CONTROL OF CASHEW STEM BORER (*Plocaederus ferrugineus* L.) BY THE DD. 136 NEMATODE (*Neoaplectana carpocapsae* WEISER 1955)

BY

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THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

FACULTY OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, TRICHUR

1989

DECLARATION

I hereby declare that this thesis entitled "Control of cashew stem borer (*Plocaederus ferrugineus* L.) by the DD-136 nematode (*Neoaplectana carpocapsae* Weiser 1955)" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara,
20.8.1989

MADHU, S.

CERTIFICATE

Certified that this thesis entitled "Control of cashew stem borer (*Plocaederus ferrugineus* L.) by the DD-136 nematode (*Neoaplectana carpocapsae* Weiser 1955)" is a record of research work done independently by Sri. Madhu, S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

Dr.T.S.VENKITESAN,
Chairman,
Advisory Committee
Professor of Nematology

Vellanikkara,
20.8.1989

CERTIFICATE

We, the undersigned members of the Advisory Committee of Sri. Madhu, S., a candidate for the degree of Master of Science in Agriculture with major in Agricultural Entomology, agree that the thesis entitled "Control of cashew stem borer (Plocaederus ferrugineus L.) by the DD-136 nematode (Neoaplectana carpocapsae Weiser 1955)" may be submitted by Sri.Madhu, S. in partial fulfilment of the requirements for the degree.

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ACKNOWLEDGEMENTS

I wish to place on record, my deep sense of gratitude to Dr.T.S.Venkitesan, Professor of Nematology, College of Horticulture and the Chairman of the Advisory Committee for his expert guidance, constant encouragement and constructive criticism throughout the course of the experimentation and preparation of the thesis.

I am also grateful to Dr.C.C.Abraham, Associate Dean, College of Horticulture, Sri.P.G.Veeraraghavan, Professor, AICRP on Cashew, Madakkathara and Sri.V.K.G. Unnithan, Associate Professor of Statistics, College of Horticulture, for their keen interest and valuable suggestions as members of the Advisory Committee.

I am deeply obliged to Sri.D.Seetharama Rao, Associate Professor of Entomology, College of Horticulture for his sincere help and valuable advice rendered throughout the period of experimentation.

I am greatly indebted to all my friends, especially Sri.Venugopalan, C., Miss.Jayasree, T.V., Miss.Mini S. Nair and Sri.Jayaraj, P. for their constant support and ungrudging help extended to me during the study.

The award of Junior Fellowship by Indian Council of Agricultural Research is gratefully acknowledged.

MADHU, S.

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Introduction

INTRODUCTION

Cashew (Anacardium occidentale L.) is one of the most important nut crops of the world. Cashew is cultivated in India over an extent of 5.1 lakh ha. The produce from the crop earned a foreign exchange worth Rs. 334.11 crores in 1987-88.

One of the major constraints in cashew cultivation is the attack of pests, most important of which in the cashew stem borer (Plocaederus ferrugineus L.). This pest was first reported by Ayyar (1932). It has now been recognised as the most destructive pest of cashew, killing productive trees outright. Pillai et al. (1976) reported 4-10% loss due to this pest alone, the infestation being more severe in old and neglected plantations.

Typical symptoms of attack include presence of small holes in collar region, gummosis, extrusion of frass through holes, yellowing of leaves, drying of twigs and finally death of trees.

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The adult insect is a medium sized reddish brown longicorn beetle. Eggs are laid on loose bark and exposed roots of trees. Grubs hatch out, bore into fresh tissues and feed on sub-epithelial tissue making irregular tunnels. The tunnels are fully pecked with frass and fibrous tissue. Damage to the vascular tissue check ascent of plant sap and the tree gradually dries. Fully grown grubs descent to root zone through tunnels, reaching a suitable root and there form a chamber filled with frass and fibrous tissue for the construction calcaerous cocccon. The eggs hatch in about 4-6 days and larval period is 6-7 months. Pupal period normally ranges from 2-3 months.

Attempts to control this notorious pest including insecticides have not been quite successful. The lack of external symptoms in the initial stages of infestation, concealed habits of the grubs inside the trunk and roots and the tight filling of the galleries with frass and excreta are some of the factors that render insecticidal control extremely difficult. Alternative methods of control using biological or other means will therefore be very ideal against the stem borer.

Plate 1. Yellowing of the foliage due to stem borer attack.

Plate 2. Frass material at the base of the tree.

Plate 3. Gummy exudation of the tree as a result of the attack.

Plate 4. A tree completely dried up following the attack by P. ferrugineus

Plate 5. Different life stages of P. ferrugineus
1 - egg; 2 - 1st instar larva 3 - 2nd instar larva; 4 - III instar larva; 5 - IV instar larva; 6 - V instar larva; PP - Pre pupa P - Pupa; A - Adult

Rao *et al.* (1979) recommended adoption of an integrated approach for the control of the pest. Pillai *et al.* (1976) reported that the nematode DD-136 recorded 60 per cent mortality of *P. ferrugineus* at an inoculum rate of 100 nemas per g body weight of grubs. The present study aims at exploring further, the possibility of using the entomogenous nematode, *Neoaplectana carpocapsae* for controlling the cashew stem borer. The following are the objectives of the study:

1. To find out the optimum nematode load required to bring out mortality of the different larval stages (grub instars) of the insect pest.

2. To work out the period required for obtaining maximum percentage of mortality of the larval stages.

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Review of Literature

REVIEW OF LITERATURE

The nematode, *Neoaplectana carpocapsae* (Steinernematidae : Rhabditida) was first described by Weiser from codling moth larvae (*Laspeyresia pomonella* L.) collected from Czechoslovakia. At the same time, Dutky and Hough reported a similar species from the codling moth larvae collected from United States, designating it by its accession number DD-136. The resultant confusion in taxonomy of the nematode prevailed for quite some time. Schmiege (1964) on the basis of his studies on the morphology of DD-136 nematode opined that DD-136 could probably be *N. carpocapsae*. Poinar (1967) through hybridization experiments showed that both the nematodes would interbreed and that they are two strains of *N. carpocapsae*.

The morphology and bionomics of *N. carpocapsae* has been studied in great detail by Weiser (1955), Schmiege (1964), Poinar (1967) and Stanuszek (1974) and has been reviewed by Poinar (1971, 1979), Gaugler (1981) and Wouts (1984).

2.1.1 Description

According to Poinar (1967) the cuticle of the nematode is very smooth, the head slightly rounded and lips united. There are six outer cephalic papillae and six inner labial papillae. The pharynx is muscular and the anterior region of procorpus is slightly expanded. Meta corpus is devoid of valves, and is followed by a basal bulb. Excretory pore is anterior to nerve ring. The female may range in length from less than 1 mm to up to 1.6 mm (Schmiege, 1964), the size being dependent on nutrient availability. Second and subsequent generations of females developing in host will be correspondingly smaller in size. The female tail is bluntly conical to dome shaped. A short spine at the tip is often observed. The female nematode is amphidelphic with opposite reflexed ovaries. (Fig.1A)

The male is smaller than female and has reflexed testis. Spicules are paired, symmetrical and curved. A bursa is absent. The male tail has twenty three anal papillae. (Fig.1B)

The infective stage (third stage or dauer) juveniles measure about 400-600 μm. The oral and anal openings are closed. The pharynx and intestine are collapsed. The tail is pointed. (Fig.1C)

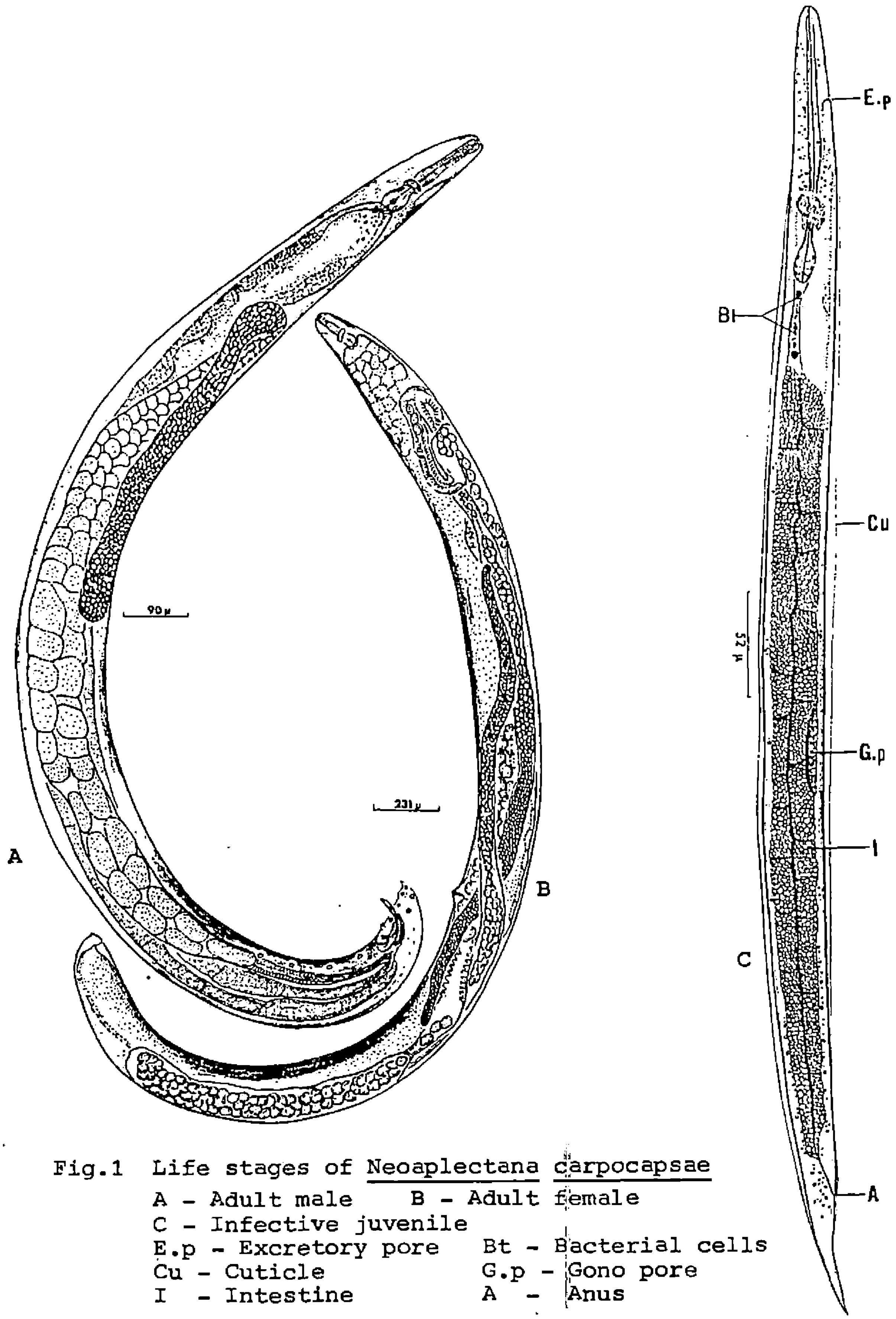

Fig.1 Life stages of Neoaplectana carpocapsae
A - Adult male B - Adult female
C - Infective juvenile
E.p - Excretory pore Bt - Bacterial cells
Cu - Cuticle G.p - Gono pore
I - Intestine A - Anus

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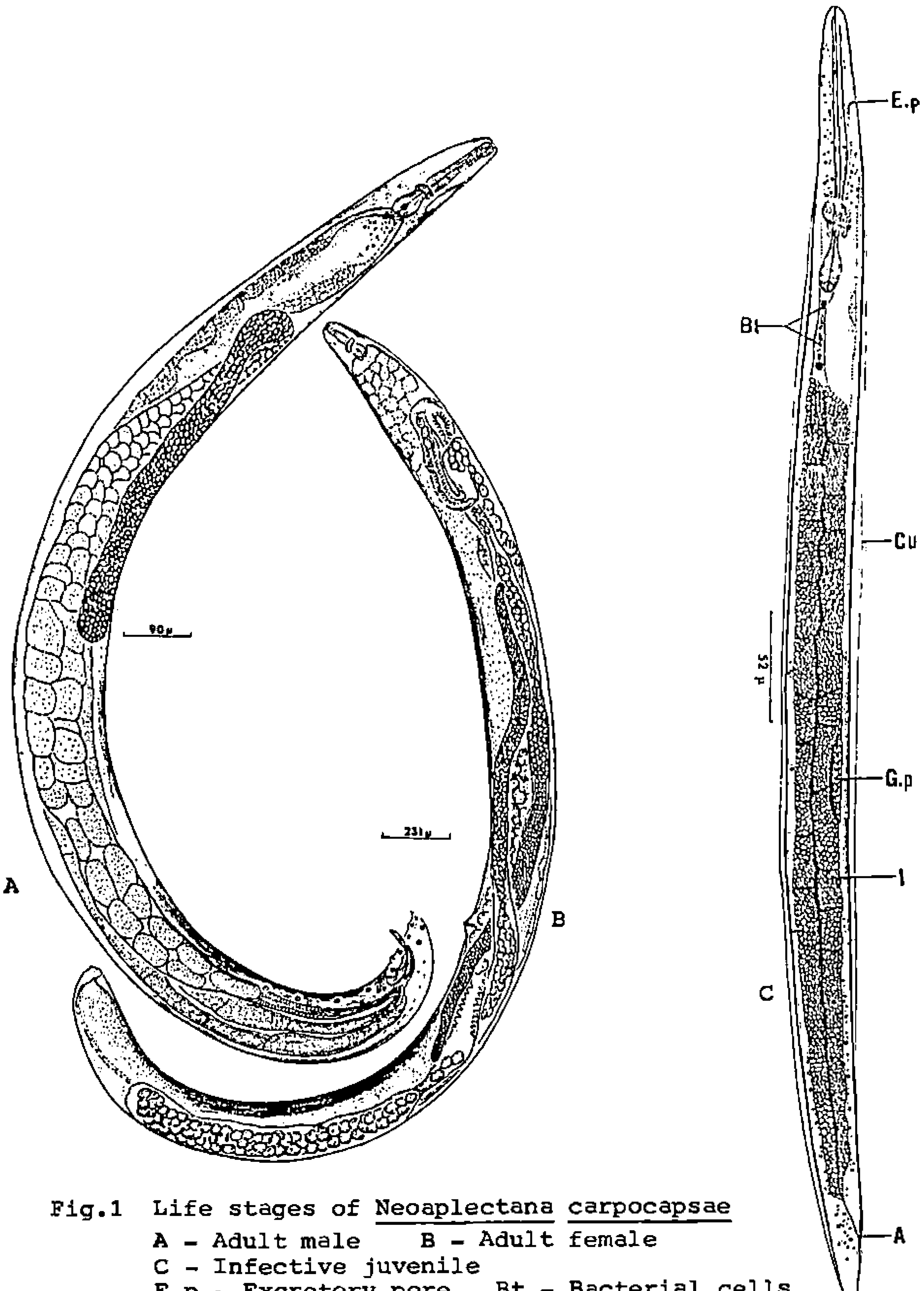

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2.1.2 Life cycle

The nematode has six stages in its life cycle, namely the egg stage, four juvenile stages and the adult stage. The infective stage juveniles are capable of entering the hosts through oral and anal openings as well as through spiracles (Triggiani and Poinar, 1976) as in the case of large bodied insects and adult lepidopterans. Wouts (1984) ruled out the possibility of entry through spiracles. Once inside, they reach the midgut, and penetrate through the gut wall and reach the haemocoel, where they develop into parasitic third stage juveniles and subsequently to fourth stage. Poinar (1979) reported that insect mortality occurred within 24 to 48 h after inoculation, while Danilov (1980) in the case of <u>Galleria mellonella</u> L. larvae found that this period was 15 h at a dosage of 5000 nemas per larva.

The nematodes continue their development inside, even after host's death, into first generation adults. These adults mate and the females lay eggs. The eggs hatch and second generation adults are produced which are smaller in size, for want of food as well as due to crowding. The second generation adults give rise to infective juveniles which emerge from depleted host into the environment, where they can survive for long periods in the absence of hosts, until they encounter a new host.

2.1.3 Associated bacterium

The first report of association between a bacterium and *N. carpocapsae* was by Weiser in 1955. Dutcky (1959) also reported this association but did not attempt to characterise the bacteria. Poinar and Thomas (1965) described the bacterium as *Achromobacter nematophilus* and reported that this bacterium was found in constant association with the nematode, stored in a pouch in the oesophageal region by the infective stages and released into the host haemocoel. The bacterium was redescribed by Poinar and Thomas as *Xenorhabdus nematophilus* in 1979. They are rod shaped, gram negative, entomopathogenic bacteria, which occur in a primary form, the most common and a secondary form, which is not as virulent as the primary form. The secondary form is not suited for nematode mass production or biological control purposes (Wouts, 1984). The bacterium was reported to be lethal when injected into haemocoel of wax moth larvae, even at a concentration of 2 to 3 cells per larvae (Poinar and Thomas, 1967).

Lysenko and Weiser (1974) studied the microflora associated with *N. carpocapsae* and reported that the bacteria

Pseudomonas maltophilia Hugh and Ryschenkow and *Xenorhabdus nematophilus* both achieved 100 per cent mortality of wax moth larvae five days after inoculation in association with *N. carpocapsae*, while axenic nematodes inoculation resulted in 80 per cent mortality, suggesting a role for the nematode other than that of being merely a living syringe. This was supported by Poinar and Thomas (1967) who reported that a single axenic nematode could kill *Galleria mellonella* larva. Burman (1982) reported toxin production by certain developmental stages of *N. carpocapsae* in axenic cultures, which could kill insects or change their behaviour and physiology. The toxin could kill insect larvae within 20 h. The infective juveniles however, did not produce any toxin.

2.1.4 Ecology

The success of DD-136 depends as in the case of most micro-organisms used in biocontrol, largely on environmental factors. Among the environmental factors, temperature and humidity have been shown to be the most important for nematode survival and infectivity. The effect of temperature and humidity as well as other abiotic components of environment have been well documented.

of the nematodes' original localities. He also reported parisitisation of insects at temperatures higher than those permitting growth and reproduction. He also reported different temperature limits for different strains of N. carpocapsae. Dunphy and Webster (1986) reported lowering of LT_{50} values for the nematodes at higher temperatures.

2.1.4.2 Moisture

Lack of adequate levels of humidity has, in most cases, been implicated as the major reason for failure of DD-136 in controlling pests under field trials. Thus Jacques in 1967 asserted that desiccation was the reason for failure of the nematode to control leaf eating pests of apple. The nematodes suffered 100 per cent mortality at 21°C and 45 RH when exposed for 5 h in the orchard. Even when exposed only for 90 minutes, 72 per cent of the nematodes had died. When RH was 30, even at 25°C, 100 per cent mortality of nematodes resulted within 90 min.

The ability of N. carpocapsae to survive gradual desiccation to levels below permanent wilting point has been demonstrated by Simons and Poinar (1973). The nematodes when exposed to gradually decreasing relative humidities,

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Schmiege (1963) reported that temperatures above 33°C were detrimental to the nematodes. While one hour exposure at 35°C killed quite a few nematodes; 100 per cent mortality occurred at 41°C for the same exposure period. That the temperature's influence is largely modified by humidity is evident from the fact that nematodes can be held in water at 50°C for several years without any loss of infectivity.

Kaya (1977 a; 1977 b) studied the growth and reproduction at various temperatures and reported that growth and reproduction took place only between 15°C and 30°C both _in-vivo_ and _in-vitro_, with 25°C being the most favoured level. At 30°C, nematodes developed into adults but did not reproduce, thereby reducing the chances of utilisation of these nematodes in environments with temperatures above 30°C.

That temperature may also help the nematode to detect its host was reported by Byers and Poinar in 1982.

Molyneaux (1985) made an interesting observation that the temperature at which nematodes became inactive were by and large determined by the climatic conditions showed more than 90 per cent survival at RH 79.5 per cent even after 12 days and more than 80 per cent survival, at RH of 48.4 per cent after four days exposure. Based on these results it was concluded that the nematodes could be best utilised for soil application since the desiccation is gradual in that habitat as against the desiccation taking place in above ground plant parts. The importance of moisture for nematode efficiency was further stressed by Lindegren et al. (1978) when they reported 55 per cent mortality of the navel orange worm *Paramyelois transitella* Walker and 34 per cent reduction in damage caused in almond trees under moist conditions, when nematodes could survive for more than 10 days.

Sledzevskaya (1980) reported that for DD-136 nematode to survive on fruit trees, rainfall should occur every third day under Transcarpathia conditions, but in damp soil of 14 to 20 per cent humidity nematode survived the whole summer.

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2.1.4.3 Soil

Not much has been studied about soil and its influence on the nematode DD-136, as compared to studies on temperature and moisture requirements. Reed and Caine (1967) reported three types of movement for the DD-136 nematodes on soil and the inability of the nematodes to penetrate the soil. Studying vertical and horizontal dispersal of *N. carpocapsae*, Moyle and Kaya (1981) reported that nematodes when placed 15 cm below showed a tendency to migrate upwards. However, nematodes placed on or near soil surface showed little dispersal. In horizontal dispersal studies, most nematodes were recovered from within 6 cm, though they did infect the wax moth larvae placed even 14 cm away.

Georgis and Poinar (1983) observed that higher clay and silt content adversely affected nematodes ability to search out and infect insect hosts. Also, the nematode showed a tendency to migrate upwards when placed at various depths in soil.

Sledzevskaya in 1980 reported that DD-136 nematodes were incapable of penetrating the soil and attacking larvae of goosabery fruit worm (*Zophodia grossulariata*).

2.1.4.4 Sunlight and ultra-violet radiation

Gaugler and Boush (1978) studied the effect of sunlight and ultraviolet radiations on *N. carpocapsae*. The authors reported that short length UV radiations (254 nm) caused high mortality, the time taken being proportional to the time of exposure. Reproduction and development were inhibited by exposure for 2.45 and 5 min respectively. Sunlight also caused a loss of pathogenicity by 95 per cent, probably due to UV portion of solar spectrum.

2.1.5 Mass multiplication of nematodes

The possibility of mass multiplication has made neoaplectanid nematodes very popular in biological control programmes. Methods have been developed which enable us the production of millions of these organisms at very little cost.

2.1.5.1 *In-vivo* methods

In-vivo methods of multiplying *N. carpocapsae* essentially involves infecting a suitable host (usually *Galleria mellonella* larvae but any large bodied lepidopterous larvae can be used), incubating them at suitable temperature for about 10 days and extracting the infective stages of the nematode. Dutky *et al.* who developed this technique in 1964, reported yields of about 200,000 nematodes per host larva. Poinar (1971) described a slight modification of the above method. Recently, Blinova and Ivanova (1980) reported that among various insects tried, the larvae of *Tenebrio molitor* L. was found best suited for *in-vivo* culturing of *N. carpocapsae* strain agriotos, owing to the easiness of rearing the insects in laboratory and also to the lack of associated nematode fauna of its own.

2.1.5.2 *In-vitro* methods

Dutky *et al.* (1964) had maintained stock cultures of DD-136, together with associated bacterium *A. nematophilus* on slants of peptone glucose agar containing a piece of cooked kidney. House *et al.* in 1965 developed a dog food medium which could yield very large quantity of infective juveniles.

Poinar (1979) opined that any protein rich material can be used for *in-vitro* production of *N. carpocapsae* provided that constant association with the bacterium and freedom from contamination is assured. The author also reported success with hanging drop technique using blood of a suitable host insect hanging in the well of a cavity slide in which nematodes develop.

Tarakanov (1980) reviewed the in-vitro production methods of neoaplectanids, especially the works done in Soviet Union.

Bedding (1981) reported that a medium constituting of homogenates of pig kidney and beef fat on crumbed polyurethane polyether yielded more than half a million nematodes per gram medium at a cost of less than two cents per million. Hara et al. (1981) reported of a method using dog food/agar medium yielding 1.25 million nematodes per petridish at a cost of 28 cents per million.

Boemare et al. (1983) reported a new method for axenic production of N. carpocapsae using larvae from aseptic eggs.

Bedding in 1984 developed methods suited for commercial scale production of neoaplectanid nematodes using chicken ofal in autoclavable plastic bags, yielding up to 1550 million infective juvenile per bag in 3 to 4 weeks at a cost of one cent per million.

Kermarrec and Mauleon (1989) reported that certain ingredients of artificial diets used for rearing host larvae may influence nematode production, as evidenced by reduction of infective juveniles produced by addition of antibiotics like nipagin and streptomycin which are commonly used to stabilise diets.

2.1.6 Storage

Storing of nematodes is an important aspect of nematode utilisation for bio-control. N. carpocapsae has been shown to be amenable for long time storage. Poinar (1971) reported that N. carpocapsae can be stored in sterile water or 0.01 per cent formalin at 5-10°C for over six years. Chu and Ching (1976) had studied the optimum conditions for storage of DD-136 and recommended storage in tap water at 7°C with adequate aeration. The authors also reported that survival of stored nematodes increased with density.

Bedding in 1984 reported that large numbers of DD-136 nematode can be stored on clean, aerated polyurethene tubes at 1 to 2°C.

2.1.6.2 Pathogenicity trials

The use of microbial agents for biocontrol necessitates the confirmation of the susceptibility of the insect in the laboratory. The methods generally employed in experimental inoculation include placing the nematode and host insect together in an infection chamber, introducing the nematodes per-os, and directly inoculating nematodes into the host haemocoel (Poinar, 1975).

The first method is adopted in cases where the method of penetration is not exactly known and we allow infection to proceed as it does in nature.

Per-os introduction allows better regulation of dosage. This can be achieved by either mixing the nematode with feed or by injecting the nematodes into the insects mouth. Poinar (1975) has described this process of inoculation in detail. Poinar and Ennik (1972) fed <u>Vespula</u> spp. sugar cubes saturated with nematodes.

Injecting nematodes into haemocoel can be useful in rearing entomogenous nematodes, to study host reaction to nematode etc. provided entry of contaminating organisms can be prevented.

In field trials, nematodes can be applied in a number of ways. Rao <u>et al</u>. (1971) applied these nematodes along with irrigation water to control yellow stem borer of rice *Scirpophaga* (*Tryporyza*) *incertulas* Walker. and observed significant mortality of the pest. In the same study the authors concluded that spray treatment was better than inoculation along with irrigation water, to control the stem borer. General methods of inoculation include spraying on to plant parts, applying the nematode suspension directly into the soil or by placing infected hosts on soil surface (Poinar, 1979). Kaya *et al*. (1984) sprayed nematodes on to corrugated paper bands attached to apple tree trunks to result upto 95 per cent mortality of the codling moth larvae.

Pye and Pye (1985) recommended dipping of young seedlings with soil into a suspension of infective juveniles of DD-136 as best method to control large pine weevil *Hylobius abietis* L. attacking pine seedlings.

Lindegren *et al*. (1987) applied nematodes into tunnels made by navel orange worms to achieve good control.

Kaya and Nelsen (1985) attempted encapsulation of entomogenous nematodes with calcium alginate and revealed that at 4°C, the nematodes survived in the capsule for up to eight months. In pathogenicity trials, however, mortality of Spodoptera exigua Hübner was humidity dependent. The authors were of the opinion that with further development, this technique could be used for protection of seeds and seedlings from soil pests.

In an improvement on this technique, Kaya et al. (1987) encapsulated tomato seeds along with nematodes and found that nematodes were released into soil as seeds germinated, killing Galleria mellonella larvae placed in soil in large numbers.

Studies on improving the performance of the nematodes with the help of the additives anti-dessicants and spreaders gave variable results.

Webster and Bronskill (1968) used Gelgard in a water thickener together with Foliocate 351^{R} and a surfactant Arlatone T^{R} to increase mortality rates from 24 to 90 per cent. Nash and Fox (1969) reported that nematode effectiveness was increased by up to 10 per cent with the additives as Glycerin, Emgard 2050, etc.

Kaya and Reardon (1982) evaluated various additives but found the results not satisfactory enough.

2.1.8 Compatibility to agrochemicals

The nematode *N. carpocapsae* enjoys considerable advantage as far as compatibility to agrochemicals are concerned. Rao *et al.* (1975) reported that a number of organophosphate insecticide killed nematode within 24 hours. Kamionek *et al.* (1979) studied influence of certain weedicides like Dual, Tribunil, Roundup etc. and reported little effect on nematode when treated directly but nematode development was poor in insects treated with these chemicals. Hara and Kaya (1982) tried a number of insecticides against nematodes and found that generally organophosphates and carbamates were detrimental for nematode growth and multiplication. Methoxychlor, fenvalerate and diflubenzuron did not have significant effects on nematodes at normal concentrations.

Report by Hara and Kaya (1983a) supported the above findings but the authors also reported that paralyzed nematodes recovered when washed in water, sufficiently enough to infect *Spodoptera exigua.*

Hara and Kaya (1983 b) reported infection and development by DD-136 in *S. exigua* larvae treated with trichlofon, mevinphos, fenvalerate or permethrin.

Compatibility of 1, 3 dichloropropene (Telone-II) with _N. carpocapsae_ was reported by Ishibashi and Kondo (1987).

2.1.9. Natural enemies

Neoaplectanid nematodes are considered as the best suited for soil inhabiting pests. But in soil, they are likely to encounter both predatory and pathogenic organisms. Poinar (1979) has given an account of the natural enemies of entomogenous nematodes. Mites, especially members of genus Macrochiles have been observed as serious predators by the author.

Among the pathogens are two protozoans, _Nosema mesnini_ Paillot and _Plistophora schubergi_ Swolfer which infected the nematodes when they attacked the lepidopteran larvae already parasitised by these protozoans (Veremtchuck and Issi, 1970). Poinar (1988) reported about a protozoan infection of _N. glaseri_ in field collected nematodes. The parasitised infective juveniles had an open intestine instead of normal collapsed intestine, filled with microsporidans.

The most important group of pathogens include fungi (Poinar, 1971). The fungus Drechmeria coniospora (Drechler) W-Gams and Jansson could not penetrate infective juveniles of N. carpocapsae. However, adults and preinfective stages of the nematode were infected (Poinar and Jansson, 1986a). The nemato phagous fungi Monacrosporium ellipsosporum (Grove) Cooke and Dickinson and Arthrobotrys oligospora Pres. were capable of infecting DD-136 and killing them within 72 h (Poinar and Jansson, 1986 b).

Bacteria has been suspected but never established as a pathogen of N. carpocapsae.

2.2.1 Host range

More experimental infections have been attempted with N. carpocapsae than with any other entomogenous nematode.(Poinar, 1979). The association of nematode with the specific bacterium which can kill insects quickly and prevent further decompositon enables the nematode attack successfully most of the insects. Gaugler in 1981 opined that Neoaplectanids kill the host they penetrate since the nematode releases the bacterium immediately on penetration. Inactivation of the nematode thereafter cannot prevent mortality of the host. Nickle and Welch (1984) opined that the more than 1000 species of insects are susceptible to N. carpocapsae and that there are very few insects that are not killed by the nematode.

Morris (1985) tested 31 species of insect pests for susceptibility to Steinernema feltiae (= N. carpocapsae) and reported susceptibility of 13 species for the first time. He also opined that as a group, Lepidotera was most susceptible to the nematode.

2.2.2 Field trials

Field trials involving N. carpocapsae have yielded mixed results. In most cases, the unfavourable environmental factors have been pointed out as the reason for failure. An upto date list of field trials provided by Poinar (1971, 1979). Gaugler (1981) stated that poor choice of target organisms and habitats were the chief reasons for failure in field trials. Generally, the nematode has been recommended against pests inhabiting moist habitats as soil or those occupying cryptic habitats. Trials against foliage feeders have mostly been disappointing due to rapid desiccation from leaf surfaces. Wouts (1984), Bedding (1984) and Kaya (1984) have reviewed field trials involving entomogenous nematodes against Lepidoptera, Hymenoptera and bark beetles respectively, up to 1980. Table 1 provides a list of field trials after 1980 and involving DD-136 nematode against major groups of pests.

2.2.3 Host defence mechanisms

Neosplectanid nematodes have a wide host range, mainly because of the association with bacteria *Xenorhabdus* sp. The immediate release of bacteria into haemocoel ensures mortality of hosts even if the nematode is countered successfully, since the bacteria are not nullified. Consequently most insects are considered susceptible to the nematodes.

Serycznska and Kamionek (1972) reported that phagocytizing haemocytes level increased in *Galleria mellonella* attacked by the nematode.

The same reaction was reported by Serycznska (1975) in Colorado potato beetle *Leptinotarsa decemlineata* Say.

Andreadis *et al.* reported in 1975 the encapsulation of the DD-136 nematodes by *Aedes aegypti* L. larvae concurrently parasitized by Reesimermis nielseni Tsai and Grudman.

Poinar (1979) reviewed insect immunity studies against entomogenous nematodes and listed host escape, celluar responses and humoral responses as the mechanisms of immunity. He described the melanisation of N. carpocapsae by Culex pipiens L. as a humoral response.

Gotz et al. (1981) reported inhibition of immunity of Hyalophora cecropia L. against X. nematophilus. This view was supported by Burman (1982).

Gotz and Gulzov (1982) reported of a substance produced by N. carpopasae which destroyed insect immunity. Matha and Mracek (1984) also reported increase in haemocyte count to a maximum at 16 h after infection and concluded the defense of wax moth larvae to be week though not altogether absent.

Dunphy and Webster (1987) examined the mechanisms by which entomphilic nematodes avoided host insects' protective mechanisms. Alikhan (1987) reported increase in haemocyte count of colorado potato beetle when bacteria or nematodes alone were introduced but not against bacteria nematode complex.

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Table 1. Field trials of N. carpocapsae against insect populations

Host	Site	Results	Reference
Order: Coleoptera			
Elm leaf beetle *Pyrrhalta luteola*	Litter	Poor control of larvae but good control of pupae	Kaya *et al*. (1981)
European elm beetle *Scolytus multistriatus*	-	10 to 66 per cent mortality	Mac Evan and Brewer, 1981
Mountain pine beetle *Dendroctonus ponderosae*	-	Upto 60% mortality	-do-
Corn root worm *Diabrotica* spp	Soil	Significant control	Poinar *et al*. 1983
Black wine weevil *Otiorhynchus sulcatus*	Soil	No significant reduction in population	Georgis and Poinar, 1984a
Strawberry, root weevil *Nemocestes incomptus*	Soil	Substantial reduction in population	Georgis and Poinar, 1984 b
Lesser meal worm *Alphitobius diaperinus*	Litter	More than 50 per cent mortality	Geden *et al*., 1985

(Contd.)

Table 1 (Contd.)

Host	Site	Results	Reference
Large pine weevil Hylobius abietis	Seedlings	80% mortality	Pye and Pye, 1985
Sugarcane borer Migdolus sp	Soil	No significant control	Arrigori et al.,1987
Crucifer flea beetle Phyllotrecta cruciferae	Soil	Nematode ineffective	Morris, 1987
Order: Diptera			
House fly Musca domestica	Manure pits	55-61% mortality	Geden et al., 1986
Manure breeding flies Fannia spp. Musca domestica	Chicken manure	No reduction in population	Mullens et al.,1987
Order: Hymenoptera			
Honey bee Apis mellifera	Bee hives	Low susceptibility	Kaya et al., 1982.
Larch sawfly Cephalia lariciphala	Larch trees	Promising against prepupae	Georgis and Hague, 1988.

(Contd.)

Table 1 (Contd.)

Host	Site	Results	Reference
Order Lepidoptera			
Lemon tree borer *Oemona hirta*	Lemon and grape fruit trees	50% reduction in population	Clearwater and Wouts, 1981
Carpenter worm *Prionoxystus robiniae*	Fig tree trunks	44-92% mortality	indagren *et al.*, 1981
Western spruce Budworm *Choristoneure occidentalis*	Western fir trees	No significant control	Kaya and Reardon, 1982
Carpenter worm	Fig tree trunks	85% control	Lindegren and Barnett, 1982
Corn ear worm *Heliothis zea*	Corn earheads	88% control	Bong and Sikorowski, 1983
Western poplar clearing moth *Paranthrene robiniae*	Poplar and Birch trees	80-90% mortality	Kaya and Lindegren, 1983
Artichoke plume moth *Platyptilia carduidactyla*	Artichoke plants	90-95% mortality	Bari and Kaya, 1984
Paranthrene tabaniformis	Poplar trees	97.5% control	Cavalcaselle and Deseo,1984
Cryptorhynchus lapathi	Poplar trees	100% mortality	-do-

(Contd.)

Table 1 (Contd.)

Host	Site	Results	Reference
Codling moth *Laspeyresia pomonella*	Base of apple trees	80% mortality of pupae and prepupae	Kaya *et al.*, 1984
Clearwing moth *Synanthedon myopaeformis*	Apple trees	74-94% control	Deseo and Miller, 1985
S. typhiaeformis	-do-	Control not significant	-do-
Cabbage butterflies *Pieris brassicae*	Cabbage plants	Successful control	Korenchenko, 1985
Grape root borer *Vitacea polistiformis*	Soil	Reduced attack	Saunders and All, 1985
Corn ear worm *Heliothis zea*	Corn ear heads	Significant reduction in damage	Bong (1986)
Raspberry crown borer *Pennisetia marginata*	Soil	Significant mortality	Capinera *et al.*, 1986
Navel orange worm *Amylois transitella*	Orange tree trunks	78% mortality	Lindegren *et al.*, 1987

Materials and Methods

MATERIALS AND METHODS

All equipments, glassware and laboratory aids were used after proper sterilisation or as otherwise stated under different methodologies described. Sterile conditions were maintained as far as possible throughout the experiment.

3.1 Mass multiplication of nematodes

The nematode nucleus culture for the study was obtained from Department of Nematology, Tamil Nadu Agricultural University and was mass multiplied in the laboratory using *in-vivo* methods.

The choice of an *in-vivo* method was chiefly based on the easiness and simplicity. Larvae of rice meal moth *Corcyra cephalonica* stain maintained in the laboratory for the purpose were used. A slightly modified version of Dutky's (1964) method was adopted. About 25-30 final instar larvae of the meal moth were washed in 0.01 per cent formalin and placed in a sterilized standard petridish containing two moist filter papers. A 3 ml suspension of the infective stages containing 300 nematodes per ml was added to the so formed infection chamber. It was then covered by a polythene bag wetted inside to keep saprozoic flies out. The insect mortality occurred within 24-48 h and the petridishes were incubated at room temperature in a water bath for ten days.

The nematodes were obtained by using a nematode collecting dish similar to the one described by White (1927). The lid of a small petridish was placed open side down on the bottom of a deep petridish and was covered with a filter paper. Formalin (0.01%) was added to reach one half of the height of the small petridish lid. The dead, parasitized insects were placed radially in the middle of filter paper. The entire collecting dish was placed in a water bath and covered by a bell-jar. The infective juveniles emerging from the cadaver migrated into the water. The nematode suspension was collected on alternate days and stored in sterilized dark brown bottles at 5°C-7.0°C in a refrigerator.

3.2 Collection, measurement and maintenance of grub stages of cashew stem borer

3.2.1 Collection of grubs

The grubs were collected from infested trees located at the Cashew Research Station and Kerala Agricultural Development Project plots, Madakkathara under

Kerala Agricultural University. Infested trees were located by observing the external symptoms and the grubs were chiselled out of the attacked and damaged portions of the trees.

3.2.2 Measurement of grubs

The grubs collected from the field were subjected to measurements of their body length (mm) maximum width of thorax (mm) width at the abdomen (mm) and body weight (mg) so as to determine and classify the instar to which they belong. The linear measurements were taken by placing the grubs on a scale graduated in millimetres. A common weighing balance was used to record the weight of the grubs.

3.2.3 Maintenance of grubs

The grubs were maintained by offering the appropriate feed material collected fresh from cashew trees in the form best suited to the particular instar stage.

The first instar grubs were offered fresh pieces of outer bark, having a size of about 7 cm x 5 cm x 1 cm after drilling a hole on the outer surface to accommodate the grub. The bark piece along with the grub was placed in a standard sterile petridish containing a moist cotton pad at the base.

The second instar grubs were offered fresh bark pieces of about 8 cm x 7 cm x 1.2 cm size, after making a small slit on the bark. The bark along with the grub was placed in black polythene bag of size 25 x 20 cm.

The third and fourth instars were offered fresh bark pieces of about 9 x 7 x 15 cm and 10 x 9 x 2.5 m respectively, in an identical manner.

The final instar grubs were given fresh root pieces of about 12-15 cm length and 3.5 - 4 cm across. The spliced roots with grubs were then covered by black polythene bags.

3.3 Preparation of nematode suspension

Nematode suspensions of concentrations 10 nemas per ml, 100 nemas per ml and 1000 nemas per ml were prepared from nematode suspensions collected from the nematode extracting dishes after counting tho nematodes extracted in a nematode counting dish and making up necessary volume with 0.01 per cent formalin.

3.4.3 Determination of threshold inoculum required for mortality of different instars

The experiment was laid out in CRD with 6 treatments including one check to each of the five instars.

T_0	-	0 nemas (Sterile water)
T_1	-	75 nemas per g body weight
T_2	-	100 " per g "
T_3	-	125 " per g "
T_4	-	150 " per g "
T_5	-	200 nemas per g body weight

Each treatment was replicated twenty times resulting in a total of 120 grubs of each instar being used for the experiment. The grubs were allowed 24 h to establish on the offered feed material, before they were inoculated with the nematodes.

3.3.1 Inoculation of first instar grubs

The tunnel made by grub was traced out and the required nematodes were added using the suspension of having 10 nemas per ml. The required quantity of suspension was made up to one ml and was added to the tunnel near the grub by using a 1 ml pipette.

3.4.2 Inoculation of second instar grubs

The tunnels were traced out and the required quantity of nematodes added to the tunnel using suspension of 100 nemas per ml. The total volume made up was two ml.

3.4.3 Inoculation of third, fourth and fifth instar

The procedure followed was identical here too, but the suspension of 1000 nemas per ml was used and made up volume was 2.5 ml.

The inoculated grubs were covered by black polythene bags and maintained under room temperature (32-36°C) and observed for mortality every alternate days. Dead grubs were removed to nematode collecting dishes described earlier.

The grubs were offered fresh, untreated feed seven days after inoculation, further changes in feed being made as and when necessary. The grubs were maintained in the lab for one month.

A number of grubs of each instar were placed in an incubator at 25-30°C, towards the later stages of the experiment to understand the influences of temperature on nematode activity and mortality of grubs.

3.4.4 Oral injection of grubs

Oral injection of the grubs with 1500 nematodes each was also studied using a plastifak(R) syringe as described by Poinar, 1975 on third, fourth and fifth instar grubs. The inoculated grubs were then dissected at 6 h, 12 h, 24 h and 48 h intervals and observed for presence of dead of live nematodes in the intestine or haemocoel.

3.5 Survival studies

Survival of nematodes on bark was studied by adding 500 nematodes on to a bask piece, kept moist by addition of water and the bark pieces were washed on first, second, third, fourth and fifth days and washings observed for live nematodes.

Results

RESULTS

The range of the measurements (both length and weight) as well as the range of nematode inoculum and the results of treatments of grubs of each instar with different doses of nematodes are given in Tables 2 to 6. The grubs were observed at every 48 h for recording mortality. The dead grubs were transferred on to a nematode collecting dish.

The length of first instar grubs ranged from 4-12 mm and body weight from 1-35 mg. The second instar grubs had a range of 12-26 mm in length and body weight of 35-451 mg. The body length ranged 27-47 mm and body weight 519-1709 mg in case of third insters. In the case of fourth instar it was 40-61 and 2106-5064 mg in length and body weight respectively. The measurement of range of fifth instar was 50-79 mm in length and 4460-1000 mg in body weight (Tables 2 to 6)

The first instar grubs recorded the maximum mortality of 15 grubs out of 120 numbers treated. Sixty out of the 120 grubs were placed in an incubator at 25-29°C, which was favourable for nematode development.

Among the grubs dead, 12 died within ten days of inoculation, with three cadavers showing symptoms similar to neoaplectanid infection. But these cadavers turned black within a couple of days. Nematodes were not obtained from any of the cadavers, showing that no nematode development occurred inside the host body. The maximum percentage (20%) of mortality recorded was in those grubs which received a dose of 200 nemas per g body weight. All of the grubs that survived fed normally and moulted into subsequent instars. Two grubs out of the 20 kept under check (T_0) also were found dead due to natural causes.

In the case of second instar grubs, 10 out of 120 grubs were observed dead. Those which were exposed to room temperature conditions were 98 in number; while 22 were placed in incubator. While seven out of the 98 placed under room temperature conditions (28-32°C) were dead, three were killed out of the 22 placed in incubator. All the three cadavers were yellowish indicating possibility of infection by nematodes. However no nematodes were recovered when the cadavers were placed for nematode extraction. The seven grubs which died under room temperature conditions all turned black and did not yield nematodes.

Treatment of the third, fourth and fifth instar grubs did not record any mortality, either in those maintained in the lab at room temperature (28-32°C) or in the incubator (25-29°C). Out of the 120 grubs in each of third, fourth and fifth instars, 57, 60 and 62 respectively were placed in incubator. All of the grubs were observed to feed and moult normally. Statistical analysis was not carried out as the mortality obtained could not be confirmed as due to nematodes; as also due to very low levels of mortality.

4.2. Per-os injection of grubs with nematodes

Oral injection of the grubs of third, fourth and fifth instar grubs with 1500 infective juveniles each did not record any mortality. The grubs dissected at 6, 12, 24 and 72 h did not reveal presence of live or dead nematodes either in the haemocoel or in the alimentary canal. When offered fresh feed the grubs fed normally and were active.

4.3 Survival of nematodes on bark

In survival studies, live, active nematodes were recovered from the bark pieces on first day but on subsequent days their number was reduced appreciably. On fifth day only very few live nematodes were recovered and they too, were not active. However, they became motile when disturbed with a nematode pick.

Table 2. Details showing the body measurements, inoculum added and percentage mortality of first instar grubs of cashew stem borer

Treatment	Length (range) mm	Weight (range) mg	Inoculum added (range)	No.of grubs treated	No.of grubs dead	Percentage mortality
T_0	5-12	1-20	0	20	2	10
T_1	4-12	5-24	1-2	20	1	5
T_2	5-12	5-29	1-4	20	2	10
T_3	5-10	2-32	1-4	20	3	15
T_4	5-12	6-35	1-6	20	3	15
T_5	4-10	1-18	1-4	20	4	20

Table 3. Details showing the body measurements, inoculum added and percentage mortality of second instar grubs of cashew stem borer

Treatment	Length (range) mm	Weight (range) mg	Inoculum added (range)	No.of grubs treated	No.of grubs dead	Percentage mortality
T_0	13-24	51-271	0	20	0	0
T_1	13-25	40-448	2-34	20	1	5
T_2	13-26	36-343	4-35	20	0	0
T_3	12-25	35-373	4-43	20	5	25
T_4	12-26	40-451	6-81	20	1	5
T_5	13-26	42-420	9-84	20	3	15

Table 4. Details showing the body measurements, inoculum added and percentage mortality of third instar grubs of cashew stem borer

Treatment	Length (range) mm	Weight (range) mg	Inoculum added (range)	No.of grubs treated	No.of grubs dead	Percentage mortality
T_0	27-47	630-2055	0	20	0	0
T_1	29-46	555-1994	42-149	20	0	0
T_2	29-43	601-2047	60-205	20	0	0
T_3	29-44	519-1924	65-241	20	0	0
T_4	28-45	875-1709	59-258	20	0	0
T_5	29-46	677-1900	135-380	20	0	0

Table 5. Details showing the body measurements, inoculum added and percentage mortality of fourth instar grubs of cashew stem borer

Treatment	Length (range) mm	Weight (range) mm	Inoculum added range	No.of grubs treated	No.of grubs dead	Percentage mortality
T_0	40-59	2306-4863	0	20	0	0
T_1	45-61	2106-5064	158-577	20	0	0
T_2	43-57	2158-4800	216-480	20	0	0
T_3	42-60	2169-4990	271-624	20	0	0
T_4	41-60	2106-4944	316-714	20	0	0
T_5	41-60	2370-4911	474-982	20	0	0

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T_1	29–46	555–1994	42–149	20	0	0
T_2	29–43	601–2047	60–205	20	0	0
T_3	29–44	519–1924	65–241	20	0	0
T_4	28–45	875–1709	59–258	20	0	0
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T_2	29-43	601-2047	60-205	20	0	0
T_3	29-44	519-1924	66-241	20	0	0
T_4	28-45	875-1709	59-258	20	0	0
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T_2	29-43	601-2047	60-205	20	0	0
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Treatment	Length (range) mm	Weight (range) mm	Inoculum added range	No.of grubs treated	No.of grubs dead	Percentage mortality
T_0	40-59	2306-4863	0	20	0	0
T_1	45-61	2106-5064	158-577	20	0	0
T_2	43-57	2158-4800	216-480	20	0	0
T_3	42-60	2169-4990	271-624	20	0	0
T_4	41-60	2106-4944	316-714	20	0	0
T_5	41-60	2370-4911	474-982	20	0	0

Table 6. Details showing the body measurements, inoculum added and percentage mortality of fifth instar of grubs of cashew stem borer.

Treatment	Length (range) mm	Weight (range) mm	Inoculum added (range)	No.of grubs treated	No.of grubs dead	Percentage mortality
T_0	54-71	5055-9520	0	20	0	0
T_1	59-70	4795-7829	359-587	20	0	0
T_2	52-70	4500-7607	450-761	20	0	0
T_3	50-71	4460-8951	558-1119	20	0	0
T_4	57-73	4925-10000	739-1500	20	0	0
T_5	59-77	5023-9083	1005-1817	20	0	0

Discussion

DISCUSSION

The cashew root and stem borer *Plocaederus ferrugineus* is one of the serious pests causing heavy casualities of grown up cashew trees. Control of the pest by the use of chemical agents has not met with complete success, due to the peculiar nature of the pest in attacking cashew trees. Several reports (Lindegren and Barnett, 1982; Cavalcaselle and Deseo, 1984 and Bong, 1986) indicate that populations of stem borer pests of trees have been successfully suppressed by the use of insect parasitic nematodes. Pillai *et al*. (1976) reported that the DD-136 nematode can cause mortality of *P. ferrugineus*. *N. carpocapsae* has been recognised as a common nematode parasite capable of parasitising insects belonging to several taxonomic groups. Hence this study was undertaken to determine the parasitic ability of the nematode on the cashew borer pest and to work out the optimum inoculum required for causing required levels of mortality of different larval instars.

The observations recorded on the different individual larval stages revealed that they vary considerably in the same stage group in their length as well as in their body weight (Table 2-6).

The parasitic nematode inoculum load given to individuals of first instar stage varied from the minimum of 1-6 numbers, to a maximum number of 359-1817 in the case of fifth instar stages of the test pest. The parasitic nematode culture used for the test was infective, as shown by periodical inoculation of <u>Corcyra cephalonica</u> larvae. Poinar and Thomas (1966) had reported that even a single axenic infective juvenile could bring the mortality of the wax moth larva. Initially the different instars of the test insect were under ambient temperature conditions (22-32°C) in the laboratory. The humidity was also maintained at required levels. However the results obtained indicated that the nematodes could not bring out significant levels of mortality of the host instars. Mortality was recorded only in case of 15 grubs out of 120 first instar grubs and 10 out of 120 second instar grubs. The rest of the grubs continued to feed normally and developed into subsequent instar stages. The dead grubs were black in colour, in contrast to the creamy yellow colour found associated with neoaplectanid infections. The third, fourth and fifth instar grubs recorded zero death.

The failure of infection by the parasitic nematodes was initially attributed to the high temperature gradients. However Schmiege (1963) and Kaya (1977a; 1977 b) had reported that nematode parasites retained infectivity up to 30°C. When the results obtained on mortality of the grubs were in negative under laboratory conditions the experiment was further continued by using a BOD incubator. The different instars treated with the required inoculum load was incubated at 25-29°C in the BOD incubator adopting the same procedures and techniques followed earlier. In this case also, most of the treated instars were not subjected to death by the nematode and they continued to feed and moult normally. Thus providing optimum temperature conditions for infection also did not help in the infectivity and parasitisation to cause mortality by the nematode on their host insects.

To further confirm the ability of the nematodes to parasitise the host insect grubs, selected grubs of third, fourth and fifth instars were taken and the nematode inoculum was added by using per-os techniques. This test also indicated that the nematode failed to bring about mortality of the host insect instar stages.

Thus all the tests carried out to find out the ability of nematode to parasitise the different instar stages indicated that the insect larvae may have some kind of natural resistance towards this nematode parasite. The incubated dead larvae which were creamy yellow in colour did not yield any nematodes, indicating that even the nematodes which entered if any, were inactivated and not allowed to feed and develop any further within the host haemocoel.

Webster and Dunphy (1987) have reviewed in detail the mechanisms of host compatibility of insects to entomogenous nematodes. According to them, location and synchronisation, host tissue tolerance, nematode insect nutritional balance, host diet and host hormone are the factors likely to be responsible for the incompatibility.

According to Poinar (1967) who had discussed on arthropod immunity to worms haemocoelic encapsulation of the nematode parasites and humural responses are the two factors which lead to restriction of the further development of the parasite in the hosts.

In the present study the results revealed that N. carpocapsae could not effectively parasitise the different instar larvae of P. ferrugineus and cause mortality. The different techniques followed from treating the nematode inoculum under simulated host environmental conditions to direct oral application had failed to induce parasitisation and bring about the death of the host insect larval stages. Several workers have reported the failure of DD-136 nematode to infect host insects even under favourable conditions (Kurashvili, 1980; Tedders *et al.*, 1982 and Poinar, 1984). These reports indicate that causes for failure of the nematode to induce significant mortality were either the small size of the insect host or the inability of the nematodes to gain entry through natural openings or by penegration. In the present studies it was not possible to establish whether the nematode had penetrated the host insect body. The grubs inoculated per-os with nematodes were dissected out to examine whether any nematodes could be covered. However the attempts failed to reveal the presence of any live or dead nematodes either in the haemocoel or in the alimentary canal. The reasons for this could not be known. Even under ideal conditions, *Steinernema feltiae* (=*N. carpocapsae*) was ineffective against *Synanthedon typhiaetormis* (Deseo and Miller, 1985). According to Zelazny (1985) *N. carpocapsae* could not infect the first instar grubs of *Oryctes rhinoceros* possibly due to host resistence.

The present studies could not confirm the findings reported by Pillai *et al.* (1976). These authors however did not clearly indicate the exact strain of *N. carpocapsae* used for their studies.

Differences among the strains of *N. carpocapsae* namely Breton strain and DD-136 strain in their infectivity to host insects had been reported by Silverman *et al.* (1982). It is not clear whether the contradictory results obtained in the present studies compared to those reported by Pillai *et al.* (1976) could be ascribed to the differences in strains of nematode parasites used in the studies. The authors have not made it clear whether they obtain infectivity and mortality of all the host instar stages. The techniques followed in the present studies were similar to those adopted by the above authors (Pillai, personal communication). The varieties in the present study and the one reported by Pillai *et al.* can perhaps be ascribed to variations in the nematode cultures used in the two investigations.

Tests carried out to study the survival of the parasite revealed that they were alive on the bark portion of cashew up to the fifth day, supporting the view that they could survive on bark material to achieve infection of the host insects. The factors of non-availability of nematode inoculum can be ruled out in the present studies because the inoculum was added directly to the host insect's larval feeding sites in the tunnels. In spite of these, it was surprising to observe that not even a single one of the third, fourth and fifth instar larval stages treated with nematode inoculum load died. Thus it gives room for the speculation that the grubs might be refractory to the nematode and its associated bacterium due to certain antagonic factors present in the alimentary canal of the grubs.

Thus the present studies have indicated that the DD-136 _N. carpocapsae_ cannot be used as a bio-control agent against populations of stem and root borer of cashew. However, studies can be undertaken to explore further the possibilities of other species of _Neoaplectana_ which can be successfully employed as a bio-control agent against _Plocaederus ferrugineus_. Poinar (1967) had reported that neoaplectarid nematodes have no natural host, and that with the aid of the associated bacteria can develop in many hosts if the infective juveniles can reach the haemocoel. Moreover, it will be worthwhile to explore whether any natural nematode enemies are parasitising the cashew infection of the host insects. The factors of non-availability of nematode inoculum can be ruled out in the present studies because the inoculum was added directly to the host insect's larval feeding sites in the tunnels. In spite of these, it was surprising to observe that not even a single one of the third, fourth and fifth instar larval stages treated with nematode inoculum load died. Thus it gives room for the speculation that the grubs might be refractory to the nematode and its associated bacterium due to certain antagonic factors present in the alimentary canal of the grubs.

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The results from the present studies call for further detailed in-depth studies on the various factors that are responsible for the host insect response to parasitic nematodes.

Summary

SUMMARY

A laboratory experiment was conducted at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, to explore the possibility of controlling the populations of cashew stem borer, Plocaederus ferrugineus L. by using the entomogenous nematode DD-136 (Neoaplectana carpocapsae Weiser). From each instar, 120 grubs were selected and inoculated with nematodes at the inoculum load of 0, 75, 100, 125, 150 and 200 nematodes per g body weight, after allow ubs the grubs 24 h to establish in the offered feed. ber of were incubated at room temperature (28-32°C). 25-29°C, grubs were later incubated in a BOD incubat on nematode to study the influence of temperature, zed per-os with infectivity. Selected grubs were inc out at regular 1500 nematodes each and were disse n bark pieces also was intervals. Survival of nemato of bark pieces with studied by inoculating a num pieces on first, second, third, nematodes, washing the b spectively and examining the fourth and fifth day, washings for live matodes.

The results indicate that the nematode could not induce mortality of the cashew stem borer grubs. The maximum mortality was observed in first instar, where 15 out of 120 grubs treated were dead. While 10 out of 120 treated grubs died in the case of second instar grubs, the third, fourth and fifth inster grubs with nematodes did not result in any mortality. The dead grubs were black in colour and did not yield any nematodes when placed in nematode extraction dish.

Per-os inoculation of the grubs too failed to bring about any mortality in the treated grubs. Live nematodes were recovered from the treated bark pieces even after five days.

Results of the present study were not in conformity to an earlier report of 60 per cent mortality at a dosage of 100 nemas per g body weight. The exact reason for the variation could not be immediately ascertained. The variable results suggest the possibility of different strains having been employed in the two studies. Suppression of the infectivity of nematode-bacterium complex by antagonistic factors in the alimentary canal is also speculated.

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A laboratory experiment was conducted at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, to explore the possibility of controlling the populations of cashew stem borer, Plocaederus ferrugineus L. by using the entomogenous nematode DD-136 (Neoaplectana carpocapsae Weiser). From each instar, 120 grubs were selected and inoculated with nematodes at the inoculum load of 0, 75, 100, 125, 150 and 200 nematodes per g body weight, after allowing the grubs 24 h to establish in the offered feed. The grubs were incubated at room temperature (28-32°C). A number of grubs were later incubated in a BOD incubator at 25-29°C, to study the influence of temperature, if any, on nematode infectivity. Selected grubs were inoculated per-os with 1500 nematodes each and were dissected out at regular intervals. Survival of nematodes on bark pieces also was studied by inoculating a number of bark pieces with nematodes, washing the bark pieces on first, second, third, fourth and fifth day, respectively and examining the washings for live nematodes.

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Thus the present studies have indicated that the nematode *Neoaplectana carpocapsae* cannot be used as a bio-control agent against stem and root borer *Plocaederus ferrugineus*.

The results of the present investigations call for in depth studies on host nematode parasite relationship as well as studies into the possible use of other organisms for the bio-control of cashew stem borer.

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CONTROL OF CASHEW STEM BORER (*Plocaederus ferrugineus*, L.) BY THE DD. 136 NEMATODE (*Neoaplectana carpocapsae* WEISER 1955).

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ABSTRACT OF THE THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

FACULTY OF AGRICULTURE

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, TRICHUR

1989

ABSTRACT

An experiment was conducted at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara to evaluate the possibility of utilisation of the DD-136 nematode, Neoaplectana carpocapsae Weiser to control the grub populations of stem borer of cashew Plocaederus ferrugineus L. The grubs, belonging to different instars were treated with different doses of the nematode under simulated field conditions.

The results of the study indicated that the nematode is incapable of inducing mortality of the grubs of cashew stem borer, even under conditions favourable for the nematode.

Detailed, in-depth studies on the various aspects of host-nematode parasite relationships are warrented. Also, it will be worthwhile to examine the possibilities of using other bio-control agents against the cashew stem borer.