PROTECTION FROM BEETLE-PREDATION IN COCHINEAL INSECTS (DACTYLOPIIDAE:HOMOPTERA)

Dissertation submitted in partial fulfillment
of the requirements for the degree of Master of Science.

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by

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January 1984

CONTENTS.

ACKNOWLEDGEMENTS.

My sincere thanks are extended to Professor V.C. Moran and Mr. G.H. Walter for their supervision, understanding and encouragement throughout this study.

The constructive criticisms, interest and helpful advice offered by many of my colleagues, especially those of Dr J.H. Hoffmann are greatly appreciated.

I gratefully acknowledge the co-operation and assistance received from the staff of the Rhodes University Electron Microscope Unit, especially Mr. A.H. Hartley, and Dr C.G. Whiteley of the Rhodes University Department of Chemistry for assistance in chemical analysis.

Finally I thank my wife for her support, patience, encouragement and for her help in preparing this manuscript.

INTRODUCTION.

In South Africa the native ladybird beetle Exochomus flaviventris Mader feeds on the introduced cochineal insect Dactylopius opuntiae (Cockerell) (Pettey, 1943, 1946, 1948; Geyer, 1947 a, b; Pettey and Marais, 1950). It has also been reported to feed on Dactylopius austrinus Lindley (Geyer, 1947 a; Pettey, 1948), but this appears to occur rarely in the field (H.G. Zimmermann and H.G. Robertson pers. comm.; Appendix 1). This thesis attempts to determine why E. flaviventris feeds on D. opuntiae in the field but not on D. austrinus.

The genus Dactylopius is host specific to cactaceous plants, particularly to those of the genus Opuntia (De Lotto, 1974). Many Opuntia species have been introduced into South Africa, several of which have become naturalized (Lansdell, 1923; Phillips, 1940 a; Annecke and Moran, 1978; Stirton, 1979; Moran and Annecke, 1979). Some of these have become problem weeds (Phillips, 1940 b; Hattingh, 1958; Taylor, 1969; Neser and Annecke 1973; Zimmermann, 1978 a, b, c, d; Zimmermann and Moran, 1982), notably prickly pear Opuntia ficus-indica (L.) (Fig. 1) and jointed cactus Opuntia aurantiaca Lindley (Fig. 2). Attempts to control these weeds have relied largely on the introduction of various species of South American cochineal insects (Pettey, 1948). Of these, Dactylopius opuntiae (Cockerell) (Fig. 3) has been successful in controlling O. ficus-indica (Pettey, 1943; 1946; 1948; 1950; Annecke and Moran 1978), and Dactylopius austrinus De Lotto (Fig. 4) has been partially successful as a biological control agent on O. aurantiaca (Moran and Annecke, 1979).

Fig. 1. The prickly pear weed Opuntia ficus-indica in the field.

Fig. 2. The jointed cactus weed Opuntia aurantiaca in the field.

Fig. 3. The cochineal insect Dactylopius opuntiae on O. ficus indica.

Fig. 4. The cochineal insect Dactylopius austrinus on O. aurantiaca.

Fig. 5. The cochineal insect Dactylopius coccus on O. ficus-indica.

Although the cochineal insect Dactylopius coccus Costa (Fig. 5) was also introduced into South Africa (Pettey, 1943; Mann, 1969), it was not introduced as a biological control agent but as a source of cochineal dye. Although all Dactylopius species produce cochineal dye, D. coccus is the most suitable for this purpose (De Lotto, 1974). At present in South Africa D. coccus is not found in the field (H.G. Zimmermann pers. comm.).

The most conspicuous character of cochineal insects is the woolly thread-like "waxy covering" which has been considered to have protective properties (Mann, 1969; Walter, 1977). The "waxy covering" of D. opuntiae is similar in appearance to that of D. austrinus (Figs 3 and 4), and these are both different from the powdery covering of D. coccus (Fig. 5).

In South Africa the native ladybird beetle E. flaviventris (Fig. 6) was found feeding on D. opuntiae within two years of the cochineal becoming established (Pettey, 1946). Both the adults and larvae feed on the cochineal insect. Two studies (Annecke, et al. 1969; Burger, 1969) indicated strongly that E. flaviventris in high numbers is capable of effectively limiting population numbers of D. opuntiae and therefore reducing the effectiveness of the insect as a biological control agent. These authors worked in different areas where prickly pear was the dominant plant, D. opuntiae numbers were low and many E. flaviventris were present. They reduced the number of coccinellids by means of low concentrations of insecticide (2 oz DDT per acre) which did not harm the cochineal insects. A dramatic rise in

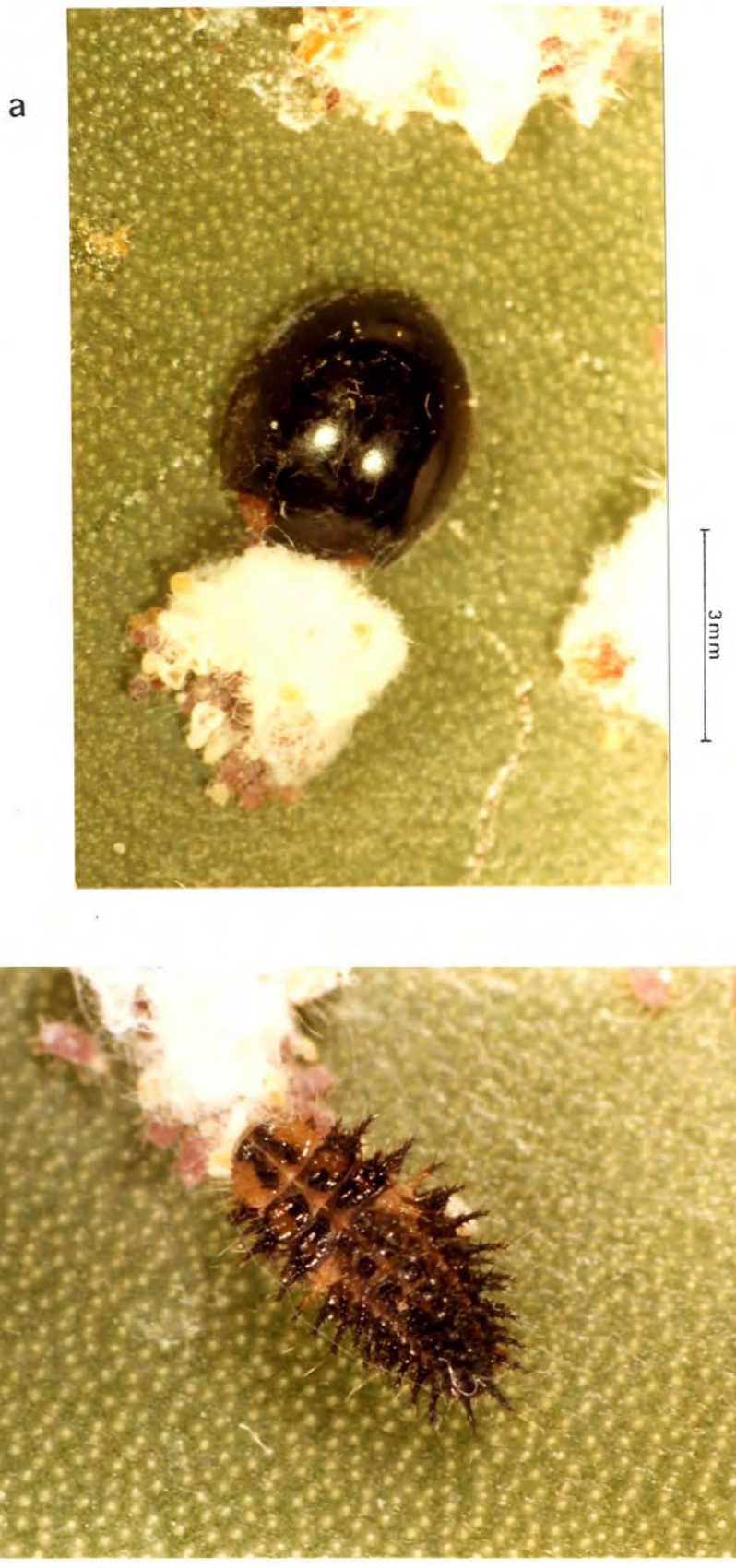


Fig. 6. The ladybird Exochomus flaviventris adult (a) and larva (b) feeding on D. opuntiae.

D. opuntiae numbers resulted and many large prickly pear plants were defoliated and killed. On the other hand E. flaviventris has been rarely observed to prey on D. austrinus in the field.

Differential predation by E. flaviventris on D. opuntiae but not on D. austrinus may be influenced by two suggested-protective mechanisms, the waxy covering of the cochineal insect (Walter, 1977) and their carminic acid (cochineal dye) content (Eisner, et al 1980). D. coccus because of its reputed high carminic acid content (Baranyovits, 1978) and its peculiar waxy covering, was included in the investigation.

The following five lines of investigation were conducted to determine which factors contribute to the differential predation on D. opuntiae and D. austrinus by E. flaviventris and reported in sequence in this thesis.

i) The ability of E. flaviventris to feed on different Dactylopius species was investigated. In one series of experiments the "waxy covering" was left intact and in others it was removed. Although E. flaviventris has been reported to feed on both D. opuntiae and D. austrinus in the laboratory (Geyer, 1947 a; b; Walter, 1976, 1977; Durrheim, 1980; Brooks, 1981; Morrice, 1981) there are no reports of E. flaviventris feeding on D. coccus in the field or in the laboratory.

ii) The structure of the wax strands secreted by the different species of cochineal insects was investigated. The non-cuticular waxes secreted by plant-feeding insects were regarded by Pollister (1938) as excretory products. However they have since been shown to be specially synthesized by the insect (Brown, 1975; Jackson and Blomquist, 1976). It is therefore reasonable to assume that the insects derive some advantage from the wax (Pope, 1983) as observed by Broadbent (1951) on "wax covered aphids".

The "waxy covering" of cochineal insects has been considered by Mann (1969) to have general protective properties and more specifically Walter (1977) showed that the "waxy covering" reduced predation by E. flaviventris. Selective predation by E. flaviventris could be the result of differences in the physical properties of the wax (Walter, 1977) or differences in composition of the wax as shown by Tulloch (1970) and Meinwald et al. (1975) for D. coccus and D. confusus.

iii) The choice of cochineal-prey species by E. flaviventris was investigated. Choice experiments were done with the "waxy covering" of the cochineal insects intact and when removed. The behaviour of the beetles when presented with each Dactylopius species separately was recorded.

iv) The protective properties of the red pigment (carminic acid) of the cochineal insects were investigated. Carminic acid is an anthraquinone (Thomson, 1971; Brown, 1975; Lloyd, 1980). Eisner et al. (1980) suggested that it acts like other quinones as a potent feeding-deterrent to predation, although Baranyovits (1978) reported

that no biological function has been demonstrated for carminic acid. Carminic acid concentration in prey individuals could influence the predation pattern of E. flaviventris.

v) Finally, the long-term effects on E. flaviventris of being fed entirely, for two generations, on each Dactylopius species was investigated. Hodek (1966) noted that the degree of food specialization of various predacious coccinellids varies, although there are no known monophagous species. Some aphid species have been found to be lethal to some coccinellid species, but not to others (Blackman, 1966; Okamoto, 1966). D. austrinus may therefore be an inadequate food (Geyer 1947 a), probably because of its carminic acid content, and this may result in selective predation of E. flaviventris on D. opuntiae.

GENERAL MATERIALS AND METHODS.

A laboratory colony of E. flaviventris was started from approximately 300 adults collected in the Grahamstown area (33°23'S; 26°29'E). The colony was kept in perspex cages (35x35x20cm). Heavy infestations of D. opuntiae on O. ficus-indica cladodes were placed in the cages as food. The colony was replaced every six months to reduce any possible inbreeding effects. The insectary, where the colony was maintained and where the experimental work was carried out, was programmed to simulate early summer conditions (Day- 14hr at 26(±1)°C and 45% (±10%) RH; night- 10hr with temperature and relative humidity slowly changing to 17°C and 90% RH).

In each experiment, newly emerged (maximum of one-day old) adult E. flaviventris were isolated and starved for three days before being used (unless otherwise stated), because adults younger than two days old do not feed (Geyer, 1947 a; and confirmed in this study). Each coccinellid was used in only one experiment and was then returned to the stock colony. In those experiments where continuous behavioural patterns were recorded, the coccinellids were starved for a period of four days, thereby enhancing the "hunger drive" and facilitating monitoring of the feeding activities.

Only adult Dactylopius females were used as a food source in these experiments because the females are conspicuous and sedentary, and together with the crawlers form the largest part of the Dactylopius

prey (Geyer, 1947 a). Walter (1976, 1977) and Durrheim (1980) reported that E. flaviventris will prey on both D. opuntiae and D. austrinus crawlers and adults in the laboratory.

In a number of the experiments the cochineal insects were "de-waxed". In the case of D. opuntiae and D. austrinus this was done by rolling the "waxy covering" off the body of the insect on to a large pin. D. coccus was "de-waxed" by brushing the powdery "waxy covering" off with a paint brush.

E. flaviventris was presented in all cases with an excess of cochineal prey obtained from growing plants to standardize the quantity and quality of the food source. The importance of the amount of food available to coccinellid fecundity and development has been demonstrated. Ives (1981) showed that egg production of Coccinella trifasciata Mulsant decreased with declining food availability, and Frazer et al. (1981) suggested, for the same species that reproduction is optimized when food supply is high. Baumgaertne et al. (1981 a) noted that access to an unlimited food supply resulted in the shortest generation time for a Hippodemia species.

All containers used for housing beetles had ventilation holes covered with muslin, and from here on will be referred to as "tops". All vials unless otherwise stated had a round paper disc which covered the floor of the vial to form a rough surface on which the beetles could easily walk.

In all experiments, results for male and female E. flaviventris were recorded separately. On analysis, there was no significant difference in any experiment between males and females. Therefore the results obtained from male and female coccinellids have been combined in all analyses.

This study consists of a number of sections (see introduction). All of these sections comprised a number of experiments each with its own methods, and these will be described when each experiment is discussed.

1. FEEDING EXPERIMENTS WITH THE "WAXY COVERING" OF THE PREY INTACT.

The ability of E. flaviventris beetles to feed on three Dactylopius species with the "waxy covering" intact.

Experiments were designed to investigate whether E. flaviventris would feed on adult female cochineal insects of the three species being examined (D. opuntiae, D. austrinus and D. coccus). Firstly E. flaviventris was presented with cochineal insects with intact "waxy coverings" to ascertain the effectiveness of the "waxy covering" in preventing predation.

Open-ended glass vials (diameter of 25mm and length of 60mm) were placed over single laboratory-reared Dactylopius adult females (i.e. leaving the "waxy covering" intact) which were still attached to, and feeding on, the host plant (Fig. 7). A single E. flaviventris was introduced into each vial and the end sealed with a top. Daily observations were made to record whether E. flaviventris had penetrated the "waxy covering" and fed on the Dactylopius female or not. The experiment ran until E. flaviventris either fed on the cochineal insect or died of starvation.

Fig. 7. E. flaviventris (arrow) confined with a D. opuntiae female to determine whether the beetle will feed on cochineal insects that have the "waxy covering" intact. The plant host is O. ficus-indica.

The results are summarized in Table 1. E. flaviventris penetrated the "waxy covering", and fed readily on D. opuntiae (39 out of 50 cases), fewer managed to penetrate the "waxy covering" and feed on D. austrinus (9 out of 50 cases) and no beetles penetrated the waxy barrier of D. coccus (0 out of 50 cases).

Table 1. The percentage survival of E. flaviventris when presented with different food sources. (N=number of beetles observed).

E. flaviventris fed on	% fed and survived	N
D. opuntiae	78	50
D. austrinus	18	50
D. coccus	0	50

These experiments showed that, in the laboratory, the "waxy covering" appeared to prevent predation. They also indicated that the "waxy covering" of different Dactylopius species does afford different degrees of protection.

The survival times of beetles that could not penetrate the "waxy covering" of the three Dactylopius species.

The results of the previous experiment where E. flaviventris was confined in a vial with a Dactylopius female on a cladode (Fig. 7), were elaborated and additional experiments were done. The longevity of the beetles that could not penetrate the intact "waxy covering" was recorded. Using the same methods the length of time that E. flaviventris could survive on the honeydew of the different Dactylopius species was recorded. As a control, vials containing beetles were placed on clean cladodes of the host plants (O. ficus-indica and O. aurantiaca) and no prey was provided.

The beetles that did not manage to feed on the cochineal prey and the beetles in the control experiments all died. However, the beetles survived for a varied period of time, living for a mean period (Table 2) of 7,9 days on D. opuntiae, 7,8 days on D. austrinus and 4,1 days when confined with D. coccus. The beetles that "starved to death" on D. opuntiae and D. austrinus lived for a similar length of time, this in turn was similar to the time that beetles lived on honeydew only (8,2 days on D. opuntiae honey dew, 8,6 days on D. austrinus honey dew and 8,3 days on D. coccus honey dew). The beetles confined with D. coccus lived (i) approximately half as long (4,1 days) as those confined with D. opuntiae and D. austrinus, and (ii) the same length of time as beetles that received no food (4,9 days on O. ficus-indica and 4,6 days on O. aurantiaca).

The beetles that could not penetrate the "waxy covering" of D. opuntiae and D. austrinus presumably fed on the honeydew of the cochineal insects and therefore lived twice as long as those beetles that had no food. On the other hand beetles that were confined with D. coccus were unable to get to the honey dew as the powdery "waxy covering" fouled up their tarsi. This resulted in loss of grip and the beetles fell on their backs and were unable to right themselves. This was not the case in the other cochineal insects. Some of the beetles that were confined with D. austrinus were also incapacitated by getting entangled in the "waxy covering", but none of those confined with D. opuntiae became entangled. D. coccus has a more effective "waxy covering" barrier against E. flaviventris predation than D. austrinus which was shown to be more effective than the "waxy covering" of D. opuntiae.

Table 2. The longevity of E. flaviventris when unable to penetrate the cochineal prey provided. ($\bar{x}$=mean and N=number of beetles observed).

	Starved to death	
Food regime	$\bar{x}$ days alive	N
No food on O. ficus-indica	4,9	15
No food on O. aurantiaca	4,6	15
D. opuntiae	7,9	11
D. austrinus	7,8	41
D. coccus	4,1	50
D. opuntiae honeydew on O. ficus-indica	8,2	15
D. austrinus honeydew on O. aurantiaca	8,6	15
D. coccus honeydew on O. ficus-indica	8,3	15

The differences between the "waxy covering" of D. coccus and the other two Dactylopius species was investigated next, and this was followed by a study of the differences between the wax of D. austrinus and that of D. opuntiae.

2. THE "WAXY COVERING".

Differences in the "waxy coverings" between the three Dactylopius species.

In the previous experiments it was shown that E. flaviventris could not penetrate the "waxy covering" of D. coccus, and found difficulty in penetrating the "waxy covering" of D. austrinus, but penetrated the "waxy covering" of D. opuntiae with relative ease. The component wax strands of D. opuntiae, D. austrinus and D. coccus were investigated in an attempt to understand why the "waxy coverings" of these Dactylopius species differ in their effect on E. flaviventris.

The "waxy coverings" of the three Dactylopius species have different properties in relation to predation by Exochomus. Superficially the appearance of the "waxy covering" of D. opuntiae and D. austrinus is similar, appearing woolly and thread-like, whereas that of D. coccus appears powdery (Fig. 5).

Under the scanning electron microscope (SEM) striking differences were noted between the "waxy covering" of D. coccus and the other two Dactylopius species (Fig, 8). D. coccus (Fig. 8 a, b) produces a large number of short tubular wax filaments that lie loosely on the body, whereas D. opuntiae and D. austrinus produce long filamentous threads (Fig. 8 c, d,) which remain attached to the body of the insect.

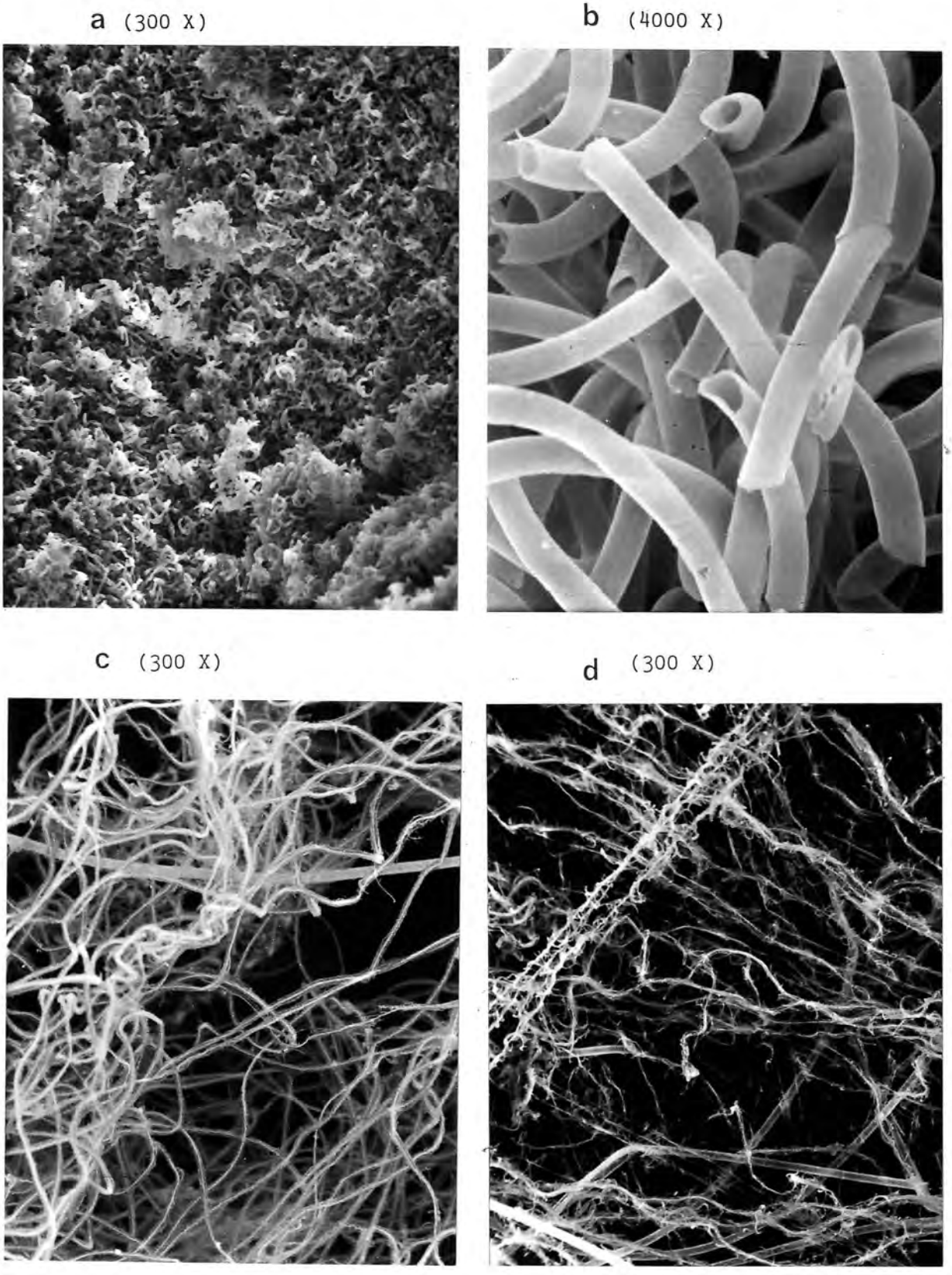


Fig. 8. SEM micrographs of the "waxy covering" of a), b) D. coccus, c) D. opuntiae, and d) D. austrinus.

The effects of the different "waxy coverings" on the tarsi of E. flaviventris.

The effects of the different "waxy coverings" on the tarsi of E. flaviventris were demonstrated with the aid of SEM micrographs of E. flaviventris tarsi before and after coming into contact with the "waxy coverings" of D. opuntiae, D. austrinus and D. coccus (Fig. 9). These results were obtained by confining a beetle with the appropriate cochineal insect in a vial for one minute. The beetles were then killed by freezing and the tarsi were examined under the SEM.

The micrographs clearly show that the powdery wax of D. coccus became clogged in the tarsal setae of E. flaviventris whereas the tarsi that came into contact with the other two species were relatively clean. SEM micrographs (Figs 9 a) show clean E. flaviventris tarsi. In Figs 9 b the clogging of the tarsal setae by D. coccus wax is shown. In Figs 9 c, d, the tarsi of E. flaviventris that had been in contact with D. opuntiae and D. austrinus wax for one minute are shown. Here there is little entanglement of the tarsal setae.

Could clogging of the tarsal setae of E. flaviventris affect the adhesion of the setae to the substrate? Many suggestions have been made as to how the adhesive setae of beetles and other animals adhere to surfaces. Stork (1980 a) regards most of these to be based on poor morphological and experimental evidence. At present the most probable mode of adhesion is that proposed by Ruibal and Ernst (1965) for

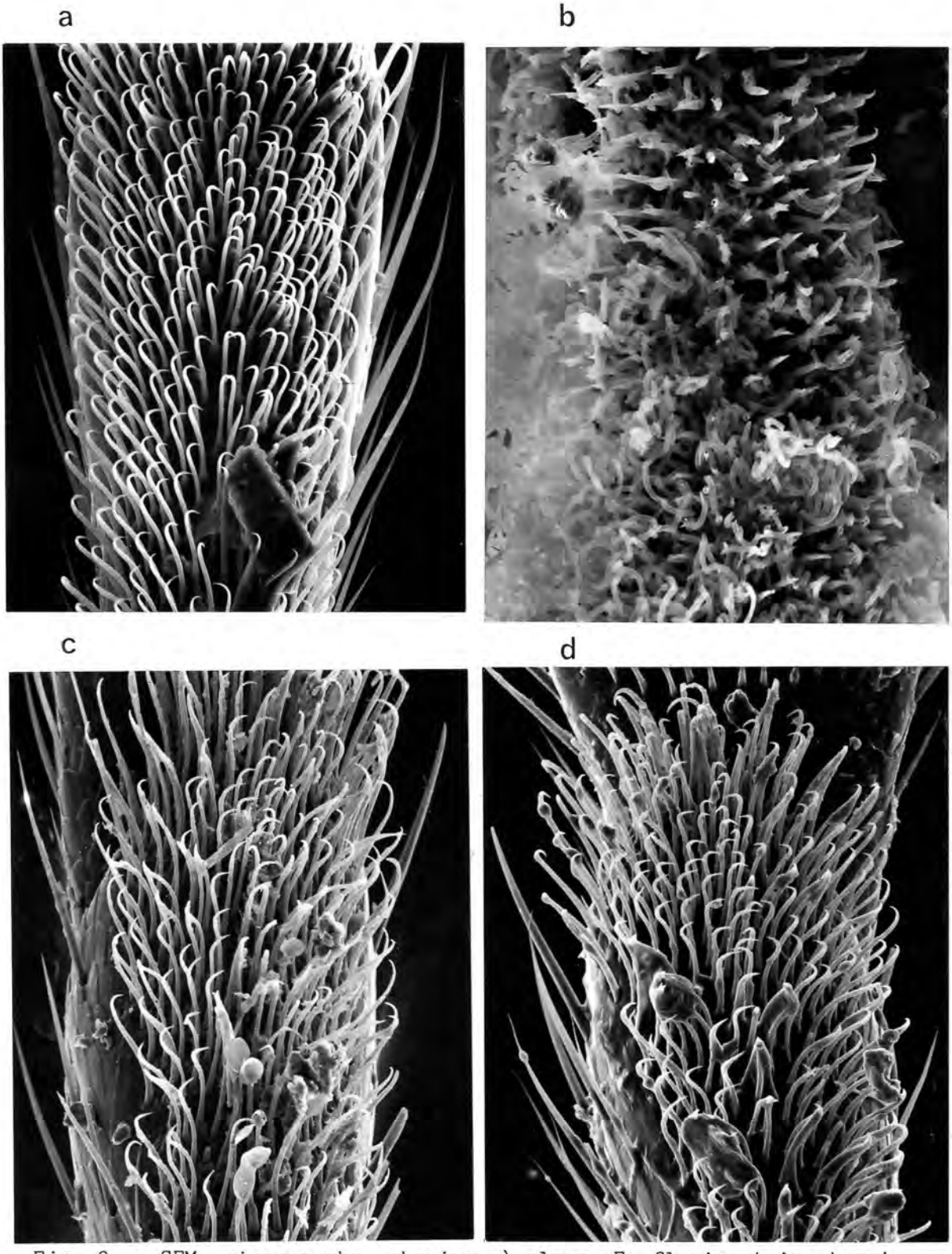


Fig. 9. SEM micrographs showing a) clean E. flaviventris tarsi. Tarsi that have come into contact for one minute with the "waxy covering" of D. coccus b), D. opuntiae c), and D. austrinus d). (Magnification 600X)

gecko tarsi, Edwards and Tarkanian (1970) for Rhodnius prolixus Stahl, Stork (1983 a) for the housefly and Stork (1980 c, 1983 b) for a number of beetle species. They propose that the mode of adhesion is direct molecular adhesion of the tarsal setae with the substratum and that the cohesion forces of a thin fluid layer would probably increase the adhesion. Stork (1980 b) showed that the wax bloom of Brussels sprouts made the plants less adhesive to Phaedon cochleariae (Fabricius)(Chrysomelidae; Coleoptera), due to clumps of powdery debris and wax blooms covering the adhesive setae of the beetle tarsi. The debris prevented the tarsal setae from coming into contact with the substrate and thereby decreased the direct molecular adhesion.

Because the tarsal setae are clogged with the D. coccus wax, the number of contact points between tarsal setae and substratum are greatly reduced, resulting in decreased cumulative cohesion forces. The powdery wax of D. coccus does decrease the adhesion of the tarsal setae to the substrate by clogging them. However, this SEM investigation showed no obvious reasons why E. flaviventris is able to penetrate D. opuntiae "waxy covering" more easily than that of D. austrinus. Therefore the differences in the "waxy covering" between D. opuntiae and D. austrinus were investigated.

The physical properties and the structures producing the "waxy covering" of D. opuntiae and D. austrinus.

The "waxy covering" of D. opuntiae and D. austrinus is thread-like and does not entangle the tarsal setae of E. flaviventris as does that of D. coccus. The beetles find it more difficult to penetrate the "waxy covering" of D. austrinus than that of D. opuntiae and do get entangled in the threads of the former. Are there physical differences between the "waxy covering" of D. opuntiae and D. austrinus which make the latter more difficult to penetrate?

The physical properties of the "waxy covering" of D. opuntiae and D. austrinus were studied by Walter (1977), who found that the initial strength of the "waxy covering" of laboratory-reared D. austrinus was nearly twice that of laboratory-reared D. opuntiae whereas the elasticity of the "waxy covering" in both species is very similar. The "waxy covering" of D. opuntiae collected in the field showed a significant increase in strength and a decrease in elasticity compared to laboratory-reared D. opuntiae. In contrast D. austrinus "waxy covering" showed little difference in strength and a small decrease in elasticity between laboratory-reared and field-collected cochineal insects (Walter, 1977). The differences were attributed to different degrees of compaction of the "waxy covering", due to weathering, as well as possible different physical and chemical properties of the "waxy covering". These differences could be due to differences in the structures that produce the "waxy coverings" of the cochineal insects (Hartley et al. 1983).

The differences in number, shape, size and distribution of the wax-producing structures are so consistent that they have been used as taxonomic features in distinguishing between different Dactylopius species (De Lotto, 1974). There are three different structures that produce the outer "waxy covering" of Dactylopius, viz. setae, quinquelocular pores (wide-rimmed or narrow-rimmed), and ducts. These structures are spread over the whole body surface of the cochineal insect (De Lotto, 1974). The differences in these structures and in their positioning between D. opuntiae, D. austrinus and D. coccus are shown in Fig 10 (Ferris, 1955; De Lotto, 1974).

D. opuntiae has four types of setae whereas D. austrinus has only two. Both these two species have a large number of wide-rimmed pores, narrow-rimmed pores and ducts. D. coccus differs from D. opuntiae and D. austrinus in having no narrow-rimmed pores or ducts, and very few setae.

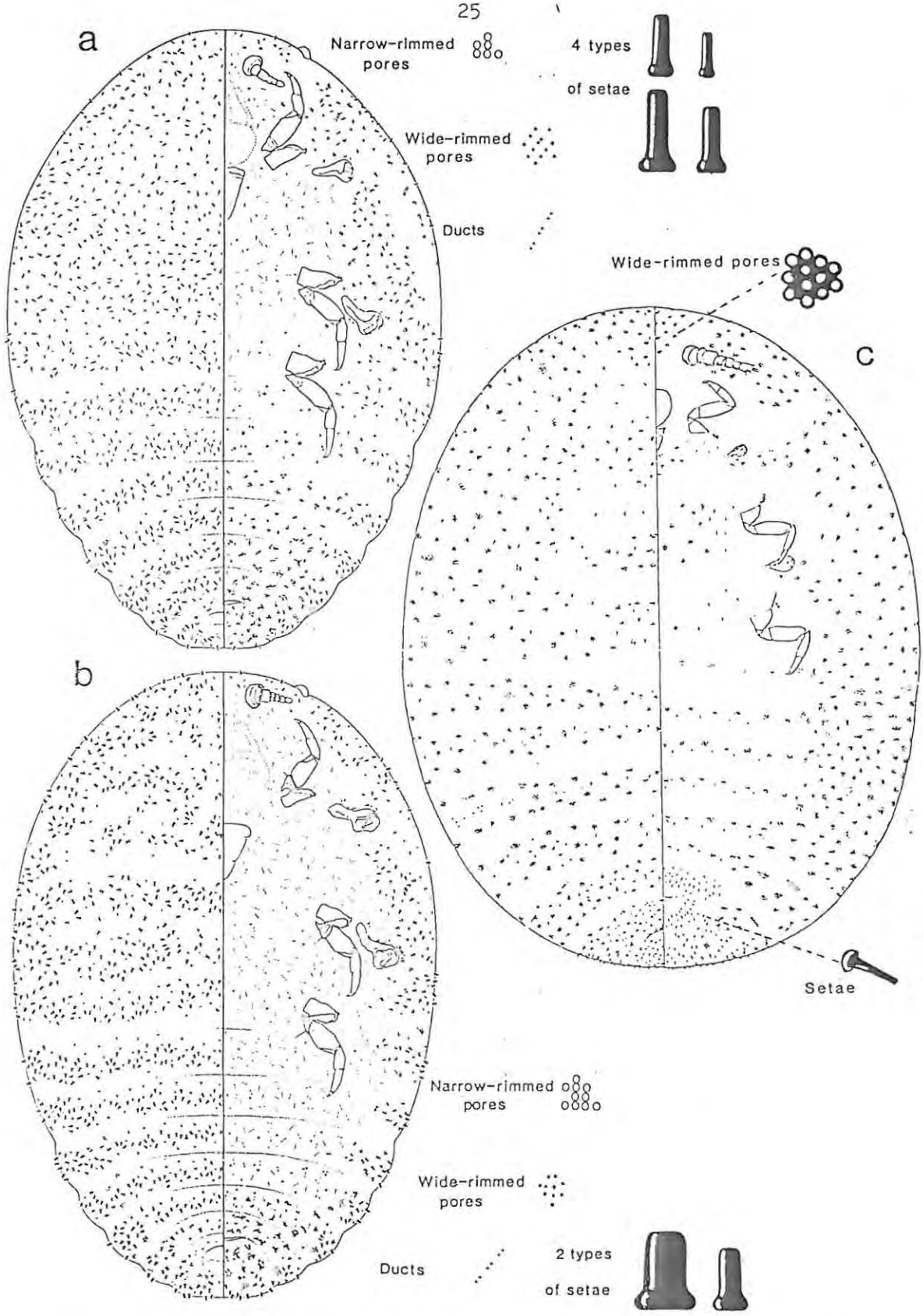


Fig. 10. a) D. opuntiae, b) D. austrinus and c) D. coccus showing the number and distribution of the "waxy covering" producing structures (from, Ferris, 1955; De Lotto, 1974).

The different structures produce different "waxy covering" components as shown by the SEM micrographs of the peg-like setae of D. opuntiae (Fig. 11 a) which produce filamentous components of the "waxy covering" which appear tubular (Fig 11 b, c). D. austrinus and D. coccus setae produce similar filaments. The quinquelocular pores (Fig. 12 a) produce threads that appear ragged (Fig. 12 b) in D. opuntiae and D. austrinus whereas D. coccus pores produce short, tubular, filaments (Fig. 12 c), which are smooth. These differences were found in an investigation with G.H. Walter and A.H. Hartley that is still in progress.

While examining the quinquelocular pores under the SEM the ducts associated with the pores (Fig. 13 a) appeared to produce a rod-like substrate to which the content of the pores adhered (Figs 13 b). Hartley et al. (1983) examined the ultrastructure of wax-secreting glands, and described the peg-like setae (Fig. 14) and possible wax-metabolising cells. The quinquelocular pores as well as their associated wax-secretory cells (Fig. 15) which appear similar in structure to the wax-secretory cells of the setae were also described. The wax-secretory cells appear similar to those of the margarodids Porphyrophora, Sphaeraspis and Eurhizococcus (Foldi, 1981), the psyllid Anomoneura (Waku, 1978) and diaspidid Aonidiella (Pesson and Foldi, 1978). The section through the quinquelocular pores also includes the associated central duct. The duct is lined with cuticle and secrets a substance which differs from that secreted by the wax-secreting cells of the setae and pores (Fig. 15).

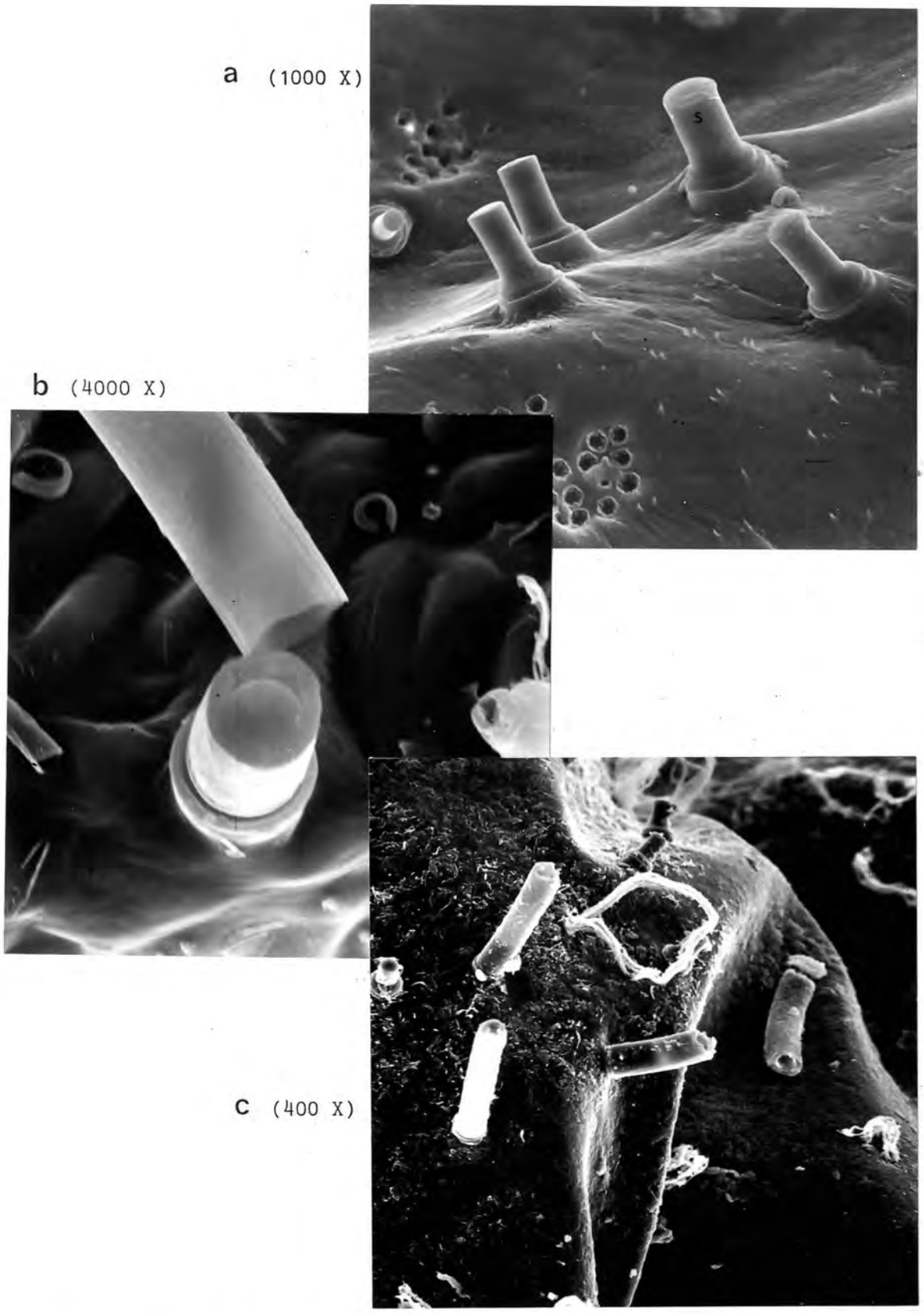


Fig. 11. SEM micrographs of the peg-like setae (s), a) "de-waxed", b) and c) with "waxy covering" attached to D. opuntiae.

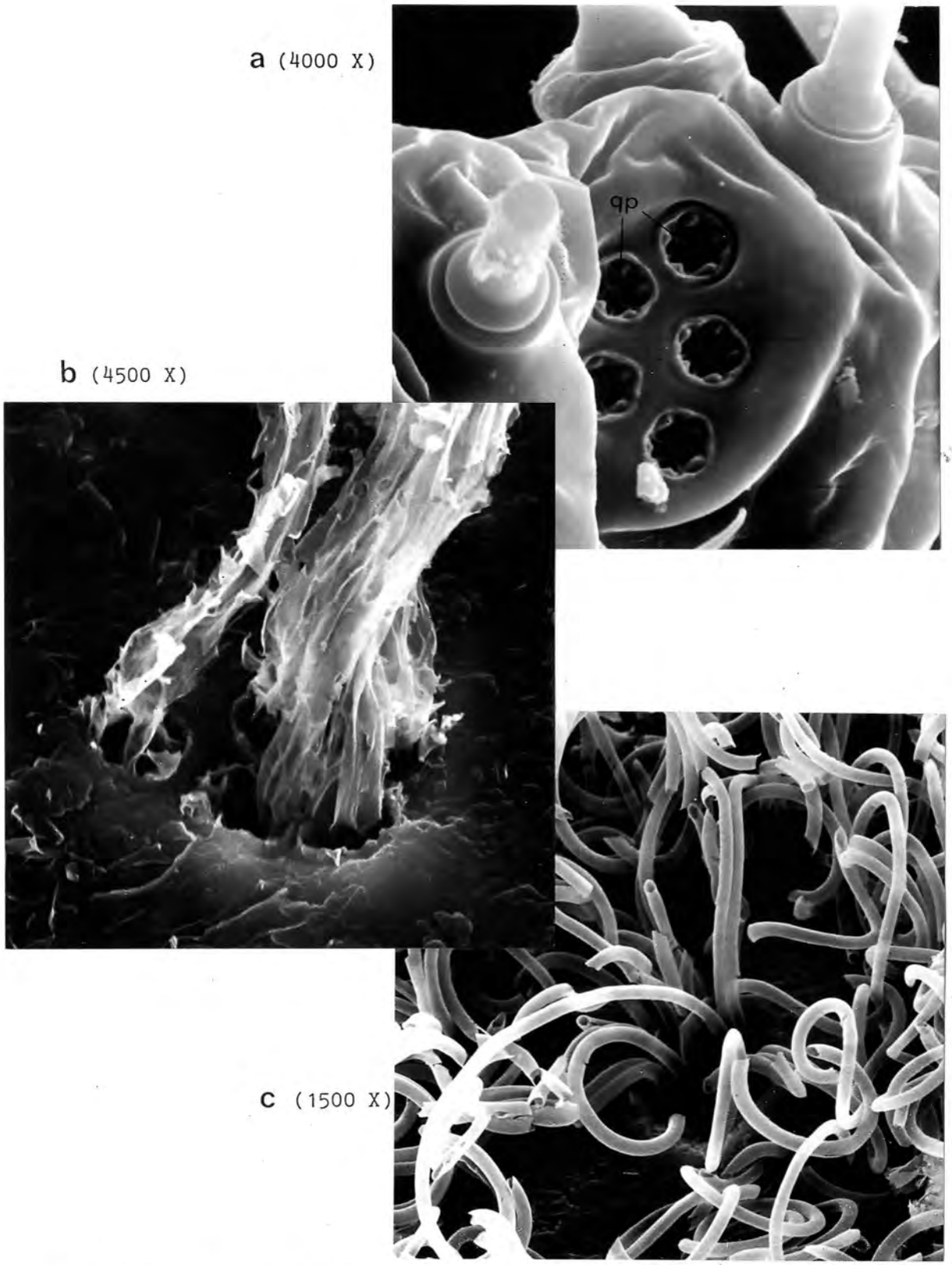
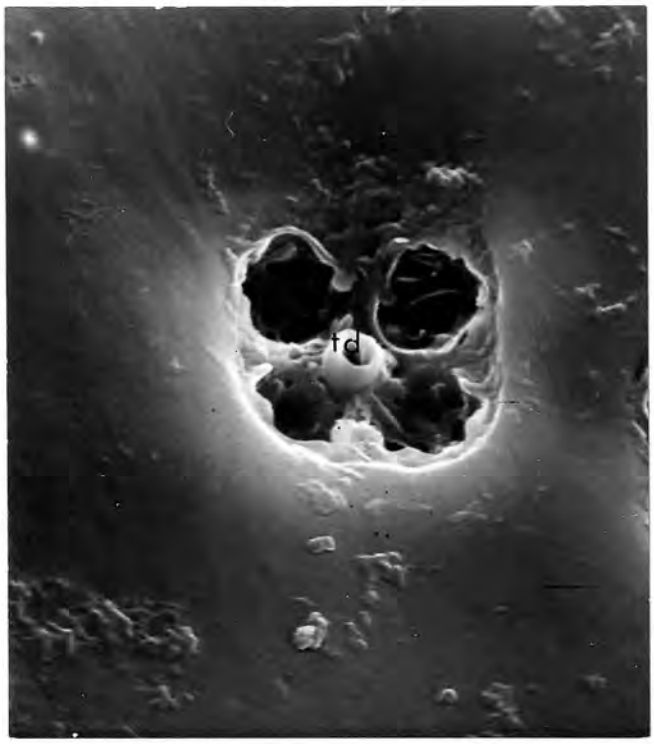


Fig. 12. SEM micrograph of the quinquelocular pores (qp), a) "de-waxed", b) with the "waxy covering" attached to D. opuntiae, and D. coccus c).

a

(8000 X)



b

(8500 X)

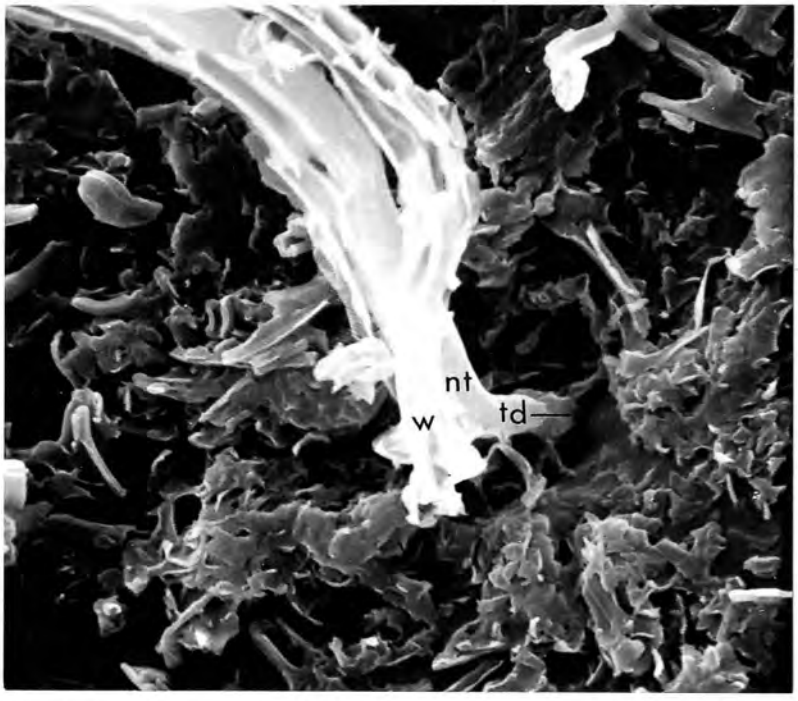


Fig. 13. A cluster of four quinquelocular pores (qp) incorporating a tubular duct (td) a) with the "waxy covering" removed b) with the "waxy covering" still attached (w=wax, nt=non-wax thread).

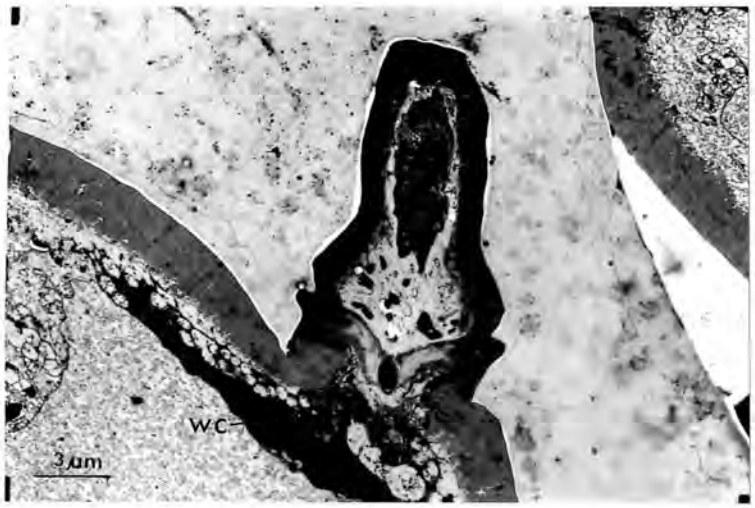


Fig. 14. A section through a seta and its wax-secreting cells (wc), (c=central column) (from, Hartley et al. 1983).

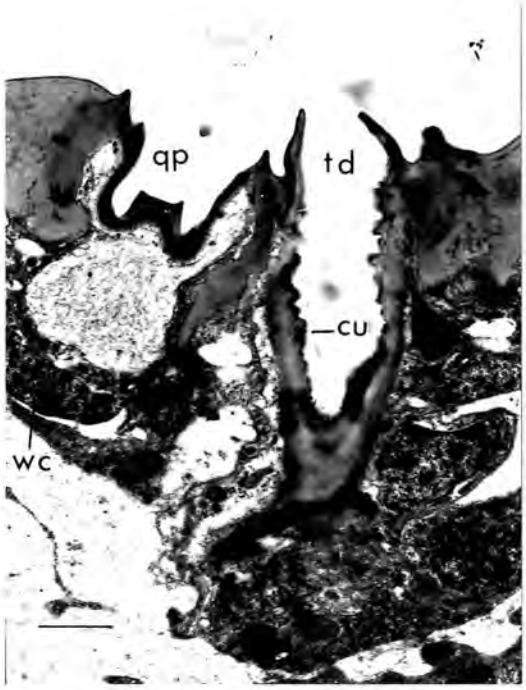


Fig. 15. A section through a quinquelocular pore (qp) and its wax-secreting cells (wc) and associated tubular duct (td) which is cuticle lined (cu) (from, Hartley et al. 1983).

To examine the structures that produce the "waxy covering", the cochineal insects were de-waxed. This was done either mechanically or by a combination of mechanical and chemical methods. In the

mechanical method a large pin was used to roll the "waxy covering" threads off the body. When necessary, the remainder of the covering was then dissolved off with benzene or hexane. In attempting to "de-wax" cochineal insects by the chemical method a thread-like mass remained and had to be removed mechanically. Attempts were then made to dissolve the "waxy covering" completely using the following methods.

i) D. opuntiae and D. austrinus with their "waxy coverings" intact were immersed in separate solvents such as hexane, benzene, carbon tetrachloride, chloroform and ethanol 80%, 90%, and a 100% . The thread-like mass remained, even after being immersed for seven days in these solvents.

ii) The thread-like mass was still present even after the insects had been subjected to refluxing solvents for one hour.

This indicates that either a highly resistant wax is produced by the cochineal insects, or more likely, a non-wax substance is produced. The presence, absence, or quantity of this substance in the "waxy covering" could influence the protective properties of the "waxy covering" on the cochineal insects.

The "waxy covering" of D. opuntiae and D. austrinus was mechanically removed, weighed and immersed in hexane for one hour at 50°C, and was agitated every 15 minutes. The "waxy covering" was removed from the hexane and dried (50°C; 10minutes) then weighed again.

In Table 3 the percentage weight loss of the "waxy covering" was recorded, showing that D. austrinus lost a mean of 20.1% and D. opuntiae lost a mean of 29,4%. These differences were significantly different at a probability of 5% ($0,05 > P < 0,01$) with the data transformed using an arcsine transformation and testing for significance by means of a t-test.

Table 3. The mean percentage weight loss of the "waxy coverings" of D. opuntiae and D. austrinus when placed in warm hexane for an hour (N=number of replicates).

	Mean % weight loss	N
D. opuntiae	29,4%	20
D. austrinus	20,1%	20

The results in Table 3 indicate that D. austrinus lost less hydrocarbon-soluble matter than D. opuntiae. Therefore D. austrinus has more of the non-wax substance than D. opuntiae. This substance appears to be produced by the ducts associated with the quinquelocular pores (see Hartley et al.1983), and could be the key to the difference in reducing E. flaviventris predation in these two Dactylopius species.

It seems that a greater component of the non-wax substance produced by the ducts reduces beetle predation, which is shown by D. austrinus and D. opuntiae where the former has more of the non-wax substance than the latter.

3. FEEDING EXPERIMENTS WITH THE "WAXY COVERING" OF THE PREY REMOVED

The ability of E. flaviventris beetles to feed on three Dactylopius species with their "waxy coverings" removed.

The "waxy covering" does afford some protection to the cochineal insects, but if this protection is removed can the contents of the cochineal insect sustain the coccinellid beetle. This was investigated by feeding individual coccinellid beetles exclusively on "de-waxed" females of one of the three Dactylopius species. A single E. flaviventris adult was placed in a glass vial (diameter of 25mm and length of 15mm) which was sealed with a top. The beetles were fed every second day so that a continuous supply of food was available, 20 beetles were fed exclusively on each of D. opuntiae, D. austrinus and D. coccus.

Observations showed that E. flaviventris could not bite through the smooth, tough, de-waxed cuticle of D. coccus and died of starvation. This was not the case with D. opuntiae or D. austrinus and their cuticle was easily pierced. In order to standardize experimental conditions, therefore, all Dactylopius species were first pierced, and then presented to E. flaviventris. Daily recordings were made to determine how long E. flaviventris lived on these diets. The experiment was run for 33 days and the results are presented in Table 4. Although E. flaviventris survived on both

D. opuntiae and D. austrinus for 33 days they were unable to survive on a diet comprised only of D. coccus. Female beetles that were fed on D. coccus lived a few days longer than male beetles (11,6 and 8,9 days respectively).

Table 4. The survival (in days) of E. flaviventris when fed on D. coccus, D. opuntiae, or D. austrinus (N=total number of beetles observed).

D. coccus	Mean survival of 10,25 days	N=10
D. opuntiae	All alive after 33 days	N=10
D. austrinus	All alive after 33 days	N=10

D. coccus, even when de-waxed and pierced, was unable to sustain E. flaviventris for any length of time (mean of 10,25 days), making D. coccus an unsuitable food source for the coccinellids. To investigate whether E. flaviventris chooses one Dactylopius species in preference to another, choice experiments were carried out.

4. CHOICE EXPERIMENTS AND FEEDING BEHAVIOUR

The choice by E. flaviventris when presented simultaneously with three Dactylopius species with their "waxy covering" intact or removed.

The experiments presented in the previous section showed that E. flaviventris could survive on D. opuntiae and D. austrinus as a food but that D. coccus did not sustain the beetles at all. Experiments were conducted to determine whether E. flaviventris chooses one Dactylopius species in preference to another. In all these experiments the coccinellids were reared, from first instar, on a species of mealybug (Pseudococcidae) that was reared on potato sprouts. Therefore the first time that the coccinellids encountered Dactylopius was at the start of each experiment. All coccinellids were starved for three days prior to the commencement of the experiment unless otherwise stated. Small containers were used so as to minimize any possible bias due to searching behaviour.

A choice of prey was presented to E. flaviventris by placing a single coccinellid in a vial (diameter of 30mm and length of 12mm). The vial contained an EPX foam floor which had three holes, into which three plugs of cactus (Fig. 16) (extracted with a cork borer) were placed. Each cactus plug supported a single specimen of a Dactylopius species (D. opuntiae, D. austrinus or D. coccus). The foam was kept damp with water so that the plugs of cactus did not dry out. The Dactylopius species were reared in the laboratory with their "waxy covering" intact. Each vial was turned through 120° to change the orientation

of the prey and reduce any possible directional stimuli. Daily observations were made on the feeding preferences of E. flaviventris. Two levels of damage were noted. Firstly minor damage occurred when the coccinellids pierced the cuticle of cochineal insects, on first encountering them, to test the food source. Secondly, extensive damage was recorded when the beetles fed on a cochineal insect, and this could take from one to three days to occur.

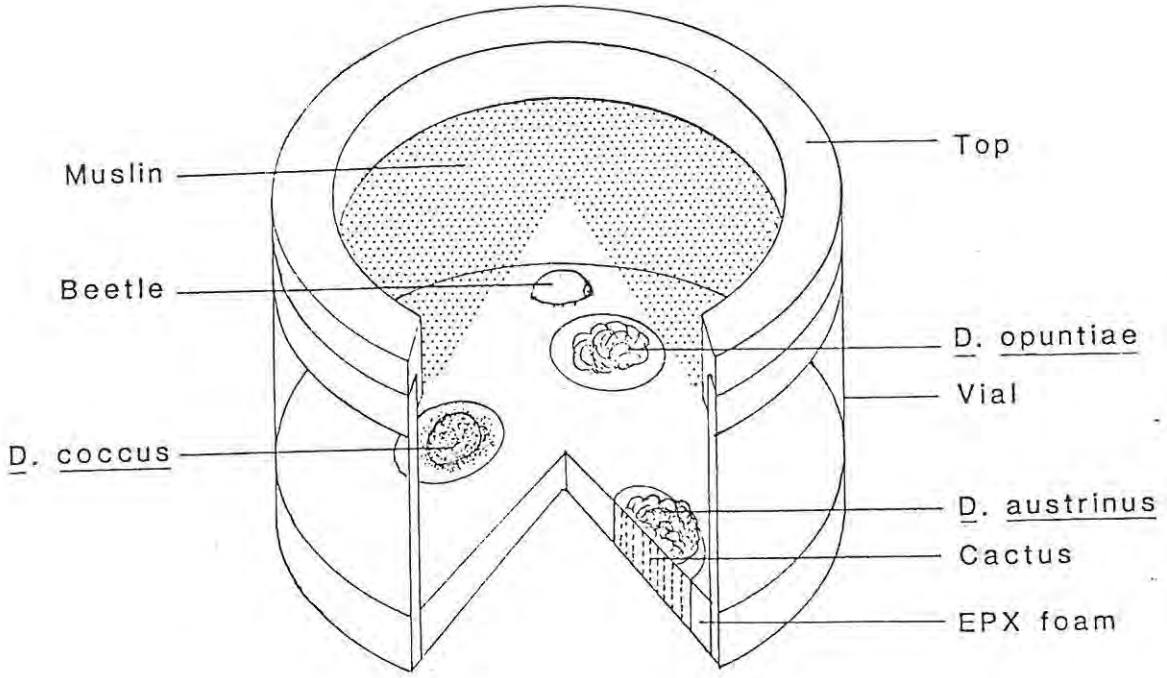


Fig. 16. The choice chamber, with D. opuntiae, D. austrinus and D. coccus as a choice of prey for E. flaviventris.

The experiment was repeated with "de-waxed" cochineal insects as prey in place of intact cochineal insects. Three depressions were hollowed out of the foam floor to hold the "de-waxed" cochineal insects, which were not attached to the host plant.

E. flaviventris showed a preference for D. opuntiae over the other two Dactylopius species when the "waxy covering" was intact and also when it was removed. During the experiment many of the prey in the arena were pierced but not necessarily extensively damaged. In Table 5 results are shown of only those cochineal insects that were extensively damaged i.e. those that had been used as food by E. flaviventris.

Table 5. The number of E. flaviventris that fed on D. opuntiae, D. austrinus and D. coccus when given a choice between the three species. Experiments were conducted with cochineal insects that had their "waxy covering" both intact and removed.

Prey species selected	"Waxy covering"	
	intact	removed
D. opuntiae only	19	21
D. austrinus only	4	5
D. coccus only	1	0
D. opuntiae and D. austrinus	3	4
D. opuntiae and D. coccus	1	0
D. austrinus and D. coccus	1	0
D. opuntiae D. austrinus and D. coccus	1	0
Total	30	30

D. coccus was pierced and extensively damaged in only four of the replicates. In previous experiments the beetles were unable to feed on D. coccus, but here their ability to penetrate the cuticle of this species appeared to be due to the apparatus used. Because E. flaviventris had a firm grip on the foam, it did manage to pierce D. coccus from below.

With the "waxy covering" intact or with it removed E. flaviventris preferred D. opuntiae to the other two species. Even with D. opuntiae as the preferred prey species, difficulty was found in observing this difference in an eight-hour continuous observation period. This difficulty stemmed from the beetles habit of attempting to feed off the first prey they encountered. To overcome this difficulty the beetles were presented with the contents of each cochineal species individually. The time for each activity (feeding, resting, walking and grooming) was then recorded.

The behaviour of E. flaviventris when presented with each Dactylopius species separately.

The fact that some of the E. flaviventris fed on both of the less suitable prey species (i.e. D. austrinus and D. coccus) may have been due to the choice of food being largely determined by the first prey encountered by the starved predator. Another series of experiments was therefore conducted to observe the behaviour of starved adult E. flaviventris. A single E. flaviventris was placed in the centre of a petri-dish which contained the contents of four D. opuntiae females arranged at the four poles around the edge of the dish. The

time that the beetles spent feeding, walking, resting, and grooming was recorded over a 75-minute period. The procedure was repeated for beetles provided with D. austrinus and D. coccus. In this investigation all the coccinellids were starved for four days prior to the experiment.

The results (Table 6) show that there was little difference between mean feeding times on D. opuntiae and D. austrinus whereas the feeding time on D. coccus was notably shorter. It is therefore clear that even when the coccinellids are starved D. coccus is seldom eaten, whereas both D. opuntiae and D. austrinus are more readily consumed.

Table 6. The mean time (minutes) spent feeding, walking, resting and grooming by E. flaviventris over a period of 75 minutes when the beetles were presented with either D. opuntiae, D. austrinus or D. coccus (N= number of beetles observed).

	Mean time (minutes)				
	Feeding	Walking	Resting	Grooming	N
D. opuntiae	55,30	6,85	11,20	1,65	15
D. austrinus	56,12	9,95	8,93	0,00	15
D. coccus	36,55	15,23	20,15	4,07	15

The results of this, and the previous experiment, show clearly that D. coccus is not acceptable to E. flaviventris as a food source. D. opuntiae is chosen in preference to D. austrinus in a long term

choice experiment, although a starved beetle will initially feed equally readily on either D. opuntiae or D. austrinus as indicated by the last experiment. The role of carminic acid was next investigated to establish whether the preference for D. opuntiae was linked to the amount of carminic acid in the body of the cochineal insects.

5. THE ROLE OF CARMINIC ACID IN PREVENTING PREDATION.

Carminic acid as a drinking and feeding deterrent.

Carminic acid, in various concentrations, was presented to E. flaviventris to ascertain whether it influenced drinking and feeding behaviour. The effect of carminic acid on drinking time of E. flaviventris was investigated by presenting coccinellids with different concentrations of an aqueous carminic acid solution ("purified" carminic acid crystals were obtained from BDH Chemicals Ltd). A single E. flaviventris was placed in a petri-dish arena (diameter of 65mm) (Fig. 17) which contained a ring of blotting paper (width 2,5mm) around the edge. The blotting paper was soaked in an aqueous carminic acid solution. The coccinellids were starved for four days before being introduced into the arena. In the arena the behaviour of E. flaviventris was observed and categorized as drinking, walking, resting, and grooming. After the coccinellid had been placed in the centre of the arena the duration of each activity was recorded. Observations in each case were continued for ten minutes as little drinking behaviour was noted after this time. The same procedures were followed using distilled water (0%), 0,5%, 1%, 2%, 4%, 8%, and 16% aqueous carminic acid solutions. Apart from the distilled water controls, a further set of controls were run by soaking blotting paper in an aqueous neutral red solution.

Fig. 17. The petri-dish arena used to observe E. flaviventris behaviour when presented with different concentrations of an aqueous carminic acid solution.

The aqueous carminic acid solutions discouraged drinking by E. flaviventris (Fig. 18), and the higher the concentration, the less time was spent drinking. Even concentrations as low as 0,5% carminic acid had a marked effect on feeding time. No significant difference in feeding time was noted between the distilled water and the neutral red solution. The increase in walking, sitting, and grooming times with the increase in carminic acid concentration can be attributed to less time being spent feeding.

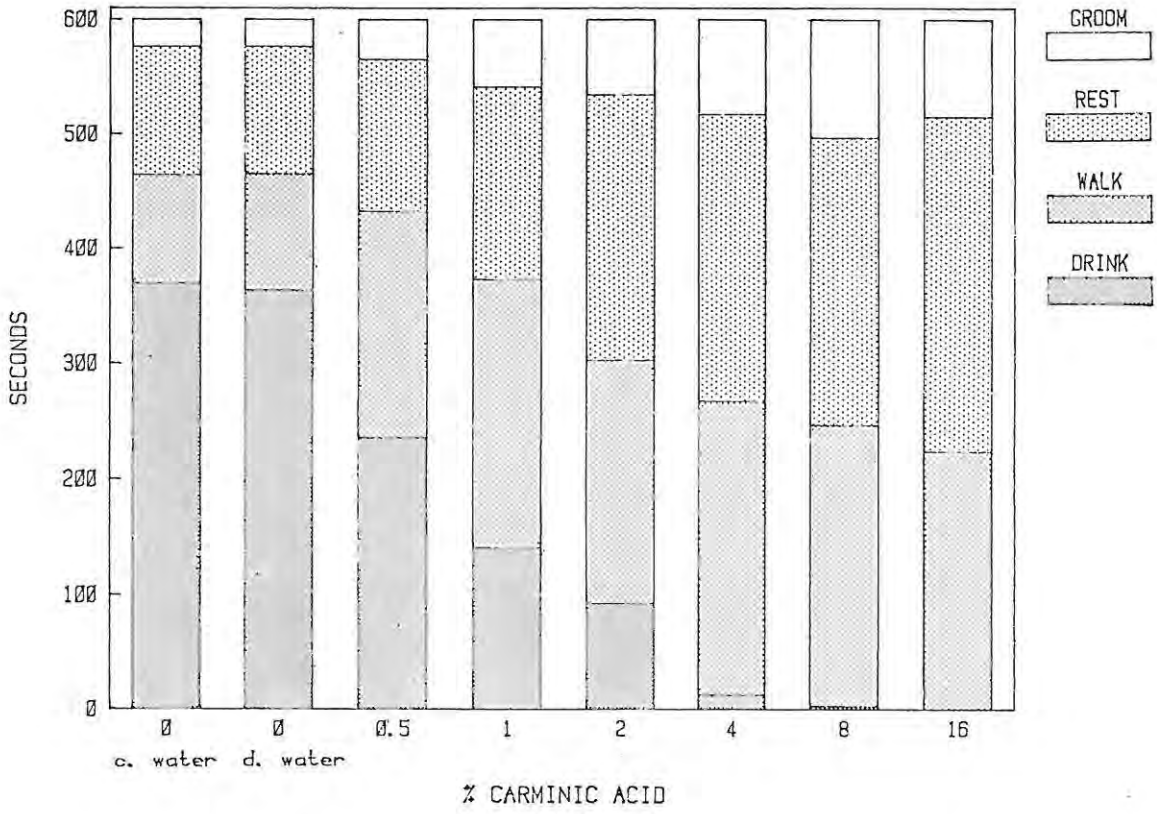


Fig. 18. The time spent drinking, walking, resting and grooming by E. flaviventris when presented with different concentrations of an aqueous carminic acid solution. The controls consisted of distilled water coloured with neutral red (c. water) and pure distilled water (d. water).

Because carminic acid decreases the drinking time of E. flaviventris the effect of carminic acid on feeding times was also investigated. This was done by weighing the cochineal insects and adding an appropriate amount of carminic acid crystals (by weight) to the body contents of the preferred cochineal species, D. opuntiae, and thoroughly mixing the two together. A single E. flaviventris was placed in the centre of a petri-dish which contained the

D. opuntiae/carminic acid mixture. Four cochineal insect individuals were used in each test and placed at the the edge of the petri dish arena at equal distances from each other. The feeding times of the beetles for 0%, 0,5%, 1%, 2%, 4%, 8%, and 16% carminic acid (over and above the carminic acid in the body of the cochineal insects) was recorded.

The feeding times of E. flaviventris (Fig. 19) decreased with increasing concentrations of carminic acid, and very little feeding occurred at concentrations of 4% carminic acid or higher.

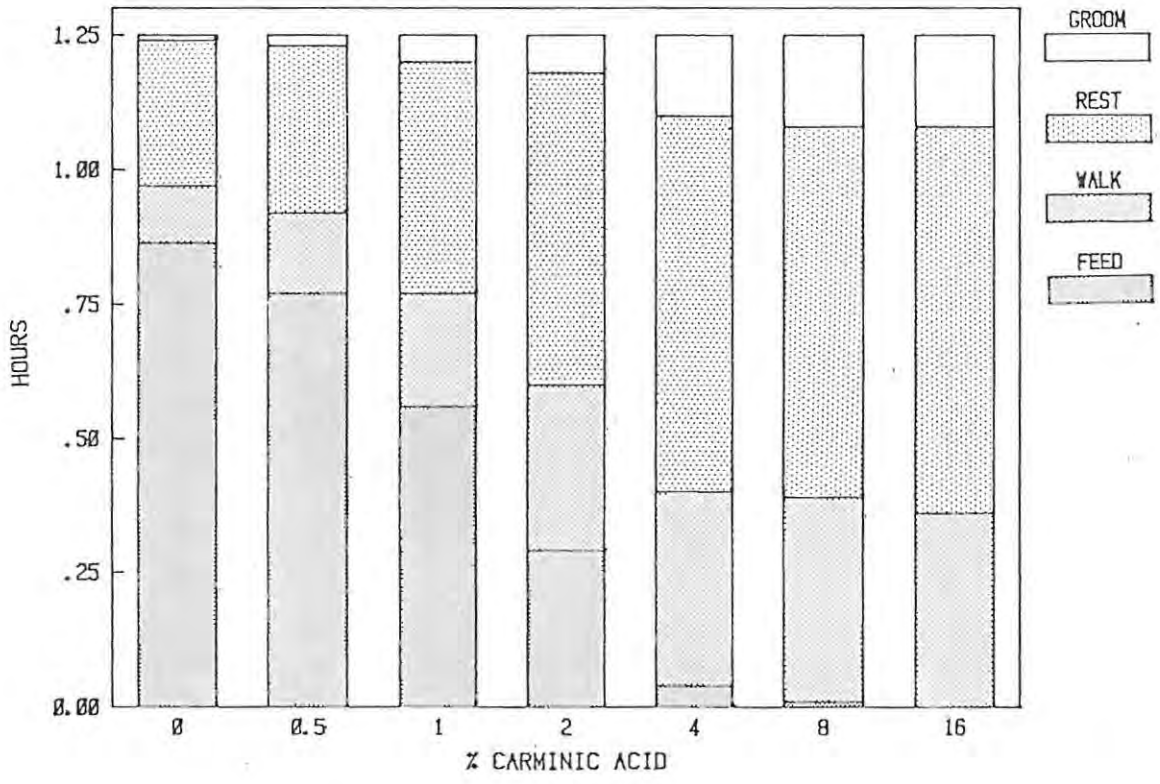


Fig. 19. The mean feeding times of E. flaviventris when fed on D. opuntiae mixed with different concentrations of carminic acid. The number of replicates in each experiment was 20.

Carminic acid therefore decreases the drinking and feeding times for E. flaviventris. To establish the possible role of carminic acid in preventing predation, the carminic acid concentration of all three Dactylopius species was determined.

Carminic acid contents of the three Dactylopius species.

The carminic acid concentration of the three Dactylopius species was measured by means of High Pressure Liquid Chromotography (HPLC) according to Eisner, et al. (1980), with a μ-Bondapak C_{18} (methanol, water; 2:1; 0.8 ml/min; ultraviolet detection at 280nm). The Dactylopius females were dried, weighed, ground and then dissolved in 66% methanol. The solution was filtered through a 0,5 um teflon membrane before an aliquot was introduced onto the HPLC column. The test solution was run against a control standard of purified carminic acid.

D. coccus had the highest concentration of carminic acid and D. austrinus the lowest (Table 7). On a differemt batch of cochineal insects the spectrophotometer results, although showing higher readings than the HPLC, also recorded that D. coccus had the highest concentration of carminic acid and D. austrinus the lowest (Table 7).

Table 7. The concentrations of carminic acid in D. austrinus, D. opuntiae, and D. coccus by dry weight (HPLC=High Pressure Liquid Chromotography, Spec=Spectrophotometer).

Species	Carminic acid concentration	
	HPLC	Spec
D. austrinus	2,8%	5,4%
D. opuntiae	6,3%	10,7%
D. coccus	11,3%	15,1%

Eisner et al. (1980) demonstrated a "feeding deterrent" effect of carminic acid on ants which were "general predators" that did not naturally feed on the prey species Dactylopius confusus (Cockerell). In contrast Baranyovits (1978) suggested with no experimental data, that the natural coccinellid predators of the cochineal insect were not deterred by carminic acid. E. flaviventris, a native of Africa, cannot be regarded as a natural predator of cochineal insects, because they have been sympatric for only about 45 years. Nevertheless high concentrations of carminic acid prevented E. flaviventris from drinking and feeding. As the percentage carminic acid increased, the feeding and drinking time decreased. If carminic acid was the only factor in deterring potential predators of Dactylopius, D. austrinus would be the preferred host as it contains the lowest concentrations of carminic acid. This is not the case, the concentration of carminic acid is only 2,8% in D. austrinus compared with 6,3% in D. opuntiae. A marked decrease in feeding time was noted after 4% carminic acid was mixed with D. opuntiae, effectively

giving 10,3% carminic acid, which is similar to the carminic acid concentration of D. coccus. This indicates that the concentration of carminic acid was too low in D. opuntiae and D. austrinus to deter feeding whereas in D. coccus the concentration appears high enough to deter feeding.

Carminic acid does not appear to play a major role in preventing predation of D. opuntiae and D. austrinus by E. flaviventris, therefore the long-term effect on E. flaviventris when fed on D. opuntiae and D. austrinus was investigated next.

6. THE LONG TERM EFFECT ON E. FLAVIVENTRIS WHEN FED ON DIFFERENT DIETS

Effects on the adults.

In the short term feeding experiments there appears to be little difference between D. opuntiae and D. austrinus as a food source, even though D. opuntiae was preferred in the choice experiments. An experiment was therefore designed to compare the suitability of D. austrinus and D. opuntiae as a food source for E. flaviventris over a number of generations.

Six newly-emerged E. flaviventris females were isolated in glass vials (diameter of 25mm and length of 15mm) for 14 days and fed on "de-waxed" D. austrinus, while another six were isolated and fed on "de-waxed" D. opuntiae (the pre-oviposition period is 13-15 days in E. flaviventris Geyer, 1947 a). After the isolation period the coccinellids were each introduced into a vial with four males that had been fed the same diet as the females they were enclosed with, and left for 24 hours during which they mated. After mating each female was isolated in a vial. Eggs were laid under the paper disc in the vial, where they were protected from adult predation. If no eggs were laid within seven days the females were mated again. These groups and their offspring were given an ad lib supply of either "de-waxed" D. opuntiae or "de-waxed" D. austrinus as food.

Daily observations were made to record the longevity of the females, the egg laying period, and the number of eggs laid per day. The incubation period of the eggs, whether the eggs developed or not, and the number of eggs that hatched was also recorded. One randomly-selected larva was taken from the hatching larvae produced by each female on each day and was put into a small gelatin capsule (diameter of 6mm and length of 18mm); daily observations were then made on these larvae to record larval mortality and the duration of each instar.

On emergence of second generation (F1) adults, sex ratios were recorded and six randomly-selected females were treated in exactly the same manner as the parental generation (P1), making sure that no sib-mating occurred.

Due to the small number of adult females, six per generation, and because there is no certainty that the data was normally distributed, the two-sample Wilcoxon test was used to analyze the data (Sokal and Rohlf, 1969). Four statistical tests were done for each treatment.

i) Beetles fed on D. opuntiae, P1 versus F1 generations.

ii) Beetles fed on D. austrinus, P1 versus F1 generations.

iii) Beetles fed on D. opuntiae (P1 generation) versus beetles fed on D. austrinus (P1 generation).

iv) Beetles fed on D. opuntiae (F1 generation) versus beetles fed on D. austrinus (F1 generation).

Due to four tests being conducted for each treatment, and the desire to keep the significant probability values for each treatment at P=0,05 (*) and P=0,01 (**) these probability values had to be divided by four in each test, and therefore P=0,0125 was substituted for P=0,05 (*) and P=0,0025 for P=0,01 (**) in each test. This is a simplification of the Bonferroni inequality (Miller, 1966).

All results for the adult beetles are grouped together in Table 8.

Table. 8. The effect on E. flaviventris of a diet comprised of D. opuntiae or D. austrinus for two generations. Only adult females are included in the analysis. The parental generation is labelled P1 and the second generation F1 ($\bar{x}$=mean, and N =number of observations).

	Prey species			
	D. opuntiae		D. austrinus	
	Generations			
The effect on	P1	F1	P1	F1
$\bar{x}$ longevity of adult (days) N Range	111 6 95-124	96 6 66-115	92 6 62-107	19 6 10-31
$\bar{x}$ number of eggs laid N Range	442 6 270-527	268 6 106-403	438 6 323-614	77 6 49-103
$\bar{x}$ number of eggs/egg laying day N Range	7 6 0-19	5 6 0-15	7 6 0-25	7 6 0-32
$\bar{x}$ egg laying period (days) N Range	99 6 78-116	83 6 61-97	77 6 59-89	15 6 7-24

Longevity of adult females.

The results in Table 8 showed that the longevity of E. flaviventris females fed on D. opuntiae for the P1 generation was not significantly different from that of the F1 generation (0,1>P>0,05 N.S.). Neither was there a significant difference between P1 beetles that fed on D. opuntiae and those P1 beetles that fed on D. austrinus (0,05>P>0,025 N.S.). However, the longevity of F1 generation beetles fed on D. austrinus was significantly lower than that in the P1 generation (P<0,0025 **) and also significantly lower than D. opuntiae in the F1 generation (P<0,0025 **).

There is thus no significant difference (P>0,05 N.S.) in longevity between P1 and F1 generation beetles fed on D. opuntiae and P1 generation beetles fed on D. austrinus. There is, however a significant difference (P<0,01**) between F1 generation beetles fed on D. austrinus and the other three groups of beetles. Therefore D. austrinus is less suitable than D. opuntiae as a food for E. flaviventris. However the deleterious effects are not expressed in beetles feeding on D. austrinus for a short period. The effect of different prey on E. flaviventris females was further investigated by recording the total number of eggs laid when P1 and F1 generation beetles were fed exclusively on D. opuntiae or D. austrinus.

Number of eggs laid.

The F1 generation beetles that fed on D. austrinus showed a decreased longevity, which was also reflected in their fecundity (Table 8). A significant difference between P1 and F1 generation beetles that fed on D. austrinus (0,0125>P>0,0025*) was shown. F1 generation beetles that fed on D. opuntiae also showed a significant decrease in the fecundity compared to P1 beetles that fed on the same diet (Table. 8, 0,0125>P>0,0025*) although these beetles were less affected than beetles that were fed on D. austrinus (0,0125>P>0,0025*). There was no significant difference between P1 generation beetles that fed on D. opuntiae and D. austrinus (0,3>P>0,20 N.S.).

The egg-laying period and oviposition rate was then analyzed to determine which was responsible for the decrease in egg production in the second generation beetles.

Egg-laying period.

The egg-laying period of E. flaviventris F1 generation fed on D. austrinus (Table 8) was significantly shorter than the P1 generation fed on D. austrinus (0,0125>P>0,0025 *). The F1 generation coccinellids fed on D. austrinus also showed a significantly shorter egg laying period than the F1 generation fed on D. opuntiae (P<0,0025 **). There was no significant difference between P1 generation beetles that fed on D. opuntiae or on D. austrinus (0,2>P>0,1 N.S.), or between P1 and F1 generation

E. flaviventris that fed on D. opuntiae (0,1> P> 0,0125 N.S.). The egg-laying period of F1 generation beetles that fed on D. austrinus clearly showed a marked decrease even though the percentage time that eggs were laid during the beetles life span was similar (Table 9). The oviposition rate was then investigated.

Table 9. The percentage time that E. flaviventris laid eggs during the females' life span when fed on D. opuntiae and D. austrinus for the F1 and P1 generations. (N=total number of beetles observed).

	Generations		
	P1	F1	N
D. opuntiae	87,4%	86,4%	6
D. austrinus	82,4%	79,8%	6

Rate of oviposition.

In Table 8 no significant difference between any of the oviposition rates was shown. This analysis does not show whether the oviposition rate has constant or whether it varied over the egg-laying period between treatments. Therefore the "five day moving averages" (Turkey, 1977) of the mean number of eggs laid per day was plotted in Fig. 20, which also summarizes the effects of the different diets on E. flaviventris. Two conclusions were drawn from Fig. 20.

i) The P1 generation beetles that fed on D. opuntiae and D. austrinus and the F1 generation beetles that fed on D. opuntiae all laid their eggs over a similar period of time, and at a similar rate over this period of time.

ii) F1 beetles fed on D. austrinus laid all their eggs over a much shorter period of time (25 days) than the females of the other treatments. This resulted in a smaller total egg production, although the egg-laying rate was the same as for the females in the other treatments.

The egg laying rates for number of eggs laid per egg laying day are similar for all treatments, however, the adult F1 generation E. flaviventris females that fed on D. austrinus showed a significantly shorter longevity, laid a smaller number of eggs, and laid for a shorter period of time. As food was the only apparent difference between the different treatments, D. austrinus as an exclusive food for two generations was unsuitable for E. flaviventris.

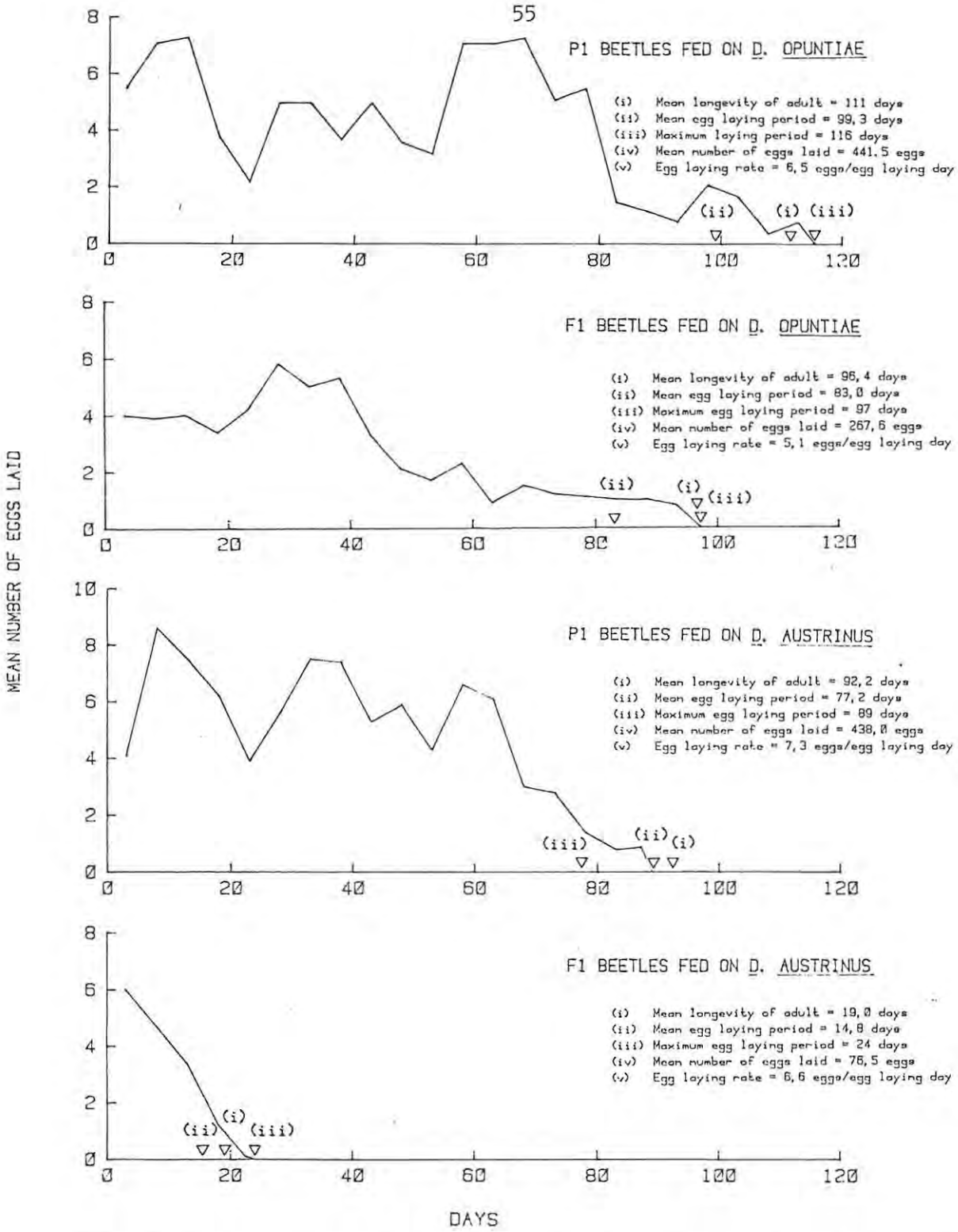


Fig. 20. The mean number of eggs laid per day by P1 and F1 generations of E. flaviventris females that fed on D. opuntiae and D. austrinus. The longevity of the females, their mean egg laying period and their total egg laying periods are also given. Six replicates in each case.

Effect on the immature stages.

The effect of different diets on the immature stages was then investigated. The incubation period, percentage egg hatch, larval mortality, the number of larval instars and their duration were investigated. The results are presented in Table 10.

Table 10. The effect on E. flaviventris immature stages when fed exclusively on D. opuntiae or D. austrinus for two generations (F1 the first filial generation and F2 the second filial generation)(N=number of observations and $\bar{x}$ =mean).

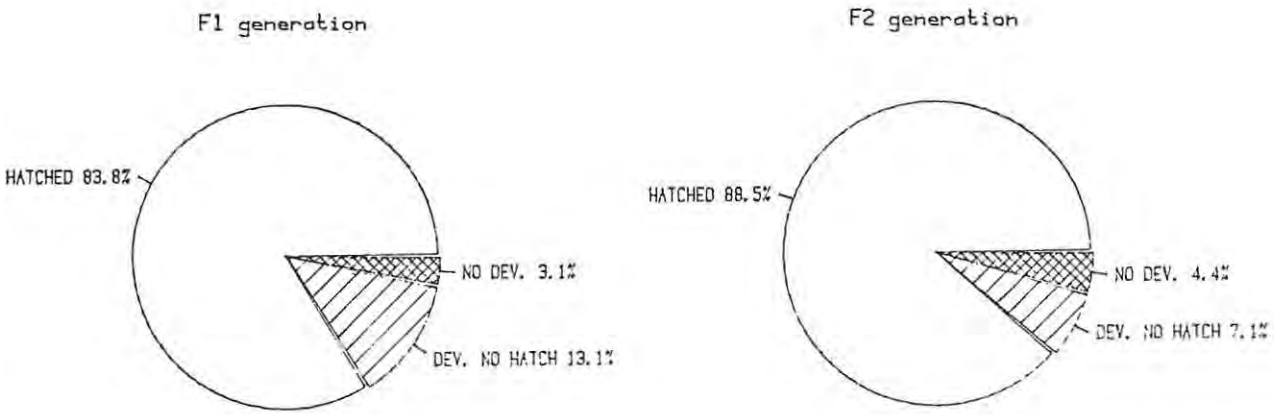
	Prey species			
	D. opuntiae		D. austrinus	
	Generations			
The effect on	F1	F2	F1	F2
$\bar{x}$ incubation period (days)	8	9	8	9
N	116	377	122	175
Standard error	0,92	0,81	0,77	0,79
% egg hatch	84	89	81	62
N	138	426	151	281
% larval mortality	15	16	40	100
N	200	200	200	66
Number of larval instars % three	13	5	19	-
before pupation % four	81	91	79	-
% five	6	4	2	-
N	171	168	121	-
Mean period, hatch to pupation (in days) of larvae with 4 instars	25	30	28	-
N	138	112	95	-
Standard error	1,96	2,11	2,06	-
Sex ratio (% females)	62	51	54	-
N	138	112	95	-

Incubation period and percentage egg hatch.

The incubation period of the eggs was not affected by the prey species (Table 10) or by the period for which the prey species was eaten.

Egg hatching, however was affected by these factors. Table 10 shows a very similar pattern for E. flaviventris egg hatch when the parental stock was fed on D. opuntiae for the F1 and F2 generations and D. austrinus fed for the F1 generation (84,1%, 88,5%, and 80,8% respectively). In the F2 generation beetles that were fed on D. austrinus, percentage egg hatch was lower (62,2%). The eggs that did not hatch were divided into categories, those that showed "no development" (indicated by the lack of colour change during the incubation period), and those that showed some development but did not hatch (these developed a red spot (Geyer, 1947 a)). The results of this analysis are shown in Fig. 21. The "hatch", "development with no hatching" and "no development" of eggs laid by E. flaviventris that fed on D. opuntiae during the F1 and F2 generations and on D. austrinus for the F1 generation was similar. However fewer eggs hatched when laid by F2 generation beetles that fed on D. austrinus. These eggs had a higher "no development" and "development with no hatching" percentage. The diet of the parental stock does appear to influence the percentage egg hatch. A poor food source appears to decreases the percentage egg hatch. The effect of diet on the larvae was investigated next.

FED ON D. OPUNTIAE



FED ON D. AUSTRINUS

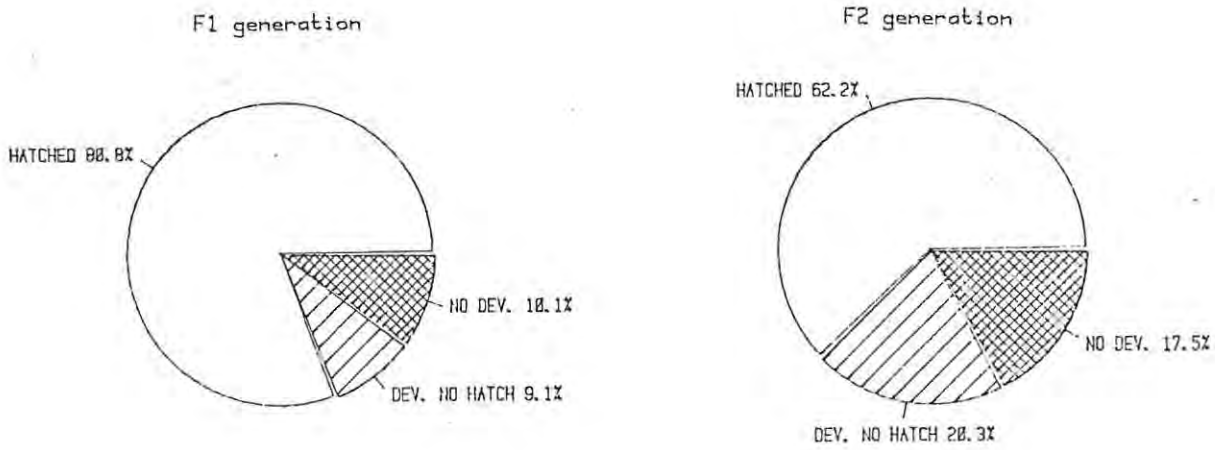


Fig. 21. The percentage hatch of eggs of E. flaviventris that were fed on D. opuntiae or D. austrinus for two generations, a first filial (F1) and a second filial (F2) generation. Unhatched eggs were classified in two ways; eggs that developed (DEV.) but did not hatch and eggs that showed no sign of development.

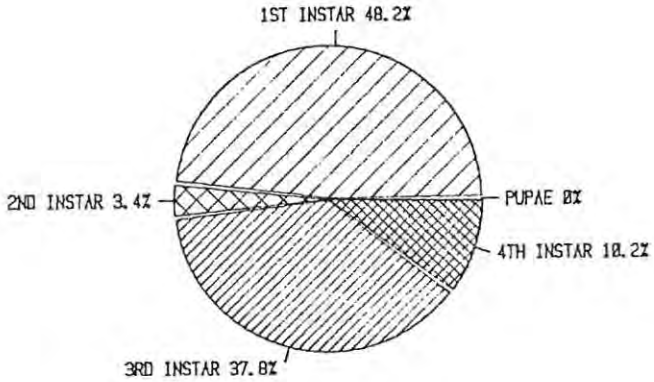
The effect of the different diets on the larvae.

The F1 generation mortality (Table 10) for E. flaviventris larvae was higher (40%) when fed on D. austrinus compared with larvae fed on D. opuntiae (15%). In the F2 generation, mortality was 100% for larvae fed on D. austrinus and no larvae developed beyond the second instar (Fig. 22). On the other hand, in the F2 generation fed on D. opuntiae there was only a 16% mortality. In all cases most mortalities occurred in the first instar (Fig. 22). The proportion of first instar deaths is higher in larvae that fed on D. austrinus than F1 and F2 larvae that fed on D. opuntiae. Only a small pupal mortality was recorded (5%) on F1 generation E. flaviventris that fed on D. austrinus (Fig. 22).

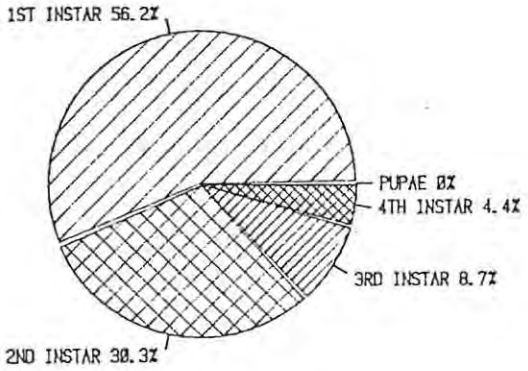
The developmental progress of the surviving larvae was followed by recording the number of larval instars and their duration. Most E. flaviventris larvae completed their development and pupated after four instars (Table 10) and feeding on D. opuntiae or D. austrinus did not affect this trend. More larvae passed through three instars than five instars.

The mean duration of larval development in those that completed development in four instars was shortest for F1 generation beetles that fed on D. opuntiae (Table 10), was slightly longer for F1 generation coccinellids that fed on D. austrinus and was longest for F2 generation coccinellids when fed on D. opuntiae. The larvae that fed on D. austrinus during the F2 generation all died, only 10 larvae survived the first instar and will not be considered further.

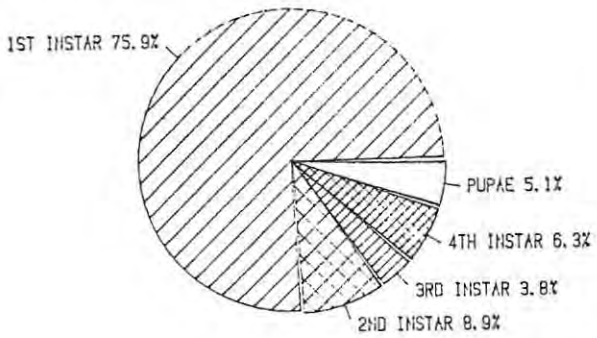
F1 larvae fed on D. opuntiae



F2 larvae fed on D. opuntiae



F1 larvae fed on D. austrinus



F2 larvae fed on D. austrinus

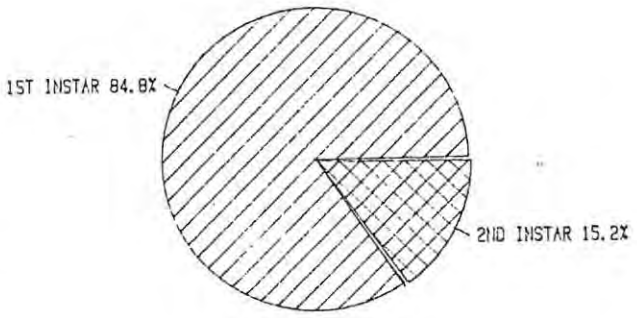


Fig. 22. The contribution (as a percentage) to the total mortality of each immature stage of E. flaviventris when fed on D. opuntiae or D. austrinus for the F1 and F2 generations.

In Fig. 23 the durations of the immature stages are shown more clearly. Several conclusions can be drawn from Fig. 23.

i) In each case F2 generation larvae took longer to develop than F1 generation larvae when fed the same diet.

ii) In the F1 generations, beetles that fed on D. austrinus took longer to develop than beetles that fed on D. opuntiae.

iii) The differences between the treatments are all statistically significant, except in the second instar where there was no significant difference between F1 generation larvae that fed on D. austrinus and F2 generation larvae that fed on D. opuntiae (the difference is regarded as significant ($P < 0,05$) when the notches in two or more boxes do not overlap (McGill et al. 1978)).

The intervals between moults were similar in all cases, as shown in Fig. 24, where the cumulative percentage of larvae moulting on a specific day is plotted. With each consecutive instar a slight spreading and flattening of the pattern is noted, this is the result of larger differences between the means and the larger standard deviations shown in Fig. 23.

These differences appear to be due to the difference in the food value of the two prey species, D. austrinus apparently having a lower food value for the coccinellids.

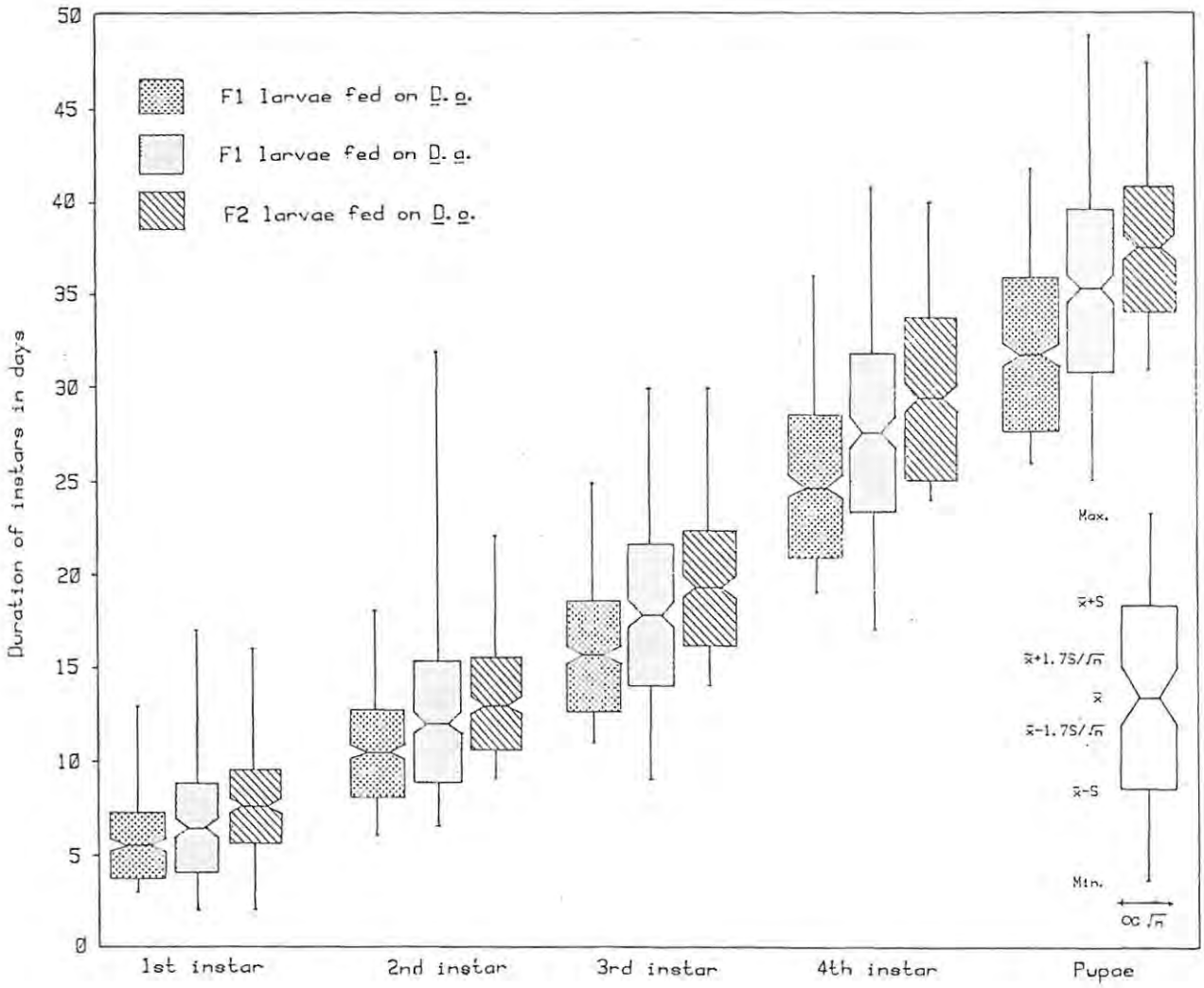


Fig. 23. The duration of the immature stages of F1 and F2 E. flaviventris that were fed exclusively on D. opuntiae (D.o.) and F1 beetles that were fed only D. austrinus (D.a.). ($\bar{x}$=mean, S=standard deviation, and n=number of observations) (McGill, et al. 1978).

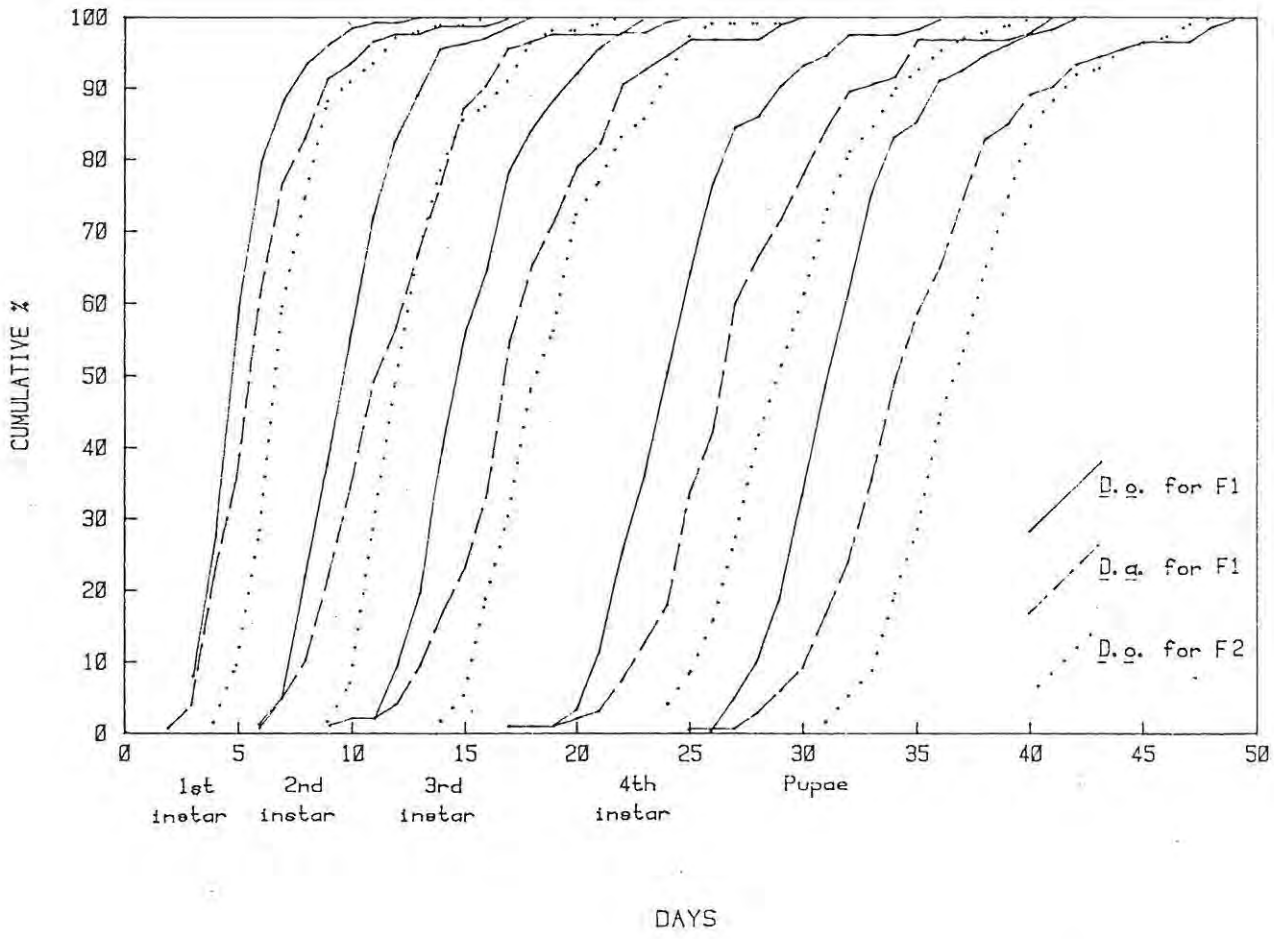


Fig. 24. The cumulative percentage of moulting intervals for E. flaviventris immature stages that fed on D. opuntiae (D.o) and D. austrinus (D.a) for the F1 and F2 generations.

The diet of the parental stock did not appear to affect the incubation period of the eggs. However, the percentage egg hatch decreased when F2 generation beetles were fed on D. austrinus. The larval mortality was also higher in F1 generation larvae that fed on D. austrinus and in the F2 generation all the larvae that fed on D. austrinus died. Diet does not appear to affect the number of larval instars but the duration of the instars was shown to be shortest for F1 generation larvae that fed on D. opuntiae and longest in the F2 generation.

The sex ratios of adults which as larvae had been fed on D. opuntiae and D. austrinus for the F1 and F2 generations were recorded and are shown in Table 10. The sex ratio of the F1 generation of E. flaviventris fed on D. opuntiae was female biased (62% females) and approximately corresponded to that recorded by Geyer (1947 a) (59% females). The change (towards 50%) in the sex ratio of the F1 generation and the F2 generation for beetles fed on D. austrinus could be due to the difference in quality of food resulting in a greater mortality of female larvae.

Of all the variations in the long term experiments only the following show a significant difference.

i) Longevity of adults, with F1 generation beetles that fed on D. austrinus living significantly shorter than the beetles fed on the other diets.

ii) Number of eggs laid, the F1 generation beetles that fed on D. austrinus laid significantly fewer eggs than the other beetles.

iii) The egg laying period of F1 generation beetles that fed on D. austrinus was significantly shorter than the beetles fed on the other diets.

iv) The percentage egg hatch of the F1 generation females that fed on D. austrinus was lower than the eggs of females feeding on the other diets.

v) The larval mortality of F1 and F2 larvae that fed on D. austrinus was higher than larvae that fed on D. opuntiae. No larvae developed beyond the second instar in the F2 larvae that fed on D. austrinus.

vi) The sex ratio of the F1 generation beetles that fed on D. opuntiae showed a bias towards the females, with no bias on the other diets.

8. DISCUSSION.

All female scale insects have piercing and sucking mouthparts and lack wings. Adults of the more primitive families tend to be quite mobile and have large, well-developed legs with numerous setae. The more advanced scale insects, however, show an increasing tendency towards complete sedentary behaviour with their legs and leg setae reduced, culminating in the apodous female pit scale insects (Miller and Kosztarab, 1979). The sedentary life of the scale insects has made them vulnerable to insect predators and parasitoids.

The coccinellids are one of the general insect predators that prey on the Coccidae, also preying heavily on Aphididae and Aleyrodidae (Clausen, 1940; De Bach, 1974). Predation by coccinellids on aphids has been well documented (see Hodek, 1966; Eastop and van Emden, 1972; Baumgaertner *et al.* 1981 b). Coccinellids have also been important in biological control. The introduction of the vedalia beetle, *Rodalia cardinalis* Mulsant, against cottony-cushion scale, *Icerya purchasi* Maskell, resulted in one of the first biological control successes. *Cryptolaemus montrouzeri* Mulsant and *Cryptognatha nodiceps* Marshall were also biological control successes, against mealybugs and coconut scale respectively (Taylor, 1935; Clausen, 1940; Hagen, 1962; De Bach, 1964, 1974).

Mann (1969) has proposed that in North and South America the relative scarcity of *D. opuntiae* in parts of its natural range (Texas and Arizona) is due to the prevalence of various predaceous insects indigenous to these areas. These predators include the adults and

larvae of many coccinellid species, larvae of several phycitid moths, of syrphid and agromyzid flies, and some neuropterans. In South Africa, the cochineal insect D. opuntiae has been shown to be controlled by coccinellid beetles (Annecke, et al. 1969; Burger, 1969).

Many scale insects develop a wax secretion that serves as protection. The scale insects appear to require a hard, high melting point wax to protect them from insect predators and from the weather (Tulloch, 1970). There is considerable variation in the manner in which the wax is attached to the insect and the form the wax takes when secreted. In some scale insects the females are covered with thick plates of wax, as occurs in some species of Ceroplastes (Coccidae). Some species such as the Pulvinaria (Coccidae) species and some of the Dactylopius species secrete a woolly mass of wax. The mealy bug, on the other hand, has a powdery wax covering its surface. In some scale insects a hard scale of wax and exuviae covers the insect and shelters the insect and its eggs, as found in the Diaspididae. In these diaspidids the scale is produced by secretions of loose fibres called the white cap secreted mainly by the pygidial glands and a glutinous liquid discharged from the anus which forms a homogeneous mass that toughens into the scale (Baranyovitz, 1953; Beardsley and Gonzalez, 1975).

The successful predation of Coccinellidae against diaspine Coccidae appears to be limited by certain physical characters of the scale covering. Species with a relatively thin covering are readily preyed

upon whereas species with a thick and tough covering, such as Lepidosaphes, Chionaspis, and Protaspis, are relatively free from attack by members of the coccinellids (Clausen, 1940).

The woolly "waxy covering" of D. opuntiae (Fig. 3) and D. austrinus (Fig. 4) was shown by Walter (1977) to afford the insects some protection from E. flaviventris (Fig. 6) predation. However, the "waxy covering" of D. opuntiae was penetrated with greater ease than that of D. austrinus (Walter, 1977; and this study). The physical properties of the "waxy covering" was also shown to be different (Walter, 1977).

The "waxy covering" of D. opuntiae and D. austrinus, contain a "non wax" substance produced by ducts associated with the quinquelocular pores, and a waxy substance produced by quinquelocular pores and setae. The different proportions of these substances could be the reason for the difference between D. opuntiae and D. austrinus "waxy coverings".

In D. coccus no ducts are found (De Lotto, 1974), and the thread-like components on to which the wax matrix adheres are not present, resulting in a powdery type "waxy covering"(Fig. 5). In D. coccus protection against predation is achieved in a completely different manner to D. opuntiae and D. austrinus. Instead of a wooly mass as a barrier, D. coccus females, and their immediate vicinities, are covered with a powdery wax. In the laboratory D. coccus females are completely protected against predation, by clogging up the tarsi of the potential predator (Fig. 9), resulting in a loss of adhesion by

the tarsi of the predator to the substrate. This is very similar to the wax blooms of brassicas which was shown to clog up the tarsi of the mustard beetle Phaedon cochleariae (Fabricius)(Stork, 1980 b).

To investigate whether the "waxy covering" was the only reason for the field observations that E. flaviventris preys predominantly on D. opuntiae, E. flaviventris was given a choice of prey (D. opuntiae, D. austrinus and D. coccus). This experiment showed that E. flaviventris preferred D. opuntiae to the other two Dactylopius species. This preference could be due to the presence of prey toxins in the less preferred prey.

A multitude of chemical defenses against a variety of animals as well as microorganisms exist in many arthropods. There are two principal ways in which arthropods may acquire defensive substances, i) from a dietary source (extrinsic) or ii) by synthesis of the substance by the arthropod itself (intrinsic)(Eisner, 1970).

i) Eisner (1980) noted that "the acquisition of defensive material from a dietary source - is wide spread among insects". The extrinsic defensive substances have been recorded in Coleoptera, Lepidoptera, Neuroptera, Hemiptera, Diptera and Orthoptera (Eisner, 1970, 1980; Rothschild, 1973; Bowers, 1980, 1981) and in some parasitoids (Vinson and Iwantsh, 1980). The vedalia beetle was noted not to develop on cottony-cushion scale growing on the ornamental Cocculus, and does poorly on others such as maple and scotch-broom (De Bach, 1974).

Host-plant-induced immunity of an insect to a parasite or predator could lead to false conclusions as to its effectiveness on feeding on the specific host or even its presence in a particular area.

ii) A large number of intrinsic chemical defense substances have been recorded and investigated (Blum, 1978, 1981). The synthesis of most of these products have not been elucidated, but most of them are well-known organic compounds with relatively simple structures. A large variety of compounds are found which suggests that a number of metabolic pathways exist in producing these varied defensive compounds (Blum, 1978, 1981). The varied defensive strategies of the Hymenoptera, Coleoptera, Hemiptera, Phasmids, to mention but a few, indicate the great variation that does exist. In some predator species extrinsic defense substances are obtained not from plants but from intrinsic defense substances from their insect prey. For example, larvae of the pyralid Laetilia, feeding on coccids containing the anthroquinone carminic acid, repel ants with the quinone-rich oral discharge from their crops (Eisner et al. 1980).

Carminic acid, which is probably synthesized in the fat body of the cochineal insect (Needham, 1978), was shown to prevent E. flaviventris from feeding when it was in high concentrations. D. coccus has a high concentration of carminic acid (11%) which could be the reason why this species was not preferred in the choice experiments. D. austrinus, on the other hand, was recorded as having the lowest carminic acid concentration (2%) of the three species and

was also not preferred in the choice experiments, thus indicating that a low concentration of carminic acid appears not to be important in predator prevention.

Carminic acid in low concentrations could prevent attacks by parasitoids. Moran (1980) points out that there have been no reports of parasitism in Dactylopius. Other Homoptera with red body contents also appear to be free from parasitoid attack, such as the pine woolly aphid Pineus pini (Macquart) (Bruzas, 1983).

On investigating the effect of different Dactylopius prey species (D. coccus, D. austrinus and D. opuntiae) on E. flaviventris, it was found that D. coccus was not suitable at all as a food and D. austrinus could sustain the beetles for only one generation. D. opuntiae was the only prey of the three species that could sustain the beetles for more than two generations. The F1 generation beetles that fed on D. opuntiae also showed that D. opuntiae, on its own, was not entirely suitable as a food. This unsuitability could be due to the food source lacking some required nutrient. Hodek (1966) noted that all coccinellids were polyphagous, and Hagen and Sluss (1966) showed that when a Hippodemia species was restricted and only allowed to feed on the aphid Terioaphis trifolii (Monell), its vigor declined compared to when it was fed a mixed diet of different species of prey and non-insect food (honey-dew and pollen).

The disappearance of D. coccus in South Africa after a period of establishment was unlikely due to predation by E. flaviventris. Likewise the partial success of D. austrinus as a biological control

agent is unlikely to be due to predation by the beetle. Other factors such as dispersal (Gunn, 1979) and weather could be responsible.

This study showed that in the laboratory E. flaviventris penetrated the "waxy covering" of D. opuntiae with greater ease than that of D. austrinus, and preferred to feed on D. opuntia which was a better food source compared to the other two Dactylopius species. These are the most likely reasons why the beetles feed on D. opuntiae in the field and not on D. austrinus.

SUMMARY.

i) D. coccus is protected against E. flaviventris predation by its "waxy covering". The "waxy covering" of D. austrinus also provides protection against E. flaviventris, though not as effective as that of D. coccus. The "waxy covering" of D. opuntiae is relatively easily penetrated by E flaviventris.

ii) The "waxy covering" of D. coccus is in a fine, powdery form which clog up the tarsi of E. flaviventris. D. opuntiae and D. austrinus have woolly "waxy coverings", which differ in their ability to protect the beetle from predation due to the physical properties of the "waxy coverings". These differences appear to be due to the number of "non-wax" threads in the "waxy covering", D. austrinus having more "non-wax" threads than D. opuntiae.

iii) E. flaviventris preferred D. opuntiae to D. austrinus or D. coccus when given a choice of the three cochineal species with intact "waxy coverings" and when the "waxy coverings" were removed.

iv) In high concentrations carminic acid prevents feeding by E. flaviventris, and this could be the reason for the coccinelid not preferring D. coccus. In concentration of 4% carminic acid and higher little feeding appears to occur.

v) D. coccus cannot sustain E. flaviventris, D. austrinus was an inferior food compared to D. opuntiae which sustained the coccinellids for more than two generations.

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