2.8 THE SUSCEPTIBILITY OF THE DEVELOPMENTAL STAGES OF TICKS (IXODIDAE) TO ENTOMOPATHOGENIC NEMATODES

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Insect parasitic nematodes, Steinernematidae and Heterorhabditidae (Gaugler 1981, 1987, Kaya 1985) appear to be especially promising as bioinsecticides. These two families of facultative parasitic rhabditoids are characterized by their association with symbiotic bacteria of the genera *Xenorhabdus* and *Photorhabdus* for Steinernemaridae and Heterobditidae respectively. The nematode infective stage (third stage juvenile) carries the bacterial symbiont monoxenically in its digestive tract (Poinar and Thomas 1966). The infective-juvenile (IJ) finds its insect host by a chemotaxis (Gaugler *et al.* 1980). After the hemocoel has been breached, the symbiotic bacteria are released, proliferate, and kill the host by septicemia, mostly within 24 to 48 h. Antibiotic compounds released by the bacteria within the insect prevent development of other microorganisms in the dead insect. The nematodes feed on the bacteria, producing two to three generations; thus thousands emerge from the insect after two to three weeks as IJ searching for new hosts (Poinar 1986).

Thousands of insect species including many of economic importance have been found to be susceptible to nematodes (Poinar 1979). The U.S. Environmental Protection Agency (USEPA) has exempted these organisms from registration and regulation requirements (Gorsuch 1982, Nickle *et al.* 1988). This fact and the successful mass rearing of entomopathogenic nematodes for commercial distribution (Bedding 1981, Georgis 1990) make them promising new biocontrol agents. Currently entomoparasitic nematodes are used commercially against pests in agriculture and in homes in many developed countries (USA, western Europe, Japan, Australia; Georgis and Manweiler 1990). The nematodes can be spread by spraying on the ground, from the air or through irrigation systems (Georgis and Manweiler 1990).

Despite the large body of evidence on pathogenicity of Steinernematidae and Heterorhabditidae to various insects, little is known about the susceptibility of other invertebrates to these nematodes (Poinar 1988, 1989).

The current methods of controlling ticks and tick borne diseases by dipping or spraying are costly and involve repeated topical applications of acaricides. The problems of chemical agents' instability, inaccessibility to treatment stations, time involved, intoxication of livestock and wildlife, resistance, and environmental contamination all contribute to reduced desirability for chemical control. Even after years of tick control programs, tick-borne diseases remain a major limiting factor in the economic development in many countries.

The biological control of plant insect pests is being rapidly developed but relatively little attention has been paid to its potential use against ticks. Several non-specific predators, pathogens and parasitoids of ticks have been described but their practical use has been generally unsuccessful to date (Addison *et al.* 1989, Arthur 1973, Barre 1991, Bowman *et al.* 1986, Cole 1965, Doube and Heath 1975, Geevarghese 1988, Hendry and Rechav 1981, Lipa 1971, Mwangi 1989, Wilkinson 1970).

Recently it was found that fully engorged *Boophilus annulatus* (Say) (Ixodidae) are highly susceptible to infection by entomopathogenic Steinernematidae and Heterorhabditidae (Samish and Glazer 1991, 1992). This paper reports on the virulence of these nematodes to various tick stages of four different tick species in order to determine their possible use in biological control.

MATERIALS AND METHODS

The experiments were performed with the entomopathogenic nematode *Steinernema carpocaosa* strain 'DT' (NC-DT) (Steinernematidae, Rhabditida, Nematoda). The one-host tick *Boophilus annulatus* was fed on three-month-old Frisian calves, while larvae and nymphs of the three-host ticks *Rhipicephalus sanguineus* (Latreille) and *Hyalomma excavatum* Koch were fed on gerbils, and the preimaginal stages of the two-host tick *Rhipicephalus bursa* C. and F. and the adults of *R. sanguineus* and *H. excavatum* were fed on rabbits, and adult *R. bursa* on sheep.

The infective juveniles of the nematodes were suspended in water and aliquots dispersed on filter paper in five cm. dia. Petri dishes. Five engorged female ticks, 10

fed or unfed males, 10 unfed females or fed nymphs or 20-40 unfed nymphs or larvae were placed in each Petri dish. Two to four dishes (at least 20 ticks) were used in each replicate. Dishes were incubated at 26-27°C and mortality of the ticks was recorded daily.

RESULTS AND DISCUSSION

The susceptibility of the various tick stages to the entomopathogenic nematode NC-DT varied considerably. Fed males were most susceptible; unfed males and fed females less so and unfed females least (Table 1). The preimaginal stages of the four tick species were resistant to this nematode strain. Larvae and nymphs of *B. annulatus* and *H. excavatum* and larvae and adults of *H. excavatum* and *R. sanguineus*, sprayed with nematodes while feeding on their hosts, were found to be resistant.

The fully engorged *B. annulatus* females became more susceptible to the NC-DT nematodes with time post repletion. When the engorged ticks were incubated for four to eight days at 26°C before they were infested with nematodes, mortality rate doubled or even tripled, whereas post repletion incubation of ticks at 14°C resulted in a lower mortality rate.

Different species and developmental stages of ticks varied conspicuously in their resistance to various entomopathogenic nematode strains. *Amblyomma variegatum* (Fabricius) was found to be resistant (Barre et al. 1991). Our study shows that the best tick model for entomopathogenic nematode NC-DT control are *B. annulatus* fully engorged females which drop off the host.

Although feeding ticks appear to be resistant to the nematodes, engorged females which dropped to the ground are susceptible and become even more so with age. The upper layer of soil is the natural habitat of the nematodes as well as of engorged ticks and therefore dispersing the nematodes on the ground seems the most promising approach. Our finding (Samish and Glazer 1992) that different nematode strains differ greatly in their virulence against *B. annulatus* shows that selection of more virulent nematode strains (NC-DT) to *B. annulatus* is badly needed.

The major reasons that nematodes could become a successful control agent of *B. annulatus* are:

1) The engorged female ticks which dropped off the host animal are susceptible to the nematodes, and they become more susceptible with aging. The engorged female ticks hide for days or weeks in the dark, humid upper layer of the soil which is also the natural habitat of the infective juvenile nematodes. The mobile nematodes can easily reach the sluggish, fully engorged ticks.

Table 1. Susceptibility of adult ticks of various species to *Steinernema carpocapsa* strain 'DT'.

	B. annulatus	*R. bursa*	*H. excavatum*	*R. sanguineus*
# hosts	1	2	3	3
Male fed	+++	+++	+++	++
Male unfed	ND	++	+	+
Female fed	+++	+	+	++
Female unfed	ND	+	+	+

ND-not done

2) Fully engorged *B. annulatus* females drop off mainly at night (Hadani et al. unpublished results) in the resting areas of the host herd. Nematodes could be applied in these limited ares.

3) The ability of entomopathogenic nematodes to remain infective in humid soil for long periods (two to three months) (Akhurst 1986, Kaya 1985) may allow long intervals between treatments which reduce expenses. If the nematode population becomes established in the habitat of the fully engorged ticks, control may become even more reliable.

4) Entomopathogenic nematodes are especially well suited for an integrated pest control regime of ticks since they are resistant to many types of insecticides (Rovesti and Deseo 1990). In addition anti-tick compounds are generally applied to the animals while nematodes will be applied on the ground, so that the two control methods are complementary.

SUMMARY

Entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae are used commercially against many insect pests. The susceptibility of *Boophilus annulatus* ticks to such nematodes was first reported by Samish and Glazer (1991). This report demonstrates susceptibility of a number of developmental stages of *B. annulatus*, *Hyalomma excavatum*, *Rhipicephalus bursa* and *R. sanguineus* to *Steinernema carpocapsa* strain 'DT'(NC-DT) nematodes. Ticks feeding on the host and their off-host preimaginal stages were resistant to NC-DT. The ticks are most susceptible to the nematodes as fed adults. The susceptibility of engorged *B. annulatus* females to nematodes was found to increase with age.

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