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RESUME

Large numbers of green lacewings have been observed on cotton in South Africa. These insects are important predators of aphids and other soft-bodied insects, mites, and their eggs. It is therefore likely that they could potentially be of considerable value in suppressing some of the major pests on cotton. Since very little work has been done in South Africa on these predators, the studies reported here were undertaken.

<u>Chrysopa</u> <u>zastrowi</u> (Esb. - Pet.) was arbitrarily chosen and is considered to be representative of the genus, and was the main species studied.

Three methods of rearing these insects in the laboratory are described. In two methods each larva was confined to its own separate enclosure to minimise cannibalism, while in the third method large numbers of larvae were confined in a common enclosure. The larvae were fed mainly on potato tuber moth eggs but aphids, mealybugs and eggs of the Angoumois grain moth and of the housefly were also used.

Since the adults are not predacious, they could be confined in the same cage for oviposition. They were fed on a diet of brewer's yeast, honey and water.

At 25[°]C and 55% RH the egg hatches in four days; the larva passes through three instars in approximately ten to eleven days and requires on average about 488 aphids

(v)

or 906 potato tuber moth eggs to mature. The mature larva spins a silken cocoon in which the third moult takes place after about four days. About ten days after spinning, the pupa leaves the cocoon by pushing off a lid. The pupal moult is accomplished outside the cocoon a short while after emergence. The adults mate and oviposit during the night only and lived in the laboratory for an average of 33 to 40 days (males and females respectively). Females showed a tendency to lay more eggs on a brown surface than on either yellow, white or green. Nipagin M (a fermentation suppressant) severely inhibited fecundity when added to the adult diet.

The seasonal occurrence of adults of <u>Chrysopa</u> spp. was studied at Roodeplaat, near Pretoria, by two methods. Three main population peaks occurred during October, December and March, the highest being in December. These peaks coincided with dry, sunny weather, suggesting an increase in activity due to the adults' search for moisture.

There are probably three generations per year, although it is possible that they breed throughout the year since embryological development can proceed slowly at 15°C.

The geographical distribution of various <u>Chrysopa</u> spp.was studied mainly in the Transvaal and South-western Cape. It is evident that certain species are probably very widespread.

Toxicological studies indicated a fair tolerance of the egg stage to seven insecticides. Only monocrotophos and endosulfan could be tested on adults. Monocrotophos was

(vi)

found to be ten times more toxic to the adults than endosulfan. However, at the recommended field dosages on cotton neither of these two insecticides should cause undue mortality.

SAMEVATTING

Groot getalle groen goudogies is op katoen in Suid-Afrika waargeneem. Hulle is belangrike roofinsekte van plantluise en ander saghuidige insekte, myte, en hulle eiers. Dit is dus waarskynlik dat hulle van aansienlike waarde in die onderdrukking van party van die belangrikste katoenplae mag wees. Aangesien min werk tot dusver op hierdie roofinsekte in Suid-Afrika gedoen is, is die studies, waaroor hier verslag gedoen word, onderneem.

<u>Chrysopa</u> <u>zastrowi</u> (Esb. – Pet.) is na willekeur gekies as verteenwoordigend van die genus, en was hoofsaaklik die spesie wat bestudeer is.

Drie metodes vir die teel van hierdie insek in die laboratorium word beskryf. In twee metodes is elke larwe afsonderlik in h hok gehou om kannibalisme te voorkom, terwyl in die derde metode groot getalle larwes in h gemeenskaplike hok gehou is. Die larwes is hoofsaaklik op aartappelmoteiers gevoer, maar plantluise, witluise en eiers van die Angoumois-graanmot en van die huisvlieg is ook gebruik.

Aangesien die volwassenes nie roofsugtig is nie, kon hulle in dieselfdehok vir eierlegging gehou word. Hulle is op 'n mengsel van brouersgis, heuning en water gevoer.

By 25°C en 55% RH broei die eier na vier dae uit. Die larwe gaan deur drie stadia in ongeveer tien tot elf dae en vereis gemiddeld omtrent 488 plantluise of 906 aartappelmoteiers om volgroeid te raak. 'n Syerige kogkon

word deur die volgroeide larwe gespin waarin die derde vervelling na vier dae plaasvind. Omtrent tien dae nadat die koøkon gespin is verlaat die papie die koøkon deur 'n deksel oop te druk. Die papievervelling geskied buite die koøkon, kort na uitkoms.

Paring en eierlegging vind slegs snags plaas, en die volwassenes het in die laboratorium in die algemeen van 33 tot 40 dae geleef (mannetjies en wyfies onderskeidelik). Wyfies was geneig om meer eiers op 'n bruin oppervlak te lê, as op óf geel, óf wit, óf groen. Nipagin M ('n gistingonderdrukker) het vrugbaarheid kwaai onderdruk nadat dit by die dieët gevoeg is.

Die seisoenvoorkoms van volwassenes van <u>Chrysopa</u> spp. is op Roodeplaat, naby Pretoria, volgens twee metodes bestudeer. Die drie vernaamste bevolkingspieke het in Oktober-, Desember- en Maart-maand voorgekom, met die hoogste in Desembermaand. Die pieke het tydens droë sonnige weer voorgekom wat 'n aanduiding is dat dit die voorliefde vir vogtigheid is wat tot toename in aktiwiteit lei.

Daar is waarskynlik drie geslagte per jaar, alhoewel dit moontlik is dat hulle dwarsdeur die jaar aanteel aangesien embriologiese ontwikkeling stadig by $15^{\circ}C$ kan voortgaan.

Die geografiese verspreiding van verskeie <u>Chrysopa</u> spp. is hoofsaaklik in die Transvaal en Suidweskaapland bestudeer. Dit is duidelik dat sekere spesies waarskynlik baie wyd verspreid is. Deur toksikologiese ondersoeke is gevind dat die eierstadium redelik weerstand teen sewe insektemiddels bied. Slegs monocrotophos en endosulfan kon op die volwassenes getoets word. Monocrotophos was tien keer meer giftig as endosulfan vir die volwassenes. Nietemin, sou geeneen van die twee middels teen die aanbeveelde dosisse op katoen egter oormatige sterftes tot gevolg hê nie.

CHAPTER 1

INTRODUCTION

1.1 MOTIVATION

With the realization that the indiscriminate use of insecticides is having an undesirable effect on our environment has come the recognition that man should now make better use of existing pest control agents proffered by nature, viz. natural enemies (parasites and predators) and diseases. These agencies occur naturally in the undisturbed environment, and form an integral part of the ecological system. In the past pesticides have been used with almost complete disregard for these beneficial agents, resulting, in many cases, in man-made pests due to unintentional destruction of these natural control agencies.

More than 20 years ago it was first realized by people such as Doutt (1948), Ullyett (1948) and Ripper, Greenslade & Hartley (1951) that the combination of chemical or physical, and biological control (integrated control) showed great possibilities in the field of pest control. It became clear that careful manipulation could turn two antagonists (chemicals and natural control agencies) into allies with a common cause --- the destruction of pests.

<u>Chrysopa</u>, or green lacewings as they are commonly called, fall into the class of predators, as the larval stage (and in a few species the adult stage) is predacious on aphids, mites and other soft-bodied insects and insect

eggs. Large populations of <u>Chrysopa</u> spp. have been observed in South Africa on cotton amongst other crops, and it therefore seems likely that these insects could potentially be of considerable value as a natural enemy of, for example, the major cotton pests, which include American bollworm, red bollworm, and aphids and red spider mite populations. This potential could however be severely hampered by current spray programmes, as it has been demonstrated that <u>Chrysopa</u> spp. are susceptible to many insecticides (Doutt, 1948; Campbell & Hutchins, 1952; Van den Bosch, Reynolds & Dietrick, 1956; Bartlett, 1964; Herne & Putman, 1966; Dinkins, Brazzel & Wilson, 1971).

Research workers have also long been aware of the possibility of large-scale releases of <u>Chrysopa</u> into the field with the intention of supplementing the existing native populations, in an attempt to control pest populations (Finney, 1948 & 1950; Ridgway & Jones, 1968). Projects such as these require that large colonies of <u>Chrysopa</u> be maintained in the laboratory, and this in turn necessitates the development of efficient and economic mass-rearing methods. Finney (1948 & 1950) pioneered research of this nature, and today, after improvements in technique and apparatus, Ridgway, Morrison & Badgley (1970) report that it is possible to produce up to 750 000 eggs per day.

As one of the limitations in the mass-rearing of <u>Chrysopa</u> is the provision of sufficient quantities of larval food, workers in America have investigated the feasibility of producing an artificial diet, which must be liquid by

virtue of the larva's suctorial mouthparts. Diets of this nature have been formulated by Hagen & Tassan (1965) and Vanderzant (1969), but according to S.W. Broodryk, 1970, Gatooma Experiment Station, Rhodesia (personal communication) the most promising diet appears to be one which is encapsulated in a thin but solid wax sphere (Ridgway, 1971, unpublished data).

Much has been written on the biology and habits of Chrysopidae. One of the earlier studies was made by Smith (1922), and others who have contributed towards this field include Balduf (1939), Toschi (1965) and Tjeder (1966).

While a lot of work has been done in overseas countries, (especially America) on many aspects relating to Chrysopa, very little has been carried out on our South African Chrysopa spp. Apart from a short study on the biology and habits of two South African Chrysopa spp. (Whitehead, 1957), it appears that no other bio-ecological studies on this genus have been attempted. If we are ever to implement these predators in integrated control programmes in the future, it is essential that we familiarize ourselves with the biology and ecology of our local Chrysopa spp. Only then, armed with better knowledge on these insects, is it possible to speculate as to how they will behave under our prevailing conditions. The species under study, Chrysopa zastrowi (Esb. - Pet.), was chosen arbitrarily, and although not as widespread as some other species, is considered by the writer to be fairly representative of the genus.

If the study presented here, although far from a complete

one, contributes towards a better understanding of these predators, and perhaps hastens the day that <u>Chrysopa</u> are incorporated into integrated control programmes, then it will have been fully justified.

1.2 TAXONOMIC POSITION OF <u>CHRYSOPA</u> <u>ZASTROWI</u> (ESB. - PET.)

The taxonomic position of <u>C</u>. <u>zastrowi</u>, based on Tjeder (1957 & 1966) is as follows:

Order:	Neuroptera
Suborder:	Planipennia
Family:	Chrysopidae (Schneider)
Subfamily:	Chrysopinae (Esb. – Pet.)
Genus:	<u>Chrysopa</u> (Leach)
Subgenus:	<u>Chrysoperla</u> (Steinm.)
Species:	<u>zastrowi</u> (Esb. – Pet.)

Tjeder (1966), in his paper on Neuroptera-Planipennia, states, "The green lace-wings were first treated as a special taxon by Schneider, 1851, under the name of Chrysopina, based on the genus <u>Chrysopa</u> Leach, 1815. In 1853 Newman changed the name into Chrysopidae, the now world-wide used family name for these well-known insects." Tjeder lists six subgenera of <u>Chrysopa</u> (<u>Ceratochrysa</u>, <u>Brinckochrysa</u>, <u>Glenochrysa</u>, <u>Saurius</u>, <u>Chrysoperla</u>, <u>Anisochrysa</u> and <u>Apertochrysa</u>), comprising a total of 33 species, which are found in South Africa (including Lesotho) and Rhodesia, in addition to a further four species which, due to the unavailability of males, cannot be referred to subgenera. The subgenus <u>Chrysoperla</u>, in which the species <u>zastrowi</u> is placed, includes four other species, <u>C</u>. <u>pudica</u> (Nav.), <u>C</u>. <u>conqrua</u> (Walk.), <u>C</u>. <u>plicata</u> (Tjed.) and <u>C</u>. <u>comans</u> (Tjed.). With the exception of the last two, which occur only in Rhodesia, they are all fairly widespread in South Africa. Tjeder also points out that <u>C</u>. <u>congrua</u> and <u>C</u>. <u>pudica</u> are " very similar and closely allied" to <u>C</u>. <u>zastrowi</u>, all three occurring in the same areas. <u>Chrysopa zastrowi</u> can however be easily separated from the other two by the short antennae and the shape of the tarsal claws, which are different in the three species.

<u>Chrysopa zastrowi</u> was first described in 1928 by Esben-Petersen, although it was known earlier as <u>C</u>. <u>vulqaris</u> (Esb. - Pet.) and <u>C</u>. <u>bequaerti</u> (Nav.). The type-specimen was collected from Grootfontein, South West Africa, and lodged in the Staatsinstitut und Museum, Hamburg. Both the Museum and the type were destroyed by fire in 1943. Tjeder therefore described a neotype, collected from Orupembe, S.W.A., close to the location of the typespecimen, which is presented in the museum of the Entomological Institute of Lund University, Sweden. A full description of Tjeder's neotype may be found in his 1966 paper.

Whereas Tjeder divides Neuroptera into three suborders (Megaloptera, Raphidioidea and Planipennia), Imms (1964) includes Raphidioidea in Megaloptera, thereby giving only two suborders. Imms further divides Planipennia up into four superfamilies --- Ithonoidea, Coniopterygoidea, Hemerobioidea and Myrmeleontoidea, of which Hemerobioidea is the best represented, comprising a total of eight families (including Chrysopidae). Imms also mentions that there are about 800 known <u>Chrysopa</u> species.

The classification down to families given by Brues, Melander and Carpenter(1954) differs slightly from that given by Imms and Tjeder, which are basically the same. Brues <u>et al</u> divide Neuroptera into the suborders Sialodea, Raphidiodea (note spelling) and Planipennia, and furthermore include an additional seven families in the superfamily Hemerobioidea. The taxonomic position of the Chrysopidae however remains unchanged from that given earlier.

CHAPTER 2

MASS - REARING

It is essential that if an insect is to be studied in the laboratory, the colony should be large enough to allow individuals to be withdrawn for experimental work, while leaving sufficient stock for regeneration purposes. <u>Chrysopa</u> spp. are amongst the more difficult species of insects to rear, not because of any external factors but rather due to an intrinsic property of <u>Chrysopa</u> larvae ---they are cannibalistic.

This cannibalistic property means that if <u>Chrysopa</u> larvae are to be efficiently reared in the laboratory, they should be kept apart in separate cages, a method which greatly complicates the rearing procedure. Furthermore, the nature of the larva's mouthparts --- two hollow sickle-shaped "fangs" formed by the apposition of the mandibles and maxillae --- necessitates a liquid diet. This in turn means that further problems are encountered in the provision of an artificial diet, as the liquid has to be contained in a material which can be easily penetrated by the larva's mouthparts.

There appear to be two main techniques in presenting liquid artificial diet to <u>Chrysopa</u> larvae. One method requires that the liquid be absorbed into small cubes of spongy material, the larva feeding by simply piercing the sponge and sucking the liquid up in the normal way (Vanderzant, 1969). In the other more sophisticated method, the liquid is encapsulated by paraffin into self-contained droplets (Hagen & Tassan 1965), or the drops of diet are surrounded by a solid wax coat which is thin enough to be penetrated by the larva's mouthparts (S.W. Broodryk, Gatooma Experiment Station, Rhodesia, 1971, personal communication; according to Ridgway, 1971, unpublished data). The last method, although more intricate in the preparation of the wax spheres, is more practical, as a stock of food "balls" can be made and stored at a suitable temperature until used. The matter of artificial diets will be further discussed in a later chapter.

The only alternative to an artificial larval diet is to provide the larva with a natural diet, although the former is for many reasons preferable. In nature, Chrysopa larvae consume virtually any soft-bodied insect or insect Tjeder (1966) lists the following egg (mites included). as being acceptable to <u>Chrysopa</u> larvae: small hemipterons including aphids, and coccids and their eggs (such as citrus mealybug, scales and the Australian bug); sawfly eggs; eggs of various moths; syrphid larvae; larvae and pupae of a coniopterygid; a thysanopteron; small coleopterous larvae; and mites. Unless a supply of the natural diet can be procurred regularly from the field, one or more of the acceptable host species has to be reared in the laboratory if a reasonable-sized colony is to be maintained. It thus becomes necessary to rear one insect to feed another, a procedure with obvious disadvantages. As the manufacture of an acceptable artificial diet was at the time impractical, the natural diet method was chosen for the purpose of this study.

The choice of insect species to be used as host material requires careful thought. In order that the larvae do not expend undue energy pursuing or overcoming their prey, it is preferable to choose a host which is fairly immobile, and one which will not offer much resistance. For this reason, insect eggs are the best type of host material, provided that the chorion can be easily penetrated by the mouthparts of even a 1st instar larva, and provided also that there are no other complicating features such as a layer of hairs covering the eggs, as found on eggs of some Lepidoptera spp.

There are two insect eggs which satisfymost criteria for mass-rearing Chrysopa. Eggs of the potato tuber moth, Phthorimaea operculella (Zeller), were used by Finney (1948 & 1950), who also immobilized last-instar potato tuber moth larvae with hot water and fed these to Chrysopa larvae. Angoumois grain moth (Sitotroga cerealella Olivier) eggs were used by Ridgway et al (1970) in an efficient and economical method of mass-producing C. carnea (Stephens). Sitotroga cerealella females will lay their eggs loose through a fine gauze screen and all that is necessary is to clean the eggs of the scales and debris which accompany the eggs through the wire-screening. This can be done by sifting the eggs through another finer wire screen, or by allowing the eggs to fall through a light air-steam. The heavier eggs fall through the air-stream, while lighter debris is carried away. A combination of the two methods was found to be the best under existing conditions.

Potato tuber moth females, on the other hand, do not lay

loose eggs, but prefer to oviposit onto a substrate such as the underside of a muslin sheet (Platner & Oatman, 1968). This has certain disadvantages in that the eggs cannot be scattered loose into <u>Chrysopa</u> larval cages as can be done with <u>S. cerealella</u> eggs. However, problems in the form of a predacious mite (<u>Pyamotes</u> sp.) in the <u>S. cerealella</u> colony arose, and egg production was so seriously affected that the potato tuber moth was chosen as the host insect.

2.1 LARVAL FOOD SOURCES

A modified version of the technique discribed by Platner & Oatman (1968) was used to produce potato tuber moth eggs in the laboratory. The breeding unit described below was that used by the Biological Control Section, Plant Protection Research Institute, Pretoria, for producing eggs for parasite studies.

Potato tubers infested with 1st instar potato tuber moth larvae were packed onto wire-mesh trays measuring 62 x 91 cm, which were accommodated in a large metal frame 180 cm high, 71 cm wide and 91 cm deep (Fig. 1). This frame could hold nine of these trays one on top of another and approximately 15 cm apart. The trays were arranged so that the lowest one contained the oldest larvae, while the youngest were on the top tray. Every two days the bottom tray was removed and the tubers discarded, and the remaining trays were moved down in succession with a new tray of freshly infested tubers being placed at the top of the frame. In this way the order of youngest larvae at the top and oldest at the bottom was

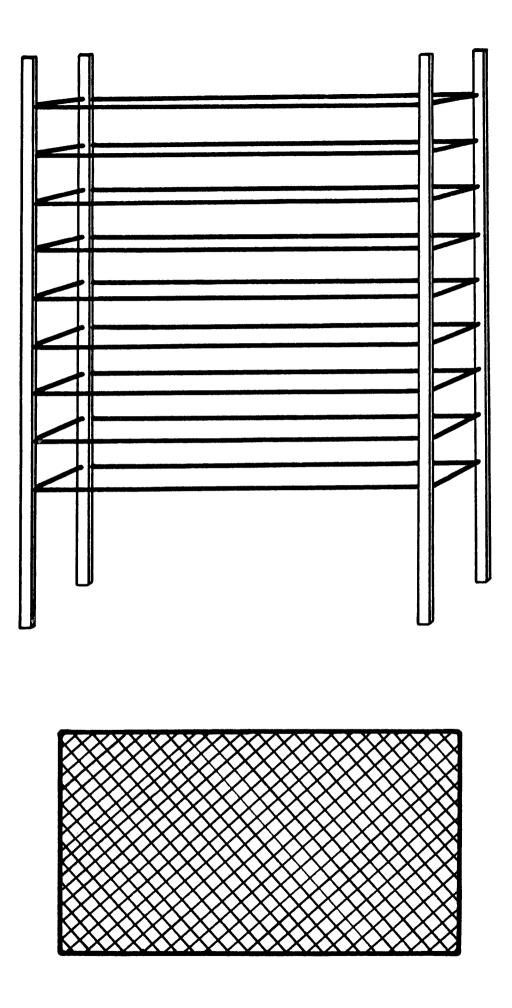


Fig. 1. The metal rack and one of nine trays used to rear the potato tuber moth P. operculella.

always maintained

As the larvae became full-grown they left the tubers and dropped onto a tray on the floor which was covered by strips of paper on which clean play-pen sand was spread. The mature larvae pupated in this sand. Every second day the pupae (in coccons) were collected, washed in 5% caustic soda solution to dissolve the coccons, rinsed in water, and when dry, placed in aluminium cake pans measuring 21 cm in diameter and 7 cm high. About 300 to 500 pupae were placed in each cake pan. Before emergence of the adults the cake pans were covered with fine nylon or cotton netting. Adults were fed plain water as well as a 10% honey-water solution, both of which were squirted onto the netting. Adults were prevented from drowning by a layer of sand in the bottom of the cake pan which absorbed the excess liquid which fell through the netting.

Eggs were laid through the netting onto filter paper circles placed on the netting for thet purpose, and which were held firmly against the netting by petri dishes placed on top of them. Of the eggs collected some were set aside for re-infesting fresh tubers while the rest were stored at 4° C until needed for feeding <u>Chrysopa</u> larvae. The eggs develop very slowly at this temperature and can be kept at 4° C for two weeks or more before hatching. At room temperature the eggs hatch after about four days.

Eggs for re-infestation purposes were placed on tubers (20 to 40 eggs per tuber, depending on size) which had previously been punctured to a depth of 1-2 mm by a flower pinholder (a spiky base used to position flowers in a vase). This was necessary otherwise newly hatched larvae infest the tuber only through the eyes and results in tubers being inadequately infested for mass- rearing purposes (Platner & Oatman, 1968). Once the eggs had hatched, the pieces of paper on which the eggs had been laid were removed as these could later provide ideal pupation sites for tuber moth larvae.

The tuber moth was not reared under strictly controlled conditions of temperature and humidity. Precautions were however taken to ensure that the temperature did not fall too low during winter. Humidity was not controlled at all.

2.2 REARING TECHNIQUES

Three methods were used to mass-rear <u>Chrysopa</u> in the laboratory, although one, the Plastic Grid method, could not be used to its full potential due to lack of a suitable food source. This will be further discussed at a later stage (section 2.2.3).

Rearing material was collected in the form of <u>Chrysopa</u> adults from the Jan Kempdorp area (Cape Province) and was later identified by Dr. Bo Tjeder, Lund University, Sweden, as <u>Chrysopa</u> (<u>Chrysoperla</u>) <u>zastrowi</u> (Esb. - Pet.).

2.2.1 The Poly Top method

This method was used for the greater part of the experimental work. It makes the best use of any larval food which is available, with little or no waste, and was therefore found to be the best method when food material was not overabundant. Larval mortality was usually low. Adults and larvae were kept under constant conditions at $25 \pm 1^{\circ}$ C and $55 \pm 5\%$ R.H.

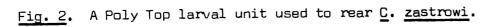
2.2.1.1 Larval Units

Each larval rearing unit consisted of 70 inverted plastic Poly Tops (internal diameter 2 cm) in five rows of 14 Poly Tops each, arranged on a masonite board of about 38 x 23 cm (fig. 2). Each pair of PolyTops was surrounded by four thin cardboard strips which kept a pair of 2,5 cm square glass lids in place, thus ensuring that the cages remained closed at all times. Glued to each glass lid was a small piece of cork into which a coloured mapping-pin was placed for identification of the occupant of the cage (green = egg; yellow = larva), and for easy handling of the lid. It was thus possible to tell at a glance the numbers of each stage present in any unit at any time. Twenty-three of these units were made, giving a total of more than 1 500 cages.

Each day two newly laid <u>C</u>. <u>zastrowi</u> eggs were placed in two of the l4-cage rows. It was found advisable to place two eggs per cage so as to ensure that a cage was not left empty due to an infertile egg, and also to provide an initial food source for the larva hatching first. Once hatched, larvae were fed every day by placing potato tuber moth eggs (on pieces of filter paper) into each cage.

While potato tuber moth eggs were generally a very good source of food for the larvae, it occasionally occurred





that the tuber moth colony suffered set-backs. One such occasion, which occurred more than once, was an infection by the bacterium <u>Streptococcus faecalis</u> var. <u>liquefaciens</u> (determined by G.M. Thomas, Agricultural Experiment Station, Division of Entomology, Berkeley, California, U.S.A.). During these occasions egg production slumped to practically zero, and then virtually any source of food that could be procurred was used to feed the larvae. These included the cabbage aphid <u>Brevicoryne brassicae</u> (L.), the wheat aphid <u>Schizaphis graminum</u> (Rond.), the citrus mealybug <u>Planococcus</u> <u>citri</u> (Risso) and any eggs that were available from the <u>S. cerealella</u> colony.

The <u>C</u>. <u>zastrowi</u> larvae were fed every day and were allowed to mature and finally pupate in the Poly Top cages. The mature larva, which by now has undergone two moults and is now in the prepupal stage, spins a white silken cocoon, usually between a piece of filter paper and the floor of the cage. Pupae were left in the cages until adult emergence, and then later the adults were transferred to the oviposition cage.

2.2.1.2 Oviposition Cage

This was constructed from 3 mm perspex and measured approximately 33 cm square by 12 cm high. The floor and roof of the cage were removable. Ventilation was by two vents, 14 x 5 cm, cut in opposite sides of the cage and covered by mosquito netting (Fig. 3).

The adult diet consisted of plain water as well as a mixture of finely ground, dry, live yeast (<u>Saccharomyces</u>

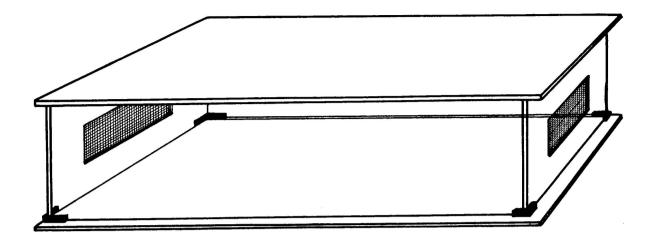


Fig. 3. The perspex oviposition cage used to rear C. zastrowi by the Poly Top method.

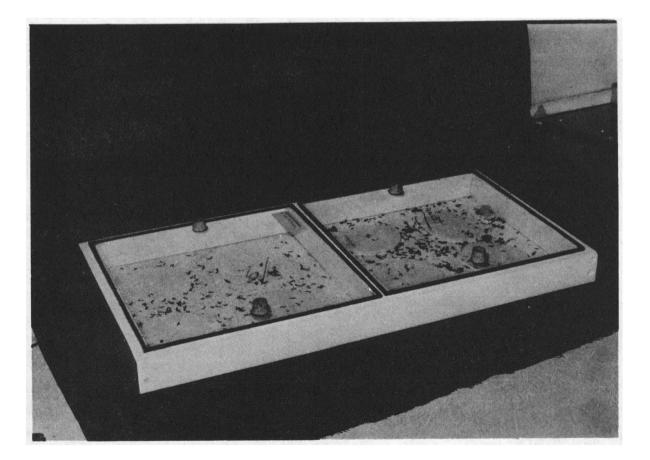


Fig. 4. One of the larval units used to rear C. zastrowi larvae by the Tray method.

<u>utywala africana</u>), honey and water in a ratio of 5:6:15 by volume. The yeast was killed by mixing it with the water and bringing it to the boil for a few seconds. This was necessary to prevent fermentation of the diet. The honey was added after the mixture had cooled, as some of the constituents of honey break down at the boiling point of water. This mixture, and the plain water, were placed separately in glass sample tubes 2,5 cm high and 2,5 cm in diameter. A cotton wool roll protruded through a hole in the Poly Top lid provided, and served as a wick up which the liquid diet was absorbed. The adults fed by drinking directly from the saturated wick.

<u>Chrysopa</u> females lay most of their eggs on ventral surfaces, and consequently a piece of white paper was placed underneath the roof of the cage, forming a ceiling. As most eggs were laid on the paper ceiling, their removal was greatly facilitated.

Each day fresh food and water was prepared and newly emerged adults collected together in one or more sample tubes. These adults, as well as those in the oviposition cage, were anaesthetized with carbon dioxide, the paper ceiling and old food tubes replaced with new ones, the newly emerged adults added to the cage and any dead adults removed. The eggs which are laid on stalks about 4 to 5 mm long were removed from the paper ceiling by shaving them off with a safety razor. This method was found to be less time-consuming and tedious than the method described by Finney (1950), and wes less damaging to the eggs than the method of Ridgway et al (1970).

2.2.2 The Tray method

At one stage during the rearing of <u>C</u>. <u>zastrowi</u> it was necessary to increase the size of the colony by a considerable amount. Due to the lack of loose, debris-free eggs as a larval food source for use with the Plastic Grid rearing units, and due to the lack of sufficient manpower which would be required to increase production by the Poly Top method, the Tray method of rearing was evaluated as a possible alternative method.

2.2.2.1 Larval Units

Finney (1948) describes a method in which wooden trays are used to rear a number of larvae in a common cage, and it was on this basis that this method was developed. The trays used in this experiment were modified slightly from Finney's and comprised a wooden frame 60 cm long, 30 cm wide and 2,5 cm deep, with a masonite floor. Each unit was divided into two square compartments by a piece of wood across its width. Lids for each compartment were of 3 mm glass, 29 x 28 cm (Fig. 4). To make the lids larvaeproof, against even 1st instar Chrysopa larvae, thin strips of felt were glued underneath the edges of the lids, forming an effective seal when the lids were placed in position. Two corks were glued to the upper surface of each lid to facilitate easier handling. Each compartment was lined with paper to facilitate cleaning the cages. When all the larvae in one compartment had pupated and had been removed. the tray could be cleaned of debris simply by replacing the

lining.

In view of the fact that the larvae were not kept apart in this method, it was essential to provide an excess of larval food in order to reduce to a minimum the chances of cannibalism. It was calculated from observations made from the Poly Top method that about 90 000 potato tuber moth eggs would be needed to successfully rear 100 <u>Chrysopa</u> larvae to maturity. Allowing for this, it is clear that an enormous supply of larval food is required to rear a reasonable sized colony by this method.

Seventy five to 100 <u>C</u>. <u>zastrowi</u> eggs of the same age were evenly distributed over the floor of each compartment and on the day prior to hatching were covered by filter paper circles on which tuber moth eggs had been laid. The filter paper served the additional purpose of providing suitable hiding places for the <u>Chrysopa</u> larvae, which invariably settled underneath these papers.

The larvae were only fed once during the lst instar but thereafter it was necessary to add fresh eggs each day in order to maintain the food excess mentioned earlier. Despite this cannibalism occurred and larval mortality was often 50%, and higher on occasions. This figure was very difficult to reduce appreciably.

Eggs of the housefly, <u>Musca domestica</u> (Linné), were sometimes used as an alternate larval food source when tuber moth eggs were in short supply. In the absence of direct moisture or of very high relative humidities, these eggs desiccate within hours, so to prevent this they were first

spread over moist filter paper circles. By this method, the eggs readily adhered to the paper, which was then placed egg-side down in the units while still damp.

Freshly-killed adults of the vinegar fly, <u>Drosophila</u> <u>melanoqaster</u> (Meigen), were also found to be an alternative to tuber moth eggs. The flies were very easy to rear on an artificial diet originally developed for rearing the American bollworm <u>Heliothis armiqera</u> (Hübner) (described by Bot, 1966), and were able to sustain 2nd instar and older <u>C. zastrowi</u> larvae through to the adult stage. The larvae fed by sucking out the liquid contents of the fly, which had a body soft enough to be easily penetrated by the mouthparts of older larvae. First instar larvae were usually unable to pierce the flies, and therefore had to be fed tuber moth eggs or housefly eggs.

Adults of <u>D</u>. <u>melanoqaster</u> worked well as a food source for a short while, but problems were later encountered which necessitated rejecting this diet. <u>Chrvsopa</u> larvae became infected by pathogens later diagnosed (by G.M. Thomas, Agricultural Experiment Station, Division of Entomology, Berkeley, California, U.S.A.) to be the spore-forming bacterium, <u>Bacillus cereus</u>, and another closely related <u>Bacillus</u> sp. These bacteria are normally considered to be potential pathogens, and, according to a communication with Thomas (1972) under certain conditions in a particular host may act as a primary pathogen. Sometimes this disease only manifested itself in the pupal stage, but in both cases the contents of the body were liquefied and dark $20 \, 7 5 \, 26 \, 1$

brown in colour with an accompanying foul odour. In some instances larval mortality was 100%, but as soon as the use of <u>D</u>. <u>melanogaster</u> was discontinued as a larval host for <u>C</u>. <u>zastrowi</u>, rearing returned to normal.

Cocoons were removed as soon as they had been completed as it was found that <u>C</u>. <u>zastrowi</u> larvae were preying on them. The cocoons were held in one-pint cream cartons to await emergence, after which the adults were anaesthetized and transferred to the oviposition cage.

2.2.2.2 Oviposition Cage

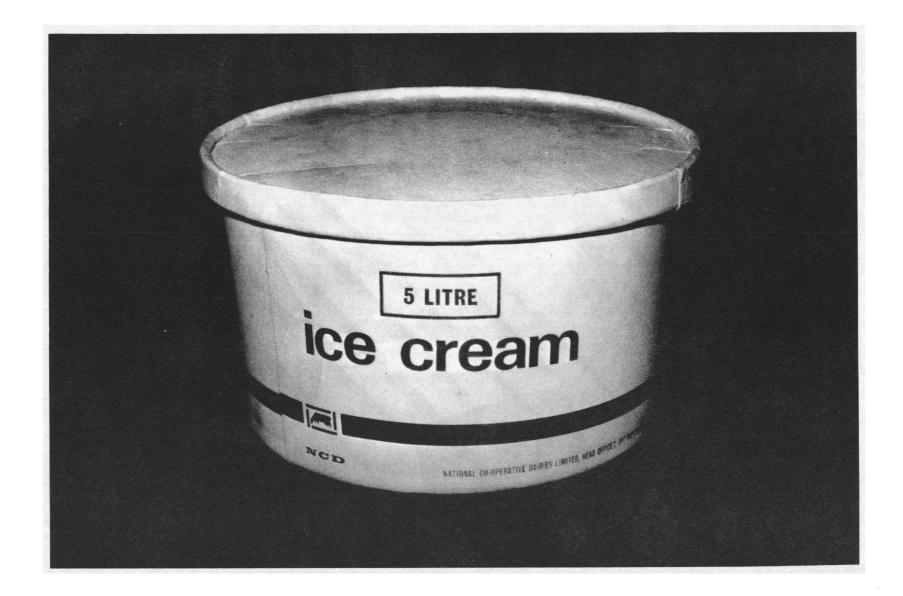
In this case the oviposition cage consisted of a five litre waxed cardboard ice-cream carton, 14 cm high and 23,5 cm in diameter with its accompanying lid (Fig. 5). The basic difference between this cage and the one used in the Poly Top method was that it was not ventilated (although the lid was not air tight), and although it would not have been totally dark inside during daylight conditions, the light would have been fairly subdued.

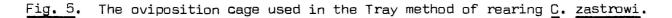
A removable paper lining placed in the lid served as the oviposition site. The adult diet, and the method of presenting it, as well as the method of removing the eggs from the paper lining, remained as for the Poly Top method.

2.2.3 The Plastic Grid method

This method is considered to hold the best potential for mass-rearing <u>C</u>. <u>zastrowi</u>, but in order to be practical a large supply of loose, debris-free eggs is required for

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larval rearing. Due to the difficulty in obtaining eggs of this nature (<u>S</u>. <u>cerealella</u>), this method was only used on a small scale. The potentialities were obvious, so it will nevertheless be described.

2.2.3.1 Larval Units

The larval rearing method was a modified version of the one described by Ridgway, <u>et al</u> (1970). Each rearing unit consisted of a plastic criss-cross grid of the type often used in neon illumination of offices etc. Each unit measured 20 cm long, 15 cm wide and 1 cm high, and was divided up into 150 compartments each measuring 1,3 cm square and 1 cm high (Fig. 6). Each compartment was used as a separate cage. A piece of organdie was glued over the bottom of the grid forming the floor of each cage, while a piece of 4 mm thick perspex, 22 x 15 cm, acted as the lid for the unit, and was held in place by tape or rubber bands.

Eggs of <u>C</u>. <u>zastrowi</u> were evenly distributed (two or three per cage) amongst the cages by using a shaker such as a pepper pot, with suitable holes. Food for the larvae can be presented in one of two ways. The simplest is to use the shaker again to distribute the loose eggs amongst the cages. The larvae are first anaesthetized by placing the unit over a wooden frame about 2,5 cm high and with internal measurements slightly smaller than the size of the units (i.e. about 19,5 x 14,5 cm). Carbon dioxide is then introduced into the frame through a hole in one side (see Fig. 6), and passes up through the organdie floor of the

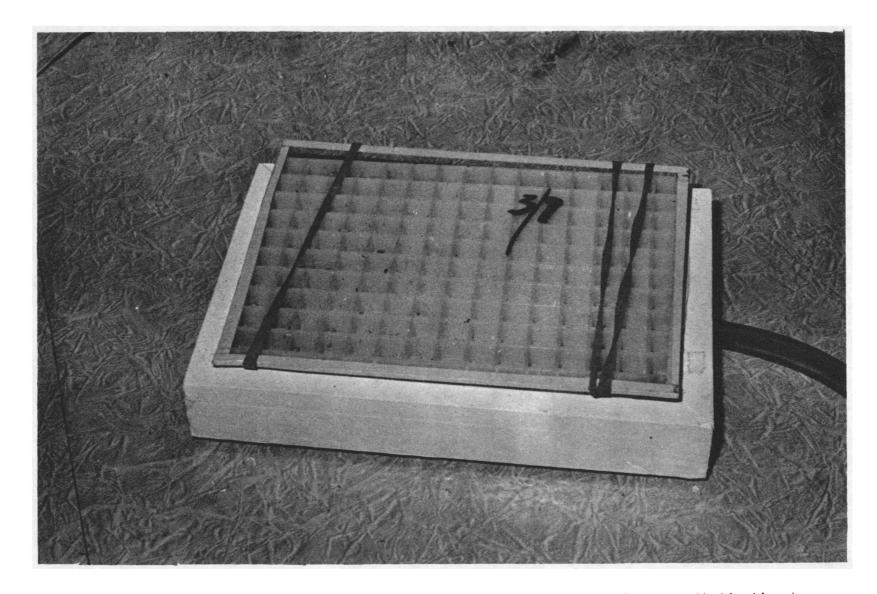


Fig. 6. Chrysopa zastrowi larvae, reared in a Plastic Grid unit, undergoing anaesthetization by carbon dioxide. The unit has been placed on a wooden frame for administration of the gas.

unit, thus immobilizing the larvae. The perspex lid is then removed and the eggs added.

The second method of presenting the food to the larvae is less messy as it avoids a build up of empty egg shells at the bottom of the cages. Small sponge-rubber discs about 9 mm in diameter and about 3 mm thick are glued to a sheet of perspex of the same dimensions as the lid of the cage, in such a manner that one disc corresponds to the position of each cage in the unit. This piece of perspex is then lowered into a shallow sticky solution such as gum tragocanth or a honey-water solution so that only the discs come into contact with the liquid. The discs, now wet, are pressed onto a clean unit lid, leaving the imprint of the discs on the lid. Eggs are then sprinkled over the sticky patches, and the excess eggs shaken off. This lid is then exchanged for the old unit lid while the larvae are under the anaesthetic. The end result is that each cage has its own supply of food stuck to the lid.

The advantage of rearing the larvae by this method is obvious. The man-power needed to feed the larvae is reduced considerably, while larval mortality due to cannibalism is eliminated. The compact units are small and easy to handle.

2.2.3.2 Oviposition Cage

The oviposition cage used for this method was the same as the ventilated, perspex cage used in the Poly Top method. It appears that any type of oviposition cage can be used,

as neither of the two cages already mentioned seemed to have any adverse effects on the adults.

CHAPTER 3

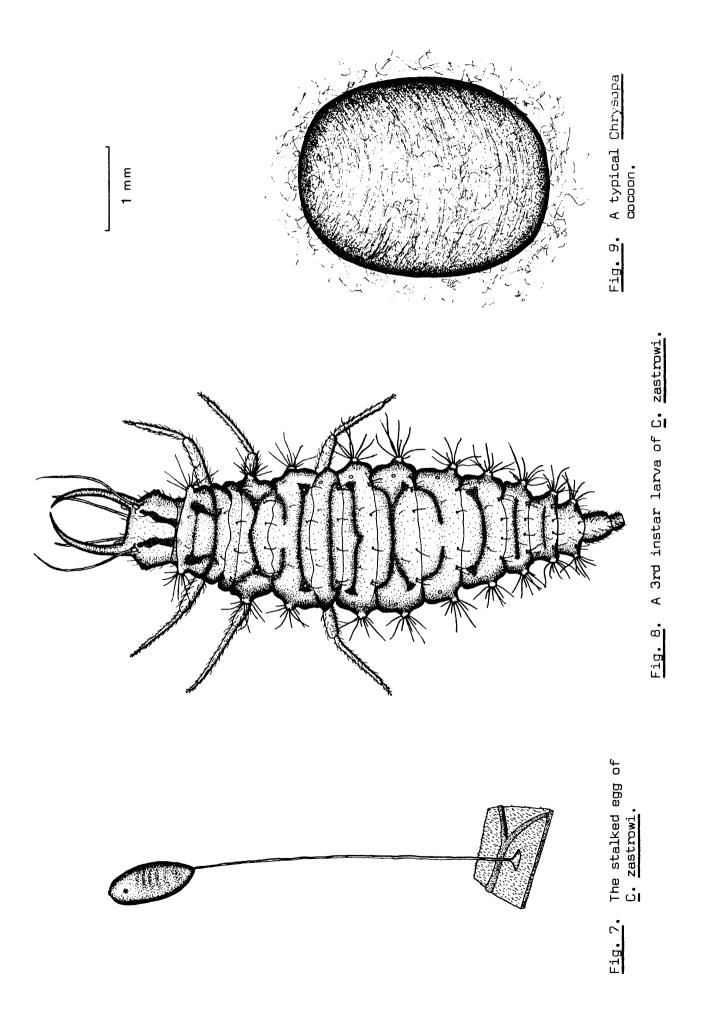
LABORATORY STUDIES ON GENERAL BIOLOGY OF C. ZASTROWI

During these studies a variety of food material was fed to the larvae, the particular host species being determined by the abundance of sources available. While potato tuber moth eggs were the main source of food, these were supplemented by those species mentioned in Chapter 2, namely <u>B. brassicae, P. citri, S. graminum</u> and eggs of <u>S. cerealella</u> and <u>M. domestica</u>. It is therefore stressed that the description and data given in this account are based on the breeding of <u>C. zastrowi</u> on more than one host species.

3.1 EGG STAGE

The elongate-ovate shaped eggs are a light green in colour when laid, often with faint patches of yellow-green especially around the posterior end, and are about 1 mm long. The posterior end is attached to the substrate by a thin hyaline stalk, usually about 3,5 to 6 mm long (Fig. 7). Eggs of <u>C</u>. <u>zastrowi</u> are laid singly, unlike some other species which may either have a number of eggs supported by a single stalk (Smith, 1922) or single eggs on stalks so closely laid that the stalks fuse for part of their length (Tjeder, 1966).

As in other species, oviposition abnormalities do occur in \underline{C} . <u>zastrowi</u>, but this was mainly as a result of the densely laid eggs which arose from mass-rearing. Stalks were often seen attached to the tops of other eggs, or on



occasions to the stalks of other eggs, giving what appeared to be a branching stalk. There were also usually a few loose unstalked eggs lying in the bottom of the oviposition cage, but these developed normally. The occurrence of unstalked eggs is not however peculiar to \underline{C} . <u>zastrowi</u>, as Smith (1922) also describes it in other species.

As already mentioned, most eggs were laid on the ceiling of the oviposition cage, although some were also laid on the walls of the cage and on the feeding dishes. In nature the females seemed to be less discriminating in their choice of oviposition sites, and eggs were found on dorsal and ventral surfaces of leaves, stalks, flowers, and cotton bolls etc.

The only noticeable feature of the egg just after it has been laid is the micropylar region, a small flattened disc rather like a coin, or a button, whitish in colour and situated on the distal tip of the egg. It remains white and noticeable throughout the egg's development.

During development of the egg, differentiation becomes obvious during the first day as the yellow patches increase in size. Reddish-brown stripes, probably indicating segmentation of the embryo and development of the legs, become noticeable on the second day. By the end of the second day the yellow areas begin to turn a light beige colour, and the reddish-brown stripes become more evident. Patches of green are still present at this stage. On the third day the egg is beige overall, and the six separate but closely positioned stemmata can be seen disto-laterally on

each side. Segmentation is obvious at this stage, and the legs can be seen neatly folded down the front of the embryo. Both sides of the egg often collapse slightly, longitudinally, probably as the yolk-sac becomes depleted with the progression of development.

3.2 ECLOSION

Three to four days after oviposition, just before the egg hatches, the embryo can be seen (with the aid of a microscope) moving slightly within the egg. At this stage the embryo is bent double, with the head pressed against the ventral part of the thorax, and the mouthparts and legs all lying together, parallel to the abdomen. The hatching process commences with a pulsating swelling in the region of the embryo's head. An egg-burster (Smith, 1922; Toschi, 1965), which is forced against the inside of the chorion by the embryo, probably causes this swelling. The eggburster makes a small longitudinal split in the chorion in front of the head. The pulsating continues, with six or seven minor pulses followed by a major pulse, which lengthens the split.

As the split lengthens, the head begins to emerge and is followed by the thorax. As the larva eases itself out of the shell, the abdomen, mouthparts and legs follow until the larva, still doubled up in an inverted U position is perched on top of the egg with the tip of the abdomen, legs and mouthparts still in the shell. The legs and mouthparts then come free and the larva begins to flex its legs. The antennae, which up to now have lain exactly superimposed over the mouthparts, are the next to be freed. Leg flexing continues until the larva can grasp the egg, and the tip of the abdomen is then extricated. On infrequent occasions the larva is unable to remove some or all of its legs and mouthparts from the egg, in which case it dies. These parts quite likely become entangled in the embryo skin, which together with the egg-burster, remains in the egg.

The hatching process up to this stage usually lasts from 15 to 20 minutes. The larva clings to the egg for anything from half an hour to an hour or more, usually in an inverted position, after which it descends the stalk and begins its search for food. If it hatches in the close vicinity of other <u>Chrysopa</u> eggs it will quite likely ascend the stalks and feed on them. Larvae are capable of surviving without food for about 24 hours after hatching, during which time they are very active. Fleschner (1950) found that a 1st instar larva of <u>C</u>. <u>californica</u> (Coq.) (= <u>C</u>. <u>carnea</u>) could crawl more than 200 metres from hatching until it starved to death.

3.3 LARVAL STAGES

3.3.1 <u>Description</u>

3.3.1.1 First Instar

First instar larvae, which are spindle-shaped and slightly more than a millimetre long, are covered with relatively long curved and transparent setae which occur on dorso-

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lateral tubercles on each segment. Shorter setae also occur over the rest of the body and legs. To the naked eye the larva appears whitish in colour, but when seen through a microscope it is in fact a very light beige. The legs and mandibles are slightly darkened, especially at the joints and tips respectively, and the characteristic head markings are plainly visible. These markings, which become darker and more obvious as the larva grows older, are, on \underline{C} . <u>zastrowi</u>, in the form of an incomplete V, the two component lines not quite meeting. The open base of the V is at the occipital margin while the arms run forward to the base of the antennae. These markings are fairly characteristic, and differ to various degrees between species. Another set of lines run laterally from the stemmata back to the occipital margin.

On eating its first meal the larva picks up its first colouring, as the contents of the gut are visible in the anterior region of the abdomen. If for example a potato tuber moth egg is eaten, the gut takes on a pale yellow colour; if a fresh <u>Chrysopa</u> egg is the first victim, a green patch is noticeable in the gut. Very soon the whole abdomen becomes coloured in this manner.

First instar larvae are capable of preying on their victims with amazing voracity considering their size, and have been observed to overcome aphids more than twice their size. They seem to have no difficulty in lifting their prey off the substrate and in so doing prevent their victims from escaping. Immediately after hatching, larvae have no difficulty in ascending the stalks of other <u>Chrysopa</u> eggs, which are then readily devoured.

3.3.1.2 Second Instar

After approximately four days the 1st instar larva, now quite dark in colour, moults. The 2nd instar larva measures about 3 mm in length and is at first almost white in colour, but with the head markings still visible. The extremeties of the body, i.e. tips of mouthparts, legs and abdomen, are the first to darken slightly, and after a while the body assumes a light brown colour with translucent setae.

When the gut of the larva is not discoloured by ingested prey, the colour of the abdomen is reddish-brown, interspersed with creamy-yellow lines and patches running across the width of the abdomen. The thorax appears slightly grey in colour, although the brown patches are still evident. All tubercles are creamy-yellow in colour, and setae are translucent throughout. The overall colour of the larva is nevertheless very much influenced by the colour of the ingested prey. The pulsating dorsal blood vessel is also plainly seen. Ventrally the abdomen and thorax are predominantly light yellow, probably due to the presence of fat bodies visible beneath the cuticle.

It is noticeable that larvae of all three instars show a preference for the underside of an object, and they were thus frequently found underneath pieces of paper in the cage, on which tuber moth eggs had been laid. Although this tendency occurred in all three instars, it was found to be more prevalent during the first two instars. It is probably a defence reaction --- in the field larvae are very rarely seen and appear to crawl into leaf sheaths and other protective cavities --- but it could also be related to the fact that many aphids and mites are found mainly on the ventral surfaces of leaves.

The larva grows very rapidly during this instar, which normally lasts two days, and when it has attained a length of about 6 mm, it moults a second time.

3.3.1.3 Third Instar

This instar again lasts about four days, and during this time the larva grows more in volume than in length. Food consumption increases substantially. Whereas a 2nd instar larva consumes about two or three times as much as in the lst instar, consumption by 3rd instar larvae increases by a factor of eight or nine over 2 nd instar larvae.

Young 3rd instar larvae (Fig. 8) are similar in colour to late 2nd instar larvae, and once again the ingested prey is largely responsible for the larval colour. The fat body is again plainly visible, as is the pulsating dorsal blood vessel running down the length of the larva.

The raised tubercles on the lateral part of all thoracic and abdominal segments each have about six to eight setae arising from them. A second series of smaller raised tubercles, half-way between the lateral tubercles and the dorsal blood vessel of each segment, have two setae arising from each tubercle, while several very small tubercles on each segment usually have only one seta. Short hairs are also found ventrally, and on the legs.

3.3.2 Feeding Behaviour

Observations on the feeding behaviour of <u>C</u>. <u>zastrowi</u> indicate that the larvae are not aware of the presence of their prey until physical contact has been made. This is done mainly by the mandibles, but contact can also be made by setae on the thorax and abdomen, and by the legs. Fleschner (1950) noted this phenomenon in the case of <u>C</u>. <u>californica</u>, and he went so far as to determine the "area of perception" for this species. He calculated it to be 1,02 mm, which he said is the average distance between the tips of the mandibles. Considering that larvae can detect prey by means of contact made with any part of the body, and that the width of any best early 1st instar larvae is greater than 1,02 mm, Fleschner's definition of "area of perception" is a little vague.

The feeding behaviour of Chrysopidae was also studied by Bänsch (1966), who found that the movement of newlyhatched chrysopid larvae over leaves was random, and not influenced by the presence of aphids unless they happened to come into contact with one. He found that there was no optic or olfactoric orientation by the larvae. Larvae probe any obstacle that is encountered, and if any prey is encountered, it is lifted off the substrate. This latter practice is, according to Bänsch, common to the other sucking predators, Hemerobiidae and Syrphidae, as well.

His observations on prey perception by Chrysopidae support what was found in the present study.

<u>Chrvsopa zastrowi</u> larvae show no discrimination in the choice of feeding site on the prey, unless some parts of the prey's body are obviously too hard for penetration of the mandibles to take place. <u>C. zastrowi</u> larvae have been seen feeding on aphids through the head, abdomen, and even through the legs. One or both "fangs" may pierce the prey. While there is still a reasonable amount of body fluid left in the prey, the larva remains fairly passive and merely feeds, but when the prey's body contents become depleted, the feeding process becomes more active, with the mouthparts probing every nook and cranny in an effort to get every drop of food. The mouthparts may be withdrawn and re-inserted into another part of the body until not a trace of body fluid remains.

Larvae are very active when searching for prey and are capable of moving at a surprising speed. Fleschner (1950) found that the average speed of ten different <u>C</u>. <u>californica</u> larvae was about 1,9 cm/second (measured over a period of one hour). No mention was made of the age of the larvae, but it may be assumed that they were in the 3rd instar.

As mentioned in the previous chapter, the mouthparts consist of two sickle-shaped fang-like tapering tubes, formed by the apposition of the dorsal mandible and the ventral maxilla. A groove on the ventral surface of the mandible is opposed by one on the dorsal surface of the lacinia of the maxilla. A channel is thus formed down the inside length of the resulting "fang" which in effect represents a suctorial tube (Smith, 1922; Imms, 1964).

There appear to be two actions in feeding. One involves the sliding backwards and forwards of the maxilla against the mandible, accompanied by the passage of fluid up either one or both of the jaws. Smith (1922) suggests that this action may help in the mechanical breakdown of the internal tissues of the prey. In the other method of feeding the jaws are held quite still during feeding, while the juices move just as rapidly up the tubes. Smith mentions a muscular pharyngeal pump in the head of the larva, as well as muscles down the whole length of the maxilla which assist by increasing the volume of the tube.

From observations on older larvae it is obvious that extraoral digestion does not play a part in the feeding of \underline{C} . zastrowi. In other words, digestive juices are not first injected into the victim through the mouthparts with the purpose of pre-digesting the body fluids before they are sucked out. Killington (1936) suggested that external digestion did to some extent take place in <u>Chrysopa</u>, but this was not the case with <u>C</u>. <u>zastrowi</u>. This fact was easily established by observing the entire feeding process under a dissecting microscope. The passage of the prey's body fluids up the larva's mouthparts is easily seen, and begins <u>immediately</u> the mouthparts pierce the body. Furthermore, no inflation of the prey was noticed at any This normally occurs when extra-oral digestion takes time. place, due to the digestive juices of the predator flowing

into the prey.

3.3.3 Cannibalism

As mentioned earlier, <u>Chrysopa</u> larvae are cannibalistic by nature, and <u>C</u>. <u>zastrowi</u> is no exception. They will not hesitate to attack other <u>Chrysopa</u> larvae but due to avoidance and defence reactions displayed by the accosted individuals, other food, if present, is generally consumed first. But it often occurs that larvae, especially young ones, are attacked and consumed despite the presence of other defenceless prey.

<u>Chrysopa</u> eggs and pupae are also likely to be preyed upon if present. Recently hatched and older larvae will consume many <u>Chrysopa</u> eggs and become quite green in colour as a result, while older 2nd instar, as well as 3rd instar larvae · have no trouble in piercing <u>Chrysopa</u> cocoons with their mandibles and consuming the defenceless occupant. Adult <u>Chrysopa</u> have on occasions also been observed to be subject to attack by <u>C</u>. <u>zastrowi</u> larvae, although understandably with little success. <u>C</u>. <u>zastrowi</u> larvae have also been seen to feed on dead adults.

Virtually all cannibalism was excluded by using the Poly Top method of rearing. It will be remembered however that two <u>C</u>. <u>zastrowi</u> eggs were placed in each cage, so that the possibility for cannibalism did exist to this extent. It usually happened that both larvae hatched, and in such cases the chances of cannibalism taking place during the lst instar were small, provided food was present. These chances however increased as the larvae increased in size and age, and it was indeed very rare that both pupated.

It was possible to study the effect of cannibalism on a population when the Tray method of rearing was used. For this particular study the larvae were reared on \underline{M} . <u>domestica</u> eggs spread on damp filter paper circles, and records kept of the number of eggs placed in each cage, the number of viable pupae obtained therefrom, and the resultant per-centage mortality (which can practically speaking be equated with the percentage cannibalism).

The results are presented in Table 1.

<u>Table 1</u>. Mortality by cannibalism of <u>C</u>. <u>zastrowi</u> larvae reared on <u>M</u>. <u>domestica</u> eggs by the Tray method.

No. of eggs hatched	No. of viable pupae	% larval mortality
41	22	46,3
43	24	44,2
53	29	45,3
55	25	54,5
72	32	55 , 6
89	31	65 , 2
108	61	43,5
128	43	66,4

It is interesting to note from Table 1 that with only one exception (where 108 eggs hatched) there was a general increase in mortality (= cannibalism) with increase in the initial size of the population. In the case of the higher initial densities, larvae would have encountered each other more often, and the higher mortality figures can be interpreted as greater competition for space due to overlapping of "hunting" territories. The high mortalities, even in the case of the lower initial population densities, serve to indicate that a <u>Chrysopa</u> larva is just as apt to attack and eat one of its companions as it would its more natural prey species. The fact that a larva does display an avoidance reaction by either moving away when accosted, or by challenging its attacker, counts in its favour for survival, but once an attacking larva has immobilized a victim, other <u>Chrysopa</u> soon join in. Up to five larvae have been seen feeding on one victim.

It is interesting to note that when a \underline{C} . <u>zastrowi</u> larva is attacked and its initial defence is unsuccessfull, it does not offer any resistance once it has been pierced by the attacker's mouthparts, but merely remains immobile and completely at the mercy of its attacker. This would suggest that a paralyzing substance is injected into the victim through the mouthparts of the attacker, but as has been shown earlier the evidence does not support this suggestion.

3.3.4 Prey Consumption

Many authors have recorded the total consumption by <u>Chrysopa</u> larvae of various species of prey. Total prey consumption during active larval life varies tremendously, depending on the species of <u>Chrysopa</u> and prey, and is exemplified by the following cases: 71 to 142 aphids,

3780 coccids and 6487 coccid eggs, all by <u>C</u>. <u>carnea</u> larvae; 138 aphids by <u>C</u>. <u>oculata</u> (Say); 2000 aphids by <u>C</u>. <u>japana</u> (Okamoto) (Tjeder, 1966); 79,7 mealybugs by a <u>Chrysopa</u> sp. (later identified as <u>C</u>. <u>burgeonina</u> (Nav.) (Whitehead, 1957). Prey consumption by <u>C</u>. <u>zastrowi</u> larvae was therefore also investigated.

<u>Materials and Method</u>. Five larvae were kept individually from hatch to pupation in glass vials stoppered with cotton wool, and fed mature, apterous wheat aphids each day. An abundance of aphids was provided so that the larvae were never without food. The number of aphids consumed each day was recorded, as was the duration of the instars and the pupal stage. The experiment was then repeated using potato tuber moth eggs. In all cases the larvae were kept at constant conditions of $27 \stackrel{+}{=} 1^{\circ}$ C and $50 \stackrel{+}{=} 5\%$ R.H.

<u>Results and Discussion.</u> The results are presented in Tables 2 and 3 on pages 43 and 44.

The greater number of potato tuber moth eggs consumed in comparison with aphids (46%) can be explained by the fact that the latter are much larger, and less are therefore needed to complete the larval stage. This stage was completed in an average of 10,8 days on the aphid diet and 9,8 days on the egg diet, a difference of about 10%, indicating that the potato tuber moth eggs are perhaps more nutritious than wheat aphids.

A further fact emerges from Tables 2 and 3. In each case one larva completed its larval stage in one day less

Table 2. Total prey consumption by <u>C.zastrowi</u> larvae, and duration of larval and pupal stages, when fed mature, apterous wheat aphids, <u>S.graminum</u>.

	1st In	star	2nd In	star	3rd In	star	Total	Dura	ation
Larva	Duration (days)	Aphids eaten	Duration (days)	Aphids eaten	Duration (days)	Aphids eaten	aphids eaten	Larvae (days)	Pupae (days)
1	4	36	2	41	5	445	522	11	8
2	4	28	2	54	5	408	490	11	8
3	4	39	2	56	4	299	394	10	8
4	4	30	2	49	5	423	502	11	9
5	4	33	2	56	5	444	533	11	9
Mean	4	33,2	2	51,2	4,8	403,8	488,3	10,8	8,4

Table 3. Total prey consumption by <u>C.zastrowi</u> larvae, and duration of the larval and pupal stages, when fed eggs of the potato tuber moth, <u>P.operculella</u>.

	1st In	star	2nd In	star	3rd In	star	Total	Dura	tion
Larva	Duration (days)	Eggs - Aphids eaten	Duration (days)	Eggs Aphids eaten	Duration (days)	Eggs Aphids eaten	eggs - aphids eaten	Larvae (days)	Pupae (days)
1	Died after seven days without moulting								
2	4	38	2	92	4	850	980	10	9
З	4	44	2	102	3	577	723	9	9
4	4	25	2	94	4	810	929	10	*
5	4	24	2	74	4	895	993	10	9
Mean	4	32,8	2	90,5	3,8	783,0	906,3	9,8	9

*Adult did not emerge

than the others. In both cases these particular larvae consumed more food than average during the first two instars (16% more aphids, 26% more eggs), and less than average during the third instar (30% fewer aphids, 32% fewer eggs). This gave rise to a less than average total food consumption (23% fewer aphids, 25% fewer eggs). As temperature and R.H. remained constant throughout the experiment, and all other treatment and handling of the larvae was indentical, an explanation for this occurrence is difficult to find. For want of another explanation it must be put down to the inherent variability, or heterogeneity, of the larvae. But bearing in mind the increased consumption during the first two instars, it seems that the larva's course was set at an early age.

Clearly then, <u>C</u>. <u>zastrowi</u> larvae are capable of pupating on less than the optimum amount of food, thereby ensuring survival in the field when food sources may be limited. Variation in the size of cocoons was also evident during mass-rearing, indicating that some larvae had pupated when smaller than others. There appeared to be a positive correlation between cocoon size and the size of the fullgrown larva.

Tables 2 and 3 also show that on the aphid diet, 1st instar larvae consumed 6,8% of the average total number of aphids eaten, while 2nd and 3rd instars accounted for 10,5% and 82,7% of the average total respectively; on the egg diet, the figures were 3,6%, 10%, and 86,4%

for the lst, 2nd and 3rd instars respectively. The most notable point emerging from these figures is the extraordinarily large percentage of prey consumed by the 3rd instar larvae.

3.3.5 Prey Preference

In order to investigate the possible presence in \underline{C} . <u>zastrowi</u> larvae of a preference for or against any particular prey, a series of experiments were carried out whereby larvae were provided with a variety of prey.

Materials and Method. Mature 3rd instar larvae were confined individually in 9 mm petri-dishes for a period of two hours with a variety of prey species, and records made of the order in which prey was consumed. Larvae were not starved prior to the experiments. The aphids used were of various sizes, while mealybugs were all young, half-grown nymphs. Prey was provided at different densities in order to determine whether this factor had any relation to prey preference.

Results and Discussion. The observations are presented in Table 4 on page 47.

Glancing at Table 4 it may seem surprising that there was such a variation in the numbers of prey consumed during the two hour period, i.e. between 9 and 21 individuals. It should however be borne in mind that the various prey species differ considerably in size --- many <u>S</u>. <u>cerealella</u> eggs can be eaten during the time it takes to consume one <u>P. citri</u> individual. Consequently, where a larva consumes Table 4. The sequence of consumption by 3rd instar <u>C.zastrowi</u> larvae of various prey species during a two hour period. In the case of <u>S.cerealella</u> and <u>P.operculella</u>, eggs were given (Number of prey individuals provided in parenthesis).

		Larva No.		
Prey species, and number provided	1 <u>Myzus persicae</u> (Sulz.) (6) <u>B.brassicae</u> (6) <u>P.citri</u> (6) <u>S.cerealella</u> (6) <u>P.operculella</u> (6)	2 <u>S.graminum</u> (10) <u>B.brassicae</u> (10) <u>P.citri</u> (10) <u>P.operculella</u> (10)	3 <u>S.graminum</u> (15) <u>B.brassicae</u> (15) <u>P.citri</u> (15) <u>S.cerealella</u> (15) <u>P.operculella</u> (15)	4 <u>S.graminum</u> (20) <u>B.brassicae</u> (20) <u>P.citri</u> (20) <u>S.cerealella</u> (25)
Sequence of consumption	M.persicae P.citri B.brassicae M.persicae B.brassicae " " P.citri P.operculella	B.brassicae S.graminum " B.brassicae P.citri P.operculella " " P.citri P.operculella	<u>S.cerealella</u> " " <u>S.graminum</u> <u>P.citri</u> <u>S.graminum</u> <u>P.operculella</u> " <u>B.brassicae</u> <u>P.citri</u> "	B.brassicae S.cerealella " S.graminum P.citri S.graminum S.cerealella " " " " " " " " " " " " " " " " " "

many eggs, the total number of prey consumed increases (c.f. larva No. 4).

The numbers of each prey consumed by each <u>C</u>. <u>zastrowi</u> larva is summarized in Table 5 (on P.49), together with the total numbers of each prey species consumed by all four larvae.

In all cases, every prey species that was encountered was eaten, and no prey was ever rejected at any time during the course of the experiment. Any prey that was attacked was in all cases sucked dry by the larva before moving off in search of the next prey.

Taking the above facts into consideration, as well as the data in Tables 4 and 5, the writer feels justified in saying that mature 3rd instar <u>C</u>. <u>zastrowi</u> larvae do not show any preference for any of the six species of prey that were used in this experiment. It is also felt that no preference will be exhibited for most other non-aggressive soft-bodied insects, and many insect eggs. This opinion is also based on the fact that besides the above six species, with one exception, no other non-aggressive soft-bodied prey species (including various mites, aphids and lepidopterous eggs and young larvae) was ever rejected nor was any hesitation in accepting any of the afore-mentioned group noticeable. The one exception was mature P. citri, which secrete a woolly wax-covering over their bodies. This tended to tangle up the mouthparts of the C. zastrowi larvae. This factor did not play a part in

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Table 5. The number of various prey species consumed by four 3rd instar <u>C</u>. <u>zastrowi</u> larvae during a two hour period.

	Numbers of prey consumed						
	<u>M. persicae</u>	<u>S</u> . <u>graminum</u>	<u>B</u> . <u>brassicae</u>	<u>P. citri</u>	eggs of <u>S. cerealella</u>	eggs of <u>P</u> . <u>operculella</u>	Total/ larva
Larva 1 " 2 " 3 " 4	2 (6)* - -	- 2 (10) 2 (15) 5 (20)	4 (6) 2 (10) 1 (15) 2 (20)	2 (6) 2 (10) 3 (15) 1 (20)	0 (6) - 4 (15) 13 (25)	1 (6) 4 (10) 3 (15) -	9 10 13 21
Total	2 (6)	9 (45)	9 (51)	8 (51)	17 (46)	8 (31)	

*Numbers in parenthesis refer to the number of each prey species offered.

the above experiments as the young half-grown stages used were only covered with a fine wax layer which did not hinder feeding by the larvae.

Furthermore, it seems improbable that prey density has any relation to preference for one or more prey species. In the last column of Table 5, "Total/larva", there is an apparent increase in prey consumption with increase in prey density, but this is due coincidentally to the fact that larvae 3 and 4 consumed more eggs than larvae 1 and 2, and has no real bearing on host preference.

Observations on younger larval instars of <u>C</u>. <u>zastrowi</u> indicate that these larvae do in fact exhibit a type of prey preference. In an unreplicated experiment, a 1st instar larva was confined with wheat aphids and cabbage aphids (both of various sizes), and half-grown nymphs of the citrus mealybug. During the period of observation the larva rejected three wheat aphids, six mealybugs and seven cabbage aphids before accepting two very small wheat aphids. All the individuals rejected were medium to large in size. Similar observations on older larvae show that very young 2nd instar individuals also tend to reject larger prey, but this preference decreases with age until the late 2nd instar, when it disappears altogether.

There appears therefore to be a prey preference, based on the size of the prey, in young <u>C</u>. <u>zastrowi</u> larvae. However, it should be pointed out that in the event that no other food is available, very young larvae will successวบ

fully attack even fairly large aphids. Most lepidopterous eggs provide no problem and are also penetrable by the mouthparts of young larvae.

It is however felt that <u>C</u>. <u>zastrowi</u> larvae may well exhibit a prey preference in cases where insects with tough cuticles are encountered. Lavallee & Shaw (1969) recorded a prey preference shown by <u>C</u>. <u>oculata</u>, the golden-eye lacewing. First, 2nd and 3rd instar larvae were caged with mature pea aphids, 1st instar alfalfa weevil larvae, and nymphal leafhoppers and plantbugs (genera not recorded), and number of prey killed was recorded after 12 hours.

All three <u>C</u>. <u>oculata</u> instars showed a marked preference for pea aphids, and the other prey was virtually ignored. However, the authors unfortunately did not record what proporttion of the respective prey species was investigated by the <u>Chrysopa</u> larvae, and subsequently rejected. In addition, no mention is made of the relative toughness of the prey's cuticles, and so in the light of the findings with <u>C</u>. <u>zastrowi</u> larvae, the validity of Lavallee & Shaw's experiment is considered questionable.

It is clear that a prey preference undoubtedly exists to a greater or lesser extent where the prey is able to defend itself to various degrees. This was amply demonstrated in the Poly Top rearing method, where it happened on occasions that two <u>C</u>. <u>zastrowi</u> eggs hatched in the same cage. Normally, only one larva survives in a case such as this, the other larva being sooner or later consumed by the survivor. While there was sufficient alternative food

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(e.g. potato tuber moth eggs), both larvae preferred to consume these, rather than each other. As soon as there was a shortage of food, one or other of the larvae was usually attacked and overpowered. This serves to demonstrate that when the two sources of food were available (the other larva, and eggs), eggs were preferred by virtue of the larvae's ability to defend themselves.

In conclusion, it is therefore clear that prey preference in <u>C</u>. <u>zastrowi</u>, and probably in most other <u>Chrysopa</u> spp., is a complex phenomenon involving many factors such as size, cuticle thickness and the defence ability of the prey, all of which must be taken into account in a study of this nature.

3.3.6 Artificial Diets

The matter of liquid artificial diets for <u>Chrysopa</u> larvae has been briefly discussed in Chapter 2 where mention was made of various methods which have been used to present the diet to the larvae.

An artificial diet has obvious advantages over a natural diet --- it is not necessary to breed larval food in the laboratory, thus eliminating many problems such as labour, seasonal fluctuation, incidence of disease and parasites, to mention a few. Furthermore, a stock of artificial diet can be prepared in bulk and stored under suitable conditions for future use.

With these factors in mind, and using the knowledge of

overseas workers in this field (Hagen and Tassan, 1965; Vanderzant, 1969) as a guideline, a few small-scale experiments were conducted to investigate the feasibility of producing an artificial liquid diet and successfully presenting it to \underline{C} . <u>zastrowi</u> larvae. The pre-requisites were that the constituents should preferably be easily procurable, and the method should preclude the use of expensive and sophisticated apparatus. It was not intended to make a comprehensive and thorough study of artificial diets for <u>Chrysopa</u> larvae, as this is a study on its own, but merely to try and find a satisfactory substitute for <u>C</u>. <u>zastrowi</u>'s natural larval diet.

Materials, Method, and Results. The various diets which were made up were absorbed into small 5 mm cubed sponges and placed in cages of a number of Poly Top units. Fresh diet was provided each day, as the liquid rapidly fermented and became unacceptable to the larvae. Only six-day old larvae were fed the diet, and larvae for each diet were taken at random from the units.

Initially, varying proportions of the following substances
were used: gelatine (for protein), Fray Bentos (protein),
fructose (carbohydrate), brewer's yeast (vitamins, mainly
B group), ascorbic acid (vitamin C), Nipagin M (fermentation
suppressant) and water. Five diets that were tested are
discussed below.

<u>Diet 1</u>. This consisted of the following:

Gelatine*	0,75	g
Fray Bentos	0,75	g
Yeast ⁺	1,0	g

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Fructose	5,0	g
Ascorbic acid	0,1	g
Nipagin M	0,1	g
Water	35,0	ml

* Heated in 10 ml water to dissolve.

+ Live <u>5</u>. <u>utywala</u> <u>africana</u>, mixed with 10 ml water and brought momentarily to the boil to kill yeast. This procedure was followed in all subsequent diets.

This particular diet was the most successful in the initial series of diets tested. It was capable of sustaining sixday old larvae up to the age of 25 days, although many died before pupating. Further details can be found in Table 6 below.

<u>Table 6</u> .	Details of <u>C</u> . <u>za</u>	<u>istrowi</u> larvae	fed	Diet	1	from
	the six-day old	stage.				

Larva	Larval period	Pupal period
		•
no.	(days)*	(days)*
1	28	9
2	9	-+
3	19	_+
4	15	_+
5	10	-+
6	Died wher	n 19 days old
7	Died wher	n 14 days old
8	Died wher	n 22 days old
9	Died when	n 20 days old
10	Died when	n 19 days old
11	Died when	n 19 days old
12	Died when	n 13 days old
13	Died when	n 19 days old
14	Died when	n 25 days old

- * Larvae in the same unit that were fed natural diets completed the larval and pupal stages in mean periods of 12,2 and 9,9 days respectively.
- + Adult did not emerge.

The other diets in this first series were even less successful, and although they produced no pupae, were able to sustain larvae often until 25 days old. In an attempt to improve the diet, other ingredients were either added to or substituted in Diet 1. These included liver extract (vitamin A and traces of other vitamins), honey (carbohydrates), fruit fly attractant (protein) and choline chloride (choline is required in fat metabolism and for pupation). However, survival to the adult stage was again poor, and only two diets were capable of sustaining a few six-day old larvae through to the pupal stage, as will be indicated below.

Diet 2. This consisted of:

Gelatine	0, 75	g
Fray Bentos	0, 75	g
Yeast	1,0	g
Honey	5 , D	g
Ascorbic acid	0,1	g
Nipagin M	0,1	g
Liver extract	0,1	ml
Water	35,0	ml

Results obtained with this diet can be found in Table 7.

<u>Table 7</u>. Details of <u>C</u>. <u>zastrowi</u> larvae fed Diet 2 from the six-day old stage.

Larva no.	Larval period (days)*	Pupal period (days)*
1	20	9
2	15	_+
3	Died whe	en 24 days old
4	Died whe	en 21 days old
5	Died whe	n 10 days old
6	Died whe	n ll days old

* Larvae in the same unit that were fed natural diets completed the larval and pupal stages in mean periods of 11,8 and 9,0 Diet 3. This consisted of:

Gelatine	0,75	g
Fray Bentos	0,75	g
Yeast	1,0	g
Fructose	5,0	g
Ascorbic acid	0,1	g
Nipagin M	0,1	g
Liver Extract	0,1	ml
Water	35,0	ml

The results obtained can be found in Table 8.

<u>Table 8</u>. Details of <u>C</u>. <u>zastrowi</u> larvae fed Diet 3 from the six-day old stage.

Larva no.	Larval period (days)*	Pupal period (days)*
1	15	10
2 3	15 10	_+ 9
4	Died whe	n 15 days old
5	Died whe	n 4 days old
6	Died whe	n 15 days old

- * Larvae in the same unit that were fed natural diets completed the larval and pupal stages in mean periods of 11,8 and 9,0 days respectively.
- + Adult did not emerge.

<u>Diet 4</u>. This diet is worthy of mention, as although no larvae pupated, it was capable of sustaining six-day old larvae for very long periods, in one case up to 47 days. Details of this diet are given below, and of larval survival, in Table 9.

Gelatine		0,5	g
Fray	Bentos	0,5	g

Yeast	3,5	g
Fructose	5,8	g
Ascorbic acid	0,35	g
Choline chloride	0,01	g
Fruit fly attractant	1,0	ml
Water	35,0	ml

<u>Table 9</u>. Details of <u>C</u>. <u>zastrowi</u> larvae fed Diet 4 from the six-day old stage. (No larvae pupated)

Larva no.	Larval period (days)*
1	38
2	11
3	46
4	45
5	47
6	11+

- * Larvae in the same unit that were fed natural diets completed the larval stage in a mean period of 12,5 days.
- + Larva escaped when 11 days old.

<u>Diet 5.</u> Following a publication by Vanderzant (1969) the gelatine diets were again modified. The main protein source was changed to enzymatic casein hydrolysate. Fray Bentos and Nipagin M were omitted from the diet, and soy bean lecithin was added (lecithin contains choline and fatty acids). Various combinations were tested, the best one consisting of:

Enzymatic casein hydrolysate	1,0	g
Soy bean lecithin	0,5	g
Yeast	2,0	g

Fructose	З,О	g
Ascorbic acid	0,2	g
Choline chloride	0,1	g
Water	25,0	ml

The results obtained are to be found in Table 10.

<u>Table 10</u>. Details of <u>C</u>. <u>zastrowi</u> larvae fed Diet 5 from the six-day old stage.

Larva no.	Larval period (days)*	Pupal period (days)*
1	21	9
2	10	11
3	18	10
4	Died when	8 days old
5	Died when	l6 days old
6	Died when	15 days old

* Larvae in the same unit that were fed natural diets completed the larval and pupal stages in mean periods of 12,9 and 11,1 days respectively.

<u>General Discussion</u>. In summarizing the results of these experiments it is clear that many of the diets tested are capable of sustaining six-day old <u>C</u>. <u>zastrowi</u> larvae for periods ranging from weeks to more than a month, but the few pupae and even fewer adults obtained indicate that some nutritional factor probably linked with pupation, is lacking. Of the diets tested, No. 5 emerges as the best, with No. 3 a close second. However, one should be careful in evaluating these results as the small numbers of larvae used in these tests could lead to misleading interpretations. Nevertheless, Diet 4 should be noted for its ability to sustain larvae for up to four times the normal larval period.

It is evident that the inclusion of enzymatic casein hydrolysate and/or soy bean lecithin to any diet is beneficial; these two products were not at the time available locally, and had to be imported from America. Further observations are that liver extract may also be used to good effect, and that honey could possibly b_y^g substituted for fructose. The necessity for the inclusion of choline chloride is open to question, following the relatively good results of Diets 1 and 3. Choline chloride could probably also be omitted from a diet containing soy bean lecithin.

The survival of <u>C</u>. <u>zastrowi</u> larvae and pupae on Diet 5 (three adults from six larvae) is encouraging when it is borne in mind that Vanderzant obtained an average of 66% adults from larvae reared on her artificial medium. But the main problem encountered was in the presentation of the liquid to the larvae. Despite the fact that fresh diet was provided each day, the liquid still fermented rapidly when exposed to air, and in addition the use of sponges was messy and unsatisfactory, with a number of larvae drowning in the liquid which inevitably formed around the base of the sponge.

The only other simple method of presenting the liquid to the larvae is that employed by Hagen & Tassan (1965), whereby the liquid diet was manually encased in wax droplets while the wax was still molten. Although this method was not attempted in this study it has since been demonstrated to be unsatisfactory for mass-rearing (S.W. Broodryk, Gatooma Experiment Station, Rhodesia, 1971, personal communication). The last remaining alternative is to produce wax balls such as were mentioned by S.W. Broodryk in a personal communication, 1971, (Ridgway, 1971, unpublished data), but this method involves the use of sophisticated equipment.

Bearing the above problem in mind, and considering the protracted larval periods which resulted, as well as the unsatisfactory percentage of adult recovery, the search for an artificial diet for \underline{C} . <u>zastrowi</u> larvae was discontinued. It was clear that a satisfactory substitute for live larval food, which fulfilled the requirements mentioned at the beginning of this section, would only be found with considerable difficulty.

3.3.7 Anal Substance

Although <u>Chrysopa</u> larvae do not excrete, a liquid secretion from the anal region is given off by all instars. In the lst instar of <u>C</u>. <u>zastrowi</u> the liquid is almost colourless and small in quantity and therefore not very obvious but as the larva gets older the secretion becomes more copious and conspicuous as it changes to first a straw colour and then to yellowish-brown with a tinge of red. This substance was observed to have five more or less distinct functions, discussed below.

- If the larva is disturbed or agitated, for example i) with the tip of a pencil, it will often immediately secrete a drop of this fluid, sometimes onto the irritating object itself. This action, and the fact that it has been found, through personal experience to have a bitter, unpleasant taste, suggests that this may be a defence mechanism. This suggestion is supported by an observation made by Kennett (1948). He liberated <u>C. californica</u> (= <u>C. carnea</u>) larvae onto a shrub infested with aphids, which had large numbers of the Argentine ant, Iridomyrmex humilis (Mayr) in attendance. When liberated in the presence of these ants, all larval instars of <u>C</u>. <u>californica</u> were susceptible to attack by one or more ants. Second and 3rd instar larvae succeeded in repelling attacking ants by bringing the tip of the flexible abdomen into contact with the ant's head, at which time anal substance was secreted. The secretion was very repulsive to the ants, which usually released the larvae, and if a large amount of substance was secreted the ants were apparently paralysed for up to 15 minutes. Very young larvae, by virtue of their size, had difficulty in bringing their abdomens into contact with the ants, and were more susceptible, while no 3rd instar larvae were observed to succumb, and were hardly bothered by the ants.
- ii) A drop of this substance is also secreted from the anus to anchor the larva to the substrate shortly before the first or second moult. In these cases the larva suspends itself from the sidewall or roof of the cage, a droplet of the substance is deposited onto the surface and the last abdominal segment held in the fluid. After setting, the secretion is able to support the larva when it releases its foothold to moult. After moulting the old skin is left hanging from this spot.
- iii) Dead larvae are often found suspended by the last abdominal segment from the walls or roof of the cage

as if in the process of moulting.

- iv) Smith (1922) and Spiegler (1962) mention the use of this sticky substance in ordinary locomotion by the larva and also when feeding on struggling prey. Tjeder (1966) states that the anal papilla is adhesive and functions as a seventh leg. This was also evident in the case of <u>C</u>. <u>zastrowi</u> where the anus was used to give extra traction, especially when walking on vertical or horizontal surfaces, or when extra stability was needed, for example when feeding on a large aphid.
 - About a day before pupation the mature larva secretes v) some of this fluid even though unprovoked. This was also observed by Whitehead (1957) and by Toschi (1965); the latter states that a substantial amount of secretion is produced by <u>C</u>. <u>carnea</u> shortly before all moults. Spiegler (1962) has shown that the Malpighian tubules are responsible for the production of this fluid, while the proctodaeum is responsible for its conduction into the silk reservoir which lies just in front of the rectum. The substance is then stored here until needed. Spiegler points out that silk production is also effected by the Malpighian tubules, and that silk and the adhesive substance may be closely related. He further suggests that they may both originally be the same substance, but that the physical action of spinning could change the adhesive substance into silk. Spiegler also suggests that as the adhesive substance has been shown to be proteinaceous, and therefore nitrogenous, it represents an end-product of nitrogenous metabolism. He is in other words implying that the adhesive substance could be connected with excretion.

3.3.8 Moulting

For some hours before a moult the larva becomes passive and does not feed. If provoked it merely moves out of the way without any characteristic defence or avoidance reaction. Just prior to the onset of the moulting process, the anal pad is glued to the substrate by a reddish-brown anal substance. The larva lies quite still, and it could in fact almost be mistaken for dead --- larvae frequently moulted on the roof of the cage, and similarly dying larvae were also often noticed "glued" to the roof by anal substance.

The moult is initiated by a series of intermittent, longitudinal stretching movements, and it almost seems as if the larva is trying to break its anal pad free. At this stage of the moult, the red tips of the new mouthparts can be seen inside the "old" mouthparts, about two thirds of the way down their length. It appears that as a result of the stretching movements, the new anal pad and a portion of the abdomen come free from the "old" cuticle and move up slightly within it. The empty tip of the old cuticle of the abdomen can clearly be seen as the larva continues to push away from the attached anal pad.

Suddenly, a split in the old cuticle appears just above the thorax, and spreads within a second or two to the head and to about halfway down the abdomen. As the larva rapidly arches its thorax and part of its abdomen out of the old cuticle, the head and mouthparts are forced to lie ventrad underneath the larva in a doubled up position. The head, mouthparts and legs are then extricated relatively slowly --- the larva experiences a fair amount of difficulty in this last manoeuvre. The abdomen is drawn out simultaneously, and the larva then lies prone next to the cast skin with its abdomen tip still lying in the empty shell. The whole process from the split to when the last appendage

has been drawn out, lasts less than two minutes.

The larva, still lying prone next to the skin, inflates itself immediately to expand its appendages. After flexing these parts for some five minutes, it suddenly removes the end of its abdomen from the old cuticle and stands on its legs. Its head and all appendages are translucent, except for the tips of the mouthparts, which are a light reddish-brown colour. The head-markings are not evident at this stage. The colouring of the abdomen and thorax are similar to that just before the moult , but lighter in colour. The larva remains next to the cast skin for about fifteen minutes, after which it moves hurriedly away.

The moulted larva does not eat for some time, at least until its new cuticle has hardened and its mouthparts become sclerotized.

3.3.9 Excretion

As mentioned earlier, <u>Chrysopa</u> larvae do not excrete. This is due to the fact that the lumina of the middle and hind intestines are not continuous in the larval or pupal stages. It is continuous in the adultstage, and excrement accumulated during the immature stages is voided soon after the adult emerges. It is excreted in the form of a pellet (Balduf, 1939).

3.4 PREPUPAL STAGE

3.4.1 Description

In the case of Chrysopa the term "prepupa" refers to the

mature 3rd instar larva which is ready to spin and finally pupate. The prepupal period lasts from approximately the commencement of spinning until the final larval moult which takes place in the cocoon.

The prepupa of <u>C</u>. <u>zastrowi</u> is predominantly a yellowishstraw colour, and to all intents and purposes is identical to the 3rd instar larva. Mature larvae become passive and stop feeding about a day before spinning, and frequently assume an attitude where the abdomen is drawn right up to the thorax, giving the individual a wrinkled, hunch-backed appearance. In this attitude the larva looks shorter than its about 10 mm length. If disturbed at this stage it merely shuffles out of the way without much sign of aggression or defence.

3.4.2 Behaviour

The prepupa usually seeks out a corner or some place offering shelter, such as a piece of paper. The pieces of paper on which eggs were presented to the larvae in the Poly Top method of rearing appeared to make ideal sheltering places, and cocoons were usually spun under one of these papers. In the Tray method many cocoons were spun under the filter paper circles provided. A prepupa thus chooses a place offering a satisfactory amount of anchorage and protection. In cotton fields for example, cocoons were often found on the ventral surfaces of cotton leaves in the niches formed by the junction of the well-formed leaf veins, or in partially opened cotton bolls. Once a suitable position has been located, spinning is commenced by the secretion of a white silken thread from the anus. Initially, widely spaced threads are anchored to the substrate in a rough circle of 2 to 3 mm radius around the prepupa. Often bits of trash lying around the cage, such as old aphid skins, are incorporated into the structure, although <u>C. zastrowi</u> larvae are not trash carriers. Smith (1922) and Tjeder (1966) mention that broken-off setae from the prepupae of some <u>Chrysopa</u> spp. are also incorporated into the cocoon. The tendency of prepupae to add trash to their cocoons was also observed in <u>C. carnea</u> by Toschi (1965).

When this rough framework is completed (the prepupa is still clearly visible at this stage) the inner layer, or cocoon proper, is started and it is at this stage that the dexterity of the prepupa's abdomen can be observed. It lies curled up and manoeuvres its abdomen to virtually any position within the framework of threads already constructed. The last few abdominal segments are telescopic and very flexible. After every 2 or 3 mm the abdominal apex presses the silken thread against a part of the framework, or even on occasions against the prepupa's own body, and is held there for a few seconds before moving on to the next site of attachment.

After about fifteen to thirty seconds the prepupa changes it position and spins from another angle. The whole spinning operation appears to be one of haste, with the prepupa almost impatiently seeking out the best point of

attachment for the thread. By continually changing position the prepupa spins a spherical or slightly oval cocoon, white in colour and about 4 mm in diameter, depending on the size of the prepupa at the time of spinning (see Fig. 9). After about twelve hours the prepupa can be seen curled up in a C position, with the tip of the abdomen almost touching the head, in such a way that the long axis of the prepupa lies in the longer axis of the cocoon. While in this position it moves the tip of its abdomen from side to side around the latitude of the cocoon in arcs of about 1 mm, obviously in the act of spinning. After about half a minute it changes position either laterally or longitudinally and continues spinning in the above-mentioned side to side manner. It therefore seems probable that most of the silk is laid down around the circumference of the cocoon.

The entire spinning process lasts between 24 and 48 hours. Cocoons spun underneath bits of paper are usually slightly flattened where they make contact with the paper, and often on the side making contact with the substrate.

If an empty cocoon is studied carefully under a stereomicroscope it will be seen that the cocoon, discounting the rough framework, is made up of two layers --- a coarse outer layer and a finer inner layer. The outer layer can be quite easily peeled off, leaving the fairly smooth inner layer intact. If this remaining portion is studied under a magnification of about 40X, it is quite clear that the inside surface is not just plain cocoon silk, but is actually a smooth shiny coating of some solidified gelatinous - type substance.

If one looks in the vicinity of the "poles" of the cocoon, where it seems often to be only two or three threads thick, this same gelatinous-type coating can be seen in the spaces between individual threads. Furthermore, when the outside surface of the cocoon is studied, silk threads can be observed partly embedded in this coating, where they broke off when the coarse outer layer of the cocoon was removed.

Following this evidence, the writer would like to propose that at least once during the spinning process the larva secretes, in relatively large quantities, a silk-like substance which it then spreads around the inside surface of the cocoon. This substance could either be the balance of silk remaining in the silk reservoir after spinning, if indeed there is any left, or it could be the anal substance. Ιt is quite possibly the former, considering its colour and texture, in which case it would seem that the larva keeps back a quantity of silk especially for this purpose. The secretion is probably liquid enough to seep through a few layers of silk thread, thereby giving the cocoon extra rigidity and giving rise to the two distinct layers which were observed. The deposition of this material has further implications which will be discussed under Section 3.5.2.

Some authors have reported that certain <u>Chrysopa</u> species line the inside of the cocoon with a thicker silk which causes the cocoon to appear opaque (Smith, 1922; Toschi, 1965). Observations on <u>C</u>. <u>zastrowi</u>'s cocoon indicate that this may not be silk thread, but rather a silk coating. After four days the 3rd larval moult takes place in the cocoon. Toschi (1965) also records this period as an average of four days in the case of <u>C</u>. <u>carnea</u>. As in other <u>Chrysopa</u> spp. (Burke & Martin, 1956; Toschi, 1965), the larval skin is pushed to the back of the cocoon where it appears as a dark circular disc.

3.5 PUPAL STAGE

3.5.1 Description

The external morphology of the pupa can be fairly easily seen if the cocoon is studied carefully under a microscope. The pupa is at first a light yellowish colour, but darkens to predominantly green with time. The head, with metallic bronze-coloured eyes, can be seen with thorax and abdomen curved beneath. Two wing-sacs are present laterally, but these are not always clearly visible through the cocoon. The legs lie in the curve of the thorax and abdomen.

It did occasionally occur that no cocoon was spun, the prepupa instead undergoing the final larval moult naked on the floor of the cage. Many of these pupae died, but some were capable of completing development to the adult stage. While alive these pupae usually lay quite still, although if disturbed squirmed for a few seconds.

3.5.2 Behaviour

<u>Chrysopa</u> <u>zastrowi</u> pupae are capable of limited movement inside the cocoon. If the cocoon is dented or the pupa disturbed in any way, the pupa usually wriggles sideways to another position.

Unlike many other insects, it is not the adult Chrysopa which emerges from the cocoon, but the pupa. The emergence from the cocoon is through a very neat circular, hinged lid which is made at the opposite end to the disc of larval skin. There is a certain amount of controversy as to the formation of this lid. Some authors suggest that the lid is cut by the pupa's mandibles (e.g. Balduf, 1939), while others are of the opinion that it is merely pushed off by the pupa (e.g. Clausen, 1940; Whitehead, 1957), Laboratory experiments similar to those conducted by Whitehead demonstrated that a perfectly natural lid could be pushed out manually from either end of the cocoon. In this case the wooden tip of a small paint brush, whittled down to a suitable size and rounded off at the top, was inserted into the cocoon and pressure exerted until a cap was pushed off. It was in all respects identical to lids on cocoons from which pupae had emerged naturally. Whitehead achieved similar results.

The writer is of the firm opinion that the lid is made solely by pressure from the pupa within the cocoon. Furthermore, a close look at the construction of the cocoon suggests why the tear occurs around the latitude of the cocoon and not for example across the top or down the side. If the two ends of the more or less oval cocoon are considered as the poles, the majority of the threads lie in a circular fashion around the latitude of the cocoon. It

is therefore logical that the split should occur as it does, and not down the length of the cocoon. To do the latter, it would have to break the threads across their "grain", so to speak. Smith (1922) was then probably correct when he suggested that the parting of the lid from the cocoon was connected with the spinning of the cocoon.

The writer would also like to suggest that the deposition of secreted material around the inside surface of the cocoon, as proposed in Section 3.4.2, is partially responsible for the neatness of the lid. Without a firm backing it is doubtful whether the edges of the cocoon's hole and lid would be as precise as they are. The likeness of lid formation from an ordinary cocoon without the coating, and from a cocoon with this deposition, probably compares respectively with tearing a piece of paper on the one hand, and a piece of celophane paper on the other.

After emergence from the cocoon the now mobile pupa moults, usually on a vertical surface, and this moult is essentially the same as a larval moult, with the old skin being split down the dorsal length of the thorax and spreading to the head. Some authors have mentioned that the pupal moult is a critical one in the life-cycle of various <u>Chrysopa</u> spp., and although mortality at this stage of <u>C. zastrowi</u>'s life was not uncommon, it was not as high as the 30 – 60% mentioned by Smith (1922).

3.6 ADULT STAGE

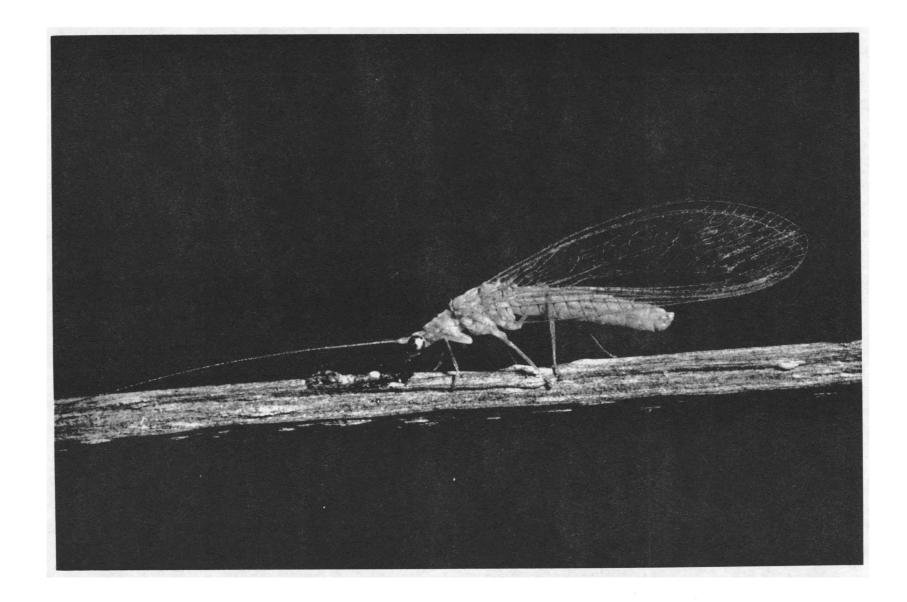
The newly emerged adult of <u>C</u>. <u>zastrowi</u> usually positions

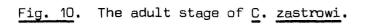
itself on a vertical surface where its crumpled wings are expanded. The pale, colourless wings take on their characteristic light green colour and gain their shape after about 20 to 40 minutes. During this time they remain quite motionless, apart from their antennae which they slowly wave around.

3.6.1 Description

The adults are very distinctive to look at, being a light yellow-green in colour, with metallic dark bronze coloured compaund eyes. When at rest the two pairs of many-celled wings, of approximately equal size and shape, are held roof-like over the abdomen and thorax (Fig 10). The total length of the body is between about 9 and 11 mm, with the abdomen 5 to 6 mm, and the filiform antennae about 8 to 10 mm in length, and consisting of about 65 segments. The wings are about 9 to 11 mm long, with hind wings slightly shorter than forewings. Wing veins are covered sparsely with short dark hairs. Legs and abdomen, especially in the genital region, are also covered with short dark hairs. Further features include a neck-like prothorax, and maxillary and labial palps which can be seen in action when the adult feeds.

It is usually quite easy to tell the two sexes apart with the naked eye especially after the females have mated and are ovipositing. Males are generally smaller (by a few millimetres) than the females, and after the females have commenced oviposition, their slightly swollen abdomens are noticeably thicker and longer than those of the males.





The sexual dimorphism of the genitalia has been adequately described by Tjeder (1966).

<u>C. zastrowi</u> adults do not give off the foul odour which has earned some species the common name "stinkflies" (Balduf, 1939).

3.6.2 Behaviour

<u>C. zastrowi</u> adults are generally nocturnal in habit, and during the hours of darkness become very active. Conversely, during daylight hours they are normally fairly passive, most of them preferring to cling to the ceiling of the cage. They tend to remain in one position for long periods, with only their antennae waving.

The adults are attracted to light, but this attraction seems to be selective, i.e. to certain wavelengths only. They are attracted to ordinary household light at night, but on two occasions when adults were being collected in a cotton field at night, they were not attracted to a car's headlights which were being used to illuminate the field. A Philips' Blue Black light trap also proved unattractive to C. zastrowi adults.

3.6.2.1 Feeding

<u>C. zastrowi</u> adults are not carnivorous, nor are they cannibalistic. (Toschi 1965, indicates that adults of some species devour their own eggs). There are some <u>Chrysopa</u> spp. in which the adults live on aphids, as intimated by Bänsch (1966), as well as other soft-bodied insects (e.g. mites) while other species will only accept an entirely liquid diet (Burke & Martin, 1956). Tjeder (1966) and Balduf (1939) also describe the carnivorous habits of some <u>Chrysopa</u> adults and Tjeder further adds that adults of certain species also feed on pollen, but it is not certain whether <u>C</u>. <u>zastrowi</u> falls into this group. Tjeder does however state that honeydew is the main diet of adults in the subgenus <u>Chrysoperla</u>, into which <u>C</u>. <u>zastrowi</u> falls. This species' mouthparts are definitely adapted for drinking, as this could clearly be observed during rearing.

Hagen, Tassan & Sawall (1970) mention a new concept in the feeding habits of <u>C</u>. <u>carnea</u>, which could also apply to other species. They found that the crops of <u>C</u>. <u>carnea</u> adults contain symbiotic yeasts of the genus <u>Torulopsis</u> (<u>C</u>. <u>carnea</u> adults are non-predacious and feed on honeydew and pollen). The authors suggest that the yeasts obtain sufficient nutrients to reproduce from the diet of <u>C</u>. <u>carnea</u> and in return provide <u>C</u>. <u>carnea</u> with the required aminoacids necessary for reproduction, and that are absent from honeydew. In view of <u>C</u>. <u>zastrowi</u>'s close relation to <u>C</u>. <u>carnea</u>, it is not unlikely that the same relationship between <u>C</u>. <u>zastrowi</u> and the symbiotic yeasts exists.

3.6.2.2 Mating and Oviposition

Mating is presumed to take place during evening or at night, as this activity was never observed during daylight hours. However, males were on various occasions during the day seen to vibrate their abdomens vertically, which

is part of the mating behaviour of some <u>Chrysopa</u> species, including the closely related <u>C</u>. <u>carnea</u>. Some <u>C</u>. <u>zastrowi</u> individuals were also seen licking or cleaning their genitalia during daylight, these also being activities normally associated with mating, and in particular the completion of copulation (Smith, 1922; Balduf , 1939; Toschi, 1965; Tjeder, 1966). Mating by <u>Chrysopa</u> spp. has been described by these authors.

After a pre-oviposition period of three to four days the females commence laying their eggs. Eggs are laid only during the hours of darkness, when the adults are most active. Eggs were never laid during daylight hours. Oviposition reaches a peak within about eight days, after which it gradually declines to zero.

The manner of oviposition is essentially the same as described by Smith (1922), i.e. a drop of colourless substance is deposited on the substrate by the tip of the female's abdomen immediately after which the abdomen is raised upwards about 4 or 5 mm. At this stage the egg is laid and becomes attached to the thread which has just been formed. The female pauses a few seconds supporting the egg while the thread hardens, and then releases it. Some stalks remained upright until the egg hatched while some bent over under the weight of the egg. Other stalks were so weak that the eggs actually lay on the substrate. It seems that these stalks either had not hardened sufficiently to support the weight of the eggs, or were thinner than the others, perhaps from being drawn out slightly more rapidly by the

female.

Oviposition abnormalities have been discussed in Section 3.1.

3.6.3 Fecundity and Longevity on Three Artificial Diets

As mentioned in Section 2.2, the standard adult diet used in this study was a mixture of brewer's yeast (<u>S</u>. <u>utywala</u> <u>africana</u> in granular form), honey and water mixed in a 5:6:15 ratio by volume. Plain water was also provided separately. For the size of the <u>C</u>. <u>zastrowi</u> colony that was maintained, female fecundity on this diet was sufficient, i.e. there was never a shortage of eggs under normal circumstances.

Fecundity was however poor when compared with results obtained by some overseas workers, who were able to achieve quite spectacular results using high sugar and protein hydrolysate concentrations. As early as 1950, Hagen (1950) obtained an average of about 25 eggs per female per day from 14 day old females which were fed honeydew and MRT, a synthetic protein hydrolysate high in amino-acid content. More recently Vanderzant (1969) obtained from 13,5 to 19 eggs per female per day over the whole life-span, using a synthetic diet of which casein hydrolysate, soy hydrolysate, Bvitamins and fructose were the main constituents. Hagen & Tassan (1970) were able to obtain an impressive average of 25 eggs per female per day over 30 days. To obtain this high fecundity they used Food Wheast (dried yeast, <u>5</u>. fragilis, plus Whey proteins), sucrose and water. As <u>C</u>. <u>zastrowi</u> fecundity was satisfactory under the conditions of this study, a detailed study of the requirements of an artificial diet was not initiated. A few smallscale experiments were however undertaken, and are described below.

<u>Materials and Method</u>. Three diets, designated Standard Diet, Fructose Diet and Casein Diet, were made up as follows:

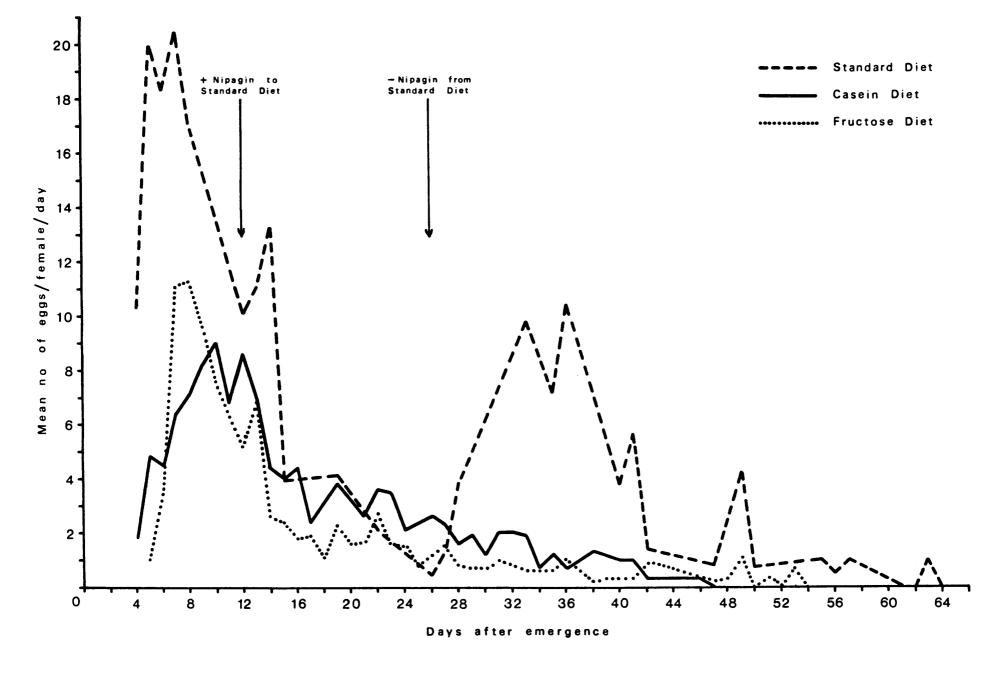
 <u>Standard Diet</u> --- Brewer's yeast (<u>S</u>. <u>utywala africana</u>), honey and water in a 5:6:15 by volume ratio. The yeast was dissolved and brought to boil as described in Section 3.3.6., and this procedure followed in all following diets.

For two weeks during this experiment, 0,5% Nipagin M was added to the diet to determine its possible fermentation suppressing effect.

- Fructose Diet --- Brewer's yeast, fructose and water in a 5:6:15 by volume mixture.
- 3. <u>Casein Diet</u> --- Brewer's yeast, enzymatic casein hydrolysate, honey and water in a 5:4:6:16 by volume mixture.

Plain water was provided separately with all diets. The liquids were presented to the adults in the glass tube and dental roll feeding tubes mentioned in Section 2.2.1.

Five males and ten females (all newly emerged) were placed with the food in cages made from inverted plastic cake covers, measuring approximately 25 cm in diameter and 12,5 cm high. A sheet of 2 mm perspex acted as the roof, and brown paper was provided on the ceiling and walls for oviposition. Fresh food was provided every two days and records kept of egg production and adult mortality. The experiment was conducted at 25 $\pm 1^{\circ}$ C and 55 \pm 5% R.H.



It is difficult to prove conclusively that it was the Nipagin alone which was responsible for the depressed fecundity. Nipagin does however cause anomalies in the rearing of other insects. Larval mortality of 100% resulted when 0,8% Nipagin was included in an artificial diet for the pink bollworm, and 0,4% Nipagin in the diet significantly lengthened the larval stage of this insect (Ouye, 1962). Larval developmental time of the cabbage looper increased when 0,3% Nipagin was added to the diet (Kishaba, Henneberry, Pangaldan & Tsao, 1968). With these facts in mind, it is not unlikely that the Nipagin was entirely responsible for the observed affect in this experiment. It is interesting to note from Fig. 11 that the oviposition-inhibiting effect of Nipagin is not lasting once the chemical has been omitted from the diet, and no permanent harm seems to be done to the process of oögenesis.

Analysis by the t-test shows that <u>C</u>. <u>zastrowi</u> females fed the Standard Diet lay significantly more eggs per female per day than those fed either the Casein or the Fructose Diet, despite the very poor oviposition which took place while Nipagin was added to the Standard Diet. The fructose and Casein Diets do not differ significantly from one another in this respect.

It is not easy to give a definite explanation for the relative success of the Standard Diet, or conversely for the poor performances of the other two diets. The failure of the Fructose Diet can perhaps be explained by the fact that while fructose is pure carbohydrate and virtually

nothing else, honey is more complex in its make-up. Besides equal quantities of glucose and fructose, it contains levulose, nitrogen, yeast and pollen (R.H. Anderson, Apicultural Section, University of Stellenbosch, Stellenbosch, 1972, personal communication). One or more of honey's constituents may be needed for satisfactory oögenesis.

The poor oviposition obtained from females fed the Casein Diet was completely unexpected. Enzymatic casein hydrolysate is a rich source of protein, something the other diets lacked, and yet it was only slightly better than the Fructose Diet, although not significantly so. It is possible that the protein concentration in the Casein Diet was too high, and that this in some way interfered with oögenesis.

In general, females fed the Standard Diet showed the potential to lay fairly well (more than ten eggs per female per day) even after a month. In the case of the Fructose and Casein Diets, oviposition dropped to a low level about two weeks after adult emergence --- to less than 4,5 eggs per female per day for Casein, and to less than 3 eggs per female per day for Fructose.

These few experiments are far from conclusive, and this aspect of mass-rearing <u>Chrysopa</u> is one which deserves a lot of attention as oviposition is obviously of the utmost importance, especially in cases where it is desirable to obtain very large numbers of eggs, such as for the field distribution of eggs with the aim of increasing the size of the natural population.

The writer feels however that in caseswhere very large numbers of eggs are not of the utmost importance, for instance where it is desired to study <u>Chrysopa</u> in the laboratory, the Standard Diet used in this study is perfectly sufficient, and has the advantage in that it consists of ingredients which are very easily procurable.

Table 12 shows the progression of mortality of the <u>C</u>. <u>zastrowi</u> adults fed the three diets. The Standard Diet was characterised by a fairly steady mortality rate over a period of 66 days in the case of the females, and a fairly rapid mortality rate over a period of 40 days in the case of the males. Adults on both the Casein and Fructose Diets showed a low mortality rate for the first five or six weeks, which then suddenly increased over the remaining two or three weeks.

The Fructose Diet was particularly notable for the fact that, except in the case of one male which died on the day of emergence, no mortality occurred during the first four weeks.

3.6.4 <u>A Colour Preference shown by Ovipositing Females</u>

During the course of this study it happened by chance that one of the ceiling papers put in the adult cage for oviposition purposes was discoloured green in one patch --- the paper in this case was plain white. When taken from the adult cage to remove the eggs it appeared that proportionately fewer eggs were laid on this green patch.

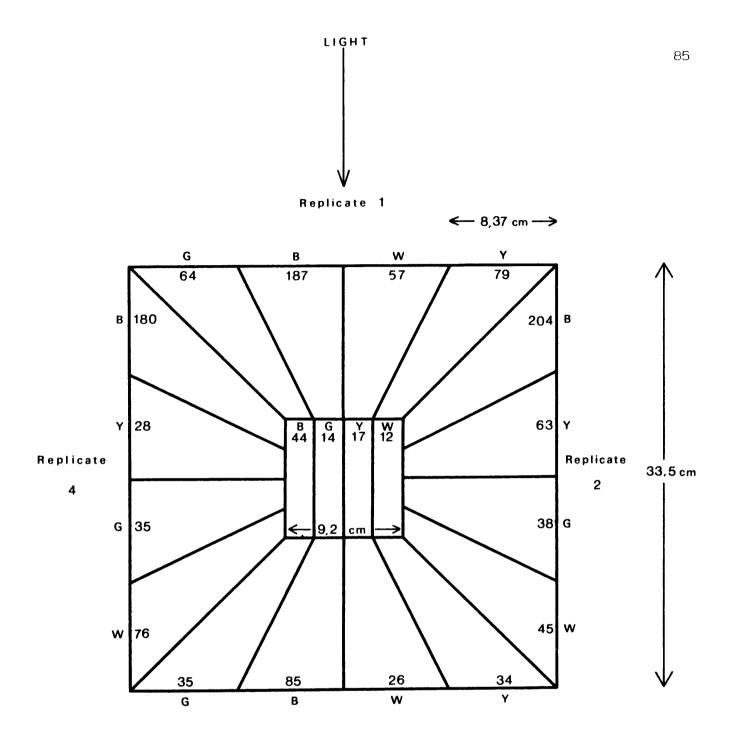
In order to test whether the females did in fact show

<u>Table 12</u>. Weekly progression of mortality of <u>C</u>. <u>zastrowi</u> adults when fed either the Standard, Casein or Fructose Diet.

	No. of survivors on each diet						
Days after	Stan	dard	Casein		Fructose		
emergence	Ŷ	07	\$	5	ę	57	
D	10	5	10	5	10	5	
7	7	3	9	4	10	4	
14	7	3	9	4	10	4	
21	7	2	8	4	10	4	
28	6	2	8	4	10	4	
35	6	l	8	4	10	3	
42	5	D	7	3	8	2	
49	3	٥	2	1	4	2	
56	2	ο	0	0	1	2	
63	1	Ο			0	0	
70	0	O					

a colour preference, an experiment was carried out whereby ovipositing females had a choice of four colours on which to lay their eggs.

<u>Materials and Method</u>. Four different colours were painted onto a sheet of white paper 33,5 cm square in the pattern shown in Fig 12. The four colours, brown, green, yellow and white poster paint (powdered) were each represented in the central block, as well as along each of the four sides of the paper. The central block of colours was included to avoid an area of convergence of colour in the centre, in which the colours would be so close together that the chances would be great that a female would see



Replicate 3

B = 700;

Totals: G = 176;

W = 216; Y = 221.

- B=Brown G=Green W=White Y= Yellow
- Plan of the coloured ceiling of the C. zastrowi oviposition Fig. 12. cage showing the arrangement of colours, the number of eggs laid on each colour wedge, and the total number of eggs laid on each colour. Digitised by the Department of Library Services in support of open access to information, University of Pretoria, 2020

one colour but lay on an adjacent one.

Disregarding the central block, the allocation of colours to each of the four sides was similar to that of a Latin Square Design with four treatments and four replicates. Table 13 shows <u>inter alia</u> how the colours would bave been allocated if arranged in a conventional Latin Square Design. Each row corresponds to a side of the paper, and the columns represent the fact that each colour was represented once only in each of the four possible positions on a side. By using the method of allocation shown in Fig. 12, errors due to any preference shown by the females for any particular part of the paper, such as the corners (which was in fact a tendency) are thus reduced considerably as is error due to the unidirectional light source.

It was for the very reason that there seemed to be a preference shown by females to oviposit in corners that the conventional Latin Square Design was not chosen. If it had been used, an error could have been introduced in favour of the colour(s) in the preferred corner(s).

The cage was left undisturbed during the experiment, and after two nights the paper was removed and counts made of the eggs on each colour wedge. Eggs laid on the central block were also counted, although this could not serve as a replication.

<u>Results and Discussion</u>. The analysis of variance of the transformed values in Table 13 can be found in Table 14. For the purpose of analysing the number of eggs laid in Table 13. Position of the colours of each replicate (= side of oviposition paper) from Fig. 13, when arranged in a conventional Latin Square Design. Numbers of eggs laid on each colour, with log. transformations in parenthesis, are also given.

Row

				Totals
G 64 (1,8062)	B 187 (2,2718)	W 57 (1,7559)	Y 79 (1,8976)	387 (7,7315)
B 204 (2,3096)	Y 63 (1,7993)	G 38 (1,5798)	W 45 (1,6532)	350 (7,3419)
Y 34 (1,5315)	W 26 (1,4150)	B 85 (1,9294)	G 35 (1,5441)	180 (6,4200)
W 76 (1,8808)	G 35 (1,5441)	Y 28 (1,4472)	B 180 (2,2553)	319 (7,1274)
378 (7,5281)	311 (7,0302)	208 (6,7123)	339 (7,3502)	1236 (28,6208)

Column

Totals

Treatment Totals

G	=	172	(6,4742)
В	=	656	(8,7661)
W	=	204	(6,7049)
Y	=	204	(6,6756)
	3	1236	(28,6208)

corner wedges versus those laid in central wedges, the two corner wedges and two central wedges of each replicate (see Fig. 12) were pooled, so that each replicate was considered as comprising one large corner wedge (e.g. G + Y in Rep. 1) and one large central wedge (e.g. B + W in Rep. 2). This data appears in Table 15, followed by the statistical analysis in Table 16.

<u>Table 14</u>. Analysis of variance of transformed values appearing in Table 14.

Source of Variation	DF	SS	MS	F
Rows Columns Treatments (colours) Error	3 3 3 6	0,2271 0,0972 0,8729 0,0711	0,2910 0,0119	44 , 5**
Total	15	1,2683		

S.E. = 0,1091 C.V. = 6,10%

Between-treatment Comparisons

Comparison	Linear Function	Divisor	DF	SS	F
Brown vs. White	8,7661 - <u>6,7049</u> 2,0612	8	1	0,2577	16,6**
Green vs. White	6,7049 <u>6,4742</u> 0,2307	8	1	0,0067	O,56 (NS)

<u>Table 15</u>. Summary of the number of eggs laid in corner and central wedges (from Fig. 13).

Wedge	No. of	No. of eq	Mean			
Position	Replications	Rep. 1	Rep. 2	Rep. 3	Rep.4	
Corner Centre	4 4	143 244	249 101	69 111	256 63	179,3 129,8

Table 16. Statistical analysis of numbers of eggs laid in corner wedges versus those laid in central wedges as indicated in Table 15 (From Snedecor, 1956,Section 4.3).

Wedge Position	No. of Reps.	DF	Mean no. of eggs	SS
Corner Centre	4 4	3 3	179,3 129,8	152747 86027
<u></u>		Total = 6	Difference = 49,5	Total = 238774

Pooled Mean Square	= 3	19795,7
Sample Standard Deviation of Difference of		
Means	=	141,067
t (6 DF)	=_	<u>49.5</u> 141,067
	=	0,35 (NS)

The analysis in Table 14 shows that the number of eggs laid on brown is highly significantly greater (at the 1% level) than those laid on either of the other three colours, while the number laid on green, yellow and white do not differ significantly from each other. Although the central block was not included in the analysis, oviposition on these colours follows the same pattern, i.e. most on brown, with little difference between the other three colours.

Further analysis of the data (Table 16) shows that there was no significant difference between the numbers of eggs laid in corner wedges and those laid in the central wedges. This demonstrates that the design of the experiment successfully eliminated any preference that might have been shown by females to lay in corners.

One can only theorize in seeking to explain this apparent phenomenon of colour preference. It has been mentioned that light, at a very low intensity, filtered into the cage during the two nights of the experiment. This means that light would have been reflected by the four experimental colours, although once again, at a very low intensity.

Insects are characteristically sensitive to the shorter wavelengths of light, i.e. at the ultra-violet end of the spectrum. Different insects also show one or more peaks of sensitivity along the course of the spectrum, some of these peaks occurring in the ultra-violet section.

It therefore seems possible that <u>C</u>. <u>zastrowi</u> females were sensitive to one or more wavelengths of light reflected by brown but not by the other colours, and that they were furthermore capable of detecting this light at very low intensities.

Although this seems the most likely explanation, it could also be argued that differential heat reflection by the

colours played a part, or that the brown paint had an attractive smell, or conversely that the other colours possessed an irritant in the paint that brown did not. The writer however doubts that the paints would have differed to this extent in their composition, as all four were of the same make and differed presumably only in the pigment used. It is however difficult to preclude the possibility that one or more of the above factors could have been involved.

As indicated earlier, oviposition by \underline{C} . <u>zastrowi</u> on plain white paper followed no particular pattern except that often there was a concentration of eggs in one or more corners.

In summing up it can be said that this experiment indicates a strong likelihood that <u>C</u>. <u>zastrowi</u> females do in fact show a colour preference. Whether the attractive colour actually stimulated oviposition, or was merely attractive to the females, is not certain.

3.6.4.1 Suggestions for Further Research

There are a number of shortcomings in this experiment. If further research were undertaken to follow up this interesting behavioural response, it will be helpful to bear these in mind when planning such an experiment. For this reason the following suggestions are offered:

 Although the Latin Square Design efficiently reduces error due to the unidirectional light source, and also due to the apparent preference of ovipositing females for corners, a circular cage mounted on a turn-table 밋그

and turning at a speed of about one revolution per half hour, is suggested. This would effectively eliminate these sources of error.

- 2. It may be advisable to experiment with a number of different brands of paint in order to ascertain the possible effect of irritants or repellents that may be present in some paint. With this aspect in mind it may also be advisable to make up the many-coloured oviposition sheet with pieces of paper already colourprocessed. This would avoid the use of paint which may be more of a source of repellent chemicals than the coloured paper.
- 3. As a control, each of the colours used should be presented to the adults separately and oviposition patterns compared with the main experiment.
- 4. An identical experiment using the many-coloured oviposition sheet should be conducted in a room from which all light is totally excluded. This will help determine what role light reflection plays in colour preference.

If, following future research, it can be determined to what stimulus ovipositing <u>Chrysopa</u> females respond, it may be of more than mere academic interest. By artificially introducing such a stimulus (if this turns out possible) into selected areas, it may for example be possible to induce females to concentrate their egglaying in a localized area where aphids or mites etc. occur in high numbers.

Implementing this type of response could also have scope in other ways --- for example to obtain high concentrations of eggs in the laboratory for the evaluation of insecticidal effects, or for the purpose of mass-releasing eggs in the field.

To these and similar ends it may therefore be well worthwhile to continue research into this behavioural response exhibited by <u>Chrysopa</u> females.

3.7 DURATION OF THE STAGES

3.7.1 Life-cycle

Notes were kept for the period January, 1969, to December, 1970, in which durations of the incubation, larval and pupal periods of <u>C</u>. <u>zastrowi</u> at 25 [±] 1[°]C and 55 [±] 5% R.H. were recorded. During this period the Poly Top method of rearing was used, and the larval food source consisted primarily of <u>P</u>. <u>operculella</u> eggs, which were supplemented from time to time by one of the following: <u>B</u>. <u>brassicae</u>, <u>S</u>. <u>graminum</u>, <u>P</u>. <u>citri</u> and <u>S</u>. <u>cerealella</u> eggs. The adult diet remained constant and comprised the mixture mentioned in Section 2.2.1.2. Details of the life-cycle are presented in Table 17.

It can be seen in Table 17 that there is a wide range in the larval and pupal durations. In the case of the larvae this can be explained by the fact that more than one species Table 17. Life-cycle and natural mortality of <u>C.zastrowi</u> reared at 25[±] 1^o C and 55[±] 5% R.H., by the Poly Top method; comparisons with <u>C.carnea</u> reared at 25^o C and unspecified R.H. are given where relevant.*

Stage of development	Duration (days)			No. of	%
	<u>C.zastrowi</u>		<u>C.carnea</u>	test	natural
	Mean	Range	Mean	insects	mortality++
Eggs	4,0	3 - 6	4,2	14918	12,4 (296)
Larvae - 1st instar	4,0	4	4,2	10	_
" - 2nd instar	2,0	2	з,0	10	_
" - 3rd instar	4,3	3 - 5	3,5	10	_
" - all instars	11,5+	7 - 20	10,6	7 106	19,5 (809)
Pupae	10,2	5 - 17	8,8	5487	6,3 (651)
Adults (male)	33,3	3 - 62	-	30	-
" (female)	39,6	2 - 66	-	14	-

* Details from Butler & Ritchie (1970). Larvae were reared on S. cerealella eggs.

+ The mean larval duration of 11,5 days was determined from the mass-rearing records, in which the determination of individual instar durations was impractical. The duration of the instars was therefore determined in a separate experiment. For this reason the sum of the separate instar durations (10,3 days) does not exactly tally with the total larval duration (11,5 days).

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++ The number of individuals used in mortality determinations is given in parenthesis.

of prey was provided, and that on one or two occasions a shortage of larval food arose with the result that the larval duration in such cases became prolonged. However, these occasions were relatively few, so that the mean larval duration was not unduly affected. The cases of prolonged pupal duration cannot however be explained as there appeared to be little or no correlation between prolonged pupal duration and prolonged larval duration.

During the investigations on the life-cycle it became evident that there was a tendency for the respective durations of the egg, larval and pupal stages to increase slightly as the colony became older. This tendency was more apparent in the case of eggs and larvae and less marked in the pupal stage. Accurate records of the life-span of mass-reared adults were not kept due to the impracticality of keeping a record of each individual adult.

A break-down of the average incubation, larval and pupal periods reveals that the incubation period increased from three to four days when the colony was about two to three months old, the larval period from about eleven to thirteen days after ten months, and the pupal period from about ten to eleven days after slightly more than a year (Table 18).

One can only speculate as to the reasons for this increase in the duration of the development of the immature stages of <u>C. zastrowi</u>. The change could have been brought about by the relaxation of certain pressures occuring under natural conditions, such pressures perhaps ensuring that

Stage	Period	Mean duration (days)	% increase
Eggs	Jan. 1969 - Feb. 1969 March 1969 - Dec. 1970	3,1 4,0	29,0
Larvae	Jan. 1969 – Oct. 1969 Nov. 1969 – Nov. 1970	10,7 13,0	21,5
Pupae	Jan. 1969 - Feb. 1970 March,1969 - Nov. 1970	9,7 10,7	10,3

<u>Table 18</u>. The increase with time in the duration of the respective developmental periods of <u>C</u>. <u>zastrowi</u>.

the insect completes its life-cycle in as short a time as possible to enable the production of the maximum number of generations per season. A large number of generations per season need not necessarily always be advantageous to an insect, but in many cases this could be so. If for example an insect were confronted with some adverse factor such as parasitism, the survivors of a multivoltine species would probably be able to recover and build up their numbers again before the end of the season --- not so a univoltine species.

A second possible cause of this increase in the duration of the life-cycle could be the continuous inbreeding that occurred in the laboratory, which tends to result in homozygosity for various traits. Homozygous individuals show less behavioural homeostasis than heterozygous individuals (Boller, 1972). It is also possible that the vigour of the wild <u>C</u>. <u>zastrowi</u> strain was reduced by introducing it to laboratory conditions which in turn could have led to the observed phenomenon. This suggestion is supported by Boller (1972) who says that when wild strains are brought into the laboratory and reared artificially, there is usually a low survival of progeny during the first few generations, followed by an increase in survival after about 5 to 7 generations. This, he says, indicates that something very drastic has happened to the insect, and it suggests that the wild strain is not adapted to artificial conditions.

Intense selection follows for individuals that survive the best under the new conditions, consequently it is not irrelevant to suggest that during this selection individuals with a longer life-cycle could be selected. To quote Boller, "..... we force the culture genetically through a bottleneck and alter and probably reduce the level of genetic variability of the laboratory populations." A reduction in genetic variability, it can be argued, could presumably lead to the homozygosity discussed by Boller, and thus to lower behavioural homeostasis.

While the aforegoing may not exactly parallel the case with <u>C. zastrowi</u>, it cannot be divorced from it entirely, and much of what Boller mentions is probably relevant. Continual inbreeding, and therefore selection could have produced a genetically and behaviourally less vigorous strain, one of the manifestations of which was an increase in the duration of development of the immature stages.

Despite attempts to keep the temperature and humidity constant, deviations inevitably occurred, and these should be considered as being a possible cause of the increase in duration of development. However, the writer feels that the deviations in these conditions which may have arisen were not drastic enough to have exerted a significant effect on the duration of the life-cycle. They may nevertheless have been a contributing factor.

The light conditions were also not kept constant for the duration of the observations, and this may have affected the life-cycle. Initially the breeding room had no time-switch, and the lights were switched on and off manually with, an $8\frac{1}{2}$: $15\frac{1}{2}$ hour light : dark regimen. Later, when a time-switch was fitted, it was adjusted to a 12 hour day-length. This may or may not have been compatible with the larvae or pupae or adults, or all three stages.

Finally, it should be remembered that parasitism, predatism and virtually all cannibalism was excluded in the Poly Top rearing method. Consequently, a less vigorous <u>Chrysopa</u> individual (larva or adult) which might have succumbed in the field, would have a better chance of survival under laboratory conditions. A less vigorous strain could thus have been selected in this way, having a longer, or "less vigorous", life-cycle.

3.7.2 Effect of Low Temperature on the Incubation Period of C. zastrowi Eggs

While most <u>Chrysopa</u> species overwinter in the prepupal

stage within the cocoon, <u>C</u>. <u>carnea</u> is recorded as overwintering as adults (Balduf,1939). Being closely allied to <u>C</u>. <u>carnea</u> it is not inconceivable that <u>C</u>. <u>zastrowi</u> may also overwinter in the adult stage. During a relatively mild winter such as we experience in South Africa, it is possible that some <u>Chrysopa</u> spp. will be able to breed at a slow rate through the winter, especially in the warmer regions. (It has been demonstrated however that <u>C</u>. <u>carnea</u> males and females are induced to enter a facultative reproductive diapause by a decreasing photoperiod (Tauber & Tauber,1969), which may well be a limiting factor as far as winter-breeding is concerned). In the case where adults may breed through the winter, it would be of interest to know what effect low temperatures have on the egg stage. With this in mind, the following experiment was carried out.

<u>Materials and Method</u>. Batches of ten one-day old <u>C</u>. <u>zastrowi</u> eggs were subjected to 5, 10, 15 and 20^oC for a period of 14 days after which they were returned to a constant temperature and humidity of 25^oC and 50% R.H. Controls were kept at 25^oC and 50% R.H. for the entire incubation period. Each temperature treatment was replicated five times and the controls replicated four times. On emergence of the larvae, the total incubation period at 25^oC was recorded for each treatment, (i.e. the one pre-treatment day plus the remaining post-treatment days), as well as the egg mortality at each temperature.

<u>Results and Discussion</u>. The results at all temperatures except 20^oC followed the same pattern, i.e. the eggs at each temperature all hatched within a few hours of each other after varying periods at 25°C. Eggs kept at 20°C, however, behaved differently, and these results will therefore be dealt with first.

At 20°C eggs started hatching before the treatment period of 14 days had passed, viz. from the sixth to the tenth day of treatment (see Table 19). No eggs hatched before the sixth or after the tenth day. Over 90% of the viable eggs hatched on the eighth and ninth days.

<u>Table 19</u>. Hatch of <u>C</u>. <u>zastrowi</u> eggs during exposure to 20⁰C. Eggs were one-day old at start of treatment.

Days at	No. of larvae emerging per replicate per day Replicates					Total no. larvae emerging
20 ⁰ C	1	2	3	4	5	per day
6	-	_	-	-	1	1
7 8	- 7	- 9	-	1 4	6	27
9	2	-	5	4	-	11
10	-	-	1	-	-	1

Mean treatment mortality = 18% Mean control mortality = 15% Incubation period of controls at 25⁰C = 4 days.

The mortality and incubation period of eggs kept at the remaining temperatures, including the condensed data from Table 19, are presented in Table 20.

<u>Table 20</u>. Total incubation period and mortality of one-day old <u>C</u>. <u>zastrowi</u> eggs exposed for 14 days to four different low temperatures and then returned to 25[°]C and 50% R.H. The total period spent at 25[°]C is also given.

Temperature	Total incubation	Total period at	Mean %
(°C)	period (days)	25 ⁰ C (days)*	mortality
5	20	6+	60
10	19	5 ,+	24
15	17	3+	6
20	6 - 10	1++	18
25(control)	4	-	15

* Includes one day pre-treatment, plus post-treatment days.
+ Total incubation period, minus treatment period of 14 days.
++ One day pre-treatment period at 25°C.

Table 20 indicates that the incubation period at 25° C alone of those eggs exposed to 15° C for the treatment period, was three days, in comparison with four days for the controls. This would seem to indicate that embryological development was not entirely arrested at 15° C, but continued at a very slow rate with a resultant shortening of the post-treatment incubation period at 25° C. At 10° C and lower, however, embryological development is arrested completely and the embryo damaged by the cold so that there is lengthening of the post-treatment period, and therefore a lengthening of the total incubation period at 25° C. Mortality throughout the experiment was not unduly high down to 10° C, but increased sharply below this temperature.

An exposure of 20°C on the other hand (Table 20) merely

has the effect of slowing embryological development down slightly, and increasing the incubation period from four days to between six and ten days. This temperature had no adverse effect on mortality.

From all these facts one can conclude that the egg stage of <u>C</u>. <u>zastrowi</u> is well able to withstand relatively short periods of low temperatures down to about 10° C. Below this temperature a high percentage mortality occurs. The embryo continues to develop down to 15° C, although at a slower rate, while below this temperature development ceases entirely or nearly so.

In a series of short unreplicated tests, one-day old \underline{C} . <u>zastrowi</u> eggs were subjected to temperatures ranging from 0° C to -8° C for short periods of one to five days. The highest mortality of 57,5% was obtained when 47 eggs were subjected to -8° C for five days; the lowest mortality of 15,8% was obtained when 120 eggs were kept at -1° C for one day.

These figures indicate that \underline{C} . <u>zastrowi</u> eggs are not much affected by very short periods at freezing point and below. The fact that there was 40% survival at -8°C for five days is perhaps worthy of mention.

On the basis of the aforegoing data, the writer is of the opinion that in general our South African winters should not prevent <u>C</u>. <u>zastrowi</u> eggs from hatching.

3.7.3 Larval Development on Different Natural Diets During the course of this study on <u>C</u>. <u>zastrowi</u> it became clear that the rate of development of the larval stage could possibly be influenced by the larval diet. The following experiment was conducted in an attempt to verify this belief.

<u>Materials and Method</u>. Batches of <u>C</u>. <u>zastrowi</u> larvae were caged individually from eclosion in Poly Top cages and fed separately on different natural diets. The five prey species used were eggs of <u>S</u>. <u>cerealella</u>, eggs of <u>P</u>. <u>operculella</u>, and nymphs and adults of <u>S</u>. <u>graminum</u>, <u>B</u>. <u>brassicae</u> and <u>P</u>. <u>citri</u>. All batches were provided with an excess of the relative prey species so that the larvae were never without food at any time. The batches were kept under constant conditions of $27 \pm 1^{\circ}$ C and $50 \pm 5\%$ R.H. Observations were made on larval duration and pupation.

Results and Discussion. Table 21 summarizes the results.

<u>Table 21</u>. Rate of development and survival of <u>C</u>. <u>zastrowi</u> larvae when fed exclusively on one of five different prey species at 27 [±] 1 ^OC and 50 [±] 5% R.H.

Prey species per batch period (days)* pupation S. cerealella 13 9,0 100 P. operculella 4 9,8 100 S. graminum 5 10,8 100 B. brassicae 12 12,9 75 P. citri 14 14,4 50		No. larvae	Mean larval	%
S. <u>cerealella</u> 13 9,8 100 P. <u>operculella</u> 4 9,8 100 S. <u>graminum</u> 5 10,8 100 B. <u>brassicae</u> 12 12,9 75	Prey species	per batch	period (days)*	pupation
	<u>P. operculella</u> <u>S. graminum</u>	4 5	9,8 10,8	100 100 75

* Determined from hatching to spinning.

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Table 21 might suggest that <u>B</u>. <u>brassicae</u> and <u>P</u>. <u>citri</u> are inferior prey species from the point of view of larval development, possibly by virtue of being lower in nutritional value that the other three. There is however a different explanation for the increase in larval developmental period which occurs when <u>C</u>. <u>zastrowi</u> larvae are fed either of these two species.

<u>Planococcus citri</u>, especially adult individuals, secrete long wax threads over the dorsal surface of the body, and this wax was found to tangle up the larva's mouthparts, thus preventing the larvae from feeding freely. Entangled larvae often went for days without feeding, and larval mortality was consequently high (50%). Wax is also secreted by <u>B</u>. <u>brassicae</u>, but in a powdery form and to a much lesser extent than <u>P</u>. <u>citri</u>, but the wax nevertheless also appeared to interfere with the larval feeding process. Mortality, considering the intensive-care conditions which existed, was also fairly high (25%).

The remaining prey species had no such drawbacks, and this is reflected both in shorter larval periods and in percentage survival. It is perhaps significant that the two shortest larval periods were obtained when eggs were fed to the larvae, suggesting that these are more nutritious than <u>5</u>. <u>graminum</u>.

The fact that the shortest larval period was obtained on <u>S</u>. <u>cerealella</u> eggs, coupled with the fact that this insect is more economical to mass-rear than <u>P</u>. <u>operculella</u>, gives good cause to recommend <u>S</u>. <u>cerealella</u> as the best source of larval food if <u>Chrysopa</u> are to be reared on a large scale.

CHAPTER 4

FIELD STUDIES ON CHRYSOPA SPP. IN THE TRANSVAAL

4.1 SEASONAL OCCURRENCE OF CHRYSOPA SPP.

The seasonal occurrence of <u>Chrysopa</u> spp. was the subject of two similar experiments run more or less concurrently at the HorticulturalResearch Station, Roodeplaat, Transvaal. Two methods (described later) were used to trap adult <u>Chrysopa</u>, one running from September 1970 to September 1971, and the other from October 1970 to May 1971.

The numbers of <u>Chrysopa</u> spp. trapped were recorded daily in one method and weekly in the other. Due to the large numbers trapped it was impossible to have all specimens identified, but all <u>Chrysopa</u> caught could be placed into one of four broad categories based mainly on colour and size --- green, brown, black (all these about 10 to 12 mm long), and a large brown species (about 25 mm long).

Samples of the four groups were classified by Dr. Bo Tjeder, Zoological Institute, Lund University, Sweden, and consisted of the following:

i)	green	species:	<u>C. pudica; C. burgeonina</u> ;
ii)	black	species:	<u>C</u> . <u>jeanneli</u> (Nav.);
iii)	small	brown species:	<u>Chrysopa</u> (new species); family Berothidae;
iv)	large	brown species:	<u>Italochrysa</u> <u>exilis</u> (Tjeder)

The last named species, although in the same subfamily as <u>Chrysopa</u> (Chrysopinae), belongs to a different genus, and

was therefore not taken into account in this study. It should therefore be stressed that the graphs which will be presented later are based on the entire range of <u>Chrysopa</u> spp. present at Roodeplaat during the experimental period, which may include additional species to those listed above.

It may be added that whereas the green, black and small brown species all reached their highest peaks at the same time, <u>I. exilis</u> appeared briefly two months before the others, and was then hardly seen again.

Weather data for the 12 months under review were obtained from the Meteorological Section, Roodeplaat. Graphs for temperature, relative humidity, rainfall, windspeed and hours of daily sunshine will be presented later and an attempt made to correlate the weather conditions with the peaks of Chrysopa activity.

4.1.1 Determination of Seasonal Abundance

As mentioned earlier, two methods were used to determine the abundance of <u>Chrysopa</u>, and these are described below.

4.1.1.1 The Trap Net method

This method was the same as that used and described by Bot (1961). A fruit tree (in this case a Jubilee peach) was given a full coverspray three times a week with the following bait:

> mercaptothion 25% W.P. 250 g brown sugar 37,5 kg water 500 l.

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The tree was also sprayed the day following any rain, if this day did not already fall on a scheduled spraying day.

The nets consisted of four frames constructed from 6 mm roundbar, standing on legs about 23 cm high, and covered with 16-gauge mosquito gauze. About 7,5 cm above each 2,5 metre square net was placed a second frame of equal size but covered with 12,5 mm chicken mesh --- this was to prevent birds from eating the dead insects which were caught by the mosquito gauze. The mosquito gauze was bent up at the edges to form a 5 cm high shield around each net to reduce the number of insects blown away by the wind. Each set of four nets was made in such a way that a small triangular piece (with a base of 33 cm) was missing from one corner, so that when the nets were arranged with all the missing corners adjacent to one another, the result was one large net 5 metres square with a hole O,l metres square in the centre to accommodate the tree trunk.

Each day all the dead insects caught by the nets were collected and placed in paper bags marked with the date. Bags were collected weekly from Roodeplaat and all the <u>Chrysopa</u> spp. sorted from each bag. Daily catches were then counted and recorded.

The peach tree began to shed its leaves during April, and when there were no longer sufficient leaves to make trapping worthwhile, the nets were moved to an evergreen keiapple tree where spraying was resumed as before. The nets remained under this tree until trapping was discontinued in the first week of September 1971.

There were unavoidable occasions when no insects were collected, such as when rain washed off the bait, and when there was wind interference despite the wind shields. This made it impossible to plot weekly totals, as these would have presented a false picture. Consequently the average number of Chrysopa caught daily during each week was calculated by dividing the total number caught per week by the number of collection-days in that week. No collections were made over weekends, but those collected each Monday were considered as comprising the catches for three days (Saturday, Sunday and Monday). The smallest number of days on which the average daily catch was based was three, of which there were two cases. There were also two cases when the average was based on four days, the remaining averages being based on five to seven days.

The daily average catch for each week (= x) was transformed to log (x + 1). The value 1 had to be added to x as in some cases x was less than 1, and thus could not be converted directly into logs. The log transformation was applied to make the results comparable with those of the Bait Pot method (see 4.1.1.2) where the daily average catch for each week had to be transformed due to the wide range of <u>Chrysopa</u> numbers caught.

<u>Chrysopa</u> catches by the Trap Net method are reflected in Fig. 13, together with other relevant data to be discussed in following sections. 108

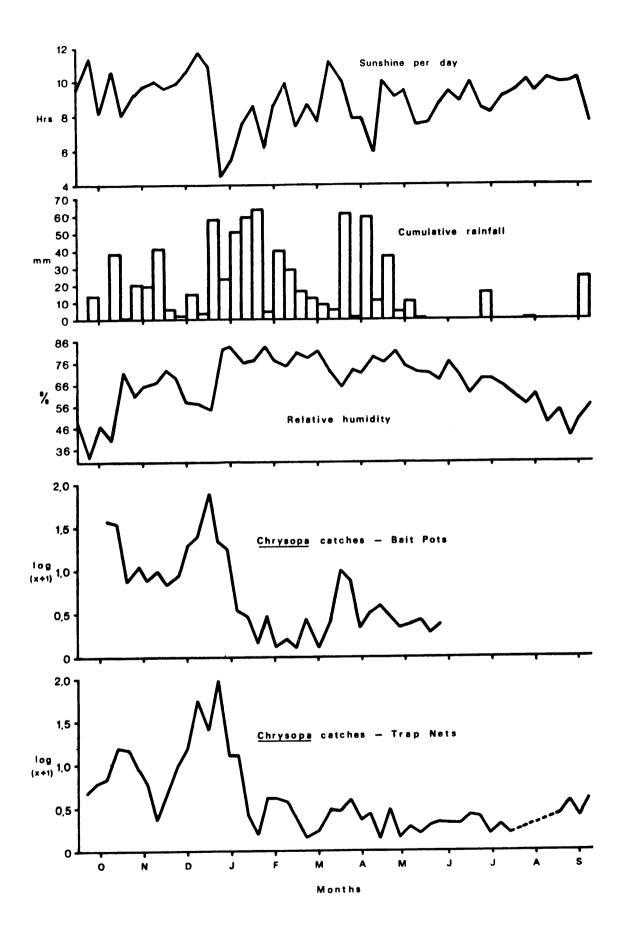


Fig. 13. Chrysopa spp. catches by the Bait Pot and Trap Net methods, compared with weekly mean weather conditions during the period September 1970 to September 1971. Daily average catches per week (x) have been transformed to log (x+1).

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4.1.1.2 The Bait Pot method

This method was also similar to that used by Bot (1961). One hundred clay pots measuring approximately 12,5 cm in diameter and 14 cm deep were filled with the following bait:

Trichlorfon	50%	W.P.	335.g	
Brown sugar			12,5	kg
Water			500 1	

The pots were distributed among ten different localities, ten pots per locality. Each locality was separated from the next by at least 200 to 300 metres. The pots were hung in trees or shrubs at a height of one to two metres from the ground and were topped up with bait once during each week. At the beginning of each week they were cleaned of insects, the old bait replaced with fresh stock, and the numbers of <u>Chrysopa</u> recorded. The results are included in Fig.13. As in the case of the Trap Net method, the daily average catch for each week was calculated, and transformed to log (x + 1) values, which were then plotted on the graph.

4.1.1.3 <u>Discussion</u>. It should at this stage be pointed out that graphs based on logarithms show proportional increases and decreases. In other words, with reference to the <u>Chrysopa</u> catches in Fig. 13, a doubling of numbers will be represented by similar sized peaks at both the top and bottom end of the scale.

Comparing the two trapping methods it can be seen from Fig. 13 that both reflect the same basic pattern of <u>Chrysopa</u> activity. A moderate peak of activity occurred around mid110

October, followed by a decline in numbers over the next four weeks.

A rapid increase in numbers from mid-November heralds what turned out to be the main peak which occurred at about the middle of December. A rapid drop in numbers followed and with one exception activity remained low thereafter except for minor sporadic peaks. The exception is evident in the Bait Pot method, where a further peak of activity occurred during March, although somewhat smaller than either of the two preceeding peaks. Furthermore, the Trap Net method reflects what could be the beginning of aspring build up during August and early September, 1971.

In summing up therefore, there were apparently three main peaks of <u>Chrysopa</u> activity during the experimental period --- an early, moderate peak in October, the main peak in December, and a smaller peak in March, with small and insignificant sporadic peaks in between.

Bot (1961), in trapping experiments at Roodeplaat, using the same two methods, to study populations of certain harmful and beneficial insects in fruit orchards, found that the highest <u>Chrysopa</u> peak occurred in the last week of November. Other significant peaks occurred in mid-October, early November, mid-December, as well as from the end of March to mid-April, with a late peak early in May. Smaller peaks occurred sporadically through the winter. Although it appears that the <u>Chrysopa</u> population was more active during Bot's survey, it should be borne in mind that his findings are based on data collected from three consecutive years of trapping.

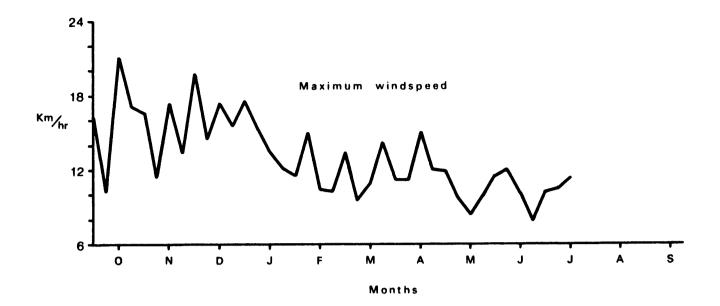
4.1.2 Prevailing Weather Conditions

In seeking an explanation for the observed fluctuations in <u>Chrysopa</u> abundance during this period, one must necessarily take the prevailing weather into consideration. Graphical representations of relative humidity, rainfall and sunshine during the experimental period at Roodeplaat are included in Fig. 13, while graphs of temperature and windspeed are presented in Fig. 14.

4.1.3 Effects of Weather on Seasonal Occurrence of Chrysopa spp.

<u>Temperature</u>. If the <u>Chrysopa</u> catches in Fig. 13 are compared with the weekly mean temperature in Fig. 14, it is fairly clear that from September/October to late March temperature has little effect on the seasonal occurrence of <u>Chrysopa</u> at Roodeplaat during the warmer months. From the end of November to the end of March the weekly mean temperature did not fluctuate widely and remained between 20 and 24^oC. However, it is quite probable that the drop in temperature from April onwards was instrumental in keeping the winter population down. This factor will be mentioned again later after the other weather conditions have been discussed. The rise in temperature from August 1971 may have been partly or wholly responsible for the small build-up apparent at that time (see Fig. 13).

<u>Windspeed</u>. Fluctuations in the windspeed over the experimental period (see Fig. 14) do not suggest any correlation



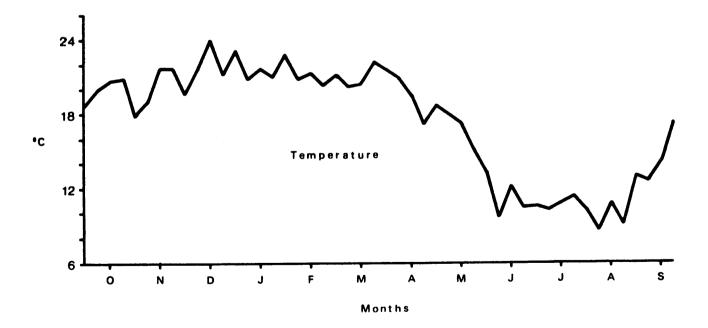


Fig. 14. Weekly mean temperature and weekly mean of the maximum windspeed for the periods September 1970 to September 1971 and September 1970 to June 1971 respectively. Digitised by the Department of Library Services in support of open access to information, University of Pretoria, 2020 between this factor and Chrysopa occurrence.

<u>Relative Humidity</u>. A close study of the relative humidity during the year under review (Fig. 13) reveals a close correlation between this and <u>Chrysopa</u> activity, especially during the summer months. The three peaks shown by the Bait Pot method, and the two main peaks from the Trap Net method all correspond to periods of low relative humidity ---- early October, mid-November to mid-December, and the first half of March. The drops in relative humidity in May and June also correspond with small peaks of <u>Chrysopa</u> activity evident by the Trap Net method. The general drop in humidity from July to late August may also (with the rise in temperature) have been responsible for the slight <u>Chrysopa</u> build-up evident in the Trap Net method.

<u>Rainfall</u>. The rainfall graph in Fig.13 indicates that from September to March <u>Chrysopa</u> activity increased during periods of little rain and decreased during wet periods. The two main peaks of <u>Chrysopa</u> activity shown by the Trap Net method, as well as the three main peaks shown by the Bait Pot method, correspond to the periods of low summer rainfall, i.e. early October, late November/ early December, and the first half of March. From the end of April onwards rainfall was low, but as mentioned earlier the limiting factor at this time of year was probably temperature.

<u>Sunshine</u>. Figure 13 indicates a direct relationship between hours of daily sunshine and the occurrence of <u>Chrysopa</u>. <u>Chrysopa</u> seemed to be most active during periods with more hours of sunshine, i.e. early October, early December and mid-March. (The long periods of sunshine towards the end of September 1970 did not, however, result in notable <u>Chrysopa</u> activity as shown by the Trap Net method). Even the small sunshine peaks of mid- and late April, late May and mid-June correspond to periods of slightly increased <u>Chrysopa</u> activity.

4.1.3.1 Discussion

The most obvious question arising from the above data is: "Why does rainfall, relative humidity and sunshine have the observed effect on <u>Chrysopa</u> activity during the warmer months?" In the writer's opinion these three factors are closely linked, and the answer lies in the adults' need for water or moisture. During rainy periods, apart from the effect of rain hampering flight activity, there is obviously no lack of moisture, consequently <u>Chrysopa</u> adults do not have to seek it out. On the other hand, during periods of low humidity, which inevitably occur during periods when the daily sunshine is longest, <u>Chrysopa</u> adults will actively have to search for water or moisture, and this probably accounts for their greater activity in the field. One possibility that should be borne in mind is that rain washing the bait off the tree and thereby resulting in small catches may confuse the interpretation of its effect on Chrysopa numbers.

During autumn and winter, or in this case after April, the mean temperatures are probably too low for normal activity irrespective of rainfall and humidity, and so temperature therefore most likely becomes the limiting factor. In colder climates most species overwinter in the cocoon as either prepupa or pupae, although as mentioned before some (e.g. <u>C</u>. <u>carnea</u>) overwinter as adults (Balduf, 1939). Our relatively mild climate may allow many of our species to continue breeding slowly throughout the winter, and this could explain the fact that adults were always present throughout the year at Roodeplaat.

The abundance of prey in the habitat (see Section 4.1.4.) is considered as not having a marked influence on the seasonal occurrence of <u>Chrvsopa</u>. Besides the fact that adults of most <u>Chrvsopa</u> spp. are not predacious (Tjeder, 1966; Hagen <u>et al</u>, 1970), it is mainly larval development that is influenced by the presence of prey species, and so prey abundance probably plays a smaller role in the occurrence of <u>Chrvsopa</u> adults. The writer also feels that the correlation between trapped <u>Chrvsopa</u> and rainfall, humidity and sunshine is strong enough to overrule the possibility that it was due to coincidence. It would be revealing, perhaps, to conduct an experiment in which immature stages are sampled in addition to adults.

To summarize, <u>Chrysopa</u> activity from September/October to about the end of March appears to be regulated by rainfall, relative humidity and the amount of sunshine, which in turn determine the need of the adults for moisture. During the cooler months from April to August/September, temperature probably regulates the population by perhaps causing some species to overwinter as immature stages, as well as by its direct influence on general activity, e.g. flight, etc.

4.1.4 Abundance of Prey

The Chrysopa spp. at Roodeplaat would seem to have a relative-

ly good supply of a variety of aphid and acarine hosts, particularly during the warmer months (September to March), but also to a lesser extent through the winter (C.C. Daiber, 1973, personal communication). The main source of food during winter is probably three aphid spp. occurring on cabbage and one aphid sp. on strawberry, although the cabbage aphids are in greater numbers

During the remainder of the year aphids also occur on cucurbits, peas (mainly in spring), beans, potatoes, strawberries, groundnuts, cotton and ornamentals (mainly roses). Red spider mites are found on strawberries and roses (mainly in spring), carnations, cotton, peas, beans, cucurbits and tomatoes. With the exception of cotton, potatoes and groundnuts, which are grown on adjacent ground, all the above crops and ornamentals are grown on Roodeplaat.

Number of Generations per year. 4.1.5

Considering the possibility that Chrysopa may breed throughout the year under our mild conditions, and the fact that generations inevitably overlap, it is difficult to determine the number of generations per year. At best one can only estimate the number of generations during the normal breeding season, which is probably from September/October to March. Allowing, under field conditions, for an incubation period of 5 days, a larval period of 20 days, a prepupal and pupal period of 14 days and a pre-oviposition period of about 4 days, the life-cycle totals about 6 weeks from egg to egg. This would allow three generations during the normal breeding season. It must be emphasized however that this is purely an estimate.

4.2 GEOGRAPHICAL DISTRIBUTION OF CHRYSOPA IN SOUTH AFRICA

Immature and adult stages of <u>Chrysopa</u> spp. were collected from various parts of the country during the course of this study. The immature stages were reared to the adult stage and representative samples from all localities sent to Dr. Bo Tjeder, Sweden, for identification. It was not possible to send all specimens collected due to the volume of work to which Dr. Tjeder was subjected at the time. All in all twelve localities were represented, nine from the Transvaal, two from the Cape Province, and one each from the Orange Free State and Natal.

According to Dr. Tjeder's determinations, the chrysopids consisted of six known Chrysopa spp., one as yet undetermined Chrysopa sp., three new Chrysopa spp.(not yet described), two spp. from the family Berothidae and one sp. from the family Hemerobiidae. In addition, H.D. Catling (P.O. Box 143, Seoul, Korea; 1970, personal communication) has informed the writer of the presence of five Chrysopa spp. in Swaziland, and one Chrysopa sp. in the Transvaal. Of the five spp. in Swaziland, four were also common to South Africa, while one was peculiar to Swaziland (<u>C</u>. <u>handschini</u> Esb. – Pet). This is not implying that <u>C</u>. <u>handschini</u> is never found in South Africa --- on the contrary, it has also been recorded in the Cape Peninsula (Tjeder, 1966). V.B. Whitehead (Fruit & Fruit Technology Research Institute, Stellenbosch, 1973, communication) has also informed the writer of personal three Chrysopa spp. which occur in the Cape Province.

Bearing in mind that this study is only concerned with

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Chrysopidae, the representatives from the families Berothidae and Hemerobiidae have been excluded from the discussion, and will not be mentioned again, apart from stating that the two undetermined Berothidae occurred at Zebediela and Roodeplaat (Transvaal), while the hemerobiid, <u>Hemerobius reconditus</u> (Nav.) occurred at Mooi River (Natal).

Table 22 indicates the localities from which <u>Chrysopa</u> were collected and recorded, the species concerned, and other relevant information (see page 120).

Figure 15 indicates geographically the occurrence of the species mentioned in Table 22 (see Page 122).

4.2.1 Discussion.

The preponderance of species in the Transvaal is not necessarily an indication of the possible favourability for <u>Chrysopa</u> spp. to the type of habitat or climate in that Province. It is due to the fact that the writer's headquarters were at that time in the Transvaal, making locally occurring species more easy to procure. However, it is apparent from large-scale surveys carried out in South Africa (Tjeder, 1966) that the Transvaal and Zululand support the greatest number of different <u>Chrysopa</u> spp. (16 and 14 respectively), followed by the south western Cape (13), the eastern Cape (12) and the northern Cape (9). Tjeder lists 32 different <u>Chrysopa</u> spp. which occur in South Africa. (None were recorded in Swaziland).

In his report Tjeder divides South Africa into the south

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LEGEND

8-Beffelspoort 8T-Barbetoa C-Citrusdal D-Dendron H-Hex River J-Jan Kompdorp L-Letaba Estates M-Malkerns MR-Mooi River P-Pretoria PL-Paari R-Roodeplast RD-Rust der Winter S-Stellenbesch V-Viljeenskroon W-Wolmaransstad Z-Zebediels Estates

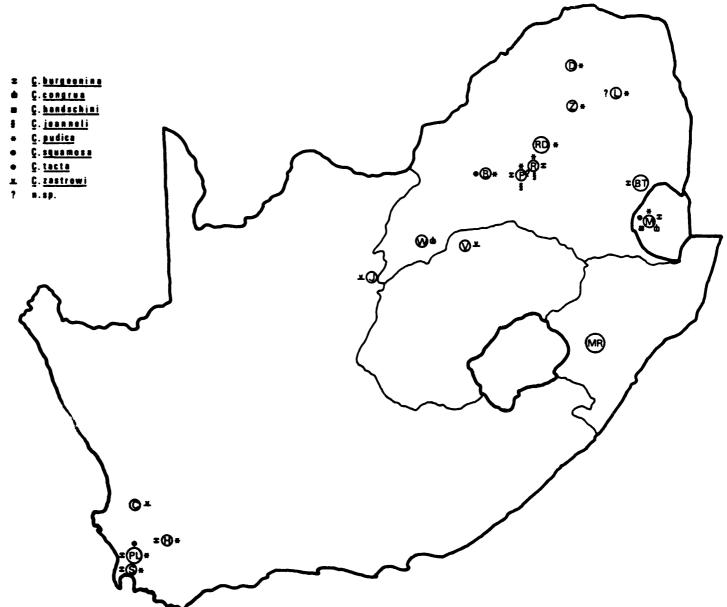


Fig. 15. Geographical distribution of some Chrysopa spp. occurring in South Africa.

western Cape, the eastern Cape, the northern Cape, the Orange Free State, Lesotho, Natal, Zululand, Swaziland and the Transvaal. He also lists South Africa's northern neighbours such as Rhodesia and Mocambique, but these have not been included in this discussion. On the basis of the above division, Tjeder lists the most widespread species as <u>C. jeanneli</u> and <u>C. congrua</u>, followed by <u>C. squamosa</u>, C. zastrowi and C. burgeonina, all equally widespread. According to Table 22 and Fig. 15, the most widespread species was <u>C. pudica</u>, occurring in seven out of twelve localities (excluding Swaziland), followed by <u>C</u>. <u>zastrowi</u> and <u>C. burgeonina</u> in three localities each, <u>C. jeanneli</u> in two localities and <u>C</u>. <u>congrua</u> and <u>C</u>. <u>squamosa</u> in one locality However, in no way can the findings of the present each. survey be directly compared with Tjeder's report, as the present survey was far from comprehensive, 85% of the specimens submitted for identification coming from the Transvaal.

It is interesting to note that there is a fair amount of overlapping of zones occupied by the various species, with as many as five different species occurring in each of two different localities (Roodeplaat and Malkerns, Swaziland). This is also very evident from Tjeder's report, as indicated earlier on in this discussion, where for example Zululand and the south western Cape --- both comparitively small areas ---support 14 and 13 different <u>Chrvsopa</u> spp. respectively. Also evident from Tjeder's report is the fact that some species are confined to definite belts or zones, although these may be fairly extensive in area.

The wide overlapping of zones makes it difficult to deter-

mine what factors limit certain species to a particular area. Prey specificity could possibly account for the distribution of certain species, but in the case of <u>C</u>. <u>zastrowi</u>, for example, there was no evidence of a prey preference. On the other hand certain species may have become adapted to certain climatic conditions or habitats ----<u>C</u>. <u>kannemeyeri</u> (Esb. - Pet.) for example is limited mainly to Lesotho and its surrounding areas. Many other species however do not show this tendency; <u>C</u>. <u>jeanneli</u> for example is found almost all over the country in a variety of climatic conditions. It is probably not any one factor alone which accounts for the distribution, but most likely a combination of two or more factors.

CHAPTER 5

SUSCEPTIBILITY OF C. ZASTROWI TO SOME INSECTICIDES

For some years now people, and especially farmers, have started to appreciate the value of certain insects which form a part of any insect complex --- natural enemies. In recent years the spotlight has been turned more and more onto integrated control, which strives to combine effectively the beneficial presence of predators and parasites with the use of one or more physical or chemical means of pest control. Insecticides are specifically designed to kill insects, and many do not differentiate between "good" and "bad" insects. It is therefore of the utmost importance when embarking on an integrated control programme to choose as far as possible an insecticide or insecticides with selective action --- killing the pests while sparing the parasites and predators.

Before such a choice can be made, candidate insecticides first have to be tested to evaluate their effects on natural enemies which might be present on, or in the vicinity of, the crop to be sprayed. With this in mind the following insecticide evaluations were carried out on <u>C</u>. <u>zastrowi</u> and although they are by no means comprehensive, they should serve as a guideline to anyone intending to undertake a more detailed study of this nature.

One of the main difficulties experienced in carrying out these evaluations --- in particular those on the adult

stage of <u>C</u>. <u>zastrowi</u> --- was maintaining a colony large enough to permit the withdrawal of sufficient adults to allow worthwhile insecticidal exposure tests. It is felt desirable to complete such tests within as short a time as possible to reduce any error which may arise due to a change in the insect colony, inherent or otherwise, or in the conditions under which the experiment is carried out. It is preferable then that a reasonably large surplus of individuals should be available within a fairly short space of time, and this necessarily calls for a large colony.

In the following two sections the results obtained in exposure tests of eggs and adults will be presented. The exposure of larvae had only just begun when this study was terminated, and no worthwhile results are therefore available.

5.1 EFFECT OF SOME INSECTICIDES ON C. ZASTROWI EGGS

Preliminary tests were initiated to determine the possible toxic effect on <u>C</u>. <u>zastrowi</u> eggs of seven insecticides, six of which are commonly used on cotton, and the seventh not yet registered.

<u>Materials & Method</u>. The insecticides were applied to the eggs by means of an endless-belt sprayer. This apparatus consists of a conveyer-type belt, mounted on rollers, with a usable length of about 2,75 metres, and can be run at different speeds. A DeVilbuss spray gun, delivering a fine mist-type spray, is fixed above the belt, and is attached via an air-flow meter and an air-pressure gauge to a compressed air cylinder. The height of the spray gun above 126

the belt can be varied, in conjunction with the air-flow and air-pressure. For insecticide evaluations it is very important that the spray pattern be as constant as possible. To prevent draughts from disturbing the spray pattern, the area around the spray gun, including about 1,5 metres of belt can be closed off almost completely. An extractor fan within the enclosed area removes any insecticide fumes present --- this is merely a safety measure for the operator.

There are four variables on this apparatus --- the belt speed, the nozzle height, the air-flow and the air-pressure --- and all affect the eventual droplet size and droplet distribution deposited. It is therefore necessary to adjust these variables until the desired spray pattern is achieved. A good deposit should have fine, evenly distributed and closely spaced droplets which should, however, not coalesce. An acceptable deposit was achieved at the following settings:

belt speed	5,8	metres/minute;
nozzle height	55,5	cm;
air-flow		litres/minute;
air-pressure	1,0	Kg/cm ²

To ensure that the apparatus was delivering a constant deposit a standardization technique similar to that used by Bartlett (1963) was used. White cardboard strips were sprayed with a gentian violet solution and the spray patterns obtained compared for evennessand constancy. By this method it was considered that the apparatus was delivering a sufficiently constant deposit.

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A paper sheet bearing one day old <u>C</u>. <u>zastrowi</u> eggs was cut into convenient sizes with about 30 to 50 eggs per piece. These pieces were taped onto 9 x 10 cm glass plates and sprayed with different concentrations of seven insecticides. When dry the eggs were cut off their stalks and placed individually into cages of the Plastic Grid rearing units previously described in Section 2.2.3. This was necessary to prevent cannibalism by newly-hatched larvae. Control batches were sprayed with distilled water by hand to avoid possible contamination from the spray gun assembly. Five days after treatment the eggs were inspected and the percentage mortality recorded.

<u>Results and Discussion</u>. The results from this experiment are shown in Table 23 on Page 129.

Due to the preliminary nature of the tests, and the fact that they were unreplicated, it would be unwise to draw too many conclusions from the results. However, considering the normal field concentrations of the insecticides used, it would seem that <u>C</u>. <u>zastrowi</u> eggs generally exhibit a fairly high tolerance to these materials. The possible exceptions to this are azinphos ethyl/endosulfan and azinphos methyl, which gave the two highest mortalities, but even in these cases it seems unlikely that there would be any significant mortality at the recommended field concentrations. This conclusion was also drawn by Bartlett (1964) who found that <u>C</u>. <u>carnea</u> eggs were notably tolerant to most insecticides, with the exception of those containing oil.

Due to circumstances beyond his control, the writer was

unable to carry these investigations further and to calculate the LD50's for the insecticides. It is nevertheless hoped that if any person undertakes a similar experiment, the procedure and results presented here will prove useful.

5.2 EFFECT OF TWO INSECTICIDES ON C. ZASTROWI ADULTS

Two insecticides, endosulfan 35% MO and monocrotophos 35% WS, were evaluated for their effect on <u>C</u>. <u>zastrowi</u> adults. Both materials are registered for controlling a variety of pests on cotton.

<u>Materials and Method</u>. Cages for exposing the adults to the insecticides were constructed from Poly Tops and Whatman No. 1 filter paper as follows.

A 2 cm diameter filter paper disc, perforated with 2 mm holes made with a leather punch, was placed into a large size Poly Top (2,7 cm diameter) with a 1,6 cm diameter hole cut out of its top. This Poly Top formed the ventilated roof of the exposure cage. A second 2,7 cm Poly Top was also modified by cutting a 1,6 cm hole into it, and into this hole was partly inserted a smaller 2 cm Poly Top filled with cotton wool saturated with the adult diet mentioned in Section 2.2.1. This assembly formed the floor of the exposure cage.

The walls of the cage were formed by a 6x8 cm rectangle of filter paper with a row of five 2 mm ventilation holes 1,5 cm from one of the longer edges of the rectangle. This was rolled into a cylinder 6 cm long and about 2 cm in diameter. The floor and roof sections, with their component parts, were then slipped over the ends of the filter paper cylinder to form a ventilated exposure cage the walls and roof of which could be treated with insecticide (see Fig. 16). Bearing in mind that food had to be provided, the maximum internal area of the cage could be treated with insecticide. It was observed that if the cage was placed upright with the food at the bottom, the adults remained on either the walls or the roof of the cage except for the short period when feeding, thus reducing to a minimum the length of time the adults are on an untreated surface.

A variation of the exposure cage was used to test for any fumigation effect which might have occurred during exposures in the above-mentioned cages. Basically the cage was constructed of two ordinary exposure cages joined end to end by two Poly Tops with 1,6 cm holes, with a piece of organdie separating the two Tops (see Fig. 17). The one cage had unperforated filter paper walls and a large intact Poly Top, containing a perforated filter paper disc, as its base, while the other cage had ventilated walls (two rows of five 2 mm holes), with a food holder fitted to the terminal Poly Top. In this case the food holder formed the roof and the intact Poly Top the base of the unit.

The lower filter paper cylinder and perforated disc of the fumigant cage were treated with the insecticide, assembled when dry, and the test insect then confined to the untreated upper cylinder. Ventilation in the upper cylinder ensured that conditions in the cage were similar to those in a

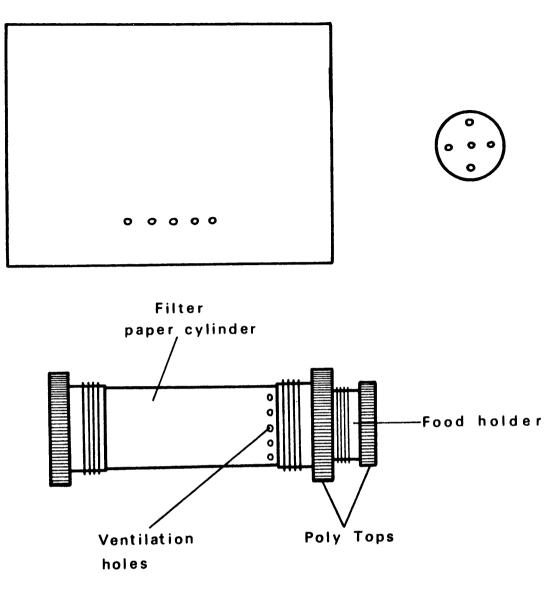


Fig. 16. The ventilated exposure cage, and its component parts, used for confining <u>C</u>. zastrowi adults to surfaces treated with insecticides.

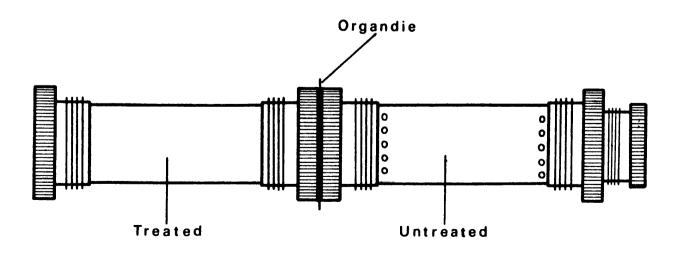


Fig. 17. The cage used for determining the fumigant effect of insecticides on <u>C</u>. <u>zastrowi</u> adults.

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normal exposure cylinder. The organdie division prevented the test insect from coming into contact with the treated surface, while allowing the passage of any toxic fumes into the upper cage.

Before the component parts of the exposure cages were sprayed, the endless-belt sprayer was first standardized by the method described in Section 5.1. It was established that a very good spray pattern and droplet distribution was achievedat the following settings:

belt speed	10,8	metres/minute;
nozzle height	66,3	cm;
air-flow	•	litres/minute;
air-pressure	1,6	Kg/cm ² .

Droplet distribution and size again remained very constant as indicated by tests with gentian violet sprayed onto white cardboard. Further spray pattern tests were carried out every few weeks to ensure that the droplet distribution remained constant.

After standardization, the component parts of the exposure cages were sprayed and set aside to dry for about 18 hours --- this was also necessary to allow the solvents and emulsifiers in the insecticide to evaporate. Surfaces for control insects were either sprayed with distilled water by hand or entirely unsprayed, for fear of contamination from the spray gun assembly. There was no difference in control mortality between either method.

Two to four day old <u>C</u>. <u>zastrowi</u> adults were then placed singly in assembled exposure cages and fresh food provided.

Due to the <u>Chrysopa</u> colony not being large enough to permit large numbers of adults to be withdrawn at regular intervals use had to be made of whatever excesses there were whenever such occasions arose. For this reason the size and number of the replicates differed. Fresh food was provided daily and all insects kept at 27^oC and 45% RH during the exposure. Mortality counts were taken after 24 and 48 hours --- an insect was considered dead if it was incapable of remaining on its legs. Such individuals did not survive if removed from the treated surface. After each exposure the filter paper components were discarded and all Poly Tops washed in acetone, thus eliminating contamination from old cages.

<u>Results and Discussion</u>. Five concentrations of each insecticide were used to determine the LD50 for endosulfan and monocrotophos. Tests with both insecticides indicated that in neither case did fumigation contribute towards mortality. Mortality of <u>C</u>. <u>zastrowi</u> adults after 24 hours is given in Table 24 on Page 135.

The data in Table 24 was subjected to probit analysis and the respective regression equations and LD50's calculated for 24 hour mortality readings. In the case of endosulfan it became clear when the log-transformed dose/mortality figures were plotted that mortality at the lowest concentration (1%) was discordant with the straightline relationship existing between the other points. Clearly then, calculations including this point would have given rise to a false regression equation, and consequently,following Bliss (1935), mortality at 1% was omitted from the calculations. Table 25 gives the results of the probit analysis (see Page 135).

et al, 1972), and expected mortalities at these concentrations obtained from the regression equations. The information is presented in Table 26.

<u>Table 26</u>. Expected mortalities of <u>C</u>. <u>zastrowi</u> adults from recommended field applications of endosulfan 35% MO and monocrotophos 35% WS, calculated from the regression equations in Table 25.

Insecticide	Pest	Recommended field Concentration % a.i.	Expected Mortality %
Endosulfan	Thrips American Bollworm Plusia looper	0,03 0,10 0,18	Nil Nil Nil
Monocroto- phos	Aphids Red spider Red bollworm Spiny bollworm Cotton stainer	0,04 0,05 0,11	2,4 2,9 8,0

Tables 24 and 25 indicate that monocrotophos is approximately ten times more toxic than endosulfan to <u>C</u>. <u>zastrowi</u> adults. This difference in toxicity is also evident in Table 26, where expected mortalities under field conditions are also given. It must be stressed that not too much weight should be placed on these expected mortalities for a number of reasons.

Firstly, filter paper could never be argued to be closely allied to a cotton leaf, and the absorption characteristics of the two substrates for insecticide is most likely considerably different. A wax layer is present in the cuticle of a leaf, and this would probably prevent any direct absorption of the insecticide into the leaf (unlike filter paper). On the other hand a certain amount of the active ingredient is probably absorbed into the wax layer itself. This may change the characteristics of the insecticide relative to that absorbed into filter paper. (It is perhaps worthy of mention at this stage that exposure cages were originally constructed from thin plastic-sheeting components, but results were so erratic that it was concluded that the plastic in some way deactivated the insecticide, possibly by absorption or some other method).

Secondly, all insects were first subjected to the residues when they were about 18 hours old. Under field conditions, no such constancy would exist. Furthermore, in the field the insecticides are exposed to all types of weathering, a factor which was not taken into account in the laboratory.

It is therefore quite obvious that it is very unwise to attempt to equate results obtained in a laboratory with what one might expect in the field. Nevertheless, the expected mortalities presented in Table 26 do, at the very least, give an indication of what might occur when these two insecticides are applied in the field.

At the risk of a barrage of criticism, the writer is of the opinion that, based on the data of this experiment and considering that laboratory conditions do not parallel field conditions, little or no mortality should be suffered by <u>C. zastrowi</u> adults exposed to endosulfan residues applied to cotton at the recommended rates. Under the same conditions, monocrotophos, being more toxic, is likely to cause low to moderate mortality, especially when applied at higher concentrations against pests such as red bollworm, spiny bollworm and cotton stainer.

SUMMARY

1. Introduction

<u>Chrysopa</u> spp. could be of considerable value as biological agents by virtue of their predacious characteristics. However current spray programmes pose a threat to this potential due to the susceptibility of <u>Chrysopa</u> to insecticides. Before incorporating this predator into integrated control programmes it is necessary to become familiar with the biology and ecology of South African <u>Chrysopa</u>.

<u>Chrysopa zastrowi</u> (Esb. - Pet.) (Neuroptera: Chrysopidae) shares the subgenus <u>Chrysoperla</u> with four other species, and together with two of these is fairly widespread in South Africa. The type-specimen was destroyed by fire in 1943, but Tjeder (1966) has described a neo-type which is preserved in the Entomological Institute of Lund University, Sweden.

2. Mass Rearing

The potato tuber moth was chosen as the host insect for \underline{C} . <u>zastrowi</u>, and was successfully reared in the laboratory. Eggs were produced in large quantities for feeding to \underline{C} . <u>zastrowi</u> larvae.

During the course of this study, three methods were used to mass-rear <u>C</u>. <u>zastrowi</u>. In the Poly Top method, larvae were confined individually to cages made from up-turned Poly Tops in 70-cage units. Larval food consisted mainly of potato tuber moth eggs, but included aphids, mealybugs and Angoumois grain moth eggs. This method worked well, but entailed a lot of labour. The Tray method of rearing utilized wood and masonite trays in which many larvae were kept together. Larval food consisted mainly of potato tuber moth eggs and housefly eggs. It was calculated that 100 <u>Chrysopa</u> larvae would need 90 000 potato tuber moth eggs to attain maturity.

The Plastic Grid method, utilizing compact units of 150 cages requires loose eggs, such as those of the Angoumois grain moth, for success. Providing such eggs are available, this is undoubtedly the best rearing method.

In all cases the adult oviposition cage consisted of either a perspex cage or a cage made from an ice-cream carton.

3. Laboratory Studies on Biology

- <u>Eqqs</u>. Green elongate-ovate eggs of <u>C</u>. <u>zastrowi</u> are laid singly on thin stalks, usually on ventral surfaces. Egg colour changes to beige before hatching, and embryological segmentation is noticeable. The larva hatches after three to four days.
- Larva. Larvae are spindle-shaped, covered with hairs which occur on tubercles, and have fang-like suctorial mouthparts. They grow from about 1 mm in length (after hatching) to about 10 mm (before spinning), and have three instars. Characteristic markings are found on the head.

There is no evidence of extra-oral digestion. Cannibalism amongst the larvae increases with increase in population density, indicating competition for space. Larvae were shown to consume an average of 488 wheat aphids, or 906 potato tuber moth eggs during their larval life. Third instar larvae showed no evidence of prey preference when offered six softbodied prey spp. Young larvae do however display a type of prey preference based on the size of the prey --- small prey was preferred to large prey.

Attemptsat providing an artificial liquid diet for the larvae were generally unsuccessful. A few diets showed limited promise, but difficulty was encountered in presenting the formulated diet to the larvae.

Larvae of all ages secrete a substance through the anus known as anal substance, which functions mainly as a repellent and as a means of anchoring itself to the substrate.

Moulting is accomplished within a short space of time, after which the larvae remain in the vicinity of the cast skin for a short while. Passive periods were noted immediately before and after a moult. <u>Chrysopa</u> larvae do not excrete. Excretion is accomplished by the adult in the form of a pellet, soon after emergence.

<u>Prepupa</u>. About eleven days after hatching the larva spins a cocoon in a sheltered place. The cocoon consists of two or three layers. Careful study indicates that a gelatinous-type substance, possibly silk, is deposited and smeared over the entire inside surface of the cocoon, probably giving rigidity and possibly facilitating the formation of a cocoon lid at pupal emergence. The 3rd larval moult takes place inside the cocoon.

- <u>Pupa</u>. The pupa is capable of limited movement inside the cocoon. Strong evidence suggests that the pupa pushes, and does not chew, a lid off the cocoon to emerge, about ten days after spinning. The pupal moult takes place out of the cocoon.
- <u>Adult</u>. The adults are green, about 10 mm in length and possess four wings which are folded roof-like over the abdomen at rest. They do not emit a foul odour, common to certain species. They are neither carnivorous nor cannibalistic. Mating is presumed to occur at night, which is the only time that eggs are laid.

The most suitable adult diet appeared to be one consisting of brewer's yeast, honey and water. The addition of Nipagin M did not suppress fermentation of the diet, and it drastically reduced female fecundity.

The females showed a definite ovipositional colour preference. Significantly more eggs were laid on brown than on either yellow, white or green. This is thought to be due to a favourable reaction to the wavelengths of light reflected by brown.

<u>Duration of the Stages</u>. The mean duration of the stages in days when reared at $25 \stackrel{+}{=} 1^{\circ}$ C and $55 \stackrel{+}{=} 5\%$ R.H. was: eggs - 4.0; larvae: 1st instar - 4,0, 2nd instar - 142

2,0, 3rd instar - 4,3; pupae - 10,2; adults: male -33,3; female - 39,6. Natural mortality was highest in the larval stage (19,5%). After rearing the colony for a number of successive generations it was noticed that there was a tendency for the duration of the stages to increase slightly. The incubation period of the egg increased by 29% after about two months, the larval duration by nearly 22% after ten months, and the pupal stage by 10% after about a year.

Eggs are able to withstand fairly short periods of low temperature down to 10° C without undue mortality. Embryological development continues slowly at 15° C, but ceases almost entirely below this temperature.

Larvae develop fastest when fed Angoumois grain moth eggs (9,0 days), followed by potato tuber moth eggs (9,8 days), wheat aphid (10,8 days), cabbage aphid (12,9 days) and citrus mealybug (14,4 days).

4. Field Studies

<u>Seasonal Occurrence</u>. Two methods were used to trap adult <u>Chrysopa</u> spp. at Roodeplaat, Transvaal -- the Trap Net method, where large nets were spread out underneath a tree sprayed with bait, and the Bait Pot method, in which pots were filled with bait and distributed over a fairly wide area.

> Three main peaks of adult <u>Chrysopa</u> activity were evident, the greatest peak occurring in December, with smaller peaks in October and March. These

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peaks appear to be regulated by rainfall, humidity and amount of sunshine. These probably determine the need of the adults for moisture, giving rise to the observed peaks during dry sunny periods.

It is estimated that under South African conditions <u>Chrysopa</u> spp. would have about three generations during the normal breeding season, although it is quite possible that they may breed throughout the year.

<u>Geographical Distribution</u>. A survey was made of the <u>Chrysopa</u> spp. occurring in different areas around the country, but mainly in the Transvaal. Specimens were identified by Dr. Bo Tjeder, Sweden, and consisted of six known species, one as yet undetermined species, three new species, two berothids and one hemerobiid. Personal information has also been received of <u>Chrysopa</u> spp. in the Cape Province and Swaziland. A distribution map has been drawn showing the occurrence of the different species.

5. <u>Susceptibility to Insecticides</u>.

- <u>Eqqs</u>. <u>C</u>. <u>zastrowi</u> eggs exhibited a fairly high tolerance to seven insecticides.
 - <u>Adults</u>. Experiments to determine the susceptibility of adults to endosulfan and monocrotophos indicate that although the latter is about ten times more toxic to adults than endosulfan, neither should cause undue mortality when applied to cotton at the recom-

mended rates. The LD50 values are 3,597% a.i. for endosulfan and 0,302% a.i. for monocrotophos.

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