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Effects of two neem insecticide formulations on the attractiveness, acceptability and suitability of diamondback moth larvae to the parasitoid, *Diadegma mollipla* (Holmgren) (Hym., Ichneumonidae)

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Abstract: Behavioural responses of female *Diadegma mollipla* to volatiles from cabbage plants and host-infested [*Plutella xylostella* (L.)] cabbage plants sprayed with two neem insecticide formulations were investigated in a Y-tube olfactometer. Parasitoids were significantly more attracted to volatiles from cabbage and host-infested cabbage sprayed with the powder formulation than to clean air. In contrast, parasitoid response to volatiles from cabbage and host-infested cabbage sprayed with the oil formulation was not significantly different from clean air. In choice tests between infested plants sprayed with water (control) or the powder formulation, parasitoids showed no preference for volatiles from either of the treatments. In similar tests with the oil formulation, parasitoids showed a preference for volatiles from control plants over plants sprayed with the oil formulation. In host acceptance and suitability tests, parasitism rates in the neem- and water-sprayed hosts were, with one exception, not significantly different. However, the neem-sprayed larvae died earlier than control larvae and were therefore not able to support parasitoid development. The implication of these findings for the combined use of neem insecticides and parasitoids in the management of *P. xylostella* is discussed.

Key words: *Diadegma mollipla*, *Plutella xylostella*, interaction, neem, parasitoid

1 Introduction

Insecticides derived from the neem tree (*Azadirachta indica* A. Juss.) have received considerable interest for the management of the diamondback moth (DBM), *Plutella xylostella* (L.), which is the most damaging pest of crucifers worldwide (Talekar and Shelton, 1993). They are effective against DBM populations (Kirsch, 1987; Schmutterer, 1992; Javaid et al., 2000) and their complex chemistry would make it difficult for the DBM to develop resistance to them (Völlinger, 1995). Parasitoids are also important natural checks of DBM populations (Talekar, 1992; Talekar and Shelton, 1993; Xu et al., 2001) and increasingly greater importance is being attached to the development and adoption of biological control-based IPM systems for DBM (Talekar, 1992; Talekar and Shelton, 1993; Shelton et al., 1996). While neem insecticides are relatively safer to natural enemies than synthetic insecticides (Schmutterer, 1995a, 1997), their selectivity varies with insect species, formulation and concentration of the neem products. In DBM management programmes where the conservation and utilization of natural enemies is sought, it is necessary to assess the non-target effects of the neem products. Furthermore, the documented effects of neem insecticides include adverse effects on insect behaviour and physiology, which include repellence, phago-deterrence, inhibited oogenesis and disrupted development (Schmutterer, 1990, 1995b). Such properties have potentially adverse implications for parasitoid functioning and could affect the ability of a parasitoid population to regulate the density of its hosts.

Two neem insecticide formulations, Neemros® and Neemroc EC®, are registered in Kenya for use against vegetable pests including the DBM. Among the hymenopteran parasitoids of DBM in eastern Africa, *Diadegma mollipla* (Holmgren) (Hym., Ichneumonidae) is important (Nyambo and Pekke, 1995). Results of our recent study suggest that direct sprays of the two neem insecticide formulations onto *D. mollipla* do not have any adverse effects on the longevity and foraging behaviour of the parasitoids (Akol et al., 2002). In the present study, we tested the hypothesis that neem-sprayed plants or plant–host complexes were unattractive to foraging *D. mollipla* females. We furthermore tested whether neem-sprayed hosts were acceptable as oviposition sites and whether duration of neem residues on host larvae and parasitoid experience influenced the oviposition decisions of the parasitoids. Additionally,

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we assessed the host suitability of neem-sprayed DBM larvae to support *D. mollipla* development.

2 Materials and methods

2.1 Plants

Cabbage (*Brassica oleracea* L. var. *capitata* L. cv. Copenhagen Market) seedlings were raised from seed in seed planters within a screened cage and 4 weeks later were individually sown to plastic pots (15 cm diameter) filled with potting soil (two parts soil, one part sand and one part manure). The plants were used in insect rearing and in the tests when 6–8 weeks old.

2.2 Insects

Plutella xylostella and *D. mollipla* were obtained from insectary colonies that had been initiated from field collected material. The DBM moths were provided with cabbage leaves partially immersed in a water-filled glass vial onto which they oviposited. Hatched larvae were transferred to potted cabbage plants on which they were subsequently reared at 22–26°C under a 14L:10D photoperiod. *Diadegma mollipla* was reared by exposing second instar DBM larvae to mated *D. mollipla* females for 24 h. The exposed larvae were subsequently reared on cabbage plants and at pupation, two parasitoid cocoons were placed in a homeopathic glass vial wherein the parasitoids emerged. Adult parasitoids were fed on a 20% honey solution and distilled water impregnated in separate pieces of cotton wool while adult moths were similarly fed on a 10% sugar solution and distilled water. The insectary colonies were regularly supplemented with field collected individuals so as to minimize loss in fitness during rearing over generations.

2.3 Insecticides

Neemroc EC® [neem seed oil (NSO)] and Neemros® [neem kernel cake powder (NKCP)] were obtained from Saroneem Ltd (Nairobi, Kenya). The content of azadirachtin in the products was 0.03% for NSO and 0.5% w/w for NKCP. They were applied as sprays using a hand-held mist sprayer at the recommended field dose rate of 15 ml/l of water for Neemroc EC® and 25 g/l of water for Neemros® (Varela, 1998; Waiganjo, 1998). Water spray alone was used as a control.

2.4 Olfactometric assays

Olfactory attractiveness of neem-treated plants and plant–host complexes to naïve *D. mollipla* females was investigated in a Y-tube olfactometer by testing individual odour sources against either clean air or another odour source. The source of test odours was placed in a glass jar (2 l capacity) with an airtight lid connected to an olfactometer arm by a Teflon® (Cole-Parmer Instrument Co., Vernon Hills, Illinois, USA) tube (0.5 cm inner diameter) (fig. 1). Two pressure pumps (Cole-Parmer Air cadet vacuum/pressure station) pumped air into and out of the system. Air from the inlet pressure pump was passed through an activated charcoal filter for purification, then through a flowmeter (Cole-Parmer Instrument Co., Vernon Hills, Illinois, USA) and finally split into two streams with each stream passing into an odour source jar. A second flowmeter was connected between the stem of the olfactometer and a second pump, which exhausted air out of the system. Airflow into the olfactometer was set at 118 ml/min and at the exit at 526 ml/min. These settings ensured the most perfect laminar flow of air within the cross-section of the Y-tube while allowing the parasitoids to move through it. Teflon® tubes conveyed air between the olfactometer, the flowmeters and charcoal filter. White Styrofoam boards (30 cm high) screened the observation arena.

Cabbage plants (uninfested and infested) with about six fully formed leaves were used in the tests. Infested cabbage plants were obtained by placing 30 second instar DBM larvae (five larvae per leaf) on individual plants and these were allowed to feed overnight (14 h). The infested and uninfested cabbage plants were then sprayed to near run-off with the neem insecticide treatments (NKCP and NSO) or water alone and allowed to air-dry before being used in the tests. Naïve, mated parasitoids (3–5 days old) were introduced singly into

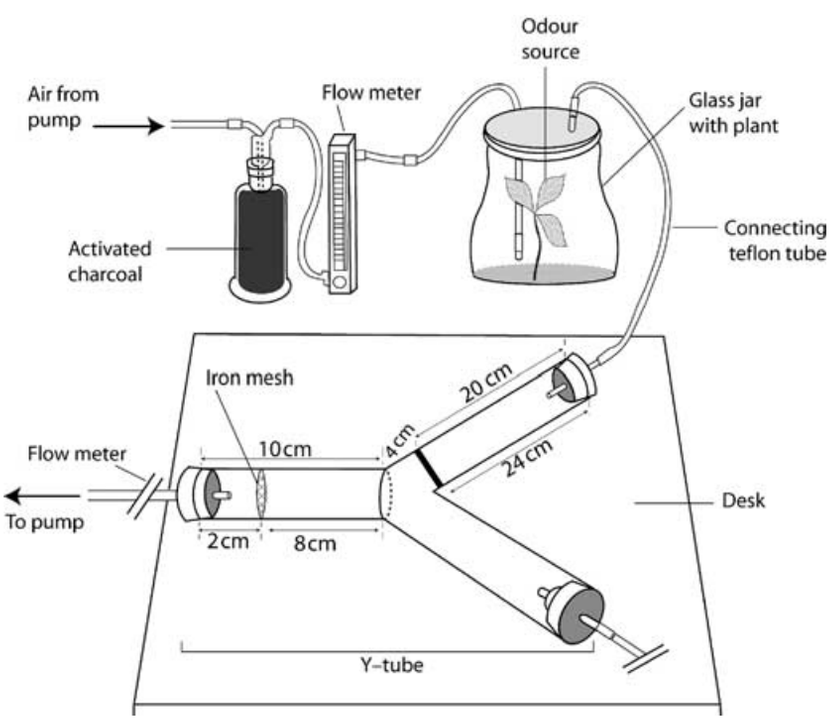

Fig. 1. *A schematic diagram of the Y-tube olfactometer and experimental set-up*

the stem of the olfactometer and allowed 3 min to choose one of the arms of the olfactometer. A disc of iron mesh (mesh size 4 mm) in the stem (2 cm from the end) of the olfactometer prevented the introduced parasitoid from flying straight into the arms of the olfactometer and encouraged it to initiate walking. Parasitoids that passed the finish line (marked 4 cm past the intersection) and remained arrested for more than 15 s in the olfactometer arm were recorded as having made a choice. Individual parasitoids were given a choice between air permeated with volatiles from the following treatments: (1) water-sprayed cabbage plant and a clean air control; (2) water-sprayed infested cabbage plant and a clean air control; (3) neem-sprayed cabbage plant and a clean air control; (4) neem-sprayed infested cabbage plant and a clean air control; (5) water-sprayed infested cabbage plant and a water-sprayed uninfested cabbage plant; (6) water-sprayed infested cabbage plant and a neem-sprayed infested cabbage plant. For the control, air was drawn through an empty jar. In all tests, each parasitoid was used only once and discarded. At least 50 parasitoids were tested for each treatment. All tests were conducted at 23 ± 2°C, 65–75% RH. Data were analysed using the log-likelihood ratio test (*G*-test) for goodness-of-fit (PROC CATMOD, SAS INSTITUTE, 1990). Parasitoids that made no choice were excluded from the analyses.

2.5 Host acceptance

A cabbage leaf infested with 25–30 second instar DBM larvae was placed into an oviposition cup (300 ml clear plastic cup with a mesh covered ventilation window inverted over a 150 ml plastic cup) and sprayed with 6 ml of either a neem insecticide (NKCP or NSO), or water. At 24 or 48 h after treatment, each group of insecticide- or water-sprayed hosts was exposed to a single 3–5-day-old parasitoid female for 24 h. The parasitoids used either had no oviposition experience (naïve) or had acquired oviposition experience (experienced) on untreated second instar DBM larvae for 24 h prior to being used in the tests 12–18 h later. Each host was subsequently dissected in a drop of water under a microscope. The percentage of hosts parasitized by each parasitoid (indicated by a parasitoid egg in the host) for each level of parasitoid status, treatment and time of exposure was determined. The percentages were arcsine transformed and subjected to a three-way ANOVA (PROC ANOVA, SAS INSTITUTE, 1990) with treatment, parasitoid status and post-treatment exposure time as factors.

2.6 Host suitability

Second instar DBM larvae were exposed to mated *D. mollipla* females at a host to parasitoid ratio of 20:1 for 24 h to allow oviposition. The parasitized larvae were then transferred to a cabbage plant and sprayed with 10 ml of water or neem insecticide at a lethal (25 g/l NKCP or 15 ml/l NSO) or predetermined sublethal dose (15 g/l NKCP or 10 ml/l NSO). The proportion of parasitoid adults that emerged was determined and used to assess the effects of the different treatments on the suitability of DBM larvae to support parasitoid development. The experiment was replicated five times with a minimum of 19 larvae in each replicate.

3 Results

3.1 Olfactometric assays

In choice tests between clean air and the NKCP-sprayed plants, whether uninfested or infested, a significantly higher proportion of parasitoids was attracted to odours from the neem-sprayed plants than to clean air (figs 2 and 3). In similar tests with NSO-sprayed plants the parasitoids' response to odours from the uninfested plants was comparable with clean air but there were no significant differences in the choices made for odours from NSO-sprayed infested plants and clean air. In the tests with water-sprayed plants, the proportion of parasitoids that moved to the arm bearing clean air (54%) was not statistically significant from the proportion (42%) of parasitoids that chose the odours from the uninfested plant. Similarly, parasitoid choice for odours from the water-sprayed infested plant (57%) was not statistically significant from that for clean air (37%). However, in pairwise comparisons between different treatments, the proportion of *D. mollipla* adults responding to neem-sprayed uninfested plants (60–61%) was significantly greater than for water-sprayed uninfested plants (42%). When the same comparisons were made with infested cabbage plants, choice distribution for the neem-sprayed plants (54–63%) was not significantly different from that for water-sprayed plants (57%).

In the choice tests involving odours from water-sprayed infested and uninfested plants, significantly more parasitoids were attracted to the former (61 and 33%, respectively). When offered a choice between odours from NKCP-sprayed infested plants and water-sprayed infested plants, choice distribution for the two odour sources (53 and 40%, respectively) were not statistically significant (fig. 4). In a choice between a water-sprayed infested plant and an NSO-sprayed infested plant, significantly more females preferred the arm with odours from the water-sprayed plant

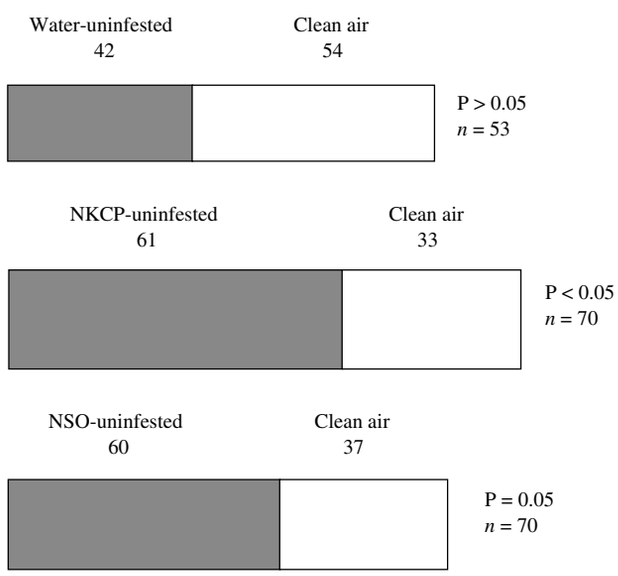

Fig. 2. *Response of naïve* Diadegma mollipla *females to odours from an uninfested cabbage plant sprayed with water or a neem insecticide. N, number of parasitoids tested individually; G-test for goodness-of-fit. Numbers next to the bars indicate the percentage of parasitoids that made a choice for one of the odour sources; the proportion of unresponsive parasitoids is not given*

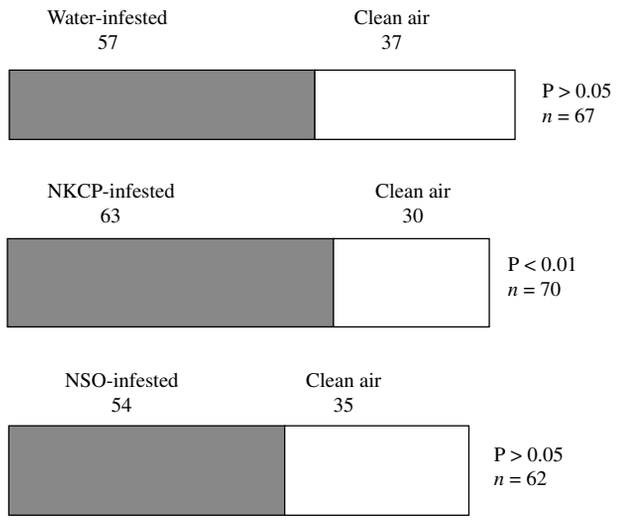

Fig. 3. *Response of naïve* Diadegma mollipla *females to odours from an infested cabbage plant sprayed with water or a neem insecticide. Other explanations as for fig. 2*

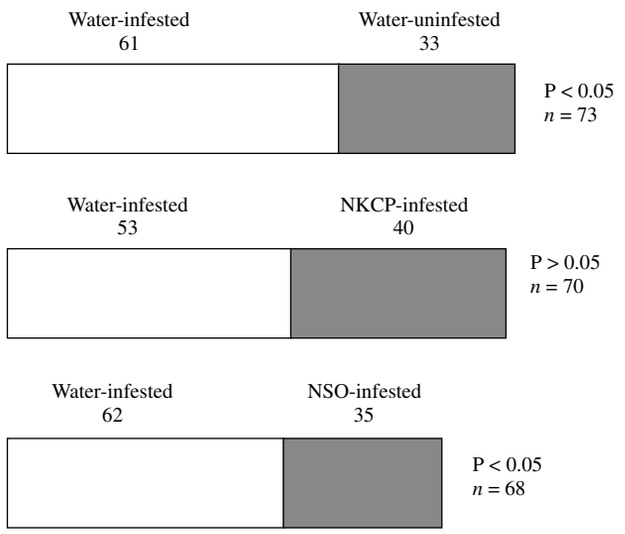

Fig. 4. *Response of naïve* Diadegma mollipla *females to odours from an infested cabbage plant sprayed with water or a neem insecticide in dual choice tests. Other explanations as for fig. 2*

(62%) to the arm with odours from the NSO-sprayed plant (35%).

3.2 Host acceptance

Host acceptance by naïve and experienced parasitoids was variable with respect to the treatment the larvae received and the time at which they were exposed to parasitism. The three-way interaction for treatment, post-exposure time and parasitoid status was highly significant. The proportion of hosts parasitized by experienced females was not significantly different between the treatments at 24 or 48 h post-treatment (table 1). Similarly, host acceptance by the experienced females did not differ significantly between the time intervals for the NKCP-sprayed hosts or for NSO-sprayed hosts. With naïve females, acceptance of hosts exposed 24 h after treatment was not significantly different between the treatments. However, significant differences between the treatments were recorded for hosts exposed to parasitism 48 h after treatment. Host acceptance of NKCP-sprayed hosts was not significantly different from the water-sprayed control hosts but both were higher than the NSO-sprayed hosts. The proportion of NKCP-sprayed hosts parasitized by the naïve females after 24 and 48 h was not significantly different. The proportion of NSO-sprayed hosts parasitized after 24 h post-treatment was however, significantly higher than that at 48 h after treatment.

3.3 Host suitability

The high mortality levels in the neem insecticide treatments precluded meaningful statistical analysis of the data. Table 2 shows the proportion of all adult insects (pooled across replicates) that emerged in each treatment and what proportion of these were parasitoids. In both cases percentage emergence from hosts that were sprayed with the lethal (NKCP 2.5% or NSO 1.5%) and sublethal doses of neem insecticides (NKCP 1.5% or NSO 1.0%) was extremely low compared with the proportion of adults that emerged from the water-sprayed hosts.

Table 1. *Parasitism of neem-sprayed diamondback moth larvae by naive and experienced* Diadegma mollipla *at two time intervals after treatment*

	Larvae parasitized (%)			
	Naïve females		Experienced females	
Treatment	24 h	48 h	24 h	48 h
NKCP	59.5 ± 3.71 aA (34)	62.1 ± 4.09 aA (31)	63.3 ± 6.32 aA (18)	58.8 ± 6.97 aA (16)
NSO	63.2 ± 3.37 aA (38)	48.3 ± 3.65 bB (28)	52.2 ± 4.69 aA (19)	65.7 ± 3.89 aA (15)
Water (control)	62.2 ± 3.15 aA (35)		57.8 ± 5.69 aA (21)	

Within columns, mean values compare treatments within a time interval for both types of parasitoid females and, mean values with the same lowercase letter are not significantly different at $P < 0.05$, Student–Newmann–Keul multiple range (SNK) test. Within rows, mean values compare time within a treatment for both types of parasitoid females and, mean values with the same uppercase letter are not significantly different at $P < 0.05$, SNK test. Figures in parentheses are number of parasitoids tested (replicates).

Table 2. *Effect of a lethal and sublethal dose of two neem insecticides on the suitability of diamondback moth larvae for* Diadegma mollipla *development*

Treatment	No. of DBM larvae exposed*	Insects emerged (%)	*D. mollipla* emerged (%)*
NKCP 25 g/l	146	5.5	2.1
NKCP 15 g/l	146	11.0	4.8
NSO 15 ml/l	201	3.5	2.5
NSO 10 ml/l	185	7.6	4.3
Water (control)	109	52.3	40.4

* Data pooled across replicates.

4 Discussion

Volatile infochemicals play an important role in insect communication in tritrophic systems (Vinson, 1976; Nordlund et al., 1981; Dicke and van Loon, 2000). Intact cabbage plants release a blend of volatiles (isoprenoids, isothiocyanates, aldehydes and acetates) to which DBM adults respond and use to locate the host plants (Auger et al., 1989; Pivnick et al., 1994). It has been amply demonstrated that parasitoids show specific responses to volatile blends (synomones) from plants or substrates in which their hosts commonly occur and make use of these volatiles in host-finding (Schuster and Starks, 1974; Shahjahan, 1974; Loke and Ashley, 1984; Auger et al., 1989; Noldus et al., 1990; Turlings et al., 1990; Agelopoulos and Keller, 1994b; Ngi-Song et al., 1996, 2000). Frass is also an important source of host-finding cues (Elzen et al., 1984; Loke and Ashley, 1984; Thibout et al., 1993; Ngi-Song et al., 1996).

In the present study, *D. mollipla* was unresponsive to odours from water-sprayed uninfested cabbage plants and showed a marked preference for odours from water-sprayed infested plants when the two odour sources were presented together. Infested cabbage plants release additional volatiles including methyl and allyl isothiocyanate (Agelopoulos and Keller, 1994a; Pivnick et al., 1994), which are most likely used by DBM natural enemies in locating their hosts (Reddy et al., 2002). The parasitoids' preference for odours from infested plants was probably a response to the additional volatiles released as a result of the feeding action of the larval hosts. A similar observation was made for *Cotesia rubecula* (Marshal) (Hym., Braconidae), which responded strongly to the altered volatile profile from host-damaged cabbage but was apparently unresponsive to intact plants (Agelopoulos and Keller, 1994b).

In the choice tests between NKCP-sprayed uninfested plants and clean air, the parasitoids showed a stronger attraction to the volatiles from the sprayed plant than to clean air. In contrast, the parasitoids' response to odours from the NSO-sprayed uninfested plants was similar to clean air. As the parasitoids did not respond to volatiles from uninfested plants, this suggests that parasitoids were able to detect and respond, albeit differently, to volatiles from the two neem formulations. In the choice tests between NKCP-sprayed infested plants and clean air, parasitoids were strongly attracted to odours from the plants. This may have been because of the parasitoids responding to the additional volatile components released by larval feeding and, probably others from this neem formulation. In tests between NSO-sprayed infested plants and clean air, parasitoids did not show a marked attraction to volatiles from the treated plants. This may have been because of the presence of other volatile components in the neem oil formulation that contaminated the volatile blend from the infested plant, thus, interfering with the detection of the latter by the parasitoids. This was confirmed by the results from the choice tests involving this formulation and water-sprayed infested plants, which also suggest that the NSO formulation affected the foraging of the parasitoids negatively as they were significantly more attracted to volatiles from the water-sprayed infested plants. Parasitoids are able to discriminate between odours from different sources and/or from a host plant under various treatments (Turlings et al., 1990; Ngi-Song et al., 1996; Takabayashi et al., 1998; Ngi-Song et al., 2000) and 'select' the more preferred odour source. In the choice tests between the water- and NKCP-sprayed infested plants, the parasitoids showed no clear preference between the two odour sources. This result and those of the single choice tests suggest that this formulation had no apparent adverse effect on the parasitoids foraging responses and may have even rendered the uninfested plants more attractive to the parasitoids. This could be an example of an interaction between a plant and a botanical pesticide formulation that enhances natural enemy activity.

Repellence of neem seed derivatives has been established for a range of insect pest species (Fagoonee, 1981; Saxena et al., 1981; Saxena and Rembold, 1984; Coudriet et al., 1985; Jilani et al., 1988) but very little information exists for beneficial arthropods. In this study with *D. mollipla* no repellent effect was observed in tests with the NKCP oil-free powder formulation although the oil (NSO) formulation while not markedly repellent, was not as innocuous as the former. Similar observations were also evident in the host acceptance tests. Acceptance levels of NKCP-sprayed hosts by naïve and experienced parasitoids were not different from those of control hosts after 24 or 48 h. At the 24 h interval, acceptance levels of NSO-sprayed hosts by naïve females were not different from the control and NKCP-sprayed hosts. However, at the 48 h interval hosts were not as acceptable as in the other treatments. It is possible that in the NKCP treatments and in the NSO-24 h treatments there was no significant change in the cues that are emitted by the larvae or their by-products such as frass, and which are in turn utilized by the parasitoids in selecting hosts for attack. Auger et al. (1989) found that DBM larval frass contained crucifer-derived disulphides to which *Diadromus pulchellus* Wesmael (Ichneumonidae) responded. Probably as time increased after treatment, the oil formulation effected significant changes in the hosts' physiology that may have interfered with the production of cues that mediate host acceptance. The fact that experienced parasitoid females showed acceptance levels of the NSO-treated hosts after 48 h that were comparable with the other treatments, may mean that experienced females are less discriminating

probably because of a lower threshold of acceptability. This may have enabled them to 'decide' to oviposit in treated larvae in the absence of more suitable (untreated) hosts. A separate study measuring time to first oviposition and level of parasitization of both neem insecticide-treated and untreated hosts offered simultaneously to experienced parasitoids for parasitization would help to elucidate the underlying reasons for the reduced host acceptance of NSO-48 h hosts by naïve parasitoids.

The impaired development of neem-treated host larvae demonstrates the potentially adverse effects neem insecticide formulations can have on parasitoid fitness. Schmutterer (1992) suggested that the delayed action of neem insecticides on DBM larvae would allow parasitoids to develop within neem insecticide-treated hosts. The observations made in the present study do not support this and indicate that a healthy host is necessary for successful development of parasitoid progeny. Other researchers have also demonstrated that early host death because of infection or toxins such as insecticides invariably killed the parasitoids contained therein (Irabagon and Brooks, 1974; Outram, 1974; Mcneil, 1975).

Our results show that Neemros® would not significantly impair the processes of host habitat location and host acceptance by *D. mollipla* and, as suggested in a previous work (Akol et al., 2002), may complement biological control programmes for the DBM. Although Neemroc EC®, the oil formulation, does not adversely affect the survival of the parasitoids (Akol et al., 2002), it may result in undesirable effects on their efficiency of foraging. Furthermore, effective applications of both formulations onto DBM larvae would render them unsuitable hosts for propagation of the parasitoid population. We suggest that introduction of these neem insecticides into the DBM-parasitoid system for the control of DBM may require the establishment of DBM-susceptible plant refugia where the parasitoids can perpetuate their numbers. This idea however, needs follow-up studies to establish the population dynamics of the parasitoids in the refugia that would be necessary to substantially lower DBM populations in neem-sprayed fields. In addition, to evaluate whether this system would be more economically viable compared with the separate use of parasitoids and the neem sprays. The study also demonstrates the value of assessing the sublethal effects of insecticides on the trophic interactions between parasitoids and their hosts.

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