An evaluation of Mimorista pulchellalis(Dyar) (Lepidoptera: Pyraustidae) as a biocontrol agent against jointed cactus in South Africa.

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by

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INTRODUCTION.

Jointed cactus Opuntia aurantiaca has proved to be South Africa's most expensive and serious weed (Serfontein 1961; Neser & Annecke 1973; Zimmermann & van de Venter 1981). Since its first introduction into the country an estimated R20 000 000 has been spent in eradication attempts by the State alone (Moran & Annecke 1979), while the current annual expenditure totals approximately R2 000 000 (G.Burger in Zimmermann 1981). In addition, large tracts of land are infested by the weed in all four provinces in the Republic, an estimated 3 000 properties totalling 1,6 X 10^6 hectares being reported infested in the eastern Cape and Karoo regions alone (Zimmermann et al. 1974).

Although the current herbicidal control program has succeeded in clearing the dense jointed cactus infestations, the actual area still affected today totals 830 000 ha (Moran & Annecke 1979; Zimmermann & Moran 1982). Much of this infested area is maintained below a tolerable economic threshold but the current intensive control program must be sustained for fear that the weed may again increase to undesirable levels (Zimmermann 1981; Zimmermann & Moran 1982). Despite the intensive efforts to control jointed cactus, the weed is still increasing and expanding its range at the rate of 8 000 ha per annum (G.Burger in Zimmermann 1981).

The gradual realization that eradication by chemical means was unrealistic (Slabber 1964; Moran & Annecke 1979), coupled with the rising costs of herbicides and labour, has stimulated renewed research in the possibilities of biological control (Moran & Annecke 1979). Biological control of jointed cactus was attempted previously in 1935 with the release of the cochineal insect Dactylopius austrinus De Lotto, but was generally assumed to have failed (Pettey 1948). Chemical control was reintroduced in

1947 and all further biological control attempts with cochineal abandoned.

It is now realized that a biological control agent is seldom capable of controlling a host species over its entire range and current research is therefore primarily aimed at the development of an effective integrated control system, a line of research which necessitates a detailed knowledge of the ecology of both the weed and biocontrol agent used for its control (Zimmermann & Moran 1982). Work in this regard has progressed and resulted in the recent registration of a new water-based herbicide - MSMA- which is non-toxic to beneficial insects (Zimmermann et al. 1981). Two main directions of research are followed: attempts to enhance the effectiveness of available natural enemies and the importation of new biocontrol agents from abroad. Results presented in this dissertation report on the second alternative, the evaluation of a recently introduced pyraustid moth Mimorista pulchellalis from South America.

The work on Mimorista is divided into two sections: a laboratory orientated study which describes the biology and rearing techniques employed, and a field orientated study where the establishment of the insect and its impact on jointed cactus populations are examined. In the final chapters the integration of this insect in the current control program are discussed and recommendations regarding its future in South Africa are given.

For the benefit of those readers not familiar with the jointed cactus problem, a brief review is given of the weed, its introduction into South Africa and the efforts needed to control it.

JOINTED CACTUS IN SOUTH AFRICA

1. Plant description and phenology

Two morphological types of jointed cactus (Opuntia aurantiaca Lindley) may be distinguished in South Africa. Typically, in unshaded conditions, jointed cactus is a low growing, many branched, inconspicuous succulent. The plant consists of numerous, short, cylindrically shaped cladodes (3-15cm long) adorned with large, barbed spines (fig.1). Cladodes are easily dislodged and may be dispersed by animals, by floods and to a lesser extent by wind. The dislodged cladodes are longlived and are all capable of giving rise to new plants. Unsupported, plants may attain a height of 20-50cm, but with support from other plants, a height of 1,5m may be reached. Many of these plants comprize 100 cladodes or more.

An aberrant and less common form of jointed cactus occurs in more shaded areas, including the well-shaded sites along watercourses and in the dense shade under large bushes. Cactus growing in these shaded sites is usually very etiolated (fig.2) and can easily be distinguished from the more typical form. The individual cladodes are longer than those of typical cactus plants (10-30cm) and the spines are not as numerous nor as long. Furthermore, cladodes in the shade retain their dark green colour and are not easily disloged from the plant. Etiolated plants often occur in large clumps and are capable of exceeding a height of 2m or more if supported by trees and undergrowth. Large plants often collapse under their own weight and the fallen stems may take root, which results in the formation of dense "mats" of cactus. Larger plants of both cactus types have an underground tuber which serves as a storage organ and which can produce new growth if the above ground part of the plant is destroyed.

In spring and early summer (October to January) jointed cactus

Fig.1. A typical jointed cactus plant. Such plants usually occur in open unshaded areas and are characterized by having short cladodes with long spines.

Fig.2. Etiolated jointed cactus. This type of cactus occurs in shaded areas. Plants are typically dark green in colour and are characterized by long, etiolated cladodes with relatively few spines.

plants initiate growth and produce large numbers of new cladodes (fig.3). The duration of production depends on environmental factors and may persist until June. After the initial rapid growth phase at the beginning of summer, formation of new cladodes slows down and flowers are produced. Flowers are a bright yellow in colour and not orange as the name, "aurantiaca" implies. Moran et al. (1976) discuss the possible reasons for this erroneous choice of epithet.

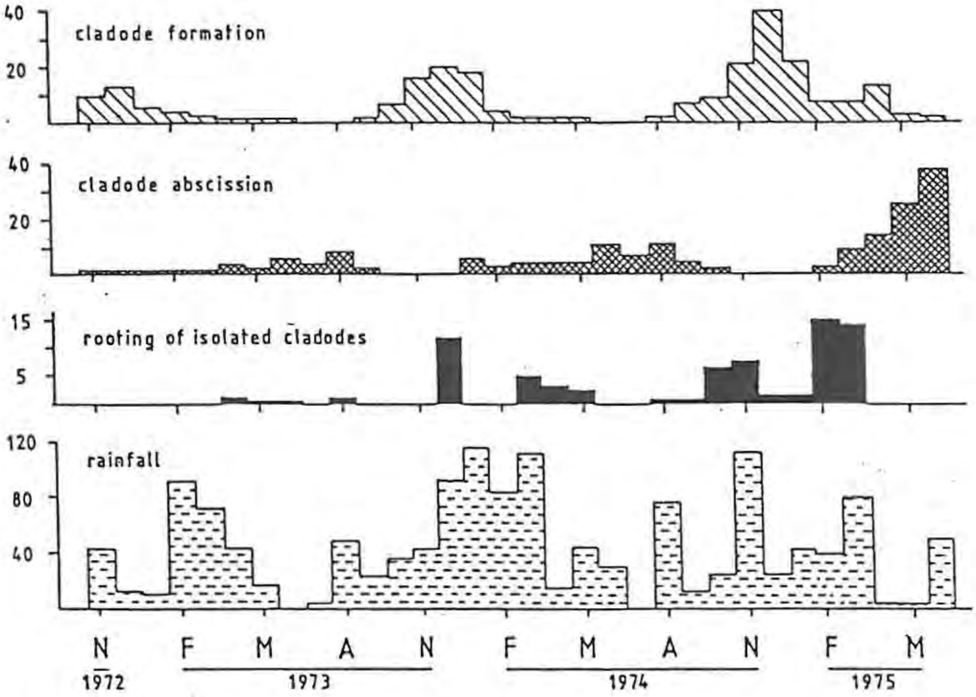


Fig.3. The phenology of jointed cactus over a three year period (from Zimmermann 1981) (N = November, F = February, M = May, A = August).

Flowers develop into red, club-shaped fruits similar in shape but somewhat smaller than the fruit of the well-known prickly pear (Opuntia ficus-indica (L.) Miller. Very few fruits are formed on etiolated cactus plants. Although numerous seeds are formed in each fruit, they are not viable (Archibald 1936; Arnold 1977) and propagation of jointed cactus is thus entirely vegetative. Fruits are also capable of vegetative growth.

Abscission of cladodes commences from April onwards and persists throughout the winter months (fig.3). Dislodged cladodes may root in the vicinity of the parent plant, but are frequently dispersed to other areas where they may give rise to new cactus infestations. Isolated cladodes may remain viable for extended periods of time and produce new growth when suitable conditions occur. Numerous accounts exist of the drought resistant properties of jointed cactus; instances are recorded of isolated joints remaining alive without moisture for periods of 3 years and longer (Schonland 1924; Phillips 1938; Serfontein 1961). Recent ecological studies on jointed cactus (Zimmermann 1981) have demonstrated that this is not the general rule however, and that desiccation accounts for a high mortality of small plants and isolated cladodes under field conditions. Nevertheless, jointed cactus can survive in areas where little rainfall occurs, especially where competition from natural vegetation is reduced (Serfontein 1961). It is for this reason that jointed cactus is often associated with agriculturally disturbed areas (Serfontein 1961).

The vegetative means of reproduction of jointed cactus may lead to the formation of dense aggregations of cactus if left unchecked. Heavy infestations may preclude grazing by livestock and can cause overgrazing of the unaffected areas. This in turn leads to soil erosion which creates conditions condusive to new cactus infestations (Serfontein 1961). Even when dense stands of cactus do not occur, low levels of jointed cactus infestation may reduce the value of a property.

Direct injury to livestock is also a common occurrence. The long spines are injurous to animals and often result in severe abscesses when they break off under the skin (Serfontein 1961), while spines that break off in the feet of animals often cause lameness. Cases have been reported where the mouths of animals are sealed off by cladodes (Morris 1957). General irritation by the spines and minute glochids may cause loss in condition and a consequent reduction in economic value while loose cladodes which become entangled in wool and mohair inconvenience handlers and generally lead to an overall loss in clip-yield (Zimmermann 1975).

2. Origin and history of introduction.

Although the true origin of the jointed cactus remains obscure, it is generally believed to be a native of Argentina and Uruguay (Britton and Rose 1919; Moran *et al*. 1976; Arnold 1977). The plant is a hybrid (as reflected by its sterility) with *O. saligna* and *O. discolor* being the possible parents (Arnold 1977). H.G.Zimmermann (pers.comm.) states however that in Argentina *O. discolor* is also sterile and can therefore not be one of the parents. Moran and Annecke (1979) give a detailed account of the taxonomic status and introduction of the plant into South Africa which is summarized here.

Jointed cactus was probably first imported into South Africa from England in 1843 (or possibly earlier) to be added to the private collection of Baron Carl Ferdinand Heinrich von Ludwig (1784-1847) in Cape Town. The Baron imported many plants from all over the world for his private collection and the Ludwig's-burg Gardens were famous at the time. After von Ludwig's death, the Cape Town Botanical Gardens were founded in 1848 from collections of plants bought from the Ludwig's-burg Gardens.

It was at about this time that jointed cactus became available to collectors and the general public for the first time (Moran & Annecke 1979). The drought resistant properties of the plant made it suitable for use as an ornamental for rockeries and cemeteries in the dry inland areas where it was taken by the early inhabitants during the middle of the last century. From these inland areas the plant gradually spread and increased its range. By the early 1890's it became evident that the plant could pose a serious threat as a noxious weed (MacDonald 1892), but unfortunately no serious eradication attempts were undertaken at this stage and the plant continued to spread. Today the distribution of cactus in South Africa is widespread with the greatest infestations in the eastern Cape (fig.4).

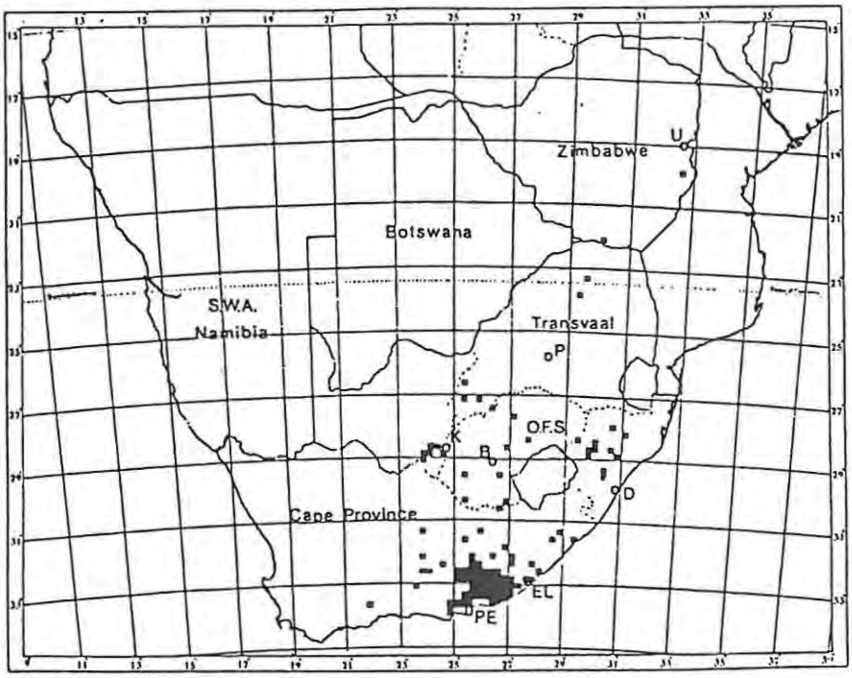


Fig.4. The distribution of jointed cactus in southern Africa on a 1/4° scale (from Zimmermann 1981) (B = Bloemfontein, D = Durban, EL = East London, K = Kimberley, O.F.S. = Orange Free State, P = Pretoria, PE = Port Elizabeth, U = Umtali).

3. History of control.

Once the possible threat of jointed cactus as a weed became evident in the early 1890's, control was initiated and the landowners were held responsible for clearing the land. At this stage control consisted of collecting the cactus into large heaps and burning (MacDonald 1892; Lansdell 1923). Gradual increase in infestations soon reached a stage however, where control measures were beyond the resources and capabilities of the landowners and in 1928 the promulgation by the Cape Provincial Council of Ordinance No.18 made jointed cactus control compulsory and placed the responsibility with the Divisional Councils. At this stage arsenical herbicides had become available and control consisted of spraying large clumps _in situ_. Individual plants and the smaller clumps were collected by hand and piled at a central area where they were sprayed and later burnt (Van der Merwe 1932).

Despite the intensified eradication program, it soon became evident that the extent of the cactus infestation at this stage had become too great for even the Divisional Councils to control (Slabber 1964). Consequently responsibility of control of the weed was placed with the State Department of Agriculture by the passing of the Union Government Act No.54 of 1934. Under the 1934 legislation, a permanent team of labourers was employed to help farmers clear their land of jointed cactus. The Government undertook to clear the infested land, after which landowners themselves were held responsible for keeping their properties free of the weed (Pettey 1948). In 1935 the Government labour force totalled 650 in 10 districts of the Cape Province. The total expentiture from 1934-1938 exceeded R340 000, while the infested area comprized approximately 200 000 morgan in the Provinces of the Cape, the O.F.S. and Natal (Pettey 1948).

It was during this period (1935) that biological control was first initiated. Stocks of the cochineal insect Dactylopius austrinus (Homoptera: Dactylopiidae) obtained from Australian cultures, were released against jointed cactus at Uitenhage in June 1935 (Pettey & Du Toit 1939; Pettey 1948). Initial results were so spectacular that mechanical and chemical control were discontinued in 1938 and the cheaper biological control method relied upon entirely for jointed cactus control. The peak of increase of the cochineal population generally occurred 12 to 18 months after release of the insect in each locality, and resulted in practically the total destruction of the above ground parts of the plant over extensive areas (Pettey 1948).

Towards the late 1940's however, it became apparent that biological control was not providing satisfactory control anymore. In certain areas cochineal did not increase or had disappeared altogether and it was generally thought that cochineal had lost its "aggressiveness". The Department of Agriculture reverted to mechanical and chemical control methods during 1947 and 1948 (Pettey 1948).

Following the failure of the biological control program, alternative more effective chemical control methods were sought. Numerous field trials conducted during the early 1950's with the new hormone-type herbicides which had become available, led to the registration of 2,4,5-T (Naude 1957). In 1957 the hormonal weed-killer scheme was announced for the control of cactus. Herbicide was issued free of charge to farmers who then had to ensure spraying of cactus, or alternatively, the State undertook the spraying responsibility and claimed 50% of the expenses from the land owners (Moran & Annecke 1979).

During the period 1957-1973 it became increasingly clear that 2,4,5-T was not providing satisfactory control of jointed cactus. The herbicide effectively destroyed all the top growth of cactus infestations, but generally failed to kill the whole plant. This was largely due to the poor translocation properties of the herbicide and as a result extensive regrowth from the underground tuber appeared within a relatively short time after application (Slabber 1964). The herbicidal formula was consequently changed in 1973 by replacing 2,4,5-T with the iso-octyl ester of picloram (Tordon M3142) (Moran & Annecke 1979).

Although the average volume of herbicides used since 1973 has averaged approximately 5 000 000 litres per annum (Zimmermann & Moran 1982), the cost of the ready-mix herbicide has risen sharply, from R38,00 to R80,00 per 200 litre drum in January 1979 alone (Zimmermann _et al._ 1981). In the 1980 calender year for example, 4 378 000 litres of herbicide were used which was valued at R1 587 000. Of this amount, R1 400 300 was accounted for by the paraffin carrier. Currently a water-based herbicide is used in jointed cactus control (Zimmermann *et al*. 1981).

Replacement of the paraffin carrier with water, not only results in a substantial annual saving, but ensures that applications of the herbicide do not directly kill the biological control agents of jointed cactus currently occurring in South Africa, namely, the cochineal insect *Dactylopius austrinus* De Lotto (Homoptera: Dactylopiidae) and the phycitid moth *Cactoblastis cactorum* (Berg). The cochineal insect remains the most important biological control agent of jointed cactus today, and is responsible for considerable destruction to the cactus (see Zimmermann 1981). *C.cactorum* was originally released against prickly pear (Pettey 1948; Annecke & Moran 1978), but attacks jointed cactus. Another phycitid moth *T.tapiacola* Dyar was also imported into South Africa in 1973 but so far has failed to become established (Hoffmann 1982).

The scheme of providing herbicide free of charge to landowners is still in use today, but as yet no reports of complete control have been received and the control program against jointed cactus remains a never ending task. Despite all efforts at reducing the increase and spread of jointed cactus infestations and the large increase of herbicidal applications, cactus continues to expand its range. It is now realized that complete eradication of jointed cactus is unrealistic and that chemical and mechanical control offer no permanent solution to the problem.

MIMORISTA PULCHELLALIS IN SOUTH AFRICA.

4. Taxanomic status

Although the moths used in this study are identical to the moths originally described by Dyar as Mimorista pulchellalis (see Mann 1969), their exact systematic position has not been finally determined. E.Munroe (Biosystematics Research Institute, Ottawa, Canada) regards the species as not being a true Mimorista, but related to Mimorista flavidissimalis (Grote) (see Mann 1969), which is properly referred to the genus Loxomorpha Amsel of which Crysobolys Munroe is a junior synonym. The larger moths Laniifera cydodes (Druce) and Beebea guglielmi Schaus may also be related as similarities in genital structure occur and both these species are also cactus feeders. The species Mimorista pulchellalis appears different enough however to require erection of a separate genus. (Letter on file to J.H.Hoffmann, Department of Zoology and Entomology, Rhodes University, Grahamstown. 1978).

5. Country of origin and history in South Africa.

All existing cultures in South Africa originate from 128 larvae collected from Parana (31.47S 60.29W), Argentina in June 1978 (Moran et al. 1979). The larvae were collected from "O.aurantiaca type" (near O.paranensis) cactus, placed in plastic vials with artificial medium and sent to South Africa. On the 29th of June the insects arrived in Grahamstown (33.23S 26.29E) and were opened under quarantine at the Department of Zoology and Entomology, Rhodes University (Moran et al. 1979).

Host specificity tests commenced in 1978 and were completed by the end of 1979. The oviposition preference of adult females was tested in "negative oviposition" experiments. Different combinations of plants occurring in South Africa were placed in a cage together with jointed cactus and the number of eggs laid on each plant recorded (Moran et al. 1979). Forty-three different plant families comprising 98 species of plants were used in the specificity tests. The fruits of the plants were also tested for specificity against the moth.

Ovipositing females showed a preference for O.aurantiaca plants in all cases. Eggs were laid only on other species of cacti occurring in South Africa, the majority of which are pest species. No insects developed to maturity on the economically important spineless varieties of cactus. In one exceptional case a single egg was laid on a pumpkin but the larva failed to develop (see Moran et al. 1979). In view of the results from the oviposition experiments, no starvation tests were carried out on the larval stages of M.pulchellalis. It seemed highly unlikely that the female would select an unsuitable host plant for oviposition and subject the developing larva to unnatural feeding conditions. The highly artificial conditions of starvation and close confinement with tested plants, resulted in the failure of a closely related species, M.flavidissimalis (Grote) (see Mann 1969) as a biological control agent against prickly pear in Australia (Dodd 1940).

The results of the host specificity tests seem to indicate that the development of Mimorista is restricted to Cactaceae. Numerous accounts are given of M.pulchellalis larvae occurring on other cacti in South America which substantiate this observation (see Mann 1969 and Zimmermann et al. 1979).

Permission for release was granted in 1979 (Moran et al. 1979 and preliminary small releases made in the vicinity of Grahamstown by J.H.Hoffmann in October of the same year. At the time when the study reported on in this dissertation commenced, M.pulchellalis had already been deemed safe for release and consequently, liberation of the insect in the field was carried out in conjunction with laboratory experiments.

I. LABORATORY STUDIES.

6. Mass-rearing.

Large numbers of insects were needed for liberation in the field and for use in experiments. It was therefore essential to develop an efficient mass-rearing program which could produce insects throughout the year.

Initially, ovipositing females were supplied with cactus collected from the field. The number of eggs laid on the plants was counted and cladodes with eggs were then placed in shallow wooden boxes (50X50X15cm), where the insects completed their development. The number of moths collected from the cages using this method became progressively smaller however, and a careful study of the survival of the different lifestages of the insect, revealed that the low return of moths was due to a high first-instar larval mortality. Many first-instar larvae apparently failed to penetrate the cactus cladodes collected from the field. Subsequent to this observation, only etiolated cactus plants were collected from the field - etiolated cactus plants are generally less desiccated than "typical" cactus plants - and supplied to ovipositing females. These measures failed to increase the success of larval penetrations and the number of adults continued to decline. Results are shown in fig.5.

By the end of June the culture had dropped to a low level and the use of cactus collected from the field was discontinued. Larval penetrations of cladodes collected from potted plants grown at the Department of Zoology and Entomology, Rhodes University, Grahamstown yielded good results (see July, fig.5). These plants were maintained under environmental conditions but, in contrast to plants growing naturally, were regularly watered. By the end of July, potted plants grown in the greenhouse had become available and resulted in an even higher survival of first-instar larvae (see August, fig.5). Plants from the greenhouse all had flush (new) growth and were subsequently used in the rearing program.

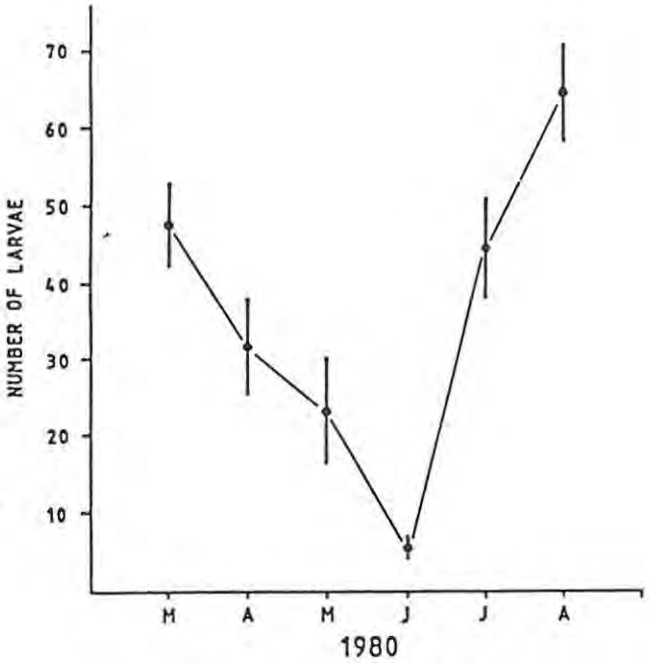


Fig.5. The mean number of M.pulchellalis larvae (per 100 eggs laid) that penetrated O.aurantiaca cladodes during each month from March to August 1980. Successful penetration appeared to be dependent on the suitability of the host plant. Prior to July, cactus had been collected from the field (see text for further details) (vertical bars = standard error).

First-instar larvae were apparently less able to penetrate cactus collected from the field toward the end of summer. Conditions were generally drier and abscission of cladodes occurred at this time (fig.3) while little new growth was present. It is possible that the cuticle of the plant may have thickened to aid in retaining water over the dry winter period. New cactus growth thus seemed to be preferable for larval penetration.

Potted plants were bulky and generally inconvenient to use in rearing procedures and a different system was developed which ensured that plants with young growth were available for ovipositing females. Cladodes collected from the field were placed in trays of vermiculite and provided with a nutrient solution (Wonder Potplant Food, 18% N, 12% P, 9% K, 0,01% Mn, 0,01% Zn, 0,03% B) at regular intervals. At a constant temperature regime of 29 ± 1 °C cladodes rooted readily, and within approximately 2 weeks produced new, flush growth that was suitable for first-instar larval survival.

Moths were maintained in oviposition cages under natural light conditions and supplied with plants that had been grown in vermiculite as described above. Plants with eggs were removed from the oviposition cages each morning and transferred to the insectary where a constant near optimum temperature (determined empirically) of 29 ± 1 °C was maintained. After the first instar larvae had burrowed into the cactus, plants with larvae were placed in wooden boxes (50X50X15cm) to complete their development. Approximately 100 larvae were placed in each box and enough cladodes added to ensure that the larvae attained their maximal size. After approximately 25 days the first adults emerged. Moths were removed in the mornings, at a time when they were least active. Glass covers over the boxes ensured that no moths escaped. Using this method 100 moths could be produced each day, the number being limited only by the space and labour available.

7. Life history and biology.

Mann (1969) gives a brief discription of the adults and larva of M.pulchellalis but furnishes little information on the biology of the insect. A knowledge of the biology is essential for the effective utilization and manipulation of a biological control agent.

Experiments to observe the activity of M.pulchellalis and to record important aspects of its biology were carried out in a greenhouse under natural light conditions. The greenhouse was programmed to simulate natural summer temperatures (28 °C day and 20 °C night) as closely as possible (see February fig.17). A total of thirty-two pairs of moths were used in the experiments. Moths were placed in clear perspex cages, 30cm X 30cm X 30cm which were screened off from adjacent cages by a double layer of newsprint (see fig.10). Isolating individual cages in this manner ensured that the behaviour of ovipositing females was not influenced by the cactus visible in neighbouring cages. An equal number of cladodes (8), was placed in each cage. Cladodes were placed equidistant from each other (see fig.10) and placed level with the floor of the cage. All cladodes were "standardized" as closely as possible (ie. similar in size and shape and locality collected) and had been grown in the greenhouse. Cladodes used in experiments all had flush (new) growth and simulated small, growing plants. Three positions were distinguised on "standardized" cladodes: a "lower" position comprising the basal third of each cladode, an "upper" position comprising the remaining two-thirds of each cladode and a "terminal" position which comprised the flush growth of each cladode.

ADULT

Adult males and females can be readily distinguished, due largely to a marked difference in colour. Males (fig.6a) are predominantly yellow except for the wings that have a large, light brown central area which extends onto the outer margin of the wing. Females (fig.6b) are almost uniformly brown in colour. Wings are brown except for a broad yellow band on the leading edge of the wing which extends over the thorax and a large dark brown central area with a lighter brown region near the apical margin. The abdomen in both males and females is always characteristically curved upwards when the moths are at rest. The abdomen of the female is more robust and less pointed than that of the male. Females were generally found to be larger than the males. Based on the mass of pupae 12 hours prior to eclosion, the mean mass (± standard error) of females was 57,4 ±

1,47mg (n=161) and of males 46,6 ± 1,15 (n=163).

Fig.6. *Mimorista pulchellalis*. a) The adult male. b) An adult female.

Eclosion of the adults usually commenced shortly after dusk and continued until the early hours of the morning (fig.7). The majority of moths (84,3%) emerged before midnight. The wings of newly emerged moths took approximately half an hour to dry and harden after which the moths remained inactive until the next morning. At first light females initiated mating. No typical calling posture was adopted and the only indication that a pheromone had been released became apparent with the sudden activity of the males. Sperm transfer lasted about 30 minutes after which the moths separated and became inactive once again for the duration of the daylight hours (fig.7). Moths of both sexes usually adopted a vertical upright position in which they remained unless disturbed (the occasional periods of day-time activity are indicated by the broken bars in fig.7).

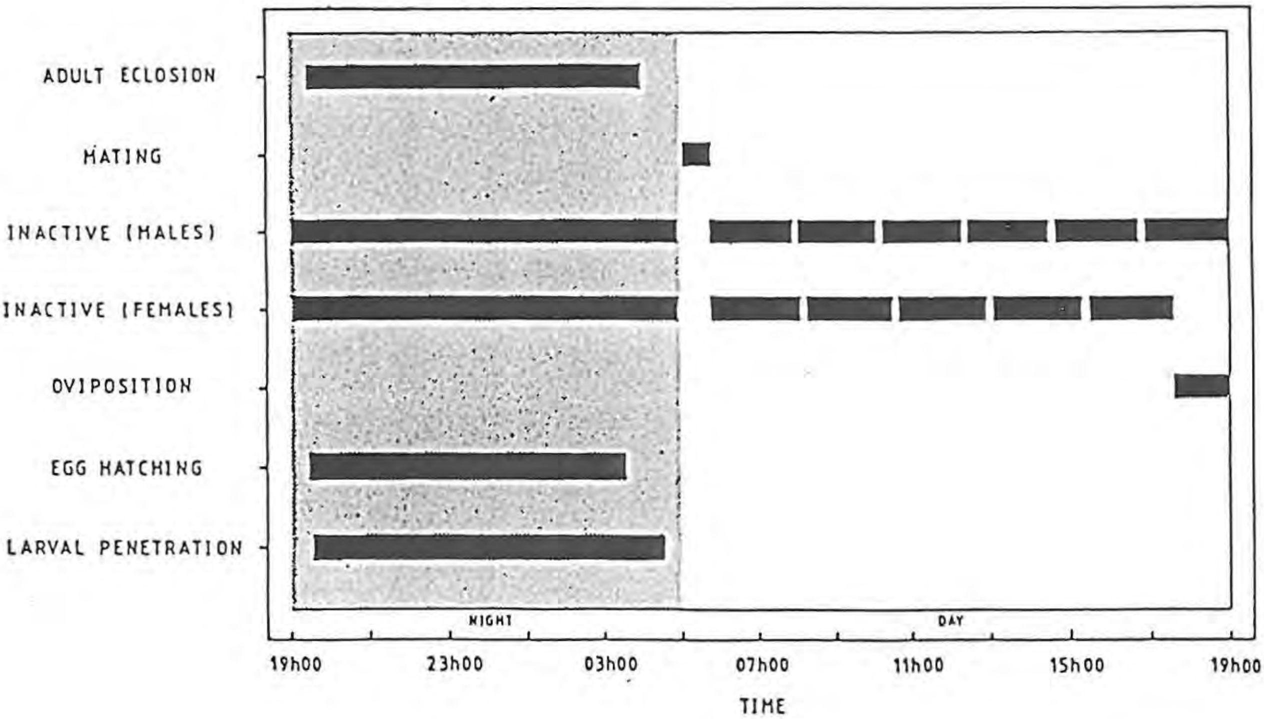


Fig.7 Activity of different lifestages of M.pulchellalis over a 24 hour period. Horizontal bars indicate when the observed behaviour took place.

In the field adults were periodically encountered and exhibited an erratic flight pattern when disturbed but quickly settled down again, usually in a place shielded from direct sunlight. Females that failed to mate remained immobile throughout the day and commenced "mate-calling" again the following dawn. After two days however, ummated females often laid infertile eggs although not all virgin females showed this behaviour. Males are capable of fertilizing more than one female.

At dusk mated females became active once again when oviposition took place (fig.7). Males remained inactive during this period. The females appeared to respond to the decrease in light intensity and oviposition occurred well before dusk on occasions when the sky became overcast. The oviposition time each day was normally short however, lasting approximately 1 hour. Temperatures also affected oviposition and females did not lay any eggs when the temperatures dropped to 15 °C or lower. This observation was made on one occasion when the heating system of the greenhouse malfunctioned. During darkness both male and female were inactive (fig.7).

A mean of 48 ± 3,5 eggs (n = 32) was laid during the lifespan of the female. The oviposition period ranged from 4-8 days (mean 5.6 ± 0,3 days, n = 32). Fifty-seven percent of the eggs were laid on day one, with 93% being laid by day four (fig.8). Most eggs were laid on the cactus cladodes, but 3% were deposited on the walls of the cages.

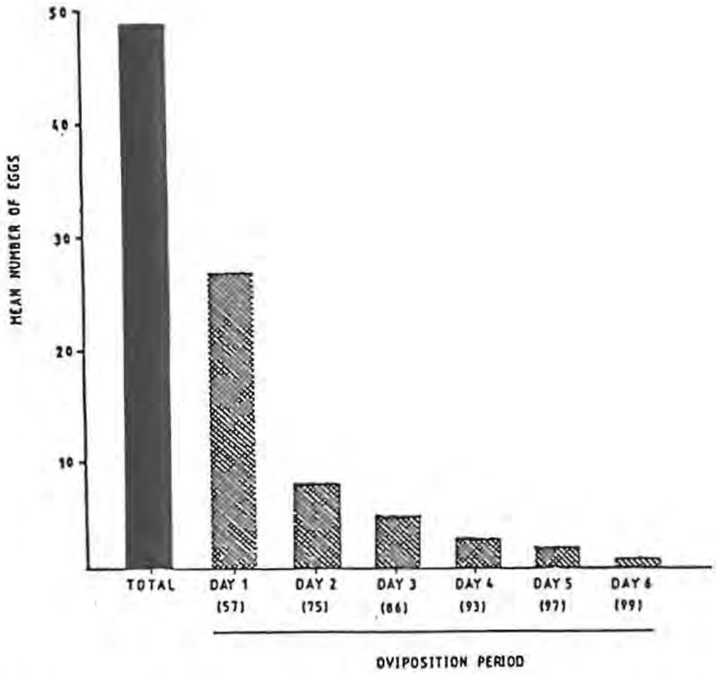


Fig.8. The mean number of eggs laid per day by 32 *M.pulchellalis* females. Figures in parenthesis represent the cumulative percentage (not indicated in the figure).

Females died shortly after their eggs had been laid. The mean longevity of females was 7,8 ± 0,21 days (n=31). Males used in the experiments had a mean longevity of 8,8 ± 0,31 days (n=30). These differences are significant ($P < 0,05$, $t = 2,63$ for 59df).

Females tended to lay eggs on the basal parts of "standardized" cladodes used in the experiments. Results are shown in fig.9. Day 1 of oviposition best illustrates this tendency (fig.9a).

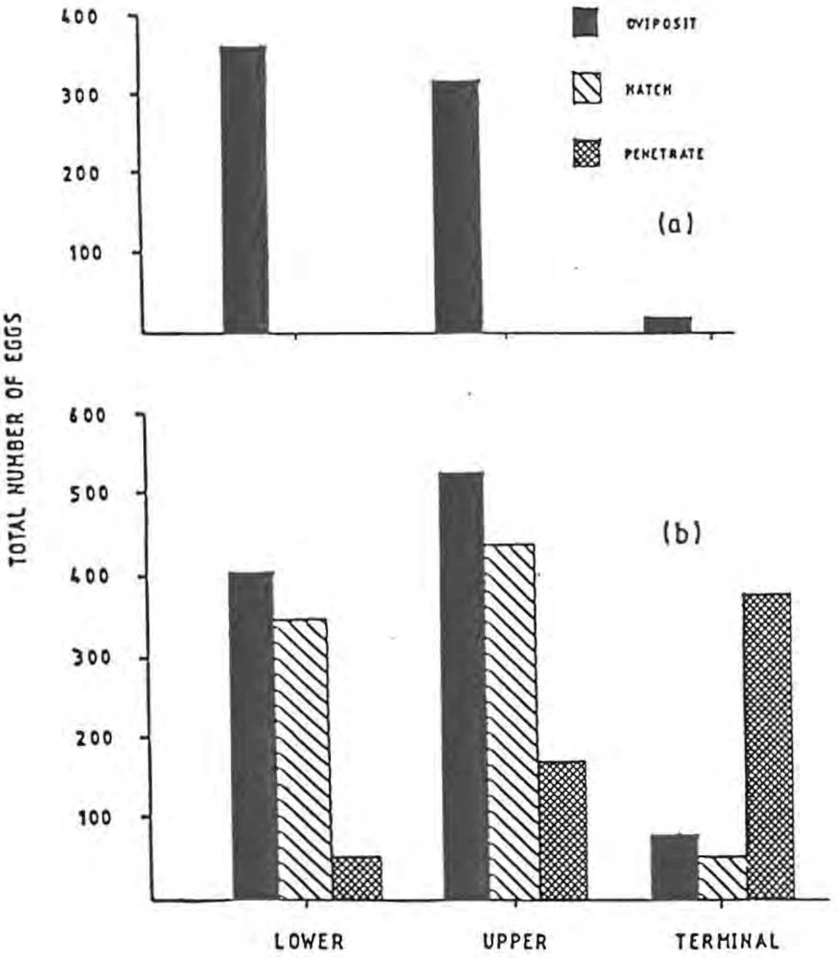
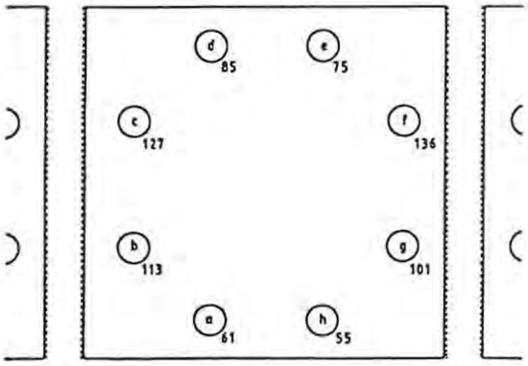


Fig.9. Positions where eggs of *M.pulchellalis* were laid and sites of larval penetration on "standardized" cladodes used in laboratory experiments (a) Day 1 of oviposition (b) Total oviposition period (lower = basal third of cladode, upper = upper two-thirds of cladode, terminal = flush (new) growth).

The difference between the number of eggs laid on the "lower" and "upper" positions of Day 1 are not significant ($P > 0,05$, $t = 0,59$ for 56df) Few eggs were laid on the "terminal" position (Day 1). After oviposition had been completed, the number of eggs laid on the "terminal" position was still low (fig.9b). The difference between the number of eggs laid on the "terminal" position and the remainder of the plant ("lower"+"upper") over the total oviposition period was highly significant ($P < 0,001$, $t = 16,1$ for 46df).

In nature, such a behavioral pattern may play an important role in protecting eggs from predators and parasitoids. This "meticulous" placing of the eggs probably has selective advantage and may be related to the relatively low number of eggs laid per female (see Moran 1980). In addition, ovipositing females seemed to show a preference for laying eggs on cladodes which had been placed close to the walls of the cage (fig.10). The difference between the number of eggs laid on cladodes placed near the walls of the cage (b,c,f and g in fig.10) and eggs laid on the remaining cladodes, was highly significant ($P < 0,001$, $t = 6.34$ for 6df)



Cladodes near side of cage		Remaining cladodes	
cladode	no. of eggs	cladode	no. of eggs
b	113	a	61
c	127	d	85
f	136	e	75
g	101	h	55
mean	119.25	mean	69.00
S.D.	13.56	S.D.	15.41

Fig.10. Diagrammatic plan of an experimental cage, showing the relative positions of two adjacent cages. The circles indicate the positions of the "standardized" cladodes placed in the cages. Numbers associated with each circle represent the total number of eggs laid each cladode by 16 females. Broken lines indicate the newsprint partitions (see text).

As all cactus had been "standardized", ovipositing females must have responded to stimuli other than suitability of host plant. The only factor which distinguished these "preferred" cladodes was their close proximity to the sides of the cage that had been screened with newsprint (fig.10). The light intensity may have been lower close to these sides of the cage, and consequently these cladodes were located in deeper shadow during the oviposition period. The fact that females seemed to prefer laying eggs on cladodes placed at a lower light intensity may have important survival value as will be examined in the discussion.

EGG

The eggs of M.pulchellalis are flat and disc-like and are laid singly on the surface of the cladodes. The diameter of the eggs ranges from 1,6 - 2,4mm with a mean and standard error of 1,97 ± 0,03mm (n = 57). Initially yellow when first laid, the eggs become progressively darker as they mature. The young larva becomes visible through the shell of the egg approximately 48 hours before hatching. Infertile eggs retain their yellow colour, dry out and eventually flake off the plant. The incubation period of the eggs ranged from 11-15 days (mean 12,5 ± 0,22 days, n = 30).

LARVA.

Hatching always commenced shortly after dusk (fig.7). First-instar larvae are light brown in colour and have a black head. Larvae wandered about the cladode in an apparently haphazard manner but usually selected a site on the terminal flush cladode for penetration. Penetration of the cactus always occurred under cover of darkness (fig.7). Terminal cladodes generally represent the new growth of cactus plants and consequently have a thinner cuticle, so making penetration easier for the first instar larvae. Most of the larvae penetrated the terminal flush growth (fig.9b).

Differences between the number of larvae that penetrated the terminal growth and the number that penetrated the remainder of the plant ("lower" + "upper"), was highly significant ($P < 0,001$ $t = 3,94$ for 46df).

After selecting a suitable penetration site on the plant, larvae "anchor" themselves by silken threads and are not easily dislodged. Before tunneling into the cactus, the larva chews a number of shallow depressions into the cuticle which radiate from the eventual entry-hole in a series of half-circles. The exact function of this procedure is unknown, although these perforations give rise to "windows" in the cuticle and may aid in gas-exchange. The larva then procedes to eat its way through the cuticle and underlying cactus tissue. The ingested material passes through the gut and is deposited on the surface of the cactus. Once the tunnel is large enough, the larva turns around and plugs the opening with a layer of silk. All further frass is deposited inside the cladode. Larvae seldom vacate the cladode in the field and complete their life-cycle inside the cactus.

Larvae slowly increase the diameter of their tunnel and move towards the base of the plant. Frass is deposited in the hollow excavated by feeding and is accumulated near the entry-hole. A wall of silk continually separates the frass from the feeding larva. New "windows" are made as feeding progresses and aid in identifying M.pulchellalis damage in the field. Initially the larvae are a dull white in colour but the cuticle becomes almost transparent in later instars, clearly showing the green ingested plant material.

Temperatures used for mass-rearing (29 ± 1 ^{o}C), resulted in a larval developmental time of 25 days. Approximate developmental times actually recorded in the field are given at a later stage.

PUPA

Pupation occurs in the hollowed husk of the damaged cladode, distal to the original entry-hole and removed from the frass deposited there (see fig.13). Final-instar larvae spin a loose cocoon in which pupation takes place.

The frass inside the cladode eventually desiccates and shrinks, often causing the dry husk of the cladode to crack open above the pupation chamber. In the field, this part of the cladode often breaks off completely or else collapses under its own weight, as indicated in fig.13.

In laboratory experiments, where many eggs may be laid on individual cladodes, numerous larvae burrow into and often totally destroyed the cladodes. In the field, only one larva occurred per plant and the damage to cactus plants was less severe. This could be due to the fact that few eggs are laid on each plant under natural conditions where oviposition responses are not altered by the restrictions of a cage and the limited number of cladodes. Alternatively, eggs in nature may be destroyed by predators and parasitoids.

Although not investigated in this study, a field trial conducted at Uitenhage showed that the egg mortality of <u>M.pulchellalis</u> could be as high as 94,5% (Richards 1982). Of a total of 250 eggs (5 eggs on each of 50 plants) monitored over a nine-month period, only 14 (5,5%) successful penetrations were recorded. Of the remaining 236 eggs, 59 (23,5%) were destroyed by predators (usually ants) and on one occasion by a parasitoid (<u>Trichograma</u> sp.) (Richards 1982). 171 eggs (68,5%) simply disappeared and could not be accounted for.

A chalcidid wasp (<u>Hockeria</u> sp.) parasitizing the <u>M.pulchellalis</u> pupae was also found on numerous occasions (both at Uitenhage, H.G.Zimmermann pers. comm., and during the course of this study). The overall effects of natural enemies on the M.pulchellalis population were not investigated and are not known.

II. FIELD STUDIES

Field studies constitute perhaps the most important aspect in any biological control program. Careful monitoring of the insect population should be carried out soon after release to ascertain whether the insect becomes established and, if so, to determine the degree of control exerted against the pest species. Results from such observations may give indications of the necessity to manipulate insect numbers or determine the need for additional imports of new natural enemies (De Bach & Bartlett 1964).

The first large-scale releases of M.pulchellalis were made in 1980. The numbers of insects released at the various localities and the release methods employed to achieve establishment, are related in the next chapter. A detailed account of the recovery and evaluation studies conducted after M.pulchellalis was released in South Africa concludes this section.

8. Releases of M.pulchellalis in South Africa.

Releases of insects were made against both morphological cactus types occurring in the eastern Cape. The "typical" variety of jointed cactus usually occurred in the low-growing False Karroid Broken Veld vegetation (Acocks 1975) which has invaded much of the eastern Cape vegetation. The etiolated jointed cactus was associated with Valley Bushveld vegetation (Acocks 1975) growing in the shade of trees and large bushes. Releasing the insects in the various localities where the cactus occurred, exposed the insects to varying climatic conditions and may have increased the chances of survival.

Initial releases were restricted to Andries Vosloo Kudu Reserve

(33.07S 26.39E), a State owned reserve 20 km north-east of Grahamstown. The vegetation of the reserve is described as Fish River Scrub (Acocks 1975) which is a variation of the Valley Bushveld. "Typical" cactus often occurred in areas of the reserve which were invaded by low-growing thorny scrub and Karroo bush (see Acocks 1975). Palmer (1981) gives a detailed description of the vegetation of the Andries Vosloo Kudu Reserve. Chemical control had not been employed for a number of years because of the adverse effects to the environment and cactus on the reserve was cleared entirely by mechanical means using teams of labourers. Arrangement with the Nature Conservation offices ensured that areas selected for biocontrol were excluded from the cactus control program.

Large numbers of insects were originally released in an area approximately 5 hectare in extent (site 1-5 AVKR, Table 1). Release of an insect in large numbers often increases the chance of establishment (Myers & Sabath 1981) while the close proximity of the release sites to one another (see fig.24) minimized emigration losses and enhanced mate-finding (De Bach & Bartlett 1964).

Larvae were frequently encountered following a release, indicating at least temporary establishment, and release areas were extended to other sites on the reserve and areas in the vicinity of Grahamstown (Table 1).

8.1 Methods of release.

Releases of M.pulchellalis were always made in the adult stage. Moths destined for release were collected from the culture every morning and accumulated until release. Both male and female moths were placed in the same container which was kept at a temperature of approximately 25 °C and exposed to natural light conditions. Moths were released every third day. This procedure ensured that the majority of moths had mated by the time they were released. Occasionally, mated females would oviposit on the walls of the container.

Table 1. Release localities of M.pulchellalis. Releases at the last three localities were made in the vicinity of Uitenhage by H.G.Zimmermann. The results of a survey conducted in January 1983, recording recent larval colonizations are shown. Insects were always released as adults (* = releases made against etiolated cactus; AVKR = Andries Vosloo Kudu Reserve; Thomas Baines Nature Reserve (33.24S 26.30E) is a State owned reserve approximately 10,5km from Grahamstown; Mosslands (33.24S 26.26E) is a privately owned farm 12,5km from Grahamstown).

Locality	Date first released	Date last released	Total no. released	New infestations January 1983
AVKR site 1	13/11/80	9/1/81	543	yes
AVKR site 2	24/3/80	29/5/80	407	yes
AVKR site 3	9/1/81	31/3/81	1013	yes
AVKR site 4	9/1/81	1/11/82	5406	yes
* AVKR site 5	19/10/81	7/1/82	60	yes
* AVKR Rhino camp	15/12/82	15/12/82	174	yes
* AVKR River Site	1/12/80	29/4/81	2050	yes
AVKR Hill Site	20/1/82	25/1/82	320	no
* Thomas Baines	29/7/81	7/12/82	3762	yes
* Mosslands	4/7/80	4/7/80	39	no
TOTAL			13774	
Doornkom	4/2/80	6/2/81	5716	yes
The Meadows	9/12/80	1/6/82	19337	yes
Springfield	23/2/81	20/10/82	24478	yes
TOTAL			49531	
GRAND TOTAL			63305	

On one occasion a large number of insects was released in the larval stage but this method was generally found to be unsuitable. Cladodes with larvae from the culture were placed in close contact with jointed cactus plants in the field. Rapid desiccation of the loose cladodes often forced the larvae to vacate the cladodes which exposed them to adverse climatic conditions and predators (mostly ants) and consequently led to a high mortality. Larvae that did complete their development in the release-cladodes were often trapped in the dried-out husk during adult eclosion.

Releases of the egg-stage were never attempted. M.pulchellalis females are reluctant to oviposit on unnatural surfaces eg. gauze strips, while placing cladodes with eggs in the field was considered impractical.

8.2 Timing of release.

Because moths ready for release were kept under natural light conditions no period of adjustment was considered necessary prior to liberation in the field. Releases were therefore made at any time of the day, barring the oviposition period (dusk, see fig.7). Recovery studies following each release always revealed a large number of early instar larvae. Moths were released on jointed cactus plants in the field but showed no preference for settling on the cactus, generally selecting any site shielded from direct sunlight.

Following the original large-scale releases made in 1980 and 1981, the insectary stock was scaled down and a small culture maintained. Surplus moths were periodically collected and released in the field. Two areas were set aside exclusively for evaluation studies (River Site & Hill Site in Table 1) and were excluded from the periodic releases. River Site was located in Valley Bushveld vegetation (Acocks 1975) where etiolated jointed cactus occurred. Hill Site was situated in the low-growing False Karroid vegetation were large clumps of "typical" jointed cactus were frequently found.

A large number of moths was released at each site over a short period of time and changes in the population density of the insect carefully monitored. Estimates were made of the jointed cactus population in both areas and were used to quantify the overall impact of M.pulchellalis damage.The number of insects released at Hill Site dwindled rapidly and after a period of three months no new larval attacks could be detected. It was therefore concluded that this cactus-type was not suitable for M.pulchellalis development and studies at this site were abandoned. All further discussion relates to field studies conducted on etiolated cactus at River Site.

9 Establishment and evaluation of M.pulchellalis.

9.1 Introduction

Studies conducted at River Site were primarily to record the survival of M.pulchellalis under South African conditions. No attempts were made to quantify the possible mortality factors affecting the insect population in the field.

The insect and cactus densities were estimated by making total counts of the number of M.pulchellalis larvae and cactus plants in randomly selected sample areas or quadrats (Seber 1973; Southwood 1978). The spatial dispersion pattern of both M.pulchellalis and jointed cactus were considered important in indicating possible host-plant preferences of the insect and were also recorded from the quadrats.

Using the system of quadrats, the temporal and spatial variation in the population density of the larvae was carefully followed and quantified over a 14-month period, from October 1981 to December 1982. The cactus density was estimated once over this same period and the number of cactus plants attacked by each generation of the insect recorded.

9.2 Materials and methods

The area selected for sampling was situated in riverine scrub on the banks of a dry watercourse. The total extent of the infestation was approximately three hectare of which 0,5 hectare was heavily infested. The remaining 2,5 hectare consisted of large isolated clumps of cactus distributed along the watercourse. The dense, luxurious growth of cactus (fig.11) made sampling extremely difficult and consequently an area 40 metres wide by 50 metres long was selected for sampling purposes. This area encompassed most of the very dense stands of cactus. To eliminate the procedure of a random selection of quadrats each time the insect population was sampled, and ensure minimal disturbance of the habitat (excessive trampling and destruction of the cactus), the selection of quadrats was made once, and these quadrats were used in all subsequent sampling.

Fig.11. Dense cactus stands at the study site. Note the etiolated growth form of the cactus.

A large number of moths (1010 males and 1040 females) were released at this site during the summer season of 1980/1981, from December 1980 to April 1981 (Table 1). No further releases were made at the site or in the immediate vicinity after April 1981. The first sample to estimate insect abundance and to establish the spatial dispersion pattern was taken in the following summer season (1981/1982) in October 1981 (see fig.16), six months after the last release. Sampling of the insect population was continued throughout the 1981/1982 summer season. Periodic surveys of the area revealed the presense of new larval colonizations, indicating the start of another generation. The number of insects in the quadrats was counted soon after at a time when all eggs were estimated to have hatched. A final sample of the insect population was taken in December 1982 (see fig. 16).

To facilitate counting of the cactus inside the quadrats and to determine insect damage, the cactus plants in the area were classified into four categories:

Catagory A --------------- Plants $\leqslant$ 10cm in height
Category B --------------- Plants $> 10 \leqslant 30$cm
Category C --------------- Plants $> 30 \leqslant 50$cm
Category D --------------- Plants $>$ 50cm

The height of plants was used as a criterion for classifying plants rather than number of cladodes. This was due to the very uniform growth habit of the cactus in the area, most of the plants having no side-branches (fig.12).

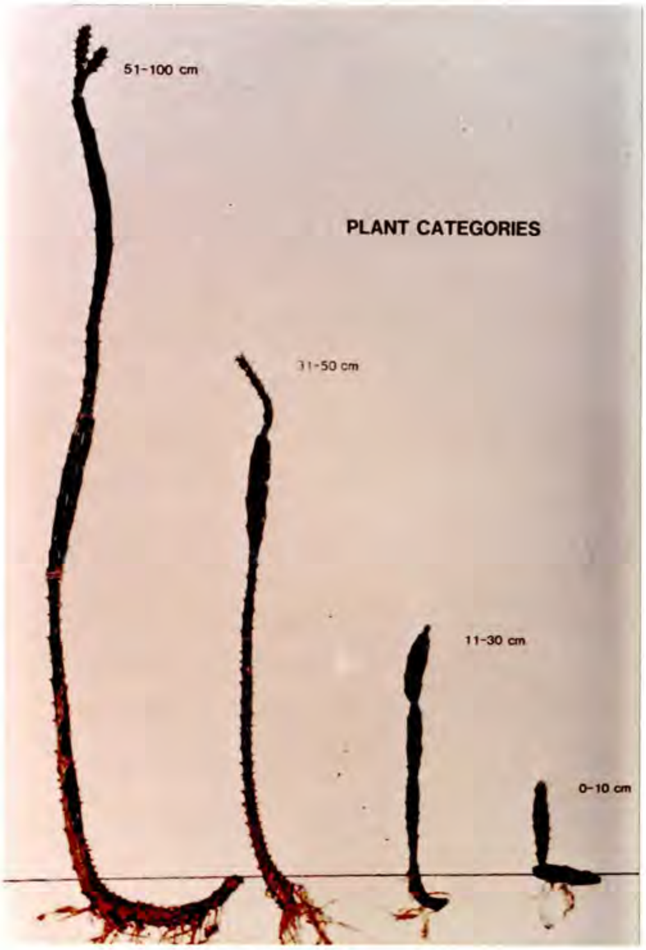


Fig.12. Plant categories of O.aurantiaca used in the study.Plants showed a very uniform growth form and produced very few side branches. Category A = 0-10cm tall plants; Category B = 10-30cm; Category C = 30-50cm; Category D = 50-100cm tall plants. Plants taller than 100cm were often encountered and included in Category D.

Using height above ground level (equal to size of plant) was both easy to measure and to visualize. Stating that a plant consisted of, for example, 5 or less cladodes was both ambiguous and confusing, including not only "small" (< 30cm tall) but also "large" (> 50cm tall) plants.

After the quadrats in the area had been selected, all the dead cactus material was removed from the quadrats. Destroyed cladodes and plants had accumulated in the area due to the combined activity of Cactoblastis cactorum and cochineal, Dactylopius austrinus. Removal of the cactus debris made the counting of jointed cactus easier and showed up any M.pulchellalis damage. Counting the cactus was further facilitated by placing a wooden rod, marked at the appropriate plant category levels in the centre of each quadrat. Quadrats containing a very dense stand of cactus were partitioned into smaller managable units which were counted separately and totalled once all the counts had been made.

M.pulchellalis colonizations (fig.13) were easily detected in the "cleared" quadrats. Only one larva was observed in each plant attacked.

Fig.13. M.pulchellalis damage to O.aurantiaca plants in the field was very characteristic. Larvae normally colonized the terminal parts of plants (see text). Note that only one cladode has been destroyed; the subterminal cladode with the pupal chamber (arrow) has only been partially destroyed and can grow again. The plant shown here is a Category B plant comprising 3 cladodes.

Besides counting the number of insects in each quadrat the following variables were also recorded: the height of each plant attacked, the number of cladodes destroyed by the larvae in such plants and the number of cladodes that were partially destroyed in each plant (see fig.13). These variables were recorded each time a sample was taken of the insect population.

In any sampling survey three factors have to be taken into consideration to make a sample scientifically valid (Elliott, 1977). These include: the size of the sampling unit (= quadrat), the number of sampling units in each sample and the location of each sampling unit.

9.2.1 Size of the sampling unit.

As the objectives of this sampling survey were to estimate the density of cactus and insect populations and to describe the dispersal pattern of both the cactus and the insect, considerable thought had to be given to the choice of quadrat size.

Generally, in sampling to determine population density, it is desirable to keep the sample size small (Elliott 1977). This results in a more representative sample of the whole area and at the same time increases the number of sampling units from which a sample may be drawn. Small sampling units would increase the accuracy of the insect and cactus estimates while reducing the variance to a minimum (Southwood 1978). On the other hand, smaller samples would be more labour intensive and more destructive of cactus due to repeated trampling to obtain the extra sample units. Because of the low insect numbers (detected by a preliminary survey), a small quadrat would exclude many insects and hence lead to an excess of zero counts in insect samples which would be difficult to analyse statistically (Southwood 1978).

In the detection of spatial patterns, quadrat size is also important (Kershaw 1975; Elliott 1977; Southwood 1978). If a population is known to be contagious, as was obvious for jointed cactus at this site, a gradual increase in the size of the sampling unit used to measure the spatial pattern would result in the apparent dispersion pattern of the population ranging from random, through contagious and finally to regular (Elliott 1977). The most marked demonstration of the clumped nature of a population would therefore be with a quadrat with an area approximately equal to the size of the clump. As the cactus clumps at this site generally covered a large area, a quadrat size of 1 metre square was regarded as being small enough to reflect spatial patterns. At the same time this size of sampling unit was convenient for estimating cactus and insect densities; it was small enough to obtain an accurate count of the cactus while being large enough to include sufficient larval numbers. Using a metre square quadrat as a sampling unit, the total sampling population that could be sampled in the area selected (40mX50m) thus consisted of 2 000 sampling units.

9.2.2 Number of sampling units

Due to the great variability in density of the cactus, the study area was divided into two unequal areas (see fig.15). The larger of the two areas (30mX40m) consisted mainly of the smaller, less densely distributed cactus clumps (sparse cactus). The remaining area (20mX40m) consisted mainly of the dense clumps of cactus (dense cactus). Each area selected was regarded as a separate community for the purpose of estimating cactus density and sampled separately.

The number of sampling units (quadrats) in each division used to estimate cactus density was determined from a method described in Kershaw (1975) and Elliott (1977). In this method the arithmetic mean of a number of quadrats is calculated and plotted against sample size. As the quadrats in this study could contain a variable number of plant sizes (see fig.11 & 12), determination of an arithmetic mean of a number of quadrats was difficult. Cactus counts for each quadrat were therefore standardized by recording the total number of cladodes per quadrat rather than the total number of plants in each category. This was made possible by marking a number of plants in each plant category in the study area and recording the number of cladodes on each plant. The average number of cladodes in each plant category recorded in this manner is shown in Table 2. Using the results from Table 2, the original counts of the quadrats - which recorded the number of plants in each plant category - could be converted to the number of cladodes per plant category and the total number of cladodes per quadrat could thus be calculated.

Table 2. The mean number of cladodes (± S.E.) recorded for each plant category of O.aurantiaca used in the study. Variables shown are based on counts of 24 plants measured at the study site. Measurements were made in January 1982 after the initial growth phase of the cactus had been completed (see fig.3).

	PLANT CATEGORY			
	A	B	C	D
Mean number of cladodes.	1,7	3,2	5,1	7,5
Standard error.	0,2	0,28	0,44	1,0

A sample consisting of 6 quadrats was initially selected from the sparse cactus while a sample of 4 quadrats was selected from the dense cactus.The mean and standard error of these quadrats were then determined and the mean plotted as a function of sample size (fig.14). Sample size was increased in multiples of the originally selected number of sample units (eg. 6, 12, 18, ... etc) and the mean and standard error of each new sample determined. Sample size was considered adequate when the mean number of cladodes recorded did not vary widely with increasing quadrat number. As this study included an estimate of insect numbers in the same area, enough quadrats with cactus (on which insects could be counted) had to be included to be able to accurately estimate the insect population.

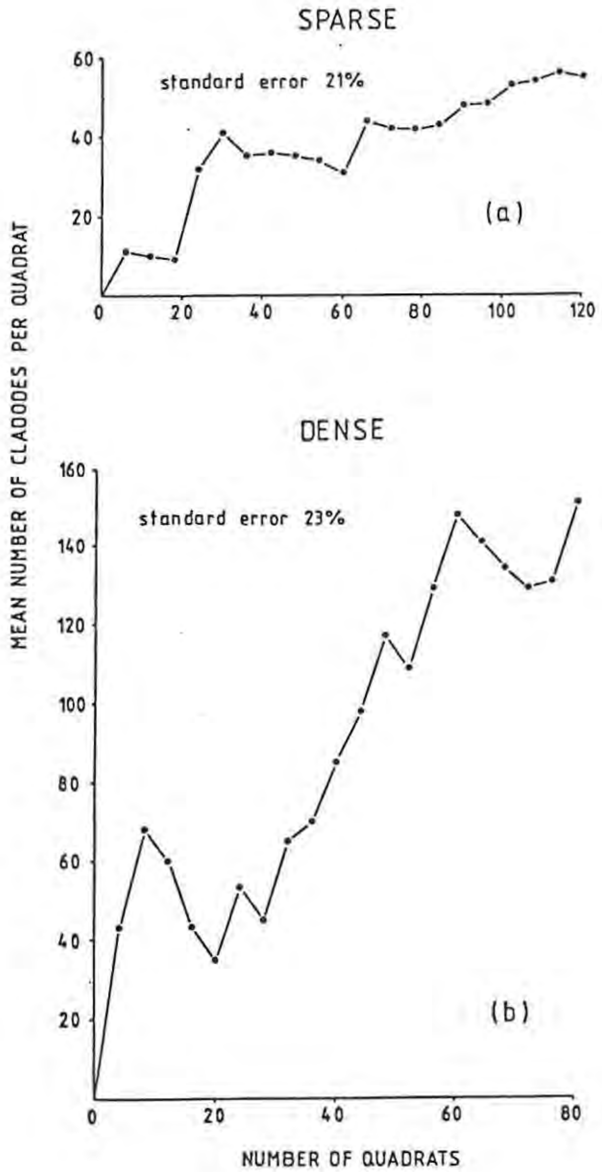


Fig.14. The density of <u>O.aurantiaca</u> (number of cladodes per quadrat) shown as a function of quadrat number for sparse and dense cactus.

Sample size thus had to maintain a balance between accuracy for cactus counts, accuracy for insect counts and the costs of sampling to increase the precision of the estimates for both the cactus and insect populations

Sampling of sparse cactus continued until 120 quadrats had been selected. At this level the standard error was 21% of the mean (fig.14). This degree of precision was considered adequate for the purposes of the study. In a similar manner, sampling was discontinued after 80 quadrats had been sampled from the dense cactus. The standard error of the mean for dense cactus after 80 quadrats was 23% (fig.14). Continued sampling to increase the level of precision of the cactus density estimate was not justified in view of the additional labour involved.

In total 200 quadrats were selected from the sampling population of 2 000 ie. 10% of the population (fig.15). Of these two hundred quadrats, 120 contained cactus and were used for estimating insect abundance. A large sample of this size was considered necessary to accurately estimate the low insect numbers (Cole 1946; Cottam et al. 1957; Elliott 1977). The same sample of 120 quadrats was used each time the insect population was sampled.

9.2.3 Location of sampling units.

To obtain an unbiased estimate of the population size of both the cactus and the insect population and to make sampling valid, samples had to be chosen at random (Stuart 1964). Furthermore, a random selection of quadrats was considered necessary to estimate particularly the insect population. Habitat preferences for the insect were not known and would not have been revealed by an objective selection of quadrat localities. Such a sampling procedure (objective) may have led to either an underestimate or an overestimate of the insect population.

Random selection of quadrats in the study area was determined uniquely for each quadrat by a system of random co-ordinates on a Cartesian grid. To achieve this, the shorter of the two boundary lines enclosing the total area (ie.40m) (fig.15) was aligned in a north-south direction while the larger of the two boundary lines (ie.50m) was aligned at right angles to the first (fig.15). Random numbers generated by a computer assigned a unique co-ordinate value to each quadrat; the first value determined the distance along the north-south boundary line (x-axis) while the second value of each quadrat co-ordinate determined the distance along the east-west boundary line (y-axis). To facilitate the location of quadrats the total area was subdivided into twenty 10 x 10 m blocks (fig. 15). Junctions between adjoining blocks were permanently marked and aided in relocating the different quadrats. These markers were also used to measure distances when quadrats were initially located. All quadrats were permanently fixed and given a number between 1 and 200. Orientation of each quadrat was the same as for the total area and determined by compass.

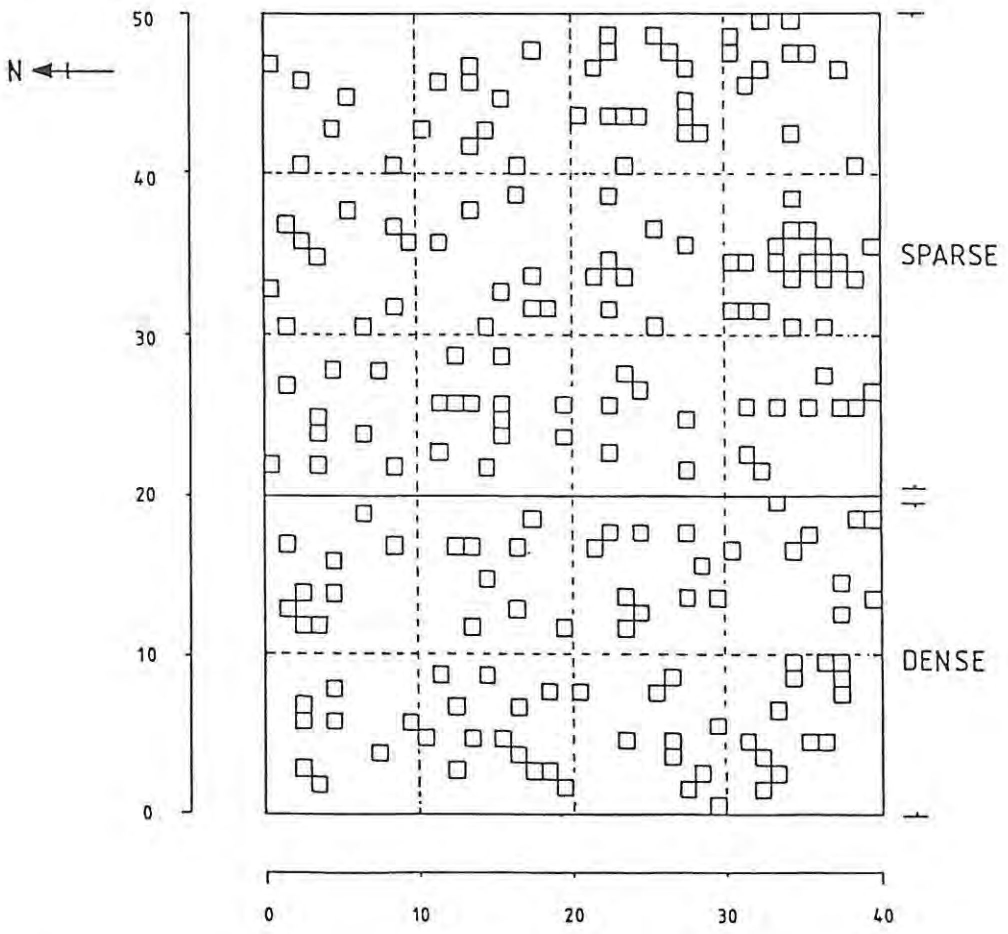


Fig.15. Location of the 200 randomly selected quadrats. The total area was divided into two unequal areas in an attempt to increase the accuracy of the cactus estimates (see text). The broken lines subdivide each division into 10X10m blocks which were used to facilitate location of the quadrats.

9.3 Results

9.3.1 Estimation of insect abundance.

The majority of insects went through three generations in the 1981/1982 summer season (fig.16). A few insects did enter a fourth generation before the winter period (illustrated by the indentation of "generation 3" in fig.16) but the numbers were so low that they were not reflected in the quadrats. It was therefore assumed that these numbers would not significantly affect the results.

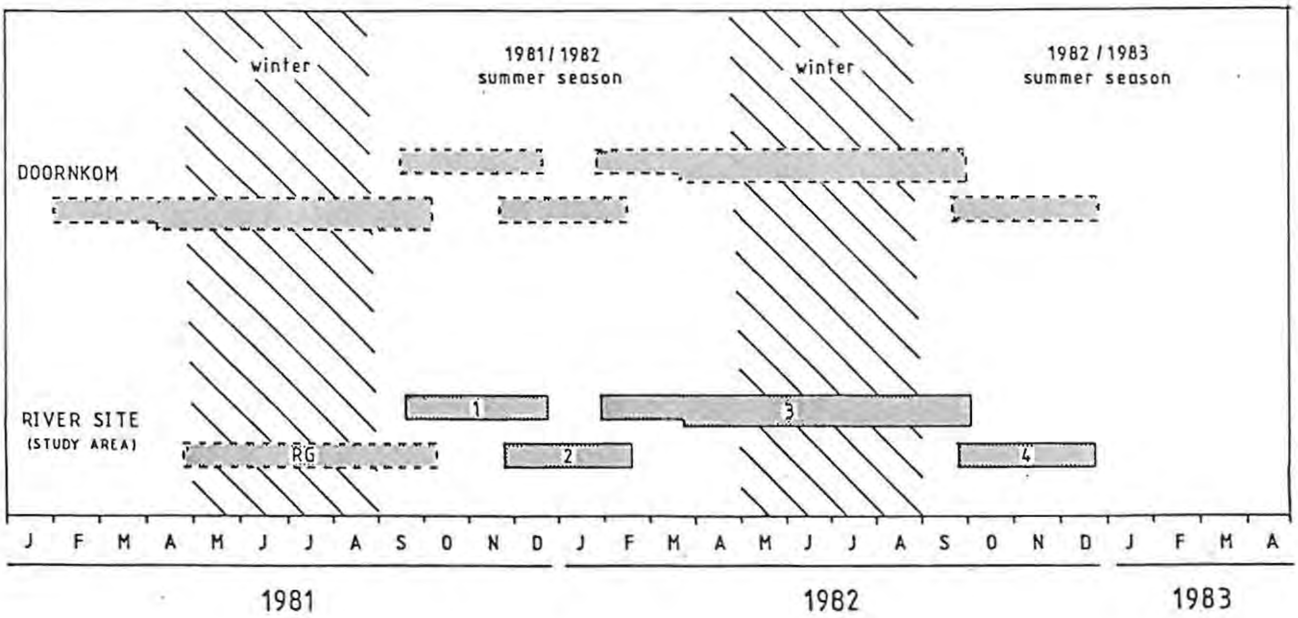


Fig.16. The number of *M.pulchellalis* generations recorded at the study site over a period of 14 months (solid lines). The broken lines indicate probable generations (not quantified). Releases at Doornkom (broken lines) were made in the vicinity of Uitenhage by H.G.Zimmermann (Table 1). The number of generations were calculated from the date of last release RG=release generation; 1,2,3 and 4 = *M.pulchellalis* generations recorded. The indentation of generation 3 indicates the small number of insects which entered a fourth generation in the 1981/1982 summer season (see text for further explanations).

Generation three overwintered and in the 1982/1983 summer season gave rise to generation four which was sampled in December 1982 (fig.16). No diapause occurred over the winter period; the development of insects slowed down and the majority overwintered as larvae. Generations overlapped to a large extent (fig.16) and it was not unusual to detect larvae (in all stages of development) as well as pupae in the field during the same survey. The temperatures recorded at the study area are shown in fig.17. It can be seen (fig.17) that extremes in temperature were often experienced by M.pulchellalis over the sampling period. Insect numbers remained at a low level throughout the study, as can be seen from the sampling results which are shown in Table 3.

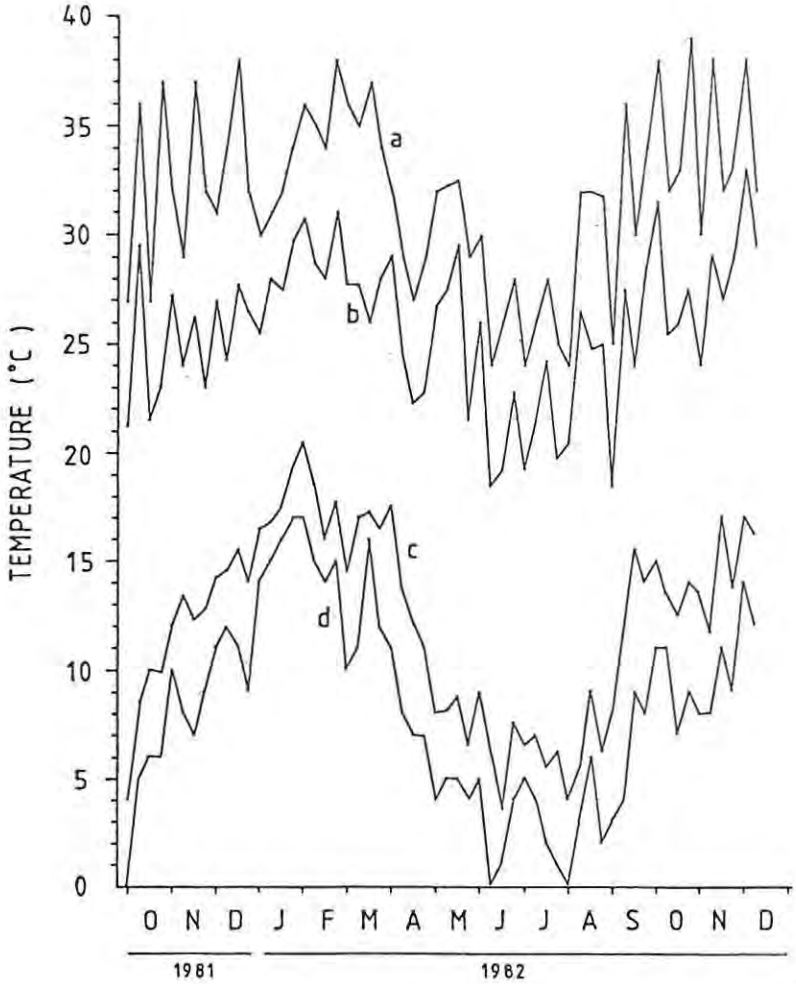


Fig.17. Air temperatures recorded in the study area over the duration of the study (a = absolute maximum, b = weekly mean maximum, c = weekly mean minimum, d = absolute minimum).

The variance was greater than the mean in all samples taken (Table 4), possibly reflecting a contagious distribution of the insect. To test whether the spatial dispersion of the larvae was clumped, the results of the sample counts were arranged in a frequency distribution and fitted to the values predicted by the negative binomial distribution as described in Elliott (1977) (see Appendix). Larvae can be regarded as being sedentary,

Table 3. The total number of *M.pulchellalis* larvae recorded for each generation in the 120 quadrats sampled. Variables represent the number of plants attacked by individual larvae in each plant category. Generations 1,2 and 3 were sampled in the 1981/1982 summer season; generation 4 was sampled in the 1982/1983 summer season (see fig.16).

	PLANT CATEGORY				
	A	B	C	D	Total
Generation 1	5	19	8	4	36
Generation 2	13	25	3	0	41
Generation 3	15	17	0	0	32
Total(season)	33	61	11	4	109
Generation 4	13	38	2	0	53

Table 4. Observed counts of *M.pulchellalis* larvae in the 120 quadrats sampled for each generation. Recorded values were successfully fitted by the negative binomial distribution (x = the number of larvae observed in quadrats; obs = observed number of quadrats; exp = number of quadrats predicted by the negative binomial distribution).

1981/1982									1982/1983		
Generation 1			Generation 2			Generation 3			Generation 4		
Total no.		36	Total no.		41	Total no.		32	Total no.		53
Mean		0.30	Mean		0.34	Mean		0.27	Mean		0.44
Variance		1.74	Variance		2.21	Variance		0.72	Variance		1.95
k		0.20	k		0.26	k		0.09	k		0.18
x	obs	exp	x	obs	exp	x	obs	exp	x	obs	exp
0	100	99.8	0	97	96.6	0	106	105.9	0	96	96.1
1	11	12.1	1	15	14.1	1	6	7.2	1	13	12.2
2	3	4.3	2	3	5.1	2	4	3.0	2	5	5.1
3	5	1.9	3	2	2.2	3	1	1.6	3	2	2.6
4	1	1.8	4	2	1.0	4	2	0.9	4	1	1.5
			>4	1	1.1	>4	1	1.1	>4	3	2.6
χ^2 (2df)=6.0			χ^2 (3df)=1.85			χ^2 (3df)=2.49			χ^2 (3df)=0.32		

Agreement with the negative binomial accepted (P>0.05) in all cases.

which made an accurate quantification of their dispersion pattern possible (Pielou 1969). The model provided a remarkably good fit (Table 4) indicating that the larvae were contagiously dispersed.

The negative binomial has two parameters, the arithmetic mean $\bar{x}$ and the exponent k. The value of k indicates the degree of contagion (low k values show contagion, high k values, a random distribution) and has frequently been used as an index of aggregation (Waters 1959). The frequency distribution of the negative binomial model is asymmetrical when values of k are low (< 2) for a large range of the arithmetic mean but approaches normality when k increases and the mean is large (Elliott 1977). The transformation of an asymmetrical frequency distribution is necessary to normalize the distribution of the counts in order to apply parametric statistical tests. These tests were necessary to detect any differences in means between the different insect generations sampled. Transformations suitable for the negative binomial distribution are described in Elliott (1977).

As the contagious distribution of the larvae would affect the validity of the statistical analysis of the samples (Elliott 1977; Southwood 1978) a suitable variance stabilizing transformation had to be found. The low number of larvae in the area and the consequent high proportion of zero counts in the samples (Table 4) made the selection of a such a transformation for the calculated values of k impossible (see Elliott 1977). Andersen (1965) has also shown that variance cannot be stabilized if low values of the mean (less than 3) and k values approaching zero are obtained in a sample (see Table 4). Instead, confidence intervals were calculated for each sample, based on the assumption that means from large sample sizes (n = 120 quadrats in this study) estimate the true population mean with a population variance that could be estimated by the sample variance (Central Limit Theorem). Estimated population density and

95% confidence intervals are shown in fig.18. This clearly shows that there was no significant difference in insect numbers between the generations.

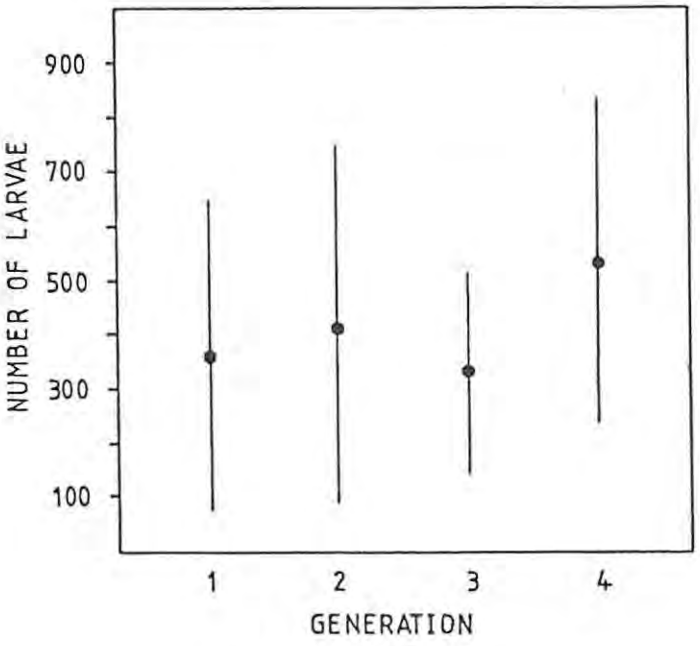


Fig.18. The estimated number of <u>M.pulchellalis</u> larvae in the study area (40X50m, fig.15) for the four generations sampled. Generation 1-3 were sampled over the 1981/1982 summer season. Generation 4 was sampled in the 1982/1983 summer season (see fig.16). Vertical bars indicate the 95% confidence intervals.

The aggregation of the larvae could also have affected the accuracy of the counts obtained (Elliott 1977) and consequently a complete census was taken of generation 4 in December 1982 (fig.16) to determine the efficiency of the sampling system employed. The variables recorded during the census were the same as were used for sampling. Results are shown in Table 5. A total of 611 larvae was counted. Twenty-nine percent of the plants colonized by <u>M.pulchellalis</u> larvae were recorded from Category A plants. The majority of the insects were recorded from Category B plants however (64% of the total number of plants attacked), while the remaining 7% comprized Category C and Category D plants.

The observed count (611) falls well within the 95% confidence levels predicted by the sample estimate (fig.18), although the latter under-estimated the true population value. This is a common phenomenon when the population being estimated is known to be highly aggregated (Cole 1946; Dice 1948; Chiang & Hodson 1959). The census showed that the sampling method used was efficient in estimating the insect population.

Table 5. The total number of M.pulchellalis larvae recorded in the study area (40X50m, fig.15) by destructive sampling. Larvae which had completed their development could easily be distinguished and had often caused considerable damage to O.aurantiaca plants colonized. The remaining larvae had suffered an early mortality and damage to plants was negligible. Each plant was attacked by only one insect.

	PLANT CATEGORY				
Development of larvae:	A	B	C	D	TOTAL
Completed	153	327	33	9	522
Uncompleted	22	61	6	0	89
Total	175 (29%)	388 (64%)	39 (6%)	9 (1%)	611 (100%)

To verify that insects did not only occur in the selected study area (40X50m), a survey was made in January 1983 to record the extent of M.pulchellalis colonizations in the total area of 3 hectare. Not all the clumps of cactus which occurred in the area were searched for M.pulchellalis attack. Results of the survey are shown in fig.19. Mimorista had dispersed to jointed cactus clumps distributed throughout the area. Both old and new larval attacks were recorded which indicated that the insect had dispersed to these areas at an earlier date. The density of the insect population and the damage to the cactus appeared to be comparable to the results obtained from the study site (40X50m).

9.3.2. Estimation of cactus density

Using the number of cladodes per quadrat was a convenient way to standardize cactus counts when the optimal sample size to estimate cactus density was to be determined. This variable (number of cladodes) was of little practical use when insect damage to the plant and infestation levels had to be described. Consequently, cactus density in the area was described in terms of the different plant categories originally recorded in the quadrats. Estimates of the total number of plants were determined from the optimal sample size (120 quadrats for sparse cactus and 80 quadrats for dense cactus) (fig.14). Results are shown in Table 6.

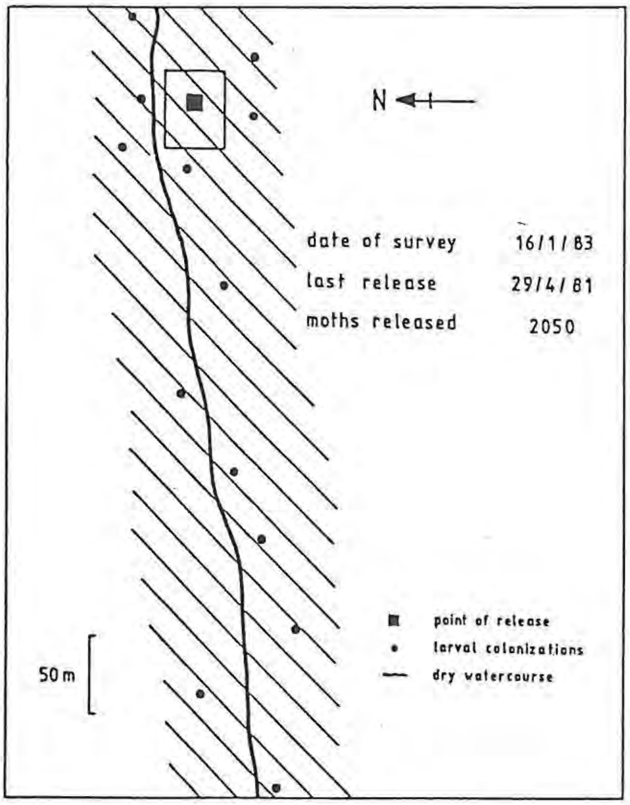


Fig.19. Sketchmap of the area (3 ha.) in which evaluation studies of M.pulchellalis were conducted. The enclosed area (40X50m) at the top of the figure represents the study site - River Site - in which the quadrats were located. Isolated clumps of O.aurantiaca occurred in the shade (hatching) along the watercourse. The marked points do not represent all the cactus clumps in the area (see text).

In the total area sampled (40X50m) the small plants (< 30cm tall) comprised 82% of the number of plants estimated (31% of Category A and 51% of Category B plants) (Table 6). The remaining jointed cactus infestations consisted predominantly of Category C plants (14%). Relatively few large (Category D) plants (3%) were recorded in the area. The large difference in the mean number of plants per plant category between the divisions (eg. 7 and 26.2 in Category B for sparse and dense cactus respectively) and the relatively small standard error associated with each mean, showed that the stratification method employed in estimating cactus density was justified. A correct stratification results in a substantial gain in the precision of the population density estimate (Stuart 1964; Snedecor & Cochran 1980).

Table 6. The estimated number of O. aurantiaca plants in each plant category for sparse and dense cactus. The estimated number for the total area is also shown. The mean number of plants per quadrat and the standard errors associated with each mean are based on 120 samples for sparse cactus and 80 samples for dense cactus.

		PLANT CATEGORY				
		A	B	C	D	TOTAL
SPARSE CACTUS	mean	5,2	7,0	1,7	0,4	
	S.E.	0,94	1,66	0,4	0,19	
(1 200m^2)	no.plants	6260	8390	1990	510	17150
DENSE CACTUS	mean	14,3	26,2	7,8	1,8	
	S.E.	2,62	5,38	2,16	0,68	
(800m^2)	no.plants	11400	20920	6260	1450	40030
TOTAL AREA (2 000m^2)	no.plants	17660 (31%)	29310 (51%)	8250 (14%)	1960 (3%)	57180 (100%)

Not all the estimates of the number of plants in the different plant categories were equally precise as can be seen when the means are plotted as a function of sample size and the standard errors calculated (fig.20). From fig.20 it appears that the sample sizes used in this study were too small to accurately estimate the density of cactus in the larger plant categories (C and D) in both sparse and dense cactus (fig. 20. e,g,f and h). This was probably due to the relatively low numbers of large plants recorded in the area (Table 6) and is related to the method of

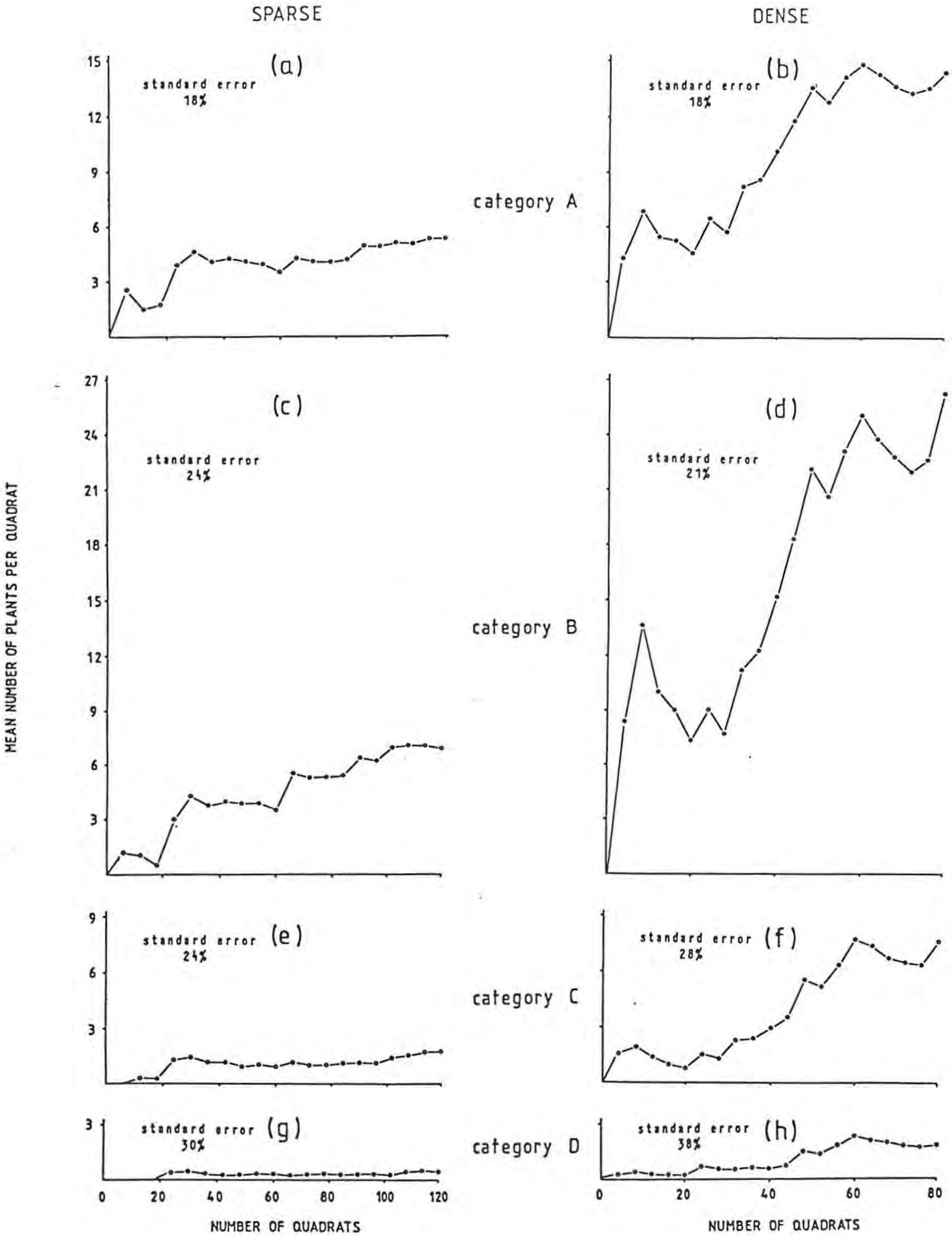


Fig.20. The density of <u>O.aurantiaca</u> (number of plants per quadrat) shown as a function of quadrat number.The standard errors of the mean for optimal sample sizes (120 quadrats for sparse and 80 quadrats for dense cactus) are shown for each plant category.

reproduction in jointed cactus. Large plants usually have a large number of cladodes which detach easily and generally fall in the vicinity of the parent plant, where they often take root and give rise to many small plants. The ratio of large plants to small plants would therefore tend to be small in jointed cactus populations.

The estimates of small plants (category A and B) (fig.20 a,c,b and d) calculated from the same sample sizes, were generally more precise than large category plants, and it may be assumed that the estimated number of plants of these sizes reflect the true population densities. Accurate population estimates of these small plant categories was considered far more important than large plant estimates as these plant categories (A and B) appeared to be predominantly attacked by Mimorista larvae (see Table 5).

The frequency distribution of the cactus counts of plants in the different plant categories recorded in the quadrats generally showed a good agreement with the frequency distribution predicted by the negative binomial distribution. This indicated a clumped spatial pattern of the cactus (Table 7). This was to be expected as it could clearly be seen that cactus in the field occurred in distinct clumps. Zimmermann (1981), showed that the negative binomial distribution provided the best fit to frequency counts obtained from quadrats used for sampling jointed cactus populations.

The model generally provided a good fit to the observed counts of the smaller plant categories (A and B)in both the sparse and dense cactus (Table 7). The larger plant categories did not provide such a good fit (see probability values in Table 7) with the observed frequency counts of both the category C and D plants (sparse cactus) differing significantly ($P < 0,05$) from the expected values predicted by the negative binomial distribution. This seems to indicate that large plants are generally less likely to have a clumped dispersion pattern than small plants.

Table 7. Calculated k-values for the negative binomial distribution fitted to the observed frequency distributions of the different plant categories. The chi square "goodness of fit" values are shown for each plant category. Figures in parenthesis are the degrees of freedom on which the chi square calculation is based. Calculated χ^2 values were rounded off to the probability values (P) indicated (* = significantly different at the 5% level).

	SPARSE			
	PLANT CATEGORY			
	A	B	C	D
value of k	0,207	0,160	0,130	0,077
χ^2	4,7(2)	0,032(2)	4,9(1)*	6,2(1)*
P	0,1	0,9	0,025	0,01
	DENSE			
	PLANT CATEGORY			
	A	B	C	D
value of k	0,268	0,192	0,118	0,033
χ^2	4,1(3)	4,0(5)	3,2(2)	3,5(1)
P	0,5	0,5	0,1	0,05

Large plants may have grown from isolated cladodes which were dispersed by animals or water, and such individual cladodes would tend to be dispersed in a random manner. In the dense cactus this tendency of random dispersion would be less obvious due to the larger number of plants present in the area (Table 7). Small plants on the other hand tend to congregate around the parent plant and hence show a clumped distribution pattern. Alternatively, sample size may have been too small to accurately estimate categories C and D (see standard error estimates in fig.20).

Counts based on a small sample size generally cannot be arranged in a frequency distribution and thus cannot provide a good fit to theoretical frequency distributions (Elliott 1977).

9.3.3 Damage to jointed cactus.

The total number of insects contributing to the destruction of jointed cactus over one season (generation 1-3) remained low, only 1,9% of the 57 180 plants in the area being attacked by M.pulchellalis larvae. Results are summarized in Table 8.

Table 8. Number of M.pulchellalis larvae colonizing O.aurantiaca plants over one growth cycle of the cactus. The estimated number of plants in the area (40X50m) was recorded in January 1982, after the growth phase was completed (Table 6). Insect numbers represent the estimated totals of generations 1,2 and 3 (from Table 3).

	PLANT CATEGORY				
Estimated no.	A	B	C	D	TOTAL
Cactus plants	17660	29310	8250	1960	57180
Insects	330	610	110	40	1090

Knowledge of the plant sizes attacked during this period was of interest because the damage to the cactus varied with plant size. Results from the total census of generation 4 were used to determine the plant sizes attacked. Estimating the number of plant sizes attacked from sample data (see Table 3) was unrealistic due to the low numbers and clumped distribution of larvae encountered in the samples.

The number of insects recorded from the different plant categories during the total census (Table 5) are shown as a proportion of the total plants attacked (611) in fig.21; the proportion in which the plant categories actually occurred in the area (from Table 6) are related to the attacked plants in the same figure.

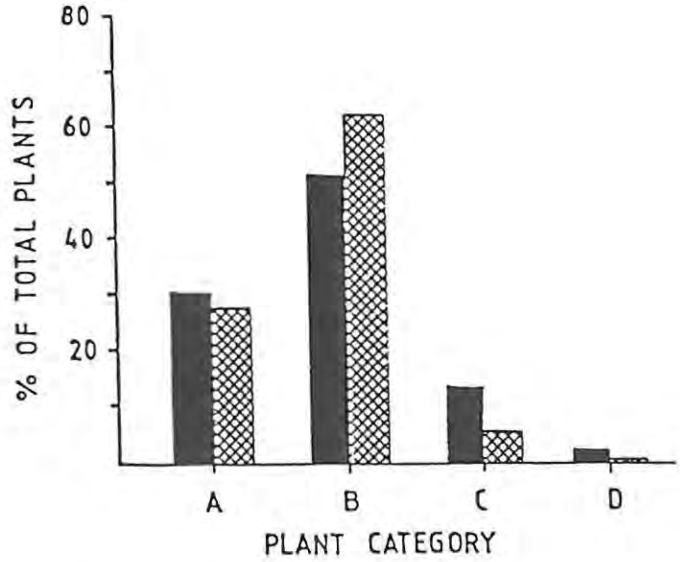


Fig.21. Plant sizes attacked by M.pulchellalis larvae, in relation to the occurrence of O.aurantiaca plants in the area sampled (40X50m). ▨ = proportion in which attacked plants were recorded in the total census of generation 4 (from Table 5). ■ = proportion of plants estimated in each plant category during sampling (from Table 6).

The observed pattern of attack of M.pulchellalis (fig.21) differed significantly from the expected pattern based on the occurrence of the plant categories in the area ($P < 0,01$; $\chi^2 = 50.66$ for 3df).

As the cladode is the basic unit of reproduction in jointed cactus, damage to plants was measured in terms of the number of cladodes totally and partially destroyed in each plant category. This gave a more realistic and easy to visualize measure of insect damage to the cactus. Jointed cactus fruits are also capable of vegetative growth but seldom occur on etiolated plants. Results recording the extent of Mimorista damage to the cactus were obtained from the total census of generation 4 and are shown in Table 9. Fig. 22 summarizes the damage of the insect in the different plant categories.

Table 9. The number of O.aurantiaca cladodes destroyed and partially destroyed by M.pulchellalis larvae in each plant category. The results were obtained from the total census of generation-4 insects. Only the larvae which had completed their development (see Table 5) are included in the analysis. The number of plants killed in each plant category is also shown.

	PLANT CATEGORY			
	A	B	C	D
Number of plants attacked	153	327	33	9
Number of cladodes in plants attacked	337	1021	159	73
Number of cladodes destroyed	205	285	14	1
Number of cladodes partially destroyed	71	303	32	10
Number of plants killed	50	12	0	0

Damage is most extensive in small plants (Category A) where a large proportion of the cladodes are destroyed; relatively few cladodes are partially destroyed (fig.22). Partially destroyed cladodes (see fig.13) are all capable of new growth. In the larger plants the number of destroyed cladodes becomes fewer while the number of partially destroyed cladodes increases (fig.22). Of greater significance is that 33% of the Category A and 4% of the Category B plants which were attacked were killed (Table 9). No plants were killed in category C and D plants, destruction being limited to individual cladodes only.

9.3.4 Recovery of M.pulchellalis larvae at other release localities.

Although quantitative evaluation of insect numbers on cactus in

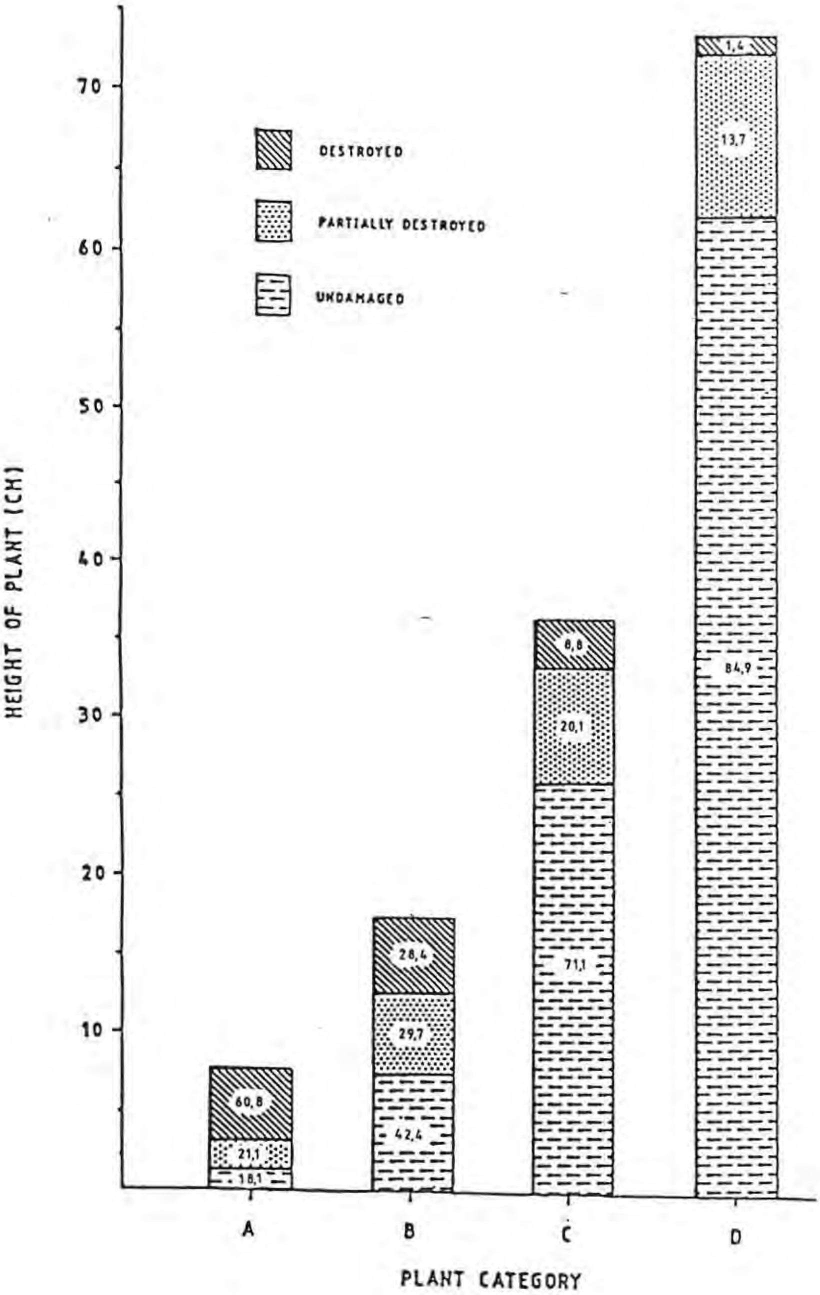


Fig. 22. The relative percentages of O.aurantiaca cladodes destroyed and partially destroyed by M.pulchellalis larvae in the different plant categories. The average height recorded for the plants attacked in each plant category is also shown. Results are summarized from Table 9.

exposed areas (Hill Site) had been abandoned due to the lack of larvae in the quadrats, a qualitative assessment made of the various release localities near Grahamstown in January 1983, showed that M.pulchellalis frequently occurred in the low-growing Karroid vegetation (Table 1). Insects in these areas usually colonized the small plants found in the centre of large cactus clumps or those that grew in the protection of large bushes. Such plants were invariably etiolated and could be compared to the small plants (Category A and B) recorded in the study area (40X50m) at River Site. Moths forced to oviposit on "typical" jointed cactus plants in exposed areas by being confined in cages, usually laid few eggs. The larvae that hatched from these eggs and successfully burrowed into the cactus often died during development. This mortality may have been caused by high temperatures which presumably prevailed in the cactus where it was exposed to the sun; air temperatures were not substantially different between exposed and shaded areas (fig.23).

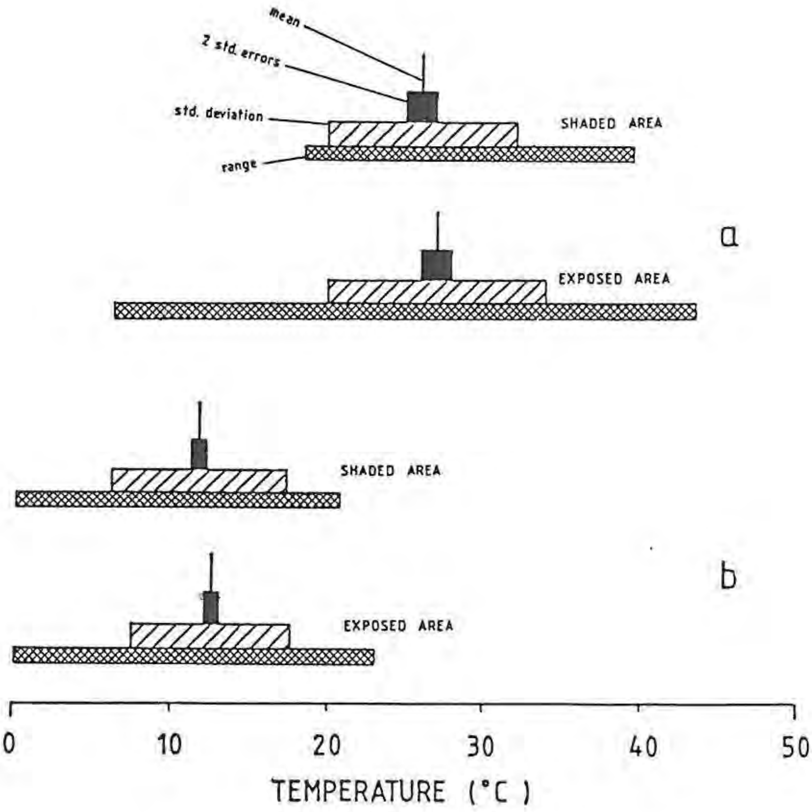


Fig. 23. Air temperatures recorded at two localities on the Andries Vosloo Kudu Reserve. (a) maximum temperatures (b) minimum temperatures (shaded area = temperatures recorded at River Site, where etiolated cactus occurred; exposed area = temperatures recorded at Hill Site, where "typical" cactus occurred).

Dispersal of the insects occurred at all the release sites. This was especially evident from releases made at sites 1-5 AVKR (Table 1), where the total cactus infestation was scattered over an area of approximately 5 hectares (fig.24). M.pulchellalis larvae could be detected in cactus clumps in both the shaded and exposed areas.

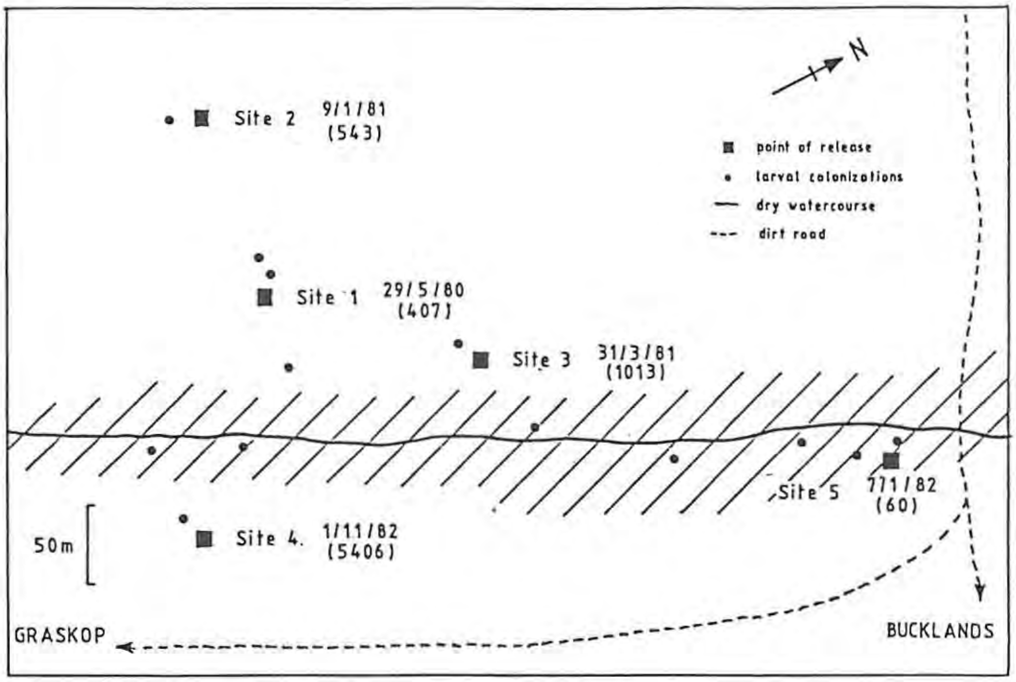


Fig. 24. Sketchmap indicating the relative positions of sites 1-5 on the Andries Vosloo Kudu Reserve (see Table 1). M.pulchellalis colonizations could be detected on O.aurantiaca plants dispersed throughout the area in a survey conducted in January 1983. The total area comprised approximately 5 ha. and had been set aside exclusively for biocontrol. Etiolated cactus occurred in the shade (hatching) along the watercourse. The date associated with each release site indicates the date of last release; the number in parenthesis represents the total number of insects released.

DISCUSSION

Establishment of M.pulchellalis in South Africa.

The M.pulchellalis population at River Site had gone through a minimum of four generations at the time the study was terminated in December 1982 (fig.16). The insects had survived two winters and one summer during which average weekly temperatures of 5 °C and 30 °C were often recorded (fig.17), and it must be assumed that M.pulchellalis has become established at this site. The aggregation of the larvae in each generation sampled over this period - indicated by the good fit of the negative binomial distribution (Table 4) - appears to have selective advantage and may directly contribute to the survival of the insect (see Waters 1959; Williams 1964 and Taylor 1971). A possible reason for the contagious dispersion pattern, and the effect this may have had on the establishment of M.pulchellalis in South Africa, is discussed below.

Although the negative binomial has successfully been fitted to insect counts to demonstrate contagion (Anscombe 1949; Evans 1953; Jenson 1959; Bliss 1971; Southwood 1978) and the estimated parameter k of the distribution may be used to indicate the degree of contagion, the ecological interpretation of k has certain limitations in its assumptions as a true population parameter and should not be used indescriminately as an index of aggregation (Taylor 1971; Taylor et al. 1979; Nachmann 1981). The value of k was found to be an inconsistent measure of aggregation as it showed a strong dependency on the mean (Taylor et al. 1979). Values of k frequently increase with the mean (Harcourt 1963) and may have the same value at two different densities (Taylor et al. 1979). Zimmermann (1981), found that values based on jointed cactus populations were generally unstable and did not relate to clumping. The high number of empty quadrats at low population densities is given as a possible reason for this inconsistency (Zimmermann 1981).

Consequently, the results of the sample estimates of generation 1 to 4 of the insect population, were fitted by the power law descibed in Taylor (1961). The power law was a consistent parameter of aggregation over a wide range of data tested (Taylor 1971; Taylor *et al*. 1978) in contrast to the parameter k of the negative binomial distribution which often gave ambiguous results (Taylor *et al*. 1978; Taylor *et al*. 1979; Nachmann 1981; Zimmermann 1981). According to Taylor (1961) the variance of a population is proportional to a fractional power of the arithmetic mean: $\sigma^2 = a\mu^b$ or $\log \sigma^2 = \log a + b\log \mu$. Both *a* and *b* are population parameters. Parameter *a* depends largely upon the size of the sampling unit while parameter *b* is an index of aggregation. The value of *b* can vary from 0 (for a regular distribution) to infinity (for a highly contagious distribution) (Taylor 1961; 1971).

Estimates of *a* and *b* from the sample data (generations 1-4) were 9.97 and 1.70 respectively. Sample size ($n = 4$) was too small for the regression coefficient ($r = 0.7$) to be significant however, and consequently the calculated values of *a* and *b* could not be used as population parameters. A large sample (of insect generations) was needed to obtain a significant fit of the regression line to the data in this study. In view of the consistent aggregation pattern displayed by the larvae over all four generations sampled (Table 4) and the stability of insect counts over this period, it must be assumed that aggregation in *Mimorista* indicates a fundamental trait, and may in part be due to the intrinsic behaviour of the species. That aggregation was not an artifact of sampling was substantiated by observations made during the total census; larvae occurred in small groups and the finding of one colonized plant usually yielded a number of colonizations in the immediate vicinity.

The distribution of the host plant is often the most important underlying cause of insect aggregation (Cole 1946; Chaing & Hodson 1959; Waters 1959; Southwood 1978), and appeared to account for the observed spatial dispersion in Mimorista. The pattern in which plants were colonized by the larvae seemed to indicate a preference for certain plant sizes, attacked plants occurring less frequently in categories C and D (fig.21). The relatively low number of larval colonizations on large plants may be due to an artifact of sampling ie. larger plants show fewer larval penetrations not because of a smaller proportion of eggs being laid on these plants, but because fewer larvae succeed in burrowing into the plant. Large plants generally have a thick cuticle (Hoffmann 1976) which impedes larval penetration of the plant. The high chi square value (50.66) which is obtained when the observed pattern of M.pulchellalis attack of jointed cactus plants is compared to the expected pattern in which the plants occurred in the area (fig.21), is partly due to this fact. Nevertheless, a z-test for proportions (Snedecor & Cochran 1980) conducted only on Category B plants gives a value of 5.807 (compared to 1.96 for the 5% level of significance), demonstrating that a signicantly larger number of small (Category B) plants is attacked.

Category B plants generally appeared to have a more clumped distribution than category C or D plants (Table 7). The ecological studies on O.aurantiaca conducted by Zimmermann (1981) also seem to indicate that a higher degree of aggregation occurred in isolated cladodes and small plants (consisting of less than 5 cladodes). Small plants are generally suitable for larval penetration and development.

Because the larvae may be regarded as sedentary, remaining in one plant throughout their development, the aggregative nature of the insect must be related to the oviposition behaviour of the adult female. During the oviposition period the female may be visually attracted to dense aggregations of plants but responds to the lower light intensity (see fig. 10) prevalent near the base of the parent plant where a dense stand of smaller, recently rooted plants occur. Numerous eggs are laid singly on individual plants before the female moves off. Laying eggs in this manner the adult female could also ensure that the eggs and larvae are hidden from predators and parasitoids.

Releases made of M.pulchellalis at other release localities (Table 1), also indicated establishment at low numbers. Unfortunately, definite establishment could not always be confirmed at all sites due to recent releases made in the areas concerned (see Table 1). Only the insects released at Doornkom by H.G.Zimmermann, may be regarded as having become permanently established (see fig.16). Moths released at site 4 in the Andries Vosloo Kudu Reserve on 1/11/82 may have accounted for the larvae recorded at sites 1,2,3 and 5 (AVKR) in the January 1983 survey. Because of the circumscribed area in which these release localities occurred (fig.24), the possibility of dispersal could not be excluded, and permanent establishment can therefore not be assumed.

In view of the survival of M.pulchellalis for at least four generations at two of the release localities (fig.16), and the ease with which the insect had dispersed and colonized areas where no direct releases had been made (see fig.19 and fig.24), it must be assumed that M.pulchellalis has become established in South Africa at all the localities indicated in Table 1.

Impact on jointed cactus populations

The behavioural development of ovipositing females has a direct bearing on the damage to jointed cactus and relates to M.pulchellalis as a biological control agent of jointed cactus in South Africa. In this study 50 (33%) of the 153 Category A plants and 12 (4%) of the 327 Category B plants that were attacked, were killed (Table 9). Assuming that the small plants (Category A) represent the seasonal increment of new plants, this destruction is of importance in preventing the increase and spread of jointed cactus. The estimated number of category A plants in the area was approximately 17 660 (Table 6) of which 50, (0.28%) were totally destroyed by generation 4 larvae. The classification of the different plant categories was largely artificial and the Category B plants killed - usually the 10-12cm tall plants - could consequently be regarded as constituting part of the increment in new plants and could therefore be included in Category A. This would increase the total number of small (Category A) plants killed by M.pulchellalis in the area (40X50m) to 62, which is equivalent to a mortality of 0,35%.

Not all detached cladodes take root and produce new growth in the first season (Serfontein 1961) which may be related to rainfall in the area (see fig.3). The number of newly rooted plants may therefore vary from season to season. In general however, one large growth phase may be expected (fig.3). If each generation of M. pulchellalis is assumed to attack the same proportion of small (Category A) plants and in approximately the same numbers (based on the results of the total census), then the total destruction to the new jointed cactus plants per season may be as high as 3 times the calculated figure for generation 4, or approximately 1%. A few insects did enter a fourth generation in the 1981/1982 summer season (fig.16) but it is assumed that the number of small plants killed by the larvae would not greatly alter the estimated mortality of 1%.

Damage to Category B plants that were not destroyed was restricted mainly to the terminal cladodes, a large number which were destroyed (fig.22). Terminal cladodes are the cladodes most likely to be detached and dispersed at the end of the summer season. Destruction of these cladodes thus also plays an important part in restricting the spread of cactus. At least three generations of M. pulchellalis occur each season (fig.16) and the number of terminal cladodes destroyed may therefore be three times as high as the estimated figure of 28.4% (Table 9). In categories C and D the damage to the plants is not severe, only a few small cladodes being destroyed (fig.22). Nevertheless colonizations of large plants may curb the plant's ability to grow and reproduce (Harris 1981).

Potential of M.pulchellalis as a biocontrol agent of jointed cactus in South Africa.

M.pulchellalis may fulfill an important role in the current control campaign against jointed cactus. Both chemical and biological control operating against O.aurantiaca today, contribute predominantly to the destruction of large plants and clumps of cactus, especially against the cactus occurring in the exposed Karroid vegetation. Small plants are generally not adequately controlled and thus constitute a potential source of new cactus infestations.

Small cactus plants in the exposed areas of the low-growing Karroid vegetation, frequently aggregate in the shade of large clumps of cactus or bushes which support jointed cactus growth. Such small plants are often overlooked by spraying teams or protected against direct chemical contact. Although small isolated cactus plants and cladodes may also occur in exposed areas, such plants generally suffer a high natural mortality because of desiccation (Zimmermann 1981). The small, protected plants may therefore be the major cause of cactus regrowth and increase in these areas. Under the shaded conditions of the Valley Bushveld vegetation (Acocks 1975), dense stands of small and large etiolated cactus plants frequently occur. Such areas are often inaccessible and chemical control is seldom applied, largely because of the difficulty in application of herbicides and because of the detrimental side-effects on the high density of non-target plants. Cochineal insects seldom occur in this habitat. Cactoblastis occurs sporadically but damage is minimal and usually restricted to large plants. Consequently the cactus infestations remain largely untouched and provide a constant reservoir from which cactus may be distributed. Conditions in these habitats are generally conducive to the rooting and survival of isolated cladodes and small plants, although leaf litter may prevent rooting of many detached cladodes, resulting in the eventual but as yet undetermined mortality of many such joints. Generally, large numbers of small plants survive, and may eventually give rise to dense aggregations of cactus.

Mimorista larvae predominantly attack small plants and, in areas where etiolated cactus infestations are prevalent, may kill approximately 1% of the yearly increment in new jointed cactus plants. Large plants may also be attacked in shaded areas and M.pulchellalis may thus contribute to the general stress conditions operating against such plants (Harris 1981). The small protected cactus plants in the low Karroid vegetation are also subjected to M.pulchellalis attack. Numerous colonizations on such plants at the areas where insects were released accounted for the survival of the larvae in exposed conditions (see Table 1). Colonized plants in exposed areas were often killed.

By attacking and killing the small plants, M.pulchellalis restricts the establishment of new jointed cactus plants which may ultimately result in an overall decrease in the density of cactus infestations. Furthermore, plants are destroyed before they reach the stage where they start shedding cladodes (see Zimmermann 1981) and M.pulchellalis may therefore contribute to preventing the distribution of jointed cactus to new localities.

The mortality figure (1%) of small plants calculated in the study, is not high. This is largely due to the low numbers of insects recorded in the study area (40X50m) and may be related to the initial dispersal of M.pulchellalis from the release point to cactus clumps distributed throughout the area of 3 hectare (fig.19). Emigration losses from the study site could be counterbalanced by the immigration of insects once the total area (3 ha) had become colonized by M.pulchellalis, and could result in the overall increase of larvae in the quadrats. Alternatively, the confinement of insects to laboratory conditions for 2,5 years - and a possible 30 generations at 29 °C - prior to release at River Site, may have resulted in the selection of a laboratory strain (Mackauer 1976) and accounted for the low number of insects in the field; a similar period of re-adjustment may be needed to increase the competetiveness of M.pulchellalis under field conditions. The highest number of larvae was recorded from the last sample taken (despite severe drought conditions and extremes in temperatures) which may illustrate that the possibility of an increase in insect numbers is not excluded in the future.

At this stage however, insect numbers remain low and damage to the cactus is not severe. Large plants which constitute the bulk of jointed cactus infestations, are only marginally affected by M.pulchellalis attack and need to be controlled. Increase in M.pulchellalis numbers in the future may contribute to the destruction of the above ground parts of these plants (as was evident from laboratory studies), but until the insect proves itself in the field, new species of insects should be imported for the biological control of jointed cactus.

SUMMARY

1. The biology of M.pulchellalis and the methods used in rearing the insect are decribed. Laboratory studies show that young cactus growth is necessary in order to maintain large numbers of insects in the cultures.

2. A total of 63 305 moths were released against both morphological types of jointed cactus in the eastern Cape. The insect appears to have become established in the majority of localities where liberations were made. Future releases should be made of the adult stages only, preferably after the insects have mated. Large numbers of moths are not needed to achieve establishment, provided releases are made early in the summer when jointed cactus has young growth and temperatures are more suitable.

3. M.pulchellalis appears adapted to survive on etiolated jointed cactus plants. Releases should therefore preferably be made against jointed cactus occurring in the shade of the Valley Bushveld vegetation. Insects will survive in areas where low-growing Karroid vegetation predominates however, provided sheltered, etiolated plants are available.

4. Insects occur in low numbers in the field and generally go through 3 generations in a year. Damage is restricted mainly to the small jointed cactus plants of which 1% may be killed in one growth season of the cactus. Large plants are less frequently attacked and damage to these plants is minimal.

5. By predominately attacking and often killing the small jointed cactus plants M.pulchellalis may play an important part in the current control program against jointed cactus in South Africa. Small plants are generally not adequately controlled and provide a constant source of cactus growth from which new infestations may arise.

6. At the present stage, jointed cactus infestations consisting of large plants are not adequately controlled by M.pulchellalis and imports of new natural enemies against jointed cactus should therefore continue.

APPENDIX 1

Calculation of $\hat{k}$ and the expected frequencies in a negative binomial distribution (based on the methods shown in Elliott 1977).

The parameters μ and k of the insect population are estimated from the frequency distribution of the sample by the statistics $\bar{x}$ and $\hat{k}$. The results of sampling generation 3 (see Table 3) are used to estimate the parameters in this example. The frequency distribution of the insect counts of generation 3, collected from the random sample of 120 quadrats (fig.15), is given below.

	No. individuals per quadrat(x)	Frequency of occurrence(f)	Total no. counts exceeding x (A(x))
	0	106	14
	1	6	8
	2	4	4
	3	1	3
	4	2	1
	5	0	1
	6	0	1
	7	1	0
Total	32	120	

where x is a particular count, f is the frequency distribution of that particular count, and A(x) is the total number of counts exceeding x.

Arithmetic mean of sample: $$\bar{x} = \frac{\Sigma fx}{n}$$

$$= \frac{32}{120}$$

$$\underline{\bar{x} = 0{,}27}$$

Variance of sample: $$s^2 = \frac{\Sigma(fx^2) - \bar{x}(\Sigma fx)}{n - 1}$$

$$= \frac{94 - 0.27(32)}{119}$$

$$= \frac{94 - 8.64}{119}$$

$$\underline{s^2 = 0.717}$$

Using these two values calculated from the observed sample counts, a rough estimate of $\hat{k}$ may be calculated (see below). There are several methods of calculating $\hat{k}$ (Anscombe 1949, 1950; Bliss & Fisher 1953), but most of these methods are approximate and should only be used as the first step towards the more accurate method of maximum likelihood (Elliott 1977). A very simple but approximate method is derived from the equation for the variance of the negative binomial (see Elliott 1977):

$$\sigma^2 = \mu + \frac{\mu^2}{k}$$

and therefore $$k = \frac{\mu^2}{\sigma^2 - \mu}$$

The parameters μ and σ^2 are estimated by the statistics $\bar{x}$ and s^2 (from the sample) in the usual way and therefore:

$$\hat{k} = \frac{\bar{x}^2}{s^2 - \bar{x}}$$

This method provides a rough estimate of $\hat{k}$ for substitution in the maximum likelihood equation:

$$n\log_e \left(1+\frac{x}{\hat{k}}\right) = \sum \frac{(A(x))}{\hat{k} + x} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 1$$

where n is the total number of sampling units, $\log_e$ designates a natural (Napierian) logarithm, and A(x) is the total number of counts exceeding x. Different values of $\hat{k}$ are tried until the equation is balanced (Elliott

1977).

A rough estimate of $\hat{k}$ for this sample is therefore:

$$\hat{k} = \frac{\bar{x}^2}{s^2 - \bar{x}}$$

$$= \frac{(0.27)^2}{0.717 - 0.27}$$

$$\underline{\hat{k} = 0.163}$$

As $\hat{k}$ probably lies close to 0.163, values of $\hat{k} = 0.160$ and $\hat{k} = 0.166$ are solved for in the maximum likelihood equation; an accurate estimate of $\hat{k}$ is found by proportion (Elliott 1977) and is shown later.

Solve for $\hat{k} = 0.160$ in each side of the likelihood equation (1)

Left side $$n\log_e\left(1 + \frac{\bar{x}}{\hat{k}}\right) = n\log\left(1 + \frac{\bar{x}}{\hat{k}}\right) \log_e 10$$

$$= 120\log\left(1 + \frac{0.27}{0.160}\right) \times 2.303$$

$$= 276.31 \log(2.688)$$

$$= 118.63$$

The value for the left side of the equation is thus $\underline{118.63}$

Right side $$\sum \frac{(A(x))}{\hat{k} + x} = \frac{A(x=0)}{\hat{k}} + \frac{A(x=1)}{\hat{k} + 1} + \frac{A(x=2)}{\hat{k} + 2} + \ldots \frac{A(x=7)}{\hat{k} + 7}$$

$$= \frac{14}{0.160} + \frac{8}{1.160} + \frac{4}{2.160} + \ldots + \frac{1}{6.160}$$

$$= 87.5 + 6.89 + 1.85 + \ldots 0.16$$

$$= 97.78$$

The value for the right side of the equation is thus $\underline{97.78}$

Differences between the left side and the right side of the likelihood equation (1) will be zero if the two sides balance. Difference between the values of the two sides (as calculated above):

$$= 118.63 - 97.78$$

$$= \underline{20.85}$$

Next try $\hat{k}$ for the other value (0.166):

Difference between the two sides (calculation not shown):

$$= \underline{21.29}$$

The difference is bigger than the previously calculated figure (20.85) for $\hat{k} = 0.16$. $\hat{k}$ must therefore be smaller than 0.16, and a value of $\hat{k} = 0.09$ is next tried. Once again, solve for $\hat{k}$ in both sides of the maximum likelihood equation (1):

$$n\log_e \left(1 + \frac{\bar{x}}{\hat{k}}\right) = n\log \left(1 + \frac{\bar{x}}{\hat{k}}\right) \log_e 10$$

$$= 120 \log \left(1 + \frac{0.27}{0.09}\right) \times 2.303$$

$$= 276.31 \times \log 4$$

$$\text{Value for left side} = \underline{166.36}$$

$$\sum \frac{(A(x))}{\hat{k} + x} = \frac{A(x=0)}{\hat{k}} + \frac{A(x=1)}{\hat{k} + 1} + \ldots + \frac{A(x=7)}{\hat{k} + 7}$$

$$= \frac{14}{0.09} + \frac{8}{1.09} + \frac{4}{2.09} + \ldots + \frac{1}{6.09}$$

$$= 155.56 + 7.34 + \ldots + 0.16$$

$$\text{Value for right side} = \underline{166.38}$$

The difference between the two sides of the maximum likelihood equation for $\hat{k} = 0.09$ is therefore:

$$166.36 - 166.38 = \underline{-\ 0.02}$$

This value is smaller than zero and the value of $\hat{k}$ thus lies between $\hat{k} = 0.16$ and $\hat{k} = 0.09$. The true value of $\hat{k}$ is found by proportion (Elliott 1977):

$\hat{k}$	difference
0.09	- 0.02
0.16	20.85

Therefore:

$$\frac{\hat{k} - 0.09}{0.16 - 0.09} = \frac{0.02}{20.85 + 0.02}$$

$$\hat{k} = \frac{0.02 \times 0.0144}{20.85} + 0.09$$

$$= 0.00001365 + 0.09$$

$$\underline{\hat{k} = 0.09}$$

The value of $\hat{k} = 0{,}09$ and $\bar{x} = 0.27$ are used in the calculation of the expected probabilities and frequencies of the negative binomial distribution.

Individual terms of the negative binomial are derived from $(q - p)^{-k}$ (Elliott 1977) and are given by:

$$P(x) = \left(1 + \frac{\mu}{k}\right)^{-k} \frac{(k + x - 1)!}{x!\ (k - 1)!} \left(\frac{\mu}{\mu + k}\right)^{x}$$

The probability of zero (0) M.pulchellalis larvae occurring in any of the 120 quadrats sampled is:

$$P(x=0) = \left(1 + \frac{\bar{x}}{\hat{k}}\right)^{-\hat{k}} \frac{(\hat{k} + 0 - 1)!}{0!\ (\hat{k} - 1)!} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)^{0}$$

$$= \left(1 + \frac{\bar{x}}{\hat{k}} \right)^{-\hat{k}}$$

Therefore $$\log P(x=0) = -\hat{k} \log \left(1 + \frac{\bar{x}}{\hat{k}}\right)$$

$$= - 0.09 \log\left(1 + \frac{0.27}{0.09}\right)$$

$$= - 0.09 \log 4$$

$$= - 0.0541$$

$$= 1.9458$$

$$\underline{P(x=0) = 0.8827}$$

The probability of the sample (n=120) containing no Mimorista larvae is thus 0.8827. The expected (exp) frequency of the 120 quadrats in the sample containing zero larvae is therefore 0.8827 X 120

$$\underline{\exp\ (0) = 105.9}$$

The probability of one larva per sampling unit (= quadrat) is:

$$P(x=1) = \left(1 + \frac{\bar{x}}{\hat{k}} \right)^{-\hat{k}} \frac{(\hat{k} + 1 - 1)!}{1!\ (\hat{k} - 1)!} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)^{1}$$

$$= P(x=0) \frac{\hat{k}!}{1!\ (\hat{k} - 1)!} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)$$

$$= P(x=0) \quad \hat{k} \, \frac{(\hat{k} - 1)!}{(\hat{k} - 1)!} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)$$

$$= 0.8827 \quad X \quad 0.09 \quad X \quad 0.75$$

$$\underline{P(x=1) = 0.060}$$

The expected frequency of <u>one</u> larvae occurring in the 120 quadrats is therefore:

$$0.060 \ X \ 120$$

$$\underline{\exp(1) = 7.2}$$

The probability of <u>two</u> larvae per sampling unit is:

$$P(x=2) = \left(1 + \frac{\bar{x}}{\hat{k}}\right)^{-\hat{k}} \frac{(\hat{k} + 2 - 1)!}{2! \, (\hat{k} - 1)!} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)^2$$

$$= \frac{x}{x + \hat{k}} \left(1 + \frac{\bar{x}}{\hat{k}}\right)^{-\hat{k}} \frac{(\hat{k} + 2 - 1)(\hat{k} + 2 - 2)}{2} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)$$

$$= P(x=1) \quad \frac{0.09 + 1}{2} \left(\frac{0.27}{0.27 + 0.09}\right)$$

$$= 0.060 \quad X \quad 0.545 \quad X \quad 0.75$$

$$\underline{P(x=2) = 0.025}$$

The expected frequency of <u>two</u> larvae occurring in 120 quadrats is thus:

$$0.025 \ X \ 120$$

$$\underline{\exp(2) = 3.0}$$

The probability of <u>three</u> larvae per quadrat is:

$$P(x=3) = \left(1 + \frac{\bar{x}}{\hat{k}}\right)^{-\hat{k}} \frac{(\hat{k} + 3 - 1)!}{3!\ (\hat{k} - 1)!} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)^{3}$$

$$= \left(1 + \frac{\bar{x}}{\hat{k}}\right)^{-\hat{k}} \frac{(\hat{k} + 3 - 1)(\hat{k} + 3 - 2)(\hat{k} + 3 - 3)(\hat{k} - 1)!}{3 \times 2!\ (\hat{k} - 1)!} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)^{3}$$

$$= \left(1 + \frac{\bar{x}}{\hat{k}}\right)^{-\hat{k}} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)^{2} \frac{\hat{k}(\hat{k} + 2 - 1)}{2} \frac{(\hat{k} + 3 - 1)}{3} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)$$

$$= P(x=2) \frac{(\hat{k} + 3 - 1)}{3} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)$$

$$= 0.025 \frac{(0.09 + 3 - 1)}{3} \cdot \left(\frac{0.27}{0.27 + 0.09}\right)$$

$$= 0.01306$$

<u>$P(x=3) = 0.013$</u> <u>$\exp(3) = 1.6$</u>

The probability of <u>four</u> larvae per quadrat is:

$$P(x=4) = P(x=3) \frac{(\hat{k} + 4 - 1)}{4} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)$$

$$= 0.013 \frac{(0.09 + 3)}{4} (0.75)$$

<u>$P(x=4) = 0.0075$</u> <u>$\exp(4) = 0.9$</u>

The probability of <u>five</u> larvae per quadrat is:

$$P(x=5) = P(x=4) \frac{(\hat{k} + 5 - 1)}{5} \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)$$

$$= 0.0075 \quad \frac{(0.09 + 4)}{5} \quad (0.75)$$

$P(x=5) = 0.0046$ exp (5) = 0.6

The probability of six larvae per quadrat is:

$$P(x=6) = P(x=5) \quad \frac{(\hat{k} + 6 - 1)}{6} \quad \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)$$

$$= 0.0046 \quad \frac{(5.09)}{6} \quad (0.75)$$

$P(x=6) = 0.0029$ exp (6) = 0.3

The probability of seven larvae per quadrat is:

$$P(x=7) = P(x=6) \quad \frac{(\hat{k} + 7 - 1)}{7} \quad \left(\frac{\bar{x}}{\bar{x} + \hat{k}}\right)$$

$$= 0.0029 \quad \frac{(6.09)}{7} \quad (0.75)$$

$P(x=7) = 0.0019$ exp (7) = 0.2

Results of the calculations are summarized below:

No.larvae per quadrat	Probability	Expected frequency	Observed frequency
0	0.8827	105.9	106
1	0.060	7.2	6
2	0.025	3.0	4
3	0.013	1.6	1
4	0.0075	0.9	2
5	0.0046	0.6	0
6	0.0029	0.3	0
7	0.0019	0.2	1

The observed frequency distributions of the sample counts were compared with the expected frequency distribution of the negative binomial model in a chi square "goodness-of-fit" test (Elliott 1977). It is generally recommended that frequencies be combined so that the expected values are less than 5 (see Elliott 1977), but Snedecor & Cochran (1980) consider that this restriction reduces the sensitivity of the chi square test and suggest that no expected values should be less than 1.

In fitting the negative binomial model to the observed counts of the insects sampled in each generation (and to the plant categories in the cactus counts), the recommendations of Snedecor & Cochran (1980) were followed as closely as possible. Results are shown in Table 4 (insects) and Table 7 (cactus).

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