Society*. **48** (3), 258-263

Valle Y March, R. G. 1968 Importância Agrícola de Algumas Espécies Entomológicas de Moçambique. Contribuiçao para o seu Estudo. *Comunicaçoes* 16. INIA, L.Marques. 148 p.

Van Son, L 1949 *The Butterflies of Southern Africa. Part I. Papilionidae and Pieridae.* Pretoria: The Transvaal Museum. 237 p.

Varley, G.C. 1963 The interpretation of change and stability in insect populations. *Proceedings of the Research Ent. Society of London.* **27**, 52-57.

Varley, G.C. & Gradwell, G.R. 1968 Population models for the winter moth. In: *Insect Abundance.* pp 132-142. Symposium, 4. The Royal Entomological Society. (ed. T.R.E. Southwood). Surrey: Adlard And Son Limited.

Varley, G.C. & Gradwell, G.R. 1960 Key factors in population studies. *Journal of Animal Ecology.* **29**, 399-401.

Varley, G.C.; Gradwell, GR.; Hassell, M.P. 1973 *Insect Population Ecology: An Analytical Approach.* Great Britain: Blackwell Scientific Publications. 212 p.

Vesala, T. 1998 On the concept of leaf boundary layer resistance for forced convection. *J. Theor. Biol.* **194** (1), 91-100.

Wagner, T.L.; Wu, H-I.; Sharpe, P.J.H.; Schoolfield, R.M.; Coulson, R.N. 1984 Modelling insect development rates: a literature review and application of a biophysical model. *Annals of the Entomological Society of America.* **77** (2), 208-225.

Waloff, N. 1968 A comparison of factors affecting different insect species on the same host plant. In: *Insect Abundance.* pp 76-87. Symposium, 4. The Royal Entomological Society. (ed. T.R.E. Southwood). Surrey: Adlard And Son Limited.

Wardle, R.A. 1929 *The Problems of Applied Entomology.* England, Manchester University Press. 587 p.

Watanabe, M. 1976 A preliminary study on population dynamics of the swallow-tail butterfly, *Papilio xuthus*, in a deforested area. *Population Ecology Kyoto University.* **17**, 200-210.

Watanabe, M. 1979 Natural mortalities of the swallow-tail butterfly, *Papilio xuthus*, at patchy habitats along the flyways in a hilly region. *Japanese Journal of Ecology.* **29**, 85-93.

Watanabe, M. 1981 Population dynamics of the swallowtail butterfly *Papilio xuthus* L., in a deforested area. *Res. Pop. Ecol.* **23**,74-93.

Waters, W. E. 1959 A quantitative measure of aggregation in insects. *Journal of Economic Entomology.* **52** (6), 1180-1184.

Waters, W.E. & Henson, W.R. 1959 Some sampling attributes of the negative binomial distribution with special reference to forest insects. *For. Sci.* **5** (4), 397-412.

Watt, A.D.1990 The consequences of natural, stress-induced and damage-induced differences in tree foliage on the population dynamics of the pine beauty moth. In: *Population Dynamics of Forest Insects.* pp 157-168. (eds. A.D. Watt; S.R. Leather; M.D. Hunter; N.A. Kidd). Andover: Intercept Ltd.

Wellington, W.G. 1960 Qualitative changes in natural populations during changes in abundance. *Canadian Journal of Zoology.* **38,** 289-314.

Wellington, W.G. 1964 Qualitative changes in unstable environments. *Canadian Entomologist.* **96**, 436-451.

Wellington, W.G. 1965 Some maternal influences on progeny quality in the western tent caterpillar, *Malacosoma pluviale* (Dyar). *Canadian Entomologist.* **97**, 1-14.

West Coast Investment Initiative 1998 *South Africa Profile.* Cape Town: WCII.

White, G.G. 1987 Effects of temperature and humidity on the rust-red flour Tenebrionidae, in wheat grain. *Australian Journal of Zoology.* **35** (1), 43-59.

White, T.C.R. 1984 The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia.* **63**, 90-105.

White, T.C.R. 1986 Weather, *Eucalyptus* dieback in New England, and a general hypothesis of the cause of dieback. *Pacific Science.* **40**, 58-78.

Whittaker, J.B. 1971 Population changes in *Neophilaenus lineatus* (L.) Homoptera: Cercopidae) in different parts of its range. *Journal of Animal Ecology.* **40**, 425-443.

Williams, D.W. & Liebhold, A.M. 1995a Forest defoliators and climatic change: potential changes in spatial distribution of outbreaks of western spruce budworm (Lepidoptera: Tortricidae) and gypsy moth (Lepidoptera: Lymantridae). *Environ. Entom.* **24**, 1: 1-9.

Williams, D.W. & Liebhold, A.M. 1995b Influence of weather on the synchrony of gypsy moth (Lepidoptera: Lymantridae) outbreaks in New England. *Environ. Entomol.* **24** (5), 987-995.

Williams, J.G. 1969 *A Field Guide to the Butterflies of Africa.* 436 Species Described 283 Illustrated in Colour with 425 Line Drawings. Collins Clear-Type Press, London.

Wilson, F. 1968 Insect abundance: prospect. In: *Insect Abundance.* pp 143-158. (ed. T.R.E. Southwood) Symposium, 4. The Royal Entomological Society. Surrey: Adlard And Son Limited.

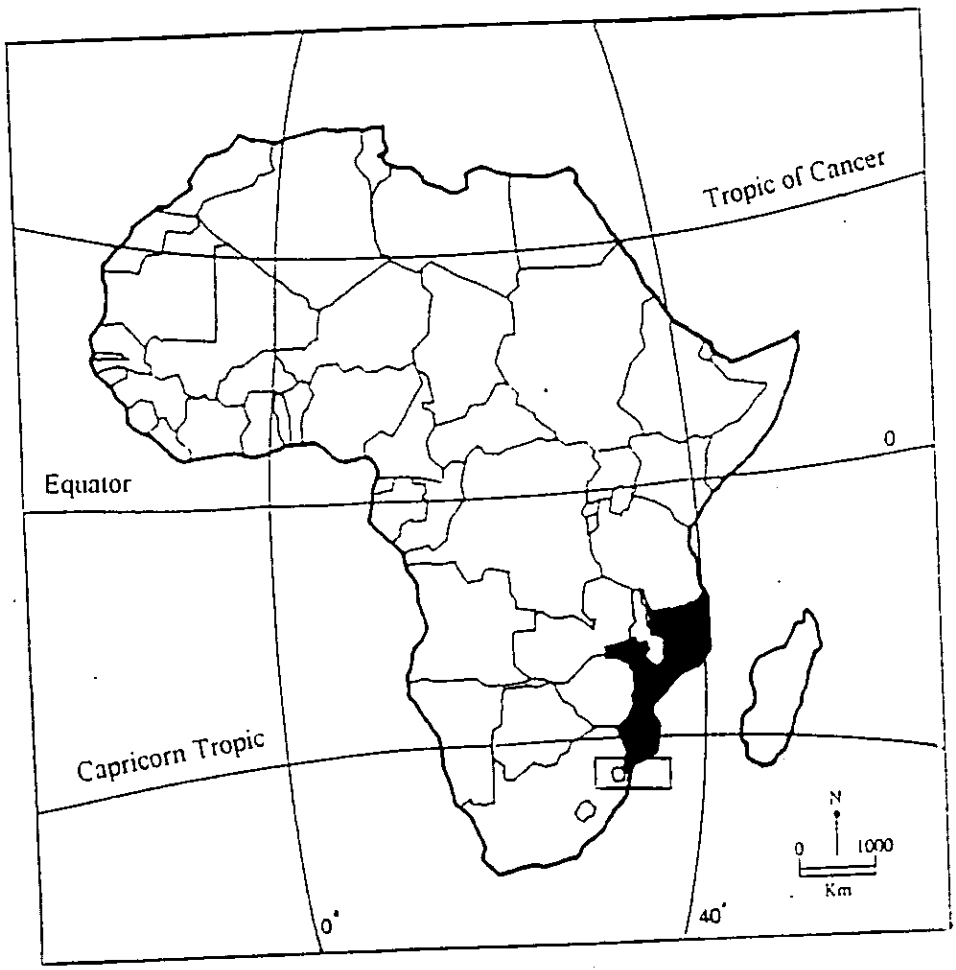
Worner, S.P. 1992 Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. *Environ. Entomol.* **21** (4), 689-699.

Wynne-Edwards, V.C. 1962 *Animal Dispersion in Relation to Social Behaviour.* New York: Hafner. 653 p.

Zhang, G.H. & Schlyter, F. 1996 High recaptures and long sampling range of pheromone traps for all web worm moth *Hyphantria cunea* (Lepidoptera: Arctiidae) males. *J. Chem. Ecol.* **22** (10) 1783-1796.

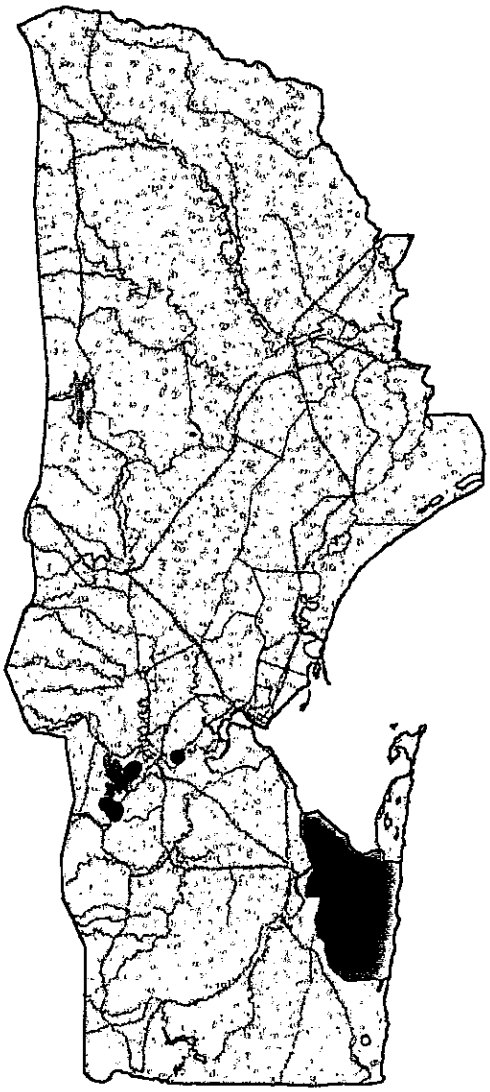
Zhang, H. ; Dotson, P. 1994 The use of microwave muffle furnace for dry ashing plant tissue samples. *Communications in Soil Science and Plant Analysis.* Vol. **25** (9&10), 1321-1327.

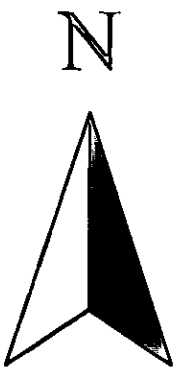
Appendix 1.1 The geographical location of Mozambique (black on map; the Maputo Province within the quadrangular line).



Adapted from Ministério da Eduçacão (1986)

Appendix 1.2 Field work sites





30000 0 30000 60000 Meters

LEGEND

Reserve

Papilio sp study areas

Rivers

Roads

Maputo province

Appendix 3.1 Summary of meteorological data during the sampling period in the Umbelúzi region

Month	Temperatures (°C)			Relat.	Wind	Rad.	Rain	Evap.
	Min.	Max.	Aver.	H.(%)	km/h	(%)	mm	mm
Feb.98	22.5	31.8	27.0	69.7	5.8	65.0	59.1	91.5
Mar.98	19.0	33.6	26.3	68.7	5.0	65.0	84.5	97.4
Apr.98	18.9	31.4	25.2	66.7	3.8	69.0	14.9	104.2
May98	14.9	30.1	22.5	48.3	3.6	45.3	82.0	121.8
Jun.98	11.6	28.1	19.8	57.0	3.5	81.0	0.0	111.9
Jul.98	12.0	27.1	19.6	59.3	5.4	72.0	4.2	112.8
Aug.98	13.4	28.3	20.9	53.0	6.0	74.0	2.9	131.4
Sep.98	16.4	29.7	23.1	58.0	6.2	58.0	19.5	129.6
Oct.98	18.1	28.5	23.4	66.0	6.6	41.0	98.0	94.8
Nov.98	20.3	29.7	25.0	69.33	6.5	51.0	142.2	94.5
Dec.98	21.4	30.3	25.8	68.67	6.7	49.0	99.5	59.2
Jan.99	22.5	31.4	26.9	72.67	5.4	61.0	175.8	92.0
Feb.99	21.4	30.6	26.0	73.33	5.3	56.0	230.0	68.7
Mar.99	22.1	30.7	26.3	72.33	4.8	66.0	58.5	79.9
Apr.99	18.5	29.4	24.0	74.67	4.3	64.0	42.1	68.8
May99	15.1	28.5	21.8	65.67	5.1	73.0	25.8	94.4
Jun.99	11.4	27.2	19.2	60.33	5.0	84.0	8.9	98.6

Appendix 3.1 (continued from previous page) Summary of meteorological data during the sampling period in the Umbelúzi region

Month	Temperatures (°C)			Relat.	Wind	Rad.	Rain	Evap.
	Min.	Max.	Aver.	H.(%)	km/h	(%)	mm	mm
Jul. 99	11.9	26.6	19.3	64.00	5.5	76.0	5.6	108.5
Aug.99	13.1	28.6	21.0	58.00	6.8	80	28.1	116.6
Sep.99	15.2	28.0	21.6	59.00	7.8	61	33.2	115.3
Oct.99	16.5	27.2	21.8	65.30	7.3	56	87.6	88.7
Nov.99	20.7	32.3	26.2	65.30	6.7	43	153.5	106.8
Dec.99	22.2	31.7	25.3	68.70	5.0	55	62.3	94.0
Jan.00	21.4	29.5	25.3	74.30	6.6	45	107.5	86.1

Min. = average minimum temperature

Max. = average maximum temperature

Aver.= average temperature

Relat. H.= average relative humidity

Wind = wind speed

Rad. = average radiation

Rain = total precipitation; Evap. = total evaporation

Appendix 3.2 Soil properties of two sites

Site/Deph (cm)	Extractable bases A	B	C	D	Organic Matter (%)	N (%)	P(Olsen) mg/100g	Texture (%) Sand	Lime	Cley	pH 1:2.5 H2O	KCl	Electric Conductivity (1:2.5)mS/cm
Olsa A/B													
0-20	8.53	3.70	0.44	1.30	2.25	0.01	0.34	45.00	28.00	26.00	7.17	6.65	0.19
20-35	6.70	3.50	0.42	0.70	2.03	0.06	0.34	60.17	15.08	24.75	6.95	6.27	0.15
35-58	3.90	3.20	0.40	0.90	1.61	0.15	0.21	37.00	45.00	18.00	7.23	5.86	0.11
58-80	5.71	3.12	1.21	1.52	1.01	0.06	0.58	78.04	7.06	14.83	8.10	6.45	0.12
+80	9.44	4.10	0.40	0.60	0.19	0.01	0.36	32.00	14.00	54.00	7.28	6.86	0.13
Olsa D													
0-20	5.20	2.00	0.50	0.60	0.82	0.06	0.32	61.00	15.00	23.00	6.41	4.95	0.12
20-40	1.00	0.24	0.00	0.00	0.91	0.02	0.35	62.00	22.00	16.00	8.13	7.52	0.09
+40	7.82	3.91	0.42	0.30	0.94	0.05	0.18	48.00	12.00	40.00	7.09	6.31	0.09

N = nitrogen

P = phosphorus

A= Ca^{2+}(Calcium); B= Mg^{2+}(Magnesium); C= Na^{+}(Sodium); D= K^{+} (Potassium)

Appendix: 3.3 Orchard age, species, cultivar and management practices (A=ground sprinkling, M= manual; Pesticides: Y= pesticides use, N=no pesticides application)

Site	field	age (years)	size (ha)	species	cultivar	plants	spacing	pesticides	irrigation
CITRUS	1	41	9.82	*C.paradisi*	Marsh	1094	8x8m	N	A
"	2	41	0.75	*C. sinensis*	Joppa	77	8x8m	N	A
"	3	41	1.98	*C. sinensis*	Joppa	249	8x8m	N	A
"	4	41	2.83	*C.paradisi*	Marsh	280	8x8m	N	A
"	5	41	3.01	*C.paradisi*	Marsh	341	8x8m	N	A
"	6	41	1.74	*C.paradisi*	Marsh	190	8x8m	N	A
"	7	30-40		*C.paradisi*	Marsh		8x8m	N	A
"	12	30-40	2.78	*C. sinensis*	Joppa	348	8x8m	N	A
"	13	30-40	2.69	*C. sinensis*	Valencia	379	8x8m	N	A
"	14	30-40	5.98	*C.paradisi*	Marsh	866	8x8m	N	A
"	15	30-40	6.05	*C.paradisi*	Marsh	860	8x8m	N	A
"	18	30-40		*C.paradisi*	Rose		8x8m	N	A
"	19	6	2.44	*C.paradisi*	Star Ruby	1141	7x3m	N	A
"	20	5	4.02	*C.paradisi*	Star Ruby	1800	7x3m	N	A
"	21	30-40	3.89	*C.paradisi*	Rose	1816	7x3m	N	A
"	22	30-40	2.19	*C. sinensis*	Valencia	306	8x8m	N	A
"	23	30-40	2.50	*C. sinensis*	Valencia	359	8x8m	N	A
"	24	30-40	3.14	*C. sinensis*	Valencia	465	8x8m	N	A
"	25	30-40	3.08	*C. sinensis*	Navel/Val.	521	8x8m	N	A
"	26	30-40	3:08	*C.paradisi*	Marsh	340	8x8m	N	A
"	27	30-40	2.65	*C.paradisi*	Marsh	247	8x8m	N	A
"	28	30-40	1.97	*C.paradisi*	Marsh	248	8x8m	N	A
"	29	30-40	3.08	*C.paradisi*	Marsh	320	8x8m	N	A
"	30	30-40	1.79	*C.paradisi*	Marsh	160	8x8m	N	A
"	31	30-40	2.05	*C.paradisi*	Marsh	240	8x8m	N	A
"	32	30-40	1.36	*C.paradisi*	Marsh	111	8x8m	N	A
"	33	6	2.14	*C.paradisi*	Rose	1012	7x3m	N	A

Appendix 3.3 (continued from previous page) : Orchard age, species, cultivar and management practices (A=ground sprinkling, M= manual; Pesticides: Y= pesticides use, N=no pesticides application)

Site	field	age (years)	size (ha)	species	cultivar	plants	spacing	pesticides	irrigation
CITRUS	34	30-40	1.33	*C. sinensis*	Valencia	634	7x3m	N	A
"	35	7	2.18	*C. sinensis*	Valencia	972	7x3m	N	A
"	36	6	4.11	*C.paradisi*	Marsh	1928	7x3m	N	A
"	37	30-40		*C.paradisi*	Marsh		7x3m	N	A
"	38	30-40	2.06	*C.paradisi*	Marsh	270	8x8m	N	A
"	39	30-40	4.16	*C.paradisi*	Marsh	518	8x8m	N	A
"	40	30-40	3.45	*C.paradisi*	Marsh	413	8x8m	N	A
"	total		94.30			18505		N	A
Olsa C.D	D2	35	6.2	*C. sinensis*	Valencia	378	8x8m	y	A
"	D3	35	6.5	*C. sinensis*	Valencia	612	8x8m	y	A
"	D4	35	6.7	*C.paradisi*	Marsh	353	8x8m	y	A
"	D5	12	6.9	*C. sinensis*	Valencia			y	A
"	D6	12	7.1	*C. sinensis*	Valencia			y	A
"	D11A	35	5.5	*C. sinensis*	Valencia	64	8x8m	y	A
"	D11B	35		*C.paradisi*	Marsh	213	8x8m	y	A
"	D12	35	4.1	*C.paradisi*	Marsh	415	8x8m	y	A
"	D21	35	2.4	*C.paradisi*	Marsh	1084	7x3m	y	A
"	D22	35	6.0	*C. sinensis*	Valencia	436	8x8m	y	A
"	D24	35	0.7	*C.paradisi*	Marsh	63	8x8m	y	A
"	s-total		52.1			7018		y	A
"	D7	≤2	5.2	*C.paradisi*	Marsh	2514	7x3m	y	A
"	D8	≤2	3.5	*C.paradisi*	Marsh	1673	7x3m	y	A
"	D9	≤2	2.2	*C.paradisi*	Marsh	1065	7x3m	y	A
"	D13	≤2	4.9	*C.paradisi*	Marsh	2335	7x3m	y	A
"	D14	≤2	3.9	*C.paradisi*	Marsh	1885	7x3m	y	A
"	D15	≤2	3.9	*C.paradisi*	Marsh	1887	7x3m	y	A

Appendix 3.3 (continued from previous page) : Orchard age, species, cultivar and management practices (A=ground sprinkling; M= manual; Pesticides: Y= pesticides use, N=no pesticides application)

Site	field	age (years)	size (ha)	species	cultivar	plants	spacing	pesticides	irrigation
Olsa C.D	D16	≤2	3.2	*C.paradisi*	Marsh	1553	7x3m	y	A
"	D17	≤2	2.6	*C.paradisi*	Star Ruby	1243	7x3m	y	A
"	D18	≤2	3.0	*C.paradisi*	Star Ruby	1433	7x3m	y	A
"	D19	≤2	2.9	*C.paradisi*	Star Ruby	1399	7x3m	y	A
"	D20	≤2	4.1	*C.paradisi*	Star Ruby	1973	7x3m	y	A
"	D23	≤2	6.0	*C.paradisi*	Oroblanco	2099	7x3m	y	A
"	D25	≤2	1.2	*C.paradisi*	Marsh	583	7x3m	y	A
"	D26	≤2	4.3	*C.paradisi*	Marsh	2058	7x3m	y	A
"	s-total	≤2	50.9			23700		y	A
"	total	≤2	103.0			30718			
Olsa A&B	A1	35	4.3	*C.paradisi*	Marsh	1097	6x3m	y	A
"	A2	35	3.7	*C.paradisi*	Marsh	1225	6x3m	y	A
"	A3	35	5.9	*C. sinensis*	Marsh	386	8x8m	y	A
"	A6	12	5.2	*C. sinensis*	Valencia	1295	8x5m	y	A
"	A7	12	5.1	*C. sinensis*	Valencia	1282	8x5m	y	A
"	A8	12	5.0	*C. sinensis*	Valencia	1265	8x5m	y	A
"	A9	12	5.1	*C. sinensis*	Valencia	1286	8x5m	y	A
"	A14	35	1.6	*C. sinensis*	Valencia	188	8x8m	y	A
"	A15	35	2.0	*C. sinensis*	Valencia	257	8x8m	y	A
"	A16	5	1.8	*C.paradisi*	Star Ruby	1000	6x3m	y	A
"	A17	5	1.7	*C.paradisi*	Star Ruby	784	6x3m	y	A
"	s-total		41.4			10065		y	A
"	A2	≤2	4.2	*C.paradisi*	Marsh	2560	6x3m	y	A
"	A4	≤2	2.2	*C.paradisi*	Star Ruby	857	7x3m	y	A
"	A5	≤2	5.3	*C.paradisi*	Star Ruby	2510	7x3m	y	A
"	s-toal		11.7	*C.paradisi*		5927		y	A

Appendix 3.3 (continued from previous page) : Orchard age, species, cultivar and management practices (A=ground sprinkling; M= manual; Pesticides: Y= pesticides use, N=no pesticides application)

Site	field	age (years)	size (ha)	species	cultivar	plants	spacing	pesticides	irrigation
Olsa A&B	B2	35	3.7	*C.paradisi*	Marsh	249	8x8m	y	A
"	B3	35	3.7	*C.paradisi*	Marsh	280	8x8m	y	A
"	B4	35	3.7	*C.paradisi*	Marsh	239	8x8m	y	A
"	B5	35	3.7	*C.paradisi*	Marsh	225	8x8m	y	A
"	B6	35	3.7	*C. sinensis*	Valencia	476	8x4m	y	A
"	B7	35	3.7	*C. sinensis*	Valencia	1041	8x4m	y	A
"	B8	35	1.8	*C.paradisi*	Marsh	147	8x8m	y	A
"	B9	35	1.8	*C.paradisi*	Marsh	134	8x8m	y	A
"	s-total		25.8			2781			
"	total		78.9			18773			
1°Maio	02	11	5.5	*C.paradisi*	Rose	1215	8x4.8m	y	A
"	03	11	2.5	*C.paradisi*	Rose	635	8x4.8m	y	A
"	05	11	6.2	*C.paradisi*	Rose	1391	8x4.8m	y	A
"	07	11	3.3	*C.paradisi*	Rose	1010	8x4.8m	y	A
"	11	11	4.4	*C.paradisi*	Rose	711	8x4.8m	y	A
"	29	11	6.06	*C.paradisi*	Rose	2213	8x4.8m	y	A
"	s-total		27.96			7175		y	A
"	04	35	6.2	*C.paradisi*	Marsh	930	7x7m	y	A
"	08	4	2.0	*C.paradisi*	Marsh	1556	6x3m	y	A
"	15	35	5.3	*C.paradisi*	Marsh	668	7x7m	y	A
"	16	35	4.04	*C.paradisi*	Marsh	744	7x7m	y	A
"	17	35	5.8	*C.paradisi*	Marsh	1465	7x3.5m	y	A
"	20	35	5.0	*C.paradisi*	Marsh	1307	7x7m	y	A
"	21	35	4.06	*C.paradisi*	Marsh	984	7x7m	y	A
"	22	35	5.1	*C.paradisi*	Marsh	1503	7x7m	y	A
"	23	35	5.5	*C.paradisi*	Marsh	1778	7x3.5m	y	A

Appendix 3.3 (continued from previous page) : Orchard age, species, cultivar and management practices (A=ground sprinkling; M= manual; Pesticides: Y= pesticides use, N=no pesticides application)

Site	field	age (years)	size (ha)	species	cultivar	plants	spacing	pesticides	irrigation
1°Maio	24	35	5.97	*C.paradisi*	Marsh	2125	6x3m	y	A
"	25	35	4.6	*C.paradisi*	Marsh	746	7x3.5m	y	A
"	26	35	4.3	*C.paradisi*	Marsh	1084	7x3.5m	y	A
"	s-total								
"	12	4	2.5	*C.sinensis*	Navel	783	8x4m	y	A
"	13	4	1.21	*C.paradisi*	Star Ruby	673	6x3m	y	A
"	24A	4	0.44	*C.paradisi*	Star Ruby	370	6x3m	y	A
"	27	4	1.7	*C.paradisi*	Star Ruby	1194	6x3m	y	A
"	s-total		5.85			3020			
"	06	35	6.02	*C.sinensis*	Valencia	656	7x7m	y	A
"	07	35	2.8	*C.sinensis*	Valencia	690	7x7m	y	A
"	10	35	3.5	*C.sinensis*	Valencia	567	7x7m	y	A
"	12	35	2.69	*C.sinensis*	Valencia	841	8x4m	y	A
"	13	35	2.98						
"	14	35	2.4						
"	18	35	5.3						
"	19	35	4.7						
"	28	35	4.0	*C.sinensis*	Valencia	846	7x7m	y	A
"	30	35	5.07	*C.sinensis*	Valencia	792	7x7m	y	A
"	31	35	7.4	*C.sinensis*	Valencia	716	7x7m	y	A
"	s-total		46.86			5108			
"	24B		11.9			460			
"	1A	1	5.0	*C.paradisi*	Marsh	2858	7x3m	y	A
"	1	2	4.095	*C.paradisi*	Marsh	1482	6x3m	y	A
"	1	2		*C.paradisi*	Marsh	1093		y	A
"	9	2	7.0	*C.paradisi*	Marsh	2083	7x3m	y	A

Appendix 3.3 (continued from previous page) : Orchard age, species, cultivar and management practices (A=ground sprinkling; M= manual; Pesticides: Y= pesticides use, N=no pesticides application)

Site	field	age (years)	size (ha)	species	cultivar	plants	spacing	pesticides	irrigation
1°Maio	11	2	3.0	*C.paradisi*	Marsh	1019	8x3m	y	A
"	23	2	2.0	*C.paradisi*	Marsh	1090	7x3m	y	A
"	24	2	1.0	*C.paradisi*	Marsh	677	6x3	y	A
"	s-total		4.113			10302			
"	27A	1		*C.paradisi*	Star Ruby		7x3m	y	A
"	27B	2		*C.paradisi*	Star Ruby		6x3	y	A
"	32	1		*C.paradisi*	Star Ruby		6x3	y	A
"	s-total		6.42			2820			
"	total		160.973			43775			

Appendix 3.4 Procedures for soil analysis (as guide of the nutrient status of the sites)

Preliminary treatments

Values of pH were measured on fresh samples with a pH meter after adding water to soil to the proportion of 1:2.5 and potassium chloride. The material was mixed and half an hour allowed for the material to settle. Readings were made when the temperature of the buffers and the sample solutions were the same to set the temperature compensator.

Soil samples were placed in a container and air dried first and oven dried until they reached a constant weight at the temperature of 40 °C degrees. A 2mm stainless steel mesh sieve was used to sieve the material. After sieving,the samples were, separately, mixed.

Extraction of elements

Cation exchange capacity

Reagentes: Ammonium acetate M (pH 7)
575 ml of glacial acetic acid
600 ml ammonium solution (0.800) to 2 litres water
dilution of 10 litres after mixing
alcohol 60% (v/v)
Potassium chloride 5% (w/v)

Ten g of air-dred sieved soil were weighed and added to 250 ml ammonium acetate. The solution was filtered. The residue was washed with 50 ml portions of alcohol. 60% until removing excess of ammonium acetate. Ten ml 10% w/v NH_4 Cl solution were added to the first portion of the alcohol. Filtrate was tested for chloride with $AgNO_3$ solution. Residue was leached with 10 ml of dilute KCl solutions followed by other portions of 5% KCl solution. Leachate was collected in a 250 ml capacity vessel. Same procedures was used to collect blank leachates. NH_4^+ -N was determined in an aliquot of the leachate by distillation and titration with M/140 HCl.

Calculation

For 1ml M/140 HCl =0.1 mg NH_4^+ -N

$$\text{CEC (cation exchange capacity) (me 100 gr}^{-1}) = \frac{\text{titre HCl (ml) x leachate volume (ml)}}{\text{1.4 x aliquot (ml) x sample wt (g)}}$$

Total major exchangeable bases and hydrogen

Extractants: Ammonium acetate M, pH 7 as in previous experiment
Acetic acid 2.5% v/v, diluting 250 ml of acetic acid to 10 litres water.
10 g soil were weighed and placed into a 500 ml polythene container
250 ml extractant were added and shaken for 1 hour on a shaker and then filtered
Blank solutions were prepared using the same procedure
Cations were determined in the filtrate.

Nitrogen

Water was used as extractant of nitrogen in fresh soil at the ratio 100:1.

Available Phosphorus according to Olsen

Principle

The sample was extracted with a sodium bicarbonate solution of pH 8.5. In the coloration process the phosphate in the extract formed a blue coloured complex with reduced molybdenum salts. This phosphorous-molybdenum blue was determined spectrophotometrically at 882 nm.
Soil samples were extracted with Olsen's reagent 0.5 M $NaHCO_3$ at pH 8.5 at the ratio 20:1 for air-dried soil samples after shaking for half an hour.

Apparatus

spectrophotometer
10 ml cuvettea and 882 nm wavelength
polythene shaking bottles of 200 or 250 ml
reciprocal shaker
volumetric flasks of 50.0 ml

Reagents

Extracting solution Olsen
Sodium bicarbonate ($NaHCO_3$) was weighd to 42 g in a 1.0 l beaker. 980 ml of distilled water was added and allowed to dissolve. The solution pH was adjusted to 8.5 by adding 1.0 M NaOH. This was made to 1000 ml with distilled water.

Sodium hydroxide 1.0 M

40.0 gr of sodium hydroxide (NaOH) were weighed into a 1.0 l beaker. Distilled water was added to dissolve. Distilled water was added to make up 1.0 l and mixed before storing the solution in a polythene bottle.

Sulphuric acid solution 5N

800 ml of distilled water were poured into a 1 l beaker. 140 ml of concentrated H_2SO_4 were added to the beaker and mixed. Distilled water was added to make up to 1000 ml.

Ammonium molybdate 1% in sulphuric acid

22 g of $(NH_4)_6MO_7O_{24}.4H_2O$ (ammonium molybdate) were weighed in a 2 l erlenmeyer flask. 500 ml of distilled water were added. The solution was heated until getting clear and was let to cool down. The solution was filtered when a precipitate remained on the bottom. 500 ml of distilled water were added. 250 ml of concentrated H_2SO_4 were added. The solution was transferred into a 2000.0 ml volumetric flask and made up to the mark with distilled water and mixed. The solution was stored in a polythene bottle.

Colouring reagent, freshly prepared (1000 ml)

2.87 g of ascorbic acid were weighed on a watch glass. 0.074 g of potassium antimonyl tartrate ($KSBOC_4H_4O_6 . 0.5 H_2O$) were weighed on a watch glass. The chemicals were transferred into a 1000.0 ml volumetric flask. 500 ml of distilled water were added. The solution was stirred with a magnetic stirrer to dissolve. 300 ml of 1% ammonium molybdate solution were added and mixed. Distilled water was added to make up the solution until the mark. The solution was then mixed and transferred to a brown bottle.

Phosphate standards

Stock solution A 500 mg P/l

2.195 gr of dry potassium dihydrogen phosphate (KH_2PO_4) were weighed on a watch glass and transferred to a 1000.0 ml volumetric flask to dissolve in distilled water. 5

drops of concentrated H_2SO_4 were added. Distilled water was added to make up the solution until the mark.

Solution B 10.0 mg P/l

5.0 ml of stock solution A were pipetted into a 250.0 ml volumetric flask. Distilled water was added to make up to the mark.

Solution C

Stated volumes of standard solution B were pipetted into 100.0 ml volumetric flasks. Distilled water was used to make up to the mark before mixing.

Standard number	Volume (ml) of Solution B	mg P/l
S0	0.0	0.0
S1	10.0	1.0
S2	20.0	2.0
S3	30.0	3.0
S4	40.0	4.0
S5	50.0	5.0

Procedure

Extraction

2.50 gr of sample were weighed in a 100 ml plastic shaking bottle. One standard sample and two blanks were included. 50.0 ml of Olsen extracting solution were added, using a dispenser. The solution was shaken for 30 minutes lengthwise in horizontal position in a reciprocal shaker at 150 rotations per minute and then filtered through a fine filter (Whatman number 5)

Colouration

5.0 ml of phosphate standard solutions (S0-S5)were pipetted into 50.0 ml volumetric flasks. 10.0 ml of the same sample filtrates and blanks were pipetted into 50.0 ml volumetric flasks. Except for the flasks containing the phospahte standard solutions, 1.0 ml of 5 N H_2SO_4 were pipetted into all other volumetric flasks and the flasks swirled. When gas CO_2 stopped, 20 ml of distilled water were added to all volumetric flasks and mixed. 15.0 ml of colouring reagent were pipetted into all volumetric flasks, mixed and made up to the marker with distilled water. The blue colour developed after 30 minutes.

Absorbance of the standards, blanks and samples were measured in a 10 mm cuvette at 882 nm.

Calculations

A calibration graph of the absorbance was plotted against the P concentration of the standards S0 - S5 (in mg P/l). The phosphate concentrations of the samples were read from the graph.
Phosphate in mg P/kg (ppm P) =

$$\frac{(A - B) \times V \times S \times Mc}{W \times F}$$

where A = mg P/l in the sample filtrate
B = average value in mg P/l of the blank filtrates
V = volume of the added Olsen extracting solution in ml
S = ml of phosphate standard solution pipetted for colouration
F = ml of filtrate pipetted for colouration
Mc = moisture correction factor

Results were recorded in whole figures.

Organic Carbon

Principle

Soil organic matter was oxidized at a temperature of approximately 120 °C with a mixture of potassium dichromate and concentrated sulphuric acid (wet combustion). The excess potassium dichromate was titrated against ammonium ferrous sulphate with dephenylamine as an indicator. Before titration, phosphoric acid was added to form a complex with the interfering iron , which provided a sharper colour change of the indicator.

Apparatus

burette, graduated in 0.01 ml

erlenmeyer flask of 500 ml
mortar and pastle
sieve of 0.5 mm
dispenser
measuring cylinder
volumetric flask of 1000.0 ml
wooden pad

Reagents

Potassium dichromate 0.1667 M (1.000 N)

55 g of $K_2Cr_2O_7$ were dried at 150-200° C for 2 hours and let to cool in a desiccator. 98.0664 g of dried $K_2Cr_2O_7$ were weighed, transferred into a 2000.0 ml volumetric flask and dissolved in distilled water. Distilled water was added to make up to the mark and mixed.

Concentrated sulphuric acid

H_2SO_4 with a concentration not less than 95%.

Concentrated 0-phosphoric acid

H_2SO_4 with a concentration of 85%.

Ammonium ferrous sulphate 0.25 M

100 gr of $(NH_4)_2\ SO_4\ .\ FeSO_4\ .\ 6H_2O$ were weighed and transferred into a 1000.0 ml volumetric flask. 700 ml of concentrated sulphuric acid were added. Distilled water was added to make up to the mark and then, mixed. The solution was stored in a brown bottle.

Each series of determinations were standardized against $K_2Cr_2O_7$.

Diphenylamine indicator 1%

20 ml of distilled water were poured into a 250 ml erlenmeyer flask. 50 ml of

concentrated sulphuric acid were added to the flask and let to cool. Other 50 ml of concentrated sulphuric acid were added to the erlenmeyer flask and let to cool. 1.0 gr of diphenylamine was added, dissolved and mixed. The solution was stored in a brown bottle.

Procedure

The series consisted of 14 samples, including one blank and one standard sample. 2 g of sample were ground in a mortar to pass a 0.5 mm sieve. 0.500 g of the sieved sample were weighed and placed into a 250 ml erlenmeyer flask, with the accuracy of 0.001 g. 5.0 ml of 0.1667 M potassium dichromate solution were added with a burette and the erlenmeyer flask was swirled to disperse the soil in the solution. For the blank, 10 ml of concentrated sulphuric acid were added with a dispenser and swirled for one minute. The flask was left for 30 minutes on a wooden pad. 80 ml of distilled water were added with a measuring cylinder. 10 ml of phosphoric acid were added with a measuring cylinder and 1.0 ml of diphenylamine indicator was added. The sample was titrated with ammonium ferrous sulphate. Near the end point the colour changed to deep violet-blue.Mmonium ferrous sulphate was added to slow down the titration. The colour changed to bright gree

For the blank

After the titration of the blank, the burette reading was recorded. 5.0 ml of 0.1667 M $K_2Cr_2O_7$ were added to the titrated blank. It was titrated again with ammonium ferrous sulphate for the standardizing the ammonium ferrous sulphate solution

Calculations

Normality of the ammonium ferrous sulphate solution

$$N = \frac{F \times K}{T}$$

where F = ml of potassium dichromate added to the titrated blank
K = Normality of the potassium dichromate
T = ml of ammonium ferrous sulphate used for the second titration of the blank
N = Normality of the ammonium ferrous sulphate. Records made in 4 decimal places.

Organic carbon content

$$5\ 0.C = \frac{(B - A) \times N \times 0.396 \times Mc}{W}$$

where B = ml ammonium ferrous sulphate used for the first blank titration
A = ml ammonium ferrous sulphate used for the sample
N = Normality of the ammonium ferrous sulphate
W = weight of the sample in grammes
Mc = moisture correction factor

0.6827 was a constant factor for the calculation of the organic matter content and results were recorded in 1 decimal place.

Appendix 3.5 Summary of data on population distribution studies (site 1= INIA, site 2= 1° Maio, site3=Olsa Citrus D, site 4=Olsa citrus A&B; the figures for months correspond to the months of the year; cultivar 1= Valencia, cultivar 2=Marsh, cultivar 3= Star Ruby, 6=Oroblanco, 7= Rose; s.error = standard error)

site	month	orchard	cultivar	mean	variance	s. error
3	4	7	2	0.12000	0.1875	0.03590
2	4	32	3	0.09600	0.1510	0.01590
2	5	1	2	0.15330	0.1844	0.03510
2	5	10	6	0.13000	0.1748	0.04180
2	5	11	2	0.00000	0.0000	0.00000
2	5	13	2	0.06500	0.0812	0.02010
2	5	23	2	0.16000	0.2569	0.05070
2	5	27	3	0.13670	0.2321	0.02780
2	5	32	3	0.07580	0.2050	0.01310
2	5	33	2	0.21000	0.4783	0.04890
2	5	9	2	0.10500	0.1447	0.02690
4	5	2	2	0.06000	0.0667	0.01830
4	5	4	3	0.09000	0.1029	0.03210
4	5	5	3	0.07000	0.0657	0.02560
3	5	13	2	0.06670	0.0628	0.02290
3	5	14	2	0.05710	0.0542	0.01970
3	5	15	2	0.17690	0.1932	0.03860
3	5	16	2	0.17860	0.2197	0.03960
3	5	17	3	0.03080	0.0301	0.01520
3	5	18	2	0.05000	0.0479	0.02000
3	5	2	1	0.00000	0.0000	0.00000
3	5	20	3	0.24000	0.2853	0.05340

Appendix 3.5 (continued from previous page) Summary of data on population distribution studies (site 1= INIA, site 2= 1° Maio, site3=Olsa Citrus D, site 4=Olsa citrus A&B; the figures for months correspond to the months of the year; cultivar 1= Valencia, cultivar 2=Marsh, cultivar 3= Star Ruby, 6=Oroblanco, 7= Rose; s.error = standard error)

site	month	orchard	cultivar	mean	variance	s. error
3	5	23	6	0.00000	0.0000	0.00000
3	5	26	2	0.09000	0.1029	0.03210
3	5	7	2	0.15000	0.1288	0.03590
3	5	8	2	0.29000	0.3292	0.05740
3	5	9	2	0.06000	0.0973	0.03120
2	6	33	2	0.08870	0.1830	0.01420
2	7	32	3	0.02020	0.0198	0.00578
2	7	33	2	0.05543	0.0702	0.00882
2	8	33	2	0.00000	0.0000	0.00000
2	9	10	1	0.04261	0.0568	0.00870
2	9	32	3	0.00000	0.0000	0.00000
2	9	33	3	0.01329	0.0132	0.00661
3	9	13	2	0.01119	0.0133	0.00386
3	9	25	1	0.00306	0.0031	0.00306
1	9	2	1	0.00000	0.0000	0.00000
1	10	2	1	0.00044	0.0004	0.00044
4	11	2	2	0.10000	0.1608	0.01540
4	11	3	1	0.26510	0.3453	0.04010
4	11	4	3	0.85880	4.0361	0.08840
4	11	3	2	0.96560	3.2267	0.05890
2	11	23	1	0.38490	0.8100	0.05280

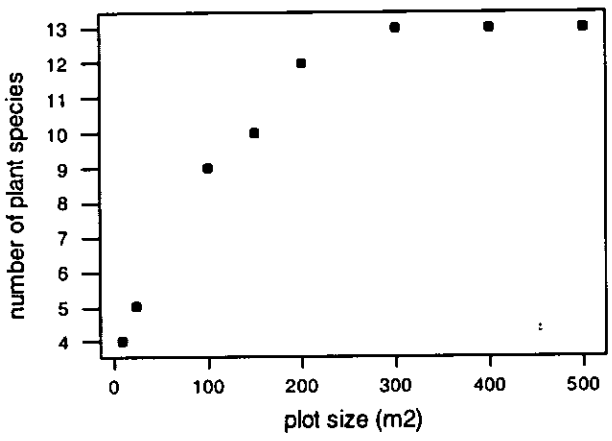
Appendix 3.5 (continued from previous page) Summary of data on population distribution studies (site 1= INIA, site 2= 1° Maio, site3=Olsa Citrus D, site 4=Olsa citrus A&B; the figures for months correspond to the months of the year; cultivar 1= Valencia, cultivar 2=Marsh, cultivar 3= Star Ruby, 6=Oroblanco, 7= Rose; s.error = standard error)

site	month	orchard	cultivar	mean	variance	s. error
4	11	1	7	0.16560	0.3440	0.01750
3	11	14	2	0.20630	0.2993	0.02440
2	12	10	1	0.39000	0.6082	0.02990
4	12	5	3	7.63800	37.1612	0.49900
4	12	5	3	0.94600	1.8333	0.11100
3	12	25	2	0.41690	1.0512	0.03990
3	12	10	1	0.34520	0.6849	0.02640
2	12	32	3	0.38240	0.7861	0.03510
2	12	33	2	1.95600	3.8064	0.14500
2	12	13	6	0.90300	2.6768	0.08610

Appendix 3.6 Summary of data on population distribution studies (N = number of sampling plots ; S.Error = standard error)

Months	N	Mean egg/plant/month	S.Error
February	2	0.12	0.02
March	2	0.48	0.40
April	2	0.12	0.30
May	24	0.10	0.01
June	2	0.09	0.01
July	2	0.04	0.02
August	2	0.00	0.00
September	6	0.01	0.01
October	2	0.01	0.03
November	6	0.35	0.10
December	8	1.44	0.71

Appendix 3.7 Graph showing the curve of plant species numbers versus sampling area



The number of plants per plot varied from hundreds to thousands (including grasses). The plant species that ocurred in the plots are shown bellow.

Family	Genus	Species
Fabaceae	*Acacia*	*Acacia karro*Hayne
Aloaceae	*Aloe*	*Aloe marlothii* Berger
Gramineae	*Melinis*	*Melinis repens* (Willd.) Zizka
Asteraceae	*Tridax*	*Tridax procumbens*
Gramineae	*Panicum*	*Panicum maximum* Jacq.
Combretaceae	*Combretum*	*Combretum apiculatum* Sonder
Fabaceae	*Afzelia*	*Afzelia quanzensis* Welw.
Anacardiaceae	*Sclerocarya*	*Sclerocarya birrea* (A. Rich) Hochst.

Appendix 3.8 Procedures for leaf nutrient analyses

(Source: Allen *et al.*, 1986; Mckillop, 1986)

Nitrogens determination

Dried leaf samples at 35 °C for 24 hours were ground with a pestle and mortar and analysed by colorimetric methods for nitrogens. Samples were weighed in a weighing flask and then the powder was transferred to a digestion test tube. Samples weight was calculated by weighing again the flask and subtracting its value. 4 ml of the digestion reagent were added to every sample for digestion at 260°C for one and half hours until the liquid became colourless. After cooling, the samples were diluted to 50 ml with distilled water and mixed. Small volume per sample were placed into cuvettes in the auto-analyser. The absorbancy control was set at 2 and the scale turned zero. A graph was produced; from this graph, the amount of nitrogen per sample solution was quantified by comparison of sample peaks with standard peaks. Combining these results with samples weights, nitrogen content was calculated as percentage of the material dry weight.

Reagents

50% hydrogen peroxide (H_2O_2); methanol (CH_3OH); sodium chloride (NaCl); sodium hydroxide solution 50% w/v (NaOH); sodium hypochlorite 5.25% (NaOCl); sodium nitroprusside ($Na_2Fe(CH)_5NO.2H_2O$); sodium phosphate, diabasic ($Na_2HPO_4.7H_2O$) or sodium phosphate, diabasic anhydrous (Na_2HPO_4); sodium potassium tartrate ($NaKC_4H_4O_6.4H_2O$); sodium salicylate (Na $C_2H_5O_3$); sulphuric acid, 95-98% (H_2SO_4); Brij-35, 30% solution

For digestion reagent 420 ml of sulphuric acid (H_2SO_4), 504 ml of hydrogen peroxide (H_2O_2) and 16.8 g of $LiSO_3$ were used. Standard solution was prepared with 20 mg/l of $(NH_4)_2SO_4$ and reagents and solutions used in colorimetric analysis were all prepared as indicated in Industrial Method No. 385-75A.

Soluble tannin determination

Standard procedures based on Association of Official Agricultural Chemists (1970) recommended by Allen (1989) were followed by colorimetric analysis of extracted tannins.

Reagents

0.05 g tannic acid were weighed in a weighing boat, dissolved in distilled water and diluted to 500 ml for standard tannic acid preparation. The Folin-Denis reagent was prepared by adding 50 mg sodium tungstate, 10 mg molybmophosphoric acid and 25 ml orthophosphoric acid to 375 ml distilled water. After heating, the solution was cooled and diluted to 500 ml. Sodium carbonate 17% w/v was prepared by dissolving 170 g sodium carbonate in 1 litre of water.

Procedure

The extracted tannins reacted with Folin-Denis reagent and sodium carbonate and formed a coloured substance, measured at a wavelength of 760 nm.

Leaf samples were oven dried at 35° C for 24 hours and ground to a fine powder. For this purpose a pestle and mortar were used. Approximately 0.1000 gr were weighed for

each sample in a boiling tube. By weighing the boiling tube and subtracting the weight to the former, the sample weight was determined.

50 ml of distilled water were added to the tubes and the samples boiled in a digestor set at 100°C for 1 hour. After cooling, the solutions were filtered in No. 40 filter papers to 50 ml flasks.

Colour development

Tannic acid standards were prepared by pipetting 0 to 30 ml of tannic acid standard into 50 ml volumetric flasks to give a standard range from 0 to 0.3 mg of tannic acid. Water blanks were prepared using the same procedures. 4 ml aliquots of the sample solutions were pipetted into 50 ml flasks and standards, blanks and samples treated in the same way afterwards. The flasks were two-thirds filled with distilled water. Folin-Denis reagent 2.5 ml and 10 ml sodium carbonate were added. The mixture was mixed and diluted to volume. Flasks were placed in water bath at 25°C for 20 minutes to allow colour development. Solution samples were poured into glasses and the optical density at the wavelength of 760 nm measured by a Spectrophotometer using water as reference. Blank readings were substracted for the determinations. After construction of the calibration curve the optical density was recorded for every sample and converted to mg tannic acid per sample aliquot.

Calculation of soluble tannin in percentage of dry weight

For C= mg tannic acid obtained from the curve

$$\text{soluble tannin (\%)} = \frac{C(\text{mg}) \times 50 \times (50/\text{aliquot (ml)})}{10 \times \text{aliquot (ml)} \times \text{sample wt (g)}}$$

Appendix 4.1 Summary of *P. demodocus* distribution studies data in old orchards (sprinkling = ground sprinling)

Site	Orchard	Age (years)	Species	Cultivar	Irrigation system	Pesticides use	Insect counts
Olsa C. A&B	B7	35	*C. sinensis*	Valencia	sprinkling	treated	0
Olsa C. D	D24	35	*C. paradisi*	Marsh	sprinkling	treated	0
1°Maio	02	11	*C. paradisi*	Rose	sprinkling	treated	1
1°Maio	05	11	*C. paradisi*	Rose	sprinkling	treated	1
1°Maio	06	35	*C. sinensis*	Valencia	sprinkling	treated	0
INIA	EAU	3	*C. sinensis*	Valencia	manual	free	3
Citrus	3	3	*C. sinensis*	Joppa	sprinkling	free	1
Citrus	26	3	*C. paradisi*	Marsh	sprinkling	free	0
Citrus	7	3	*C. paradisi*	Marsh	sprinkling	free	2
Citrus	31	3	*C. paradisi*	Marsh	sprinkling	free	0
Citrus	20	5	*C. paradisi*	Star Ruby	sprinkling	free	0
Olsa C. D	D6	12	*C. sinensis*	Valencia	sprinkling	treated	1
1°Maio	10	35	*C. sinensis*	Valencia	sprinkling	treated	0

Appendix 4.2 Summary of *P. demodocus* distribution studies data in young orchards (sprinkling = ground sprinkling)

Site	Orchard	Age (years)	Species	Cultivar	Irrigation system	Pesticides use	Insect counts
Olsa C. A&B	A4	2	*C. paradisi*	Marsh	sprinkling	treated	11
Olsa C. D	D15	2	*C. paradisi*	Marsh	sprinkling	treated	13
Olsa C. D	D20A	2	*C. paradisi*	Star Ruby	sprinkling	treated	8
Olsa C. D	D19	2	*C. paradisi*	Star Ruby	sprinkling	treated	10
Olsa C. D	D20B	2	*C. paradisi*	Star Ruby	sprinkling	treated	6
Olsa CD	D17	2	*C. paradisi*	Star Ruby	sprinkling	treated	13
1°Maio	11	2	*C. paradisi*	Rose	sprinkling	treated	7
1°Maio	10A	1	*C. sinensis*	Valencia	sprinkling	treated	0
1°Maio	1	1	*C. paradisi*	Marsh	sprinkling	treated	1
1°Maio	9	2	*C. paradisi*	Marsh	sprinkling	treated	2
INIA	EAU	2A	*C. sinensis*	Valencia	manual	free	13
INIA	EAU	2B	*C. paradisi*	Marsh	manual	free	20

Appendix 4.3 Cumulative data for static life table in < 2 years old orchards (* = orchards treated with pesticides; ** = pesticides free orchards; *** = unfertile; Larv. = larvae; sampled area: 3,600 m^2 at Olsa C A & B; 5,400 m^2 at Olsa Citrus D; 5,400 m^2 at 1° Maio and 2,500 m^2 at INIA)

Stage	Olsa C D*	Olsa C A & B*	1° Maio *	Total *	Proportion *		INIA **
Eggs ***	45	2	36	83			6
Yellow eggs	56	1	18	74			46
Dark eggs	25	1	27	53			35
Pinkish eggs	22	2	46	69			29
1st instar lar.	10	4	37	51	11.9		22
2nd instar lar.	11	6	14	32	7.4		28
3rd instar lar.	11	4	9	24	5.6		28
4th instar lar.	9	0	6	15	3.5		15
5th instar lar.	2	1	6	13	3.0		15
pupae	3	0	3	6	1.4		5

Appendix 4.4 Survival curve parameters for *P. demodocus* in different sites, cultivars and seasons (location 6=UEM, location 3=INIA, location 23= 1° Maio; cultivar 1=Valencia, cultivar 2= Marsh, cultivar 3=Star Ruby; season 1=dry season, season 2=rainy season; linear, quadratic and cubic are curves functions)

location	cultivar	season	intercept	linear	quadratic	cubic
6	1	2	1.96752	-0.078400	-0.00253	-0.0000292
6	2	2	2.07960	-0.111355	0.00392	-0.0000475
6	1	2	1.82076	-0.102443	0.00327	-0.0000341
6	2	2	1.77678	-0.109994	0.00366	-0.0000383
6	3	2	2.66331	-0.174594	0.00358	-0.0000224
6	3	2	1.95263	-0.155417	0.00561	-0.0000612
3	1	2	1.69178	-0.085500	0.00317	-0.0000367
3	1	2	2.19690	-0.168446	0.00483	-0.0000450
3	2	2	2.09810	-0.144558	0.00322	-0.0000234
3	2	2	2.19987	-0.130578	0.00433	-0.0000460
3	3	2	2.62633	-0.179716	0.00451	-0.0000370
3	3	2	2.17113	-0.129975	0.00420	-0.0000445
23	1	2	1.94569	-0.130573	0.00448	-0.0000478
23	1	2	2.25145	-0.154669	0.00585	-0.0000683
23	1	2	2.61211	-0.147381	0.00537	-0.0000615
6	1	1	1.88281	-0.106986	0.00342	-0.0000371
6	1	1	2.03928	-0.187771	0.00551	-0.0000520
6	2	1	2.21161	-0.139570	0.00486	-0.0000538
6	2	1	0.95087	-0.006020	0.00174	-0.0000159
6	3	1	2.22607	-0.210753	0.00622	-0.0000567

Appendix 4.4 (continued from previous page) Survival curve parameters for *P. demodocus* in different sites, cultivars and seasons (location 6=UEM, location 3=INIA, location 23= 1° Maio; cultivar 1=Valencia, cultivar 2= Marsh, cultivar 3=Star Ruby; season 1=dry season, season 2=rainy season; linear, quadratic and cubic are curves functions)

location	cultivar	season	intercept	linear	quadratic	cubic
6	3	1	1.53056	-0.197315	0.00792	-0.0000100
3	1	1	1.62984	-0.104113	0.00293	-0.0000284
3	1	1	1.74852	-0.094100	0.00304	-0.0000395
3	2	1	1.69989	-0.087700	0.00184	-0.0000129
3	2	1	1.82274	-0.049600	-0.00119	0.0000313
3	3	1	1.57982	-0.083700	0.00220	-0.0000207
3	3	1	2.06554	-0.120395	0.00281	-0.0000261
23	1	1	1.86765	-0.167741	0.00474	-0.0000426
23	1	1	2.16454	-0.148424	0.00469	-0.0000484
23	1	1	1.88035	-0.093300	0.00210	-0.0000179

Appendix 4.5 Data on the experiment to assess the effect of different markers on the butterfly life span (S.E. = standard error)

Treatment	longevity (in days)
1(permanent pen marker)	9
1 (permanent pen marker)	10
2 (nail polish)	8
2 (nail polish)	11
3 (Tipp-Ex)	9
3 (Tipp-Ex)	10
4 (control)	9
4 (control)	11

Marker/treatment	N	Mean	S.E.Mean
nail polish	2	9.5	0.5
permanent pen marker	2	9.5	1.5
Tipp-Ex	2	9.5	0.5
control	2	10	1.0

Appendix 4.6 Significance of the contribution of different mortality factors to total K (y = relationship equation; r^2 = correlation coefficient; s = standard deviation; ** significant at P = 0.01; kx = mortality factor at a given stage; kxd = mortality factor by disappearance; lx = larval density; y. eggs = yellow eggs; p. eggs = pinkish eggs; d. eggs = dark eggs; 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} = first, second, third, fourth, fifth larval instar)

Instar	y	r^2	r^2 (adjusted)	s	$F_{(1, 28)}$	P	Relationship
y. eggs	0.321 + 0.0310x	0.4%	0.0%	0.1832	0.12	0.736	kx vs K
p. eggs	0.123 + 0.0301x	0.9%	0.0%	0.1199	0.26	0.613	kx vs K
d. eggs	0.108 + 0.0677x	4.8%	1.4%	0.1150	1.41	0.245	kx vs K
1^{st}	0.133 + 0.0081x	0.1%	0.0%	0.1282	0.02	0.899	kx vs K
2^{nd}	-0.236 + 0.267x	33.9%	31.6%	0.1420	14.38	0.001**	kx vs K
2^{nd}	-0.221 + 0.255x	31.4%	28.9%	0.1436	12.79	0.001**	kxd vs K
2^{nd}		0.00	0.00%	0.1733	0.00	0.950	kxd vs lx
3^{rd}	-0.062 + 0.141x	9.9%	6.7%	0.1626	3.07	0.09	kxd vs K
4^{th}			47.2%	0.1549	26.93	<0.01**	kx vs K
4^{th}	-0.480 + 0.399x	49.0%	46.1%	0.1452	25.83	<0.01**	kxd vs K
4^{th}		1.5%	0.0%	0.1998	0.42	0.520	kxd vs lx
5^{th}	-0.030+ 0.0780x	4.6%	1.2%	0.1347	1.36	0.253	kx vs K
pupae	-0.072 +0.121x	9.5%	6.3%	0.1423	2.94	0.097	kx vs K

Appendix 4.7 Equations for the relationship between kx values of the various developmental stages against environmental factors ($Y1_{(kx)}$ is the equation for the 2nd instar larvae and $Y2_{(kx)}$ the equation for the 5th instar larvae; $Y3_{(kx)}$ for yellow eggs; $Y4_{(kx)}$ for pinkish eggs; $Y5_{(kx)}$ for dark eggs; $Y6_{(kx)}$ for first instar larvae; $Y7_{(kx)}$ for third instar larvae; $Y8_{(kx)}$ for fourth instar larvae; $Y9_{(kx)}$ for pupae; P = probability ; a = intercept; the other symbols are coefficients for the meteorological data: c.t for for current temperature; m.t. for for mean temperature; mi.t. for minimum temperature; ma.t. for maximum temperature; c.r.h. for current relative humidity; m.r.h. for mean relative humidity; m.w. for mean wind speed; sun for solar radiation; ev. for evaporation; rain for rainfall; * significant at P = 0.05 and ** significant at P = 0.01; ni = no information)

Equation and P	a	c.t.	m.t.	mi.t.	ma.t.	c.r.h.	m.r.h.	m.w.	sun	ev.	rain
$Y1_{(kx)}$	0.09	-0.04	0.13	0.06	0.11	-0.01	0.01	-0.01	-0.02	-0.00	-0.00
P	0.86	0.09	0.86	0.87	0.76	0.07	0.07	0.14	0.05	0.90	0.97
									*		
$Y2_{(kx)}$	0.71	-0.04	-1.71	0.94	0.80	0.00	0.03	-0.09	0.00	0.07	-0.03
P	0.21	0.04	0.00	0.00	0.33	0.00	0.02	0.00	0.00	0.02	0.00
		*	**	**		**	*		**	*	**
$Y3_{(kx)}$	6.4	-0.20	33	-16	-17	-0.155	0.017	-0.26	ni	-0.46	0.052
P	0.87	0.89	0.88	0.88	0.88	0.881	0.921	0.871		0.875	0.866
$Y4_{(kx)}$	-0.05	0.065	ni	ni	-0.0	0.006	-0.008	0.027	ni	-0.037	-0.00
P	0.952	0.186			0.32	0.682	0.476	0.179		0.355	0.991
$Y5_{(kx)}$	-67.9	0.90	1.96	-0.7	-0.4	0.337	-0.05	0.85	-0.50	0.124	0.523
P	0.004	0.002	0.007	0.00	0.00	0.002	0.119	0.009	0.017	0.046	0.024
	**	**	**	**	**	**		**	*	*	*

Appendix 4.7 (continued from previous page) Equations for the relationship between kx values of the various developmental stages against environmental factors ($Y1_{(kx)}$ is the equation for the 2nd instar larvae and $Y2_{(kx)}$ the equation for the 5th instar larvae; $Y3_{(kx)}$ for yellow eggs; $Y4_{(kx)}$ for pinkish eggs; $Y5_{(kx)}$ for dark eggs; $Y6_{(kx)}$ for first instar larvae; $Y7_{(kx)}$ for third instar larvae; $Y8_{(kx)}$ for fourth instar larvae; $Y9_{(kx)}$ for pupae; P = probability ; a = intercept; the other symbols are coefficients for the meteorological data: c.t for for current temperature; m.t. for for mean temperature; mi.t. for minimum temperature; ma.t. for maximum temperature; c.r.h. for current relative humidity; m.r.h. for mean relative humidity; m.w. for mean wind speed; sun for solar radiation; ev. for evaporation; rain for rainfall; * significant at P = 0.05 and ** significant at P = 0.01; ni = no information)

Equation and P	a	c.t.	m.t.	mi.t.	ma.t.	c.r.h.	m.r.h.	m.w.	sun	ev.	rain
$Y6_{(kx)}$	-3.0	0.52	1.98	-1.31	-0.9	0.042	-0.09	0.004	-0.188	-0.47	0.0506
P	0.30	0.27	0.70	0.62	0.71	0.380	0.427	0.887	0.281	0.237	0.283
$Y7_{(kx)}$	-0.1	0.06	ni	ni	-0.04	-0.006	0.008	0.027	ni	-0.037	-0.00
P	0.95	0.19			0.325	0.682	0.476	0.179		0.355	0.991
$Y8_{(kx)}$	-4.6	0.05	0.08	ni	ni	0.028	-0.004	-0.097	ni	-0.019	-0.019
P		*				*	**			*	*
$Y9_{(kx)}$	1.72	0.00	-0.06	ni	0.029	-0.002	-0.011	-0.006	ni	0.015	0.003
P	0.42	0.99	0.51		0.682	0.831	0.573	0.796		0.830	0.690

Appendix 4.8 kx values for each developmental stage per site, generation and cultivar (Site 23 = 1° Maio; 3 = INIA; 6 = UEM; G = generation; C = cultivar: 1 = Valencia; 2= Marsh; 3 = Star Ruby; kx1 = kx for yellow egg stage; kx2 = kx for pinkish egg stage; kx3 = kx for dark egg stage; kx4 = kx for 1st larval instar; kx5 = kx for 2nd larval instar; kx6 = kx for 3rd larval instar; kx7 = kx for 4th larval instar; kx8 = kx for 5th larval instar; kx9 = kx for pupal stage; number of samples = 30)

Site	G	C	kx1	kx2	kx3	kx4	kx5	kx6	kx7	kx8	kx9
23	4	1	0.13	0.08	0.24	0.48	0.30	0.30	0.00	0.00	0.00
23	5	1	0.25	0.07	0.37	0.23	0.07	0.34	0.00	0.10	0.00
23	6	1	0.14	0.01	0.09	0.42	0.20	0.17	0.00	0.30	0.18
23	1	1	0.33	0.27	0.25	0.20	0.10	0.12	0.12	0.00	0.00
23	2	1	0.60	0.27	0.21	0.20	0.14	0.00	0.00	0.00	0.04
23	3	1	0.42	0.20	0.07	0.26	0.07	0.18	0.02	0.04	0.00
3	4	1	0.33	0.33	0.23	0.15	0.10	0.00	0.00	0.30	0.00
3	5	1	0.60	0.06	0.20	0.00	0.00	0.09	0.47	0.00	0.48
3	4	2	0.68	0.08	0.12	0.08	0.22	0.08	0.00	0.40	0.00
3	5	2	0.44	0.17	0.17	0.02	0.15	0.00	0.63	0.48	0.48
3	4	3	0.59	0.12	0.07	0.09	0.17	0.08	0.10	0.12	0.18
3	5	3	0.16	0.36	0.29	0.02	0.33	0.00	0.37	0.18	0.30
3	2	1	0.47	0.00	0.08	0.18	0.08	0.00	0.00	0.00	0.05
3	3	1	0.11	0.23	0.28	0.04	0.00	0.43	0.55	0.00	0.00
3	2	2	0.31	0.10	0.14	0.20	0.58	0.30	0.00	0.30	0.00
3	3	2	0.11	0.45	0.19	0.02	0.13	0.28	0.00	0.10	0.00

Appendix 4.8 (continued from previous page) kx values for each developmental stage per site, generation and cultivar (Site 23 = 1° Maio; 3 = INIA; 6 = UEM; G = generation; C = cultivar: 1 = Valencia; 2= Marsh; 3 = Star Ruby; kx1 = kx for yellow egg stage; kx2 = kx for pinkish egg stage; kx3 = kx for dark egg stage; kx4 = kx for 1st larval instar; kx5 = kx for 2nd larval instar; kx6 = kx for 3rd larval instar; kx7 = kx for 4th larval instar; kx8 = kx for 5th larval instar; kx9 = kx for pupal stage; number of samples = 30)

Site	G	C	kx1	kx2	kx3	kx4	kx5	kx6	kx7	kx8	kx9
3	2	3	0.17	0.07	0.34	0.07	0.10	0.28	0.40	0.00	0.07
3	3	3	0.30	0.07	0.37	0.05	0.57	0.30	0.15	0.00	0.00
6	4	1	0.38	0.12	0.29	0.09	0.07	0.09	0.10	0.15	0.00
6	5	1	0.48	0.00	0.17	0.37	0.18	0.60	0.00	0.00	0.00
6	4	2	0.16	0.16	0.26	0.15	0.20	0.13	0.00	0.05	0.06
6	5	2	0.38	0.10	0.00	0.00	0.12	0.18	0.00	0.00	0.00
6	4	3	0.51	0.32	0.25	0.10	0.07	0.48	0.30	0.00	0.30
6	5	3	0.54	0.05	0.60	0.00	0.30	0.00	0.00	0.00	0.30
6	2	1	0.33	0.33	0.18	0.01	0.00	0.25	0.00	0.15	0.30
6	3	1	0.41	0.22	0.11	0.15	0.04	0.19	0.07	0.00	0.00
6	2	2	0.36	0.28	0.20	0.15	0.00	0.15	0.07	0.04	0.30
6	3	2	0.31	0.20	0.17	0.24	0.12	0.00	0.00	0.00	0.08
6	2	3	0.25	0.25	0.20	0.27	0.68	0.34	0.70	0.00	0.00
6	3	3	0.81	0.14	0.22	0.14	0.20	0.00	0.00	0.00	0.00

Appendix 4.9a Mortality factors, survival and population density for yellow egg stage at various sites, cultivars and generations (site 23 =1°Maio; 3 = INIA, 6 = University Campus; other kx include substrate dried, death due to egg dislodgement and unknown causes)

Site	Generation	Cultivar	Desiccation	Infertility	Other kx	Stage kx	Survival	Density
23	4	Valencia	0.031	0.099	0.000	0.13	0.83	1.53
23	5	Valencia	0.128	0.122	0.000	0.25	0.56	2.03
23	6	Valencia	0.199	0.011	0.000	0.14	0.73	1.81
23	1	Valencia	0.129	0.000	0.131	0.33	0.47	2.05
23	2	Valencia	0.370	0.230	0.000	0.60	0.25	2.46
23	3	Valencia	0.285	0.107	0.028	0.42	0.37	2.28
3	4	Valencia	0.192	0.138	0.000	0.33	0.42	1.74
3	5	Valencia	0.590	0.010	0.000	0.60	0.25	2.00
3	4	Marsh	0.444	0.236	0.000	0.68	0.21	1.96
3	5	Marsh	0.179	0.261	0.000	0.44	0.37	2.06
3	4	Star Ruby	0.433	0.157	0.000	0.59	0.26	1.82
3	5	Star Ruby	0.058	0.102	0.000	0.16	0.69	2.01
3	2	Valencia	0.350	0.062	0.058	0.47	0.34	1.81
3	3	Valencia	0.104	0.006	0.000	0.11	0.77	1.94
3	2	Marsh	0.200	0.110	0.000	0.31	0.49	1.93
3	3	Marsh	0.088	0.022	0.000	0.11	0.77	2.18
3	2	Star Ruby	0.069	0.101	0.000	0.17	0.68	2.03
3	3	Star Ruby	0.180	0.120	0.000	0.30	0.50	2.36
6	4	Valencia	0.089	0.291	0.000	0.38	0.42	1.99
6	5	Valencia	0.391	0.029	0.060	0.48	0.33	1.80
6	4	Marsh	0.058	0.102	0.000	0.16	0.70	2.01
6	5	Marsh	0.145	0.235	0.000	0.38	0.42	1.08
6	4	Star Ruby	0.319	0.191	0.000	0.51	0.31	2.03
6	5	Star Ruby	0.329	0.211	0.000	0.54	0.29	1.49
6	2	Valencia	0.197	0.124	0.009	0.33	0.47	2.12
6	3	Valencia	0.194	0.113	0.103	0.41	0.39	1.97
6	2	Marsh	0.141	0.219	0.000	0.36	0.43	2.26
6	3	Marsh	0.246	0.004	0.060	0.31	0.49	1.83
6	2	Star Ruby	0.171	0.051	0.028	0.25	0.56	2.51
6	3	Star Ruby	0.315	0.009	0.486	0.81	0.16	2.21

Appendix 4.9b Mortality factors, survival and population density for pinkish egg stage at various sites, cultivars and generations (site 23 =1°Maio; 3 = INIA, 6 = University Campus; other kx include substrate dried, death due to egg dislodgement and unknown causes)

Site	Generation	Cultivar	Desiccation	Infertility	Other kx	Stage kx	Survival	Density
6	4	Valencia	0.029	0.091	0.000	0.12	0.76	1.61
6	5	Valencia	0.000	0.000	0.000	0.00	1.00	1.32
3	4	Valencia	0.124	0.206	0.000	0.33	0.46	1.41
3	5	Valencia	0.040	0.020	0.000	0.06	0.88	1.40
6	4	Marsh	0.050	0.110	0.000	0.16	0.69	1.85
6	5	Marsh	0.000	0.100	0.000	0.10	0.80	0.70
3	4	Marsh	0.000	0.080	0.000	0.08	0.84	1.28
3	5	Marsh	0.121	0.008	0.041	0.17	0.67	1.62
6	4	Star Ruby	0.122	0.198	0.000	0.32	0.48	1.52
6	5	Star Ruby	0.000	0.050	0.000	0.05	0.89	0.95
3	4	Star Ruby	0.066	0.054	0.000	0.12	0.76	1.23
3	5	Star Ruby	0.234	0.126	0.000	0.36	0.44	1.85
23	4	Valencia	0.042	0.038	0.000	0.08	0.84	1.40
23	5	Valencia	0.053	0.017	0.000	0.07	0.85	1.78
23	6	Valencia	0.000	0.000	0.010	0.01	0.98	1.67
6	2	Valencia	0.172	0.005	0.153	0.33	0.63	1.79
6	3	Valencia	0.180	0.000	0.040	0.22	0.61	1.56
6	2	Marsh	0.118	0.009	0.153	0.28	0.52	1.89
6	3	Marsh	0.058	0.000	0.142	0.20	0.64	1.52
6	2	Star Ruby	0.125	0.045	0.080	0.25	0.62	2.26
6	3	Star Ruby	0.000	0.000	0.140	0.14	0.72	1.40
3	2	Valencia	0.000	0.000	0.000	0.00	1.00	1.34
3	3	Valencia	0.149	0.000	0.081	0.23	0.59	1.83
3	2	Marsh	0.000	0.089	0.011	0.10	0.79	1.62
3	3	Marsh	0.301	0.088	0.061	0.45	0.36	2.07
3	2	Star Ruby	0.055	0.015	0.000	0.07	0.84	1.86
3	3	Star Ruby	0.050	0.020	0.000	0.07	0.85	2.06
23	1	Valencia	0.217	0.012	0.041	0.27	0.53	1.72
23	2	Valencia	0.226	0.021	0.023	0.27	0.53	1.86
23	3	Valencia	0.034	0.036	0.130	0.20	0.64	2.16

Appendix 4.9c Mortality factors, survival and population density for dark egg stage at various sites, cultivars and generations (site 23 =1°Maio; 3 = INIA, 6 = University Campus; other kx include substrate dried, death due to egg dislodgement and unknown causes)

Site	Generation	Cultivar	Desiccation	Other kx	Stage kx	Survival	Density	Total K
6	4	Valencia	0.059	0.231	0.29	0.52	1.49	1.29
6	5	Valencia	0.010	0.160	0.17	0.67	1.32	1.80
3	4	Valencia	0.000	0.230	0.23	0.58	1.08	1.44
3	5	Valencia	0.000	0.200	0.20	0.50	1.34	1.90
6	4	Marsh	0.067	0.193	0.26	0.55	1.69	1.23
6	5	Marsh	0.000	0.000	0.00	1.00	0.60	0.78
3	4	Marsh	0.024.	0.096	0.12	0.75	1.20	1.66
3	5	Marsh	0.019	0.151	0.17	0.68	1.45	2.06
6	4	Star Ruby	0.000	0.250	0.25	0.56	1.20	2.03
6	5	Star Ruby	0.122	0.478	0.60	0.25	0.90	1.49
3	4	Star Ruby	0.000	0.070	0.07	0.85	1.11	1.52
3	5	Star Ruby	0.110	0.180	0.29	0.52	1.49	2.01
23	4	Valencia	0.000	0.240	0.24	0.57	1.32	1.53
23	5	Valencia	0.119	0.251	0.37	0.43	1.71	1.43
23	6	Valencia	0.000	0.090	0.09	0.80	1.66	1.51
6	2	Valencia	0.022	0.158	0.18	0.67	1.59	1.32
6	3	Valencia	0.000	0.110	0.11	0.77	1.34	1.27
6	2	Marsh	0.054	0.146	0.20	0.63	1.61	1.55
6	3	Marsh	0.000	0.170	0.17	0.67	1.32	1.12
6	2	Star Ruby	0.197	0.003	0.20	0.54	2.06	2.51
6	3	Star Ruby	0.000	0.220	0.22	0.61	1.26	1.51
3	2	Valencia	0.000	0.080	0.08	0.82	1.34	0.91
3	3	Valencia	0.238	0.042	0.28	0.53	1.60	1.64
3	2	Marsh	0.000	0.140	0.14	0.73	1.52	1.93
3	3	Marsh	0.052	0.138	0.19	0.64	1.62	1.28
3	2	Star Ruby	0.259	0.081	0.34	0.46	1.79	1.25
3	3	Star Ruby	0.008	0.362	0.37	0.43	1.97	2.06
23	1	Valencia	0.000	0.250	0.25	0.57	1.45	1.27
23	2	Valencia	0.034	0.176	0.21	0.62	1.59	1.68
23	3	Valencia	0.000	0.070	0.07	0.85	1.96	1.32

Appendix 4.9d Mortality factors, survival and population density for 1st instar larval stage at various sites, cultivars and generations (site 23 = 1° Maio; 3 = INIA, 6 = University Campus; other kx include death of larvae due to unknown causes)

Site	Generation	Cultivar	Disappearance	Other kx	Stage kx	Survival	Density
6	4	Valencia	0.090	0.000	0.09	0.81	1.20
6	5	Valencia	0.370	0.000	0.37	0.43	1.15
3	4	Valencia	0.150	0.000	0.15	0.71	0.85
3	5	Valencia	0.000	0.000	0.00	1.00	1.04
6	5	Marsh	0.000	0.000	0.00	1.00	0.60
3	4	Marsh	0.080	0.000	0.08	0.83	1.08
3	5	Marsh	0.020	0.000	0.02	0.95	1.28
6	4	Star Ruby	0.100	0.000	010	0.78	0.95
6	5	Star Ruby	0.000	0.000	0.00	1.00	0.30
3	4	Star Ruby	0.090	0.000	0.09	0.82	1.04
3	5	Star Ruby	0.020	0.000	0.02	0.94	1.20
23	4	Valencia	0.480	0.000	0.48	0.33	1.08
23	5	Valencia	0.173	0.027	0.23	0.59	1.34
6	4	Marsh	0.150	0.000	0.15	0.70	1.43
23	6	Valencia	0.395	0.025	0.42	0.38	1.57
6	2	Valencia	0.010	0.000	0.01	0.96	1.41
6	3	Valencia	0.150	0.000	0.15	0.71	1.23
6	2	Marsh	0.150	0.000	0.15	0.69	1.41
6	3	Marsh	0.240	0.000	0.24	0.57	1.15
6	2	Star Ruby	0.000	0.270	0.27	0.21	1.79
6	3	Star Ruby	0.140	0.000	0.14	0.73	1.04
3	2	Valencia	0.180	0.000	0.18	0.67	1.26
3	3	Valencia	0.040	0.000	0.04	0.91	1.32
3	2	Marsh	0.000	0.200	0.20	0.63	1.38
3	3	Marsh	0.020	0.000	0.02	0.96	1.43
3	2	Star Ruby	0.070	0.000	0.07	0.86	1.45
3	3	Star Ruby	0.043	0.007	0.05	0.88	1.62
23	1	Valencia	0.200	0.000	0.20	0.63	1.20
23	2	Valencia	0.200	0.000	0.20	0.63	1.38
23	3	Valencia	0.221	0.039	0.26	0.55	1.89

Appendix 4.9e Mortality factors, survival and population density for 2nd instar larval stage at various sites, cultivars and generations (site 23 = 1° Maio; 3 = INIA, 6 = University Campus; other kx include death of larvae due to unknown causes)

Site	Generation	Cultivar	Disappearance	Other kx	Stage kx	Survival	Density
6	4	Valencia	0.070	0.000	0.07	0.85	1.29
6	5	Valencia	0.180	0.000	0.18	0.67	1.80
3	4	Valencia	0.100	0.000	0.10	0.80	1.44
3	5	Valencia	0.000	0.000	0.00	1.00	1.90
6	4	Marsh	0.200	0.000	0.20	0.63	1.23
6	5	Marsh	0.120	0.000	0.12	0.75	0.78
3	4	Marsh	0.220	0.000	0.22	0.60	1.66
3	5	Marsh	0.150	0.000	0.15	0.72	2.06
6	4	Star Ruby	0.000	0.070	0.07	0.86	2.03
6	5	Star Ruby	0.300	0.000	0.30	0.50	1.49
3	4	Star Ruby	0.170	0.000	0.17	0.67	1.52
3	5	Star Ruby	0.330	0.000	0.33	0.47	2.01
23	4	Valencia	0.300	0.000	0.30	0.50	1.53
23	5	Valencia	0.070	0.000	0.07	0.85	1.43
23	6	Valencia	0.200	0.000	0.20	0.64	1.51
6	2	Valencia	0.000	0.000	0.00	1.00	1.32
6	3	Valencia	0.040	0.000	0.04	0.92	1.27
6	2	Marsh	0.000	0.000	0.00	1.00	1.55
6	3	Marsh	0.120	0.000	0.12	0.75	1.12
6	2	Star Ruby	0.680	0.000	0.68	0.85	2.51
6	3	Star Ruby	0.200	0.000	0.20	0.63	1.51
3	2	Valencia	0.080	0.000	0.08	0.83	0.91
3	3	Valencia	0.000	0.000	0.00	1.00	1.64
3	2	Marsh	0.546	0.034	0.58	0.27	1.93
3	3	Marsh	0.130	0.000	0.13	0.73	1.28
3	2	Star Ruby	0.100	0.000	0.10	0.79	1.25
3	3	Star Ruby	0.556	0.014	0.57	0.27	2.06
23	1	Valencia	0.100	0.000	0.10	0.80	1.27
23	2	Valencia	0.140	0.000	0.14	0.73	1.68
23	3	Valencia	0.053	0.017	0.07	0.84	1.32

Appendix 4.9f Mortality factors, survival and population density for 3rd instar larval stage at various sites, cultivars and generations (site 23 = 1° Maio; 3 = INIA, 6 = University Campus; other kx include death of larvae due to unknown causes)

Site	Generation	Cultivar	Disappearance	Other kx	Stage kx	Survival	Density
6	4	Valencia	0.000	0.090	0.09	0.82	1.04
6	5	Valencia	0.600	0.000	0.60	0.25	0.60
3	4	Valencia	0.000	0.000	0.00	1.00	0.60
3	5	Valencia	0.090	0.000	0.09	0.82	1.04
6	4	Marsh	0.000	0.130	0.13	0.75	1.08
6	5	Marsh	0.180	0.000	0.18	0.67	0.48
3	4	Marsh	0.080	0.000	0.08	0.83	0.78
3	5	Marsh	0.000	0.000	0.00	1.00	1.11
6	4	Star Ruby	0.480	0.000	0.48	0.33	0.78
6	5	Star Ruby	0.000	0.000	0.00	1.00	0.00
3	4	Star Ruby	0.000	0.080	0.08	0.83	0.78
3	5	Star Ruby	0.000	0.000	0.00	1.00	0.85
23	4	Valencia	0.000	0.300	0.30	0.50	0.30
23	5	Valencia	0.340	0.000	0.34	0.45	1.04
23	6	Valencia	0.170	0.000	0.17	0.67	0.95
6	2	Valencia	0.230	0.020	0.25	0.56	1.40
6	3	Valencia	0.150	0.040	0.19	0.64	1.04
6	2	Marsh	0.150	0.000	0.15	0.72	1.26
6	3	Marsh	0.000	0.000	0.00	1.00	0.78
6	2	Star Ruby	0.340	0.000	0.34	0.45	1.04
6	3	Star Ruby	0.000	0.000	0.00	1.00	0.70
3	2	Valencia	0.000	0.000	0.00	1.00	1.00
3	3	Valencia	0.430	0.000	0.43	0.37	1.28
3	2	Marsh	0.300	0.000	0.30	0.50	0.60
3	3	Marsh	0.255	0.025	0.28	0.53	1.28
3	2	Star Ruby	0.280	0.000	0.28	0.53	1.28
3	3	Star Ruby	0.300	0.000	0.30	0.50	1.00
23	1	Valencia	0.120	0.000	0.12	0.75	0.90
23	2	Valencia	0.000	0.000	0.00	1.00	1.04
23	3	Valencia	0.180	0.000	0.18	0.67	1.56

Appendix 4.9g Mortality factors, survival and population density for 4th instar larval stage at various sites, cultivars and generations (site 23 = 1° Maio; 3 = INIA, 6 = University Campus; other kx include death of larvae due to unknown causes)

Site	Generation	Cultivar	Disappearance	Other kx	Stage kx	Survival	Density
6	4	Valencia	0.100	0.000	0.10	0.78	0.95
6	5	Valencia	0.000	0.000	0.00	1.00	0.00
3	4	Valencia	0.000	0.000	0.00	1.00	0.60
3	5	Valencia	0.470	0.000	0.47	0.33	0.95
6	4	Marsh	0.000	0.000	0.00	1.00	0.95
6	5	Marsh	0.000	0.000	0.00	1.00	0.30
3	4	Marsh	0.000	0.000	0.00	1.00	0.70
3	5	Marsh	0.332	0.298	0.63	0.23	1.11
6	4	Star Ruby	0.300	0.000	0.30	0.50	0.30
6	5	Star Ruby	0.000	0.000	0.00	1.00	0.00
3	4	Star Ruby	0.000	0.100	0.10	0.80	0.70
3	5	Star Ruby	0.370	0.000	0.37	0.43	0.85
23	4	Valencia	0.000	0.000	0.00	1.00	0.00
23	5	Valencia	0.000	0.000	0.00	1.00	0.70
23	6	Valencia	0.000	0.000	0.00	1.00	0.78
6	2	Valencia	0.000	0.000	0.00	1.00	1.15
6	3	Valencia	0.000	0.070	0.07	0.86	0.85
6	2	Marsh	0.070	0.000	0.07	0.85	1.11
6	3	Marsh	0.000	0.000	0.00	1.00	0.78
6	2	Star Ruby	0.700	0.000	0.70	0.20	0.70
6	3	Star Ruby	0.000	0.000	0.00	1.00	0.70
3	2	Valencia	0.000	0.000	0.00	1.00	1.00
3	3	Valencia	0.550	0.000	0.55	0.29	0.85
3	2	Marsh	0.000	0.000	0.00	1.00	0.30
3	3	Marsh	0.000	0.000	0.00	1.00	1.00
3	2	Star Ruby	0.400	0.000	0.40	0.40	0.70
3	3	Star Ruby	0.150	0.000	0.15	0.70	1.00
23	1	Valencia	0.120	0.000	0.12	0.75	0.90
23	2	Valencia	0.000	0.000	0.00	1.00	1.04
23	3	Valencia	0.020	0.000	0.02	0.96	1.38

Appendix 4.9h Mortality factors, survival and population density for 5th instar larval stage at various sites, cultivars and generations (site 23 = 1° Maio; 3 = INIA, 6 = University Campus; other kx include death of larvae due to unknown causes)

Site	Generation	Cultivar	Disappearance	Other kx	Stage kx	Survival	Density
6	4	Valencia	0.150	0.000	0.15	0.71	0.85
6	5	Valencia	0.000	0.000	0.00	1.00	0.00
3	4	Valencia	0.300	0.000	0.30	0.50	0.60
3	5	Valencia	0.000	0.000	0.00	1.00	0.48
6	4	Marsh	0.000	0.050	0.05	0.89	0.95
6	5	Marsh	0.000	0.000	0.00	1.00	0.30
3	4	Marsh	0.400	0.000	0.40	0.40	0.70
3	5	Marsh	0.301	0.179	0.48	0.33	0.48
6	4	Star Ruby	0.000	0.000	0.00	1.00	0.00
6	5	Star Ruby	0.000	0.000	0.00	1.00	0.00
3	4	Star Ruby	0.000	0.120	0.12	0.75	0.60
3	5	Star Ruby	0.000	0.180	0.18	0.67	0.48
23	4	Valencia	0.000	0.000	0.00	1.00	0.00
23	5	Valencia	0.100	0.000	0.10	0.80	0.70
23	6	Valencia	0.300	0.000	0.30	0.50	0.78
6	2	Valencia	0.150	0.000	0.15	0.71	1.15
6	3	Valencia	0.000	0.000	0.00	1.00	0.78
6	2	Marsh	0.040	0.000	0.04	0.91	1.04
6	3	Marsh	0.000	0.000	0.00	1.00	0.78
6	2	Star Ruby	0.000	0.000	0.00	1.00	0.00
6	3	Star Ruby	0.000	0.000	0.00	1.00	0.70
3	2	Valencia	0.000	0.000	0.00	1.00	1.00
3	3	Valencia	0.000	0.000	0.00	1.00	0.30
3	2	Marsh	0.300	0.000	0.30	0.50	0.30
3	3	Marsh	0.100	0.000	0.10	0.80	1.00
3	2	Star Ruby	0.000	0.000	0.00	1.00	0.30
3	3	Star Ruby	0.000	0.000	0.00	1.00	0.85
23	1	Valencia	0.000	0.000	0.00	1.00	0.78
23	2	Valencia	0.000	0.000	0.00	1.00	1.04
23	3	Valencia	0.018	0.022	0.04	0.91	1.36

Appendix 4.9i Mortality factors, survival and population density for pupal stage at various sites, cultivars and generations (site 23 = 1° Maio; 3 = INIA, 6 = University Campus; other kx include failure of emergence due to unknown causes)

Site	Generation	Cultivar	Parasitism	Other kx	Stage kx	Survival	Density
6	4	Valencia	0.000	0.000	0.00	1.00	0.70
6	5	Valencia	0.000	0.000	0.00	1.00	0.00
3	4	Valencia	0.000	0.000	0.00	1.00	0.30
3	5	Valencia	0.480	0.000	0.48	0.33	0.48
6	4	Marsh	0.055	0.067	0.12	0.75	0.90
6	5	Marsh	0.000	0.000	0.00	1.00	0.30
3	4	Marsh	0.000	0.000	0.00	1.00	0.30
3	5	Marsh	0.480	0.000	0.48	0.00	0.00
6	4	Star Ruby	0.300	0.000	0.30	0.00	0.00
6	5	Star Ruby	0.300	0.000	0.30	0.00	0.00
3	4	Star Ruby	0.180	0.000	0.18	0.67	0.48
3	5	Star Ruby	0.300	0.000	0.30	0.50	0.30
23	4	Valencia	0.000	0.000	0.00	1.00	0.00
23	5	Valencia	0.000	0.000	0.00	1.00	0.60
23	6	Valencia	0.180	0.000	0.18	0.67	0.48
6	2	Valencia	0.300	0.000	0.15	1.00	1.00
6	3	Valencia	0.000	0.080	0.08	0.83	0.78
6	2	Marsh	0.300	0.000	0.30	0.50	1.04
6	3	Marsh	0.080	0.000	0.08	0.33	0.78
6	2	Star Ruby	0.000	0.000	0.00	1.00	0.00
6	3	Star Ruby	0.000	0.000	0.00	1.00	0.70
3	2	Valencia	0.051	0.046	0.10	0.80	1.00
3	3	Valencia	0.000	0.000	0.00	1.00	0.30
3	2	Marsh	0.000	0.000	0.00	1.00	0.30
3	3	Marsh	0.000	0.000	0.00	1.00	1.00
3	2	Star Ruby	0.070	0.000	0.07	0.86	0.85
3	3	Star Ruby	0.000	0.000	0.00	1.00	0.30
23	1	Valencia	0.000	0.000	0.00	1.00	0.78
23	2	Valencia	0.040	0.260	0.26	0.55	1.04
23	3	Valencia	0.000	0.060	0.06	0.86	1.32

Appendix 4.10 ANOVA details for *P. demodocus* nutritional indices

Efficiency of Conversion of Assimilated Food (ECD)

Source of variation	DF	Sum of Squares	Mean Squares	F	P
Cultivar	2	126	63	0.45	0.643
Error	27	3787	140		
Total	29	3913			

Assimilation Efficiency (AD)

Source of variation	DF	Sum of Squares	Mean Squares	F	P
Cultivar	2	65.2	32.6	1.99	0.156
Error	27	442.1	16.4		
Total	29	507.3			

Relative Consumption Rate (RCR)

Source of variation	DF	Sum of Squares	Mean Squares	F	P
Cultivar	2	0.0000115	0.0000057	0.76	0.477
Error	27	0.0002036	0.0000075		
Total	29	0.0002151			

Relative Growth Rate (RGR)

Source of variation	DF	Sum of Squares	Mean Squares	F	P
Cultivar	2	0.0000031	0.0000015	0.26	0.775
Error	27	0.0001622	0.0000060		
Total	29	0.0001653			

Efficiency of Conversion of Ingested Food (ECI)

Source of variation	DF	Sum of Squares	Mean Squares	F	P
Cultivar	2	29.1	14.6	0.48	0.627
Error	27	827.4	30.6		
Total	29	856.5			