

## Integration of biological control and botanical pesticides - evaluation in a tritrophic context

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### Abstract

The plant kingdom is by far the most efficient producer of chemical compounds, synthesising many products that are used in defence against herbivores. Extracts made from some plants, particularly extracts from plants within the Meliaceae (mahogany) family, have been shown to have insecticidal properties. We investigated the potential of these extracts and the possibility of integrating botanical pesticides with biological control of the diamondback moth, *Plutella xylostella*. Sub-lethal doses of botanical extracts were prepared from leaves of the syringa tree (*Melia azedarach*) and commercial preparations (Neemix 4.5<sup>®</sup>) from the neem tree (*Azadirachta indica*). In "no-choice" tests, bioassay trays were used to test the impact of three different doses on first-instar larvae. In "choice" tests, half a leaf was treated with extract and the other half left untreated. The impact that these extracts had on natural enemies was investigated using two parasitoid species, *Cotesia plutellae* and *Diadromus collaris*. Results indicated that these extracts had a significantly negative impact on first-instar larvae of *P. xylostella*. However, the extracts had no direct negative impact on their parasitoids. Therefore, it appears that biological control and botanical pesticides can be combined to control *P. xylostella*.

### Keywords

tritrophic interactions, *Melia azedarach*, *Azadirachta indica*, *Plutella xylostella*, *Diadromus collaris*, *Cotesia plutellae*

### Introduction

The plant kingdom is by far the most efficient producer of chemical compounds, synthesising many products that are used in defence against herbivores. Extracts prepared from plants have a variety of properties including insecticidal activity, repellence to pests, antifeedant effects, insect growth regulation, toxicity to nematodes, mites and other agricultural pests, also antifungal, antiviral and antibacterial properties against pathogens (Prakash & Rao 1986, 1997).

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), continues to present one of the greatest threats to crucifer production in many parts of the world, sometimes causing more than 90% crop loss (Verkerk & Wright 1996). Pesticides have dominated attempts to control *P. xylostella* for more than 40 years (Syed 1992, Shelton *et al.* 1997). Largely because of the negative impact of pesticides and the increasing difficulty encountered in controlling diamondback moth populations, much effort has been devoted to finding alternative control measures for this pest.

Biological control is widely recognised as a major component of *P. xylostella* management strategies particularly where control with chemicals has failed. A wide range of parasitoids has been associated with *P. xylostella*. Over 50 egg, larval and pupal parasitoids have been recorded in the literature. However, as a sole method of pest control in a specific target crop, biological control is seldom sufficient (Hokkanen 1997). Therefore the requirements of biological control must be integrated with the needs and uses of other control tactics such that a synergistic outcome is obtained.

Investigations into alternative control mechanisms for *P. xylostella* have led to the testing of plant extracts. Of the 1,800 plant species reported by Grainge *et al.* (1984) to possess pest control properties, only 82 species have been reported to be active against *P. xylostella* (Morillo-Rejesus 1986). Plants within the Meliaceae (the mahogany family), Asteraceae, Fabaceae and Euphorbiaceae contain most of the insecticidal plant species reported. Extracts from the neem tree, *Azadirachta indica* A. Juss. (Meliaceae) have been made from seeds and kernels and have been found to give good control of *P. xylostella* (Schmutterer 1997, Verkerk & Wright 1993, Prijono & Hassan 1993). A closely related species, the syringa tree, *Melia*

*azedarach* L. (Meliaceae) also has insecticidal properties (Ascher *et al.* 1995) and has been tested against a number of insect species including *P. xylostella*. These botanical pesticides are thought to be compatible with biological control as they have little or no impact on natural enemy species.

*Plutella xylostella* was recorded as a pest on cabbage in South Africa as early as 1917 (Gunn 1917). Twenty-three species of parasitoids and hyperparasitoids have been reported to attack *P. xylostella* in the field (Ulliyett 1947, Kfir 1998). However, control of crucifer pests in South Africa is heavily dependent on insecticides, despite the abundance of natural enemies in the country. Cabbage is an important subsistence crop in South Africa (Bell & McGeoch 1996), and it is estimated that 80% of small-scale rural farmers that have access to water are growing cabbage. Although *A. indica* does not grow in South Africa, the closely related exotic species, *M. azedarach* is common in this country.

Many farmers in developing countries do not have the resources to buy and apply chemical pesticides. Biological control in the form of locally abundant natural enemies and botanical pesticides that can be easily prepared from local trees are free to the farmer and, therefore, uniquely suited to low-input integrated pest management systems. We investigated the impact of extracts prepared from *M. azedarach* and commercial extracts from *A. indica* on *P. xylostella*, with the aim of integrating these botanical pesticides with biological control.

## Materials and methods

### The plant extracts

Leaves were collected from *M. azedarach* at Rietondale in Pretoria, South Africa (28°15'S; 25°44'E). The leaves were placed in a glasshouse (30°C ± 5°C) and left to dry. The leaves were then crushed into a fine powder and stored in an air-tight container until use. Three different extracts were prepared by using different weights of crushed leaves, 1 g, 3 g and 5 g. Each extract was made with 100 mL of distilled water. The water was heated to 48°C, and the leaves were added to the water and shaken for approximately one minute. The extract was left in a refrigerator overnight. The following morning the extract was filtered using Advantec® filter paper no. 2. Three drops of liquid detergent were added to the final extract to act as a wetting agent.

A commercial preparation of *A. indica*, Neemix 4.5®, was provided by Thermo Trilogy Corporation, Columbia, USA. Three different sub-lethal doses were prepared. 10.7 µl (low), 16 µl (medium) and 32 µl (high) per 100 mL of distilled water. These doses are thirty, twenty and ten times below the recommended doses respectively. The control used consisted of 100 mL of distilled water mixed with three drops of liquid detergent.

### Experimental plants and insects

Cabbages, *Brassica oleracea* var. *capitata* L. (Cruciferae) were bought as seedlings and planted in black plastic bags in a glasshouse at 30°C ± 5°C. *Plutella xylostella* larvae were taken from a culture started in 1993, in which several hundred larvae were collected from *B. oleracea* var. *capitata* in the field in Rietondale and an experimental farm near Brits (25°38'S; 27°47'E), South Africa. The laboratory culture is maintained on canola seedlings, *Brassica napus* L. (Cruciferae), however, for these experiments, hatchlings were removed from the canola and placed on cabbage for 24 hours before being exposed to the experimental plants. The two parasitoid species most common in the field were *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae), and *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae). These two species were chosen for the experiments and maintained in culture in the laboratory. Insect rearing and all laboratory experiments were maintained in a controlled environment (24 ± 2°C; 65% r.h., 16:8 L:D).

### No choice test

Leaf discs (30 mm x 35 mm) were cut from the cabbage plants. The leaf discs were placed in a randomised block design in bioassay trays (440 mm x 210 mm). The bioassay trays had 32 cells (4 x 8 cells). The leaf discs were cut to fit into these cells. Four blocks were used and each block had eight treatments. The treatments consisted of the three extracts prepared from *M. azedarach*, the three extracts prepared from *A. indica*, and two control treatments. The leaves were dipped into the treatment and left to dry for approximately 60 minutes. First instar larvae were used in the experiment. One larva was exposed in each cell. The bioassay tray was sealed with "Bio CV 4" vented covers to prevent the larvae from escaping. Six trays were placed randomly on the laboratory bench for each test. The test was repeated six times. The trays

were checked every two days and the leaves replaced. The number of dead larvae or pupae and the number of moths emerging were recorded. Larvae that survived were weighed at the pupal stage.

#### Choice test

One half of the leaf was treated with plant extract or the control treatment and one half was left untreated. The treated side was marked with a felt tip pen. Seven treatments were used: the three extracts from *M. azedarach*, the three extracts prepared from *A. indica* and one control. The treated half of the leaf was dipped into the extract solution and left to dry for approximately 60 minutes. Entire leaves approximately the same size ( $\pm 6$  cm diameter) were used. Each leaf was placed into a Petri dish with a lid (9 cm diameter). For each treatment 10 leaves were used. The treatments were arranged randomly on the laboratory bench. The test was repeated six times. One first-instar larva was placed in the Petri dish, the dish was sealed and the position of the larva was recorded every hour during daylight.

#### Parasitoids

A single parasitoid was placed in a test tube with some honey and a strip of filter paper (6 cm x 1 cm). Seven treatments were used: the three extracts prepared from *M. azedarach*, the three extracts prepared from *A. indica*, and one control treatment. The filter paper was dipped in the treatments and was replaced every two days. For each test, ten parasitoids were exposed to each treatment and the tests were repeated six times for *C. plutellae* and five times for *D. collaris*.

#### Statistical methods

Data were analysed using the Genstat 5 statistical package, version 4.2 (Genstat 5 Committee 2000).

For the no-choice test, mortality was calculated as the proportion of larvae that died out of the total that survived to become moths. Proportions usually follow a binomial distribution, therefore differences between mortality for the different treatments were tested using the generalised linear model (GLM) with binomial distribution (Dobson 1990). Fisher's protected t-test of least significant differences (LSD) was applied to separate the mean proportions at the 5% level of significance. An unbalanced analysis of variance was carried out for the pupal weights, and once again Fisher's protected t-test of least significant differences (LSD) was applied to separate the treatment means at the 5% level of significance. For the choice test, a one sample chi-square test was used and comparisons were made between treatments. An unbalanced analysis of variance was used to analyse the impact of the extracts on the parasitoids. Each parasitoid species was analysed separately.

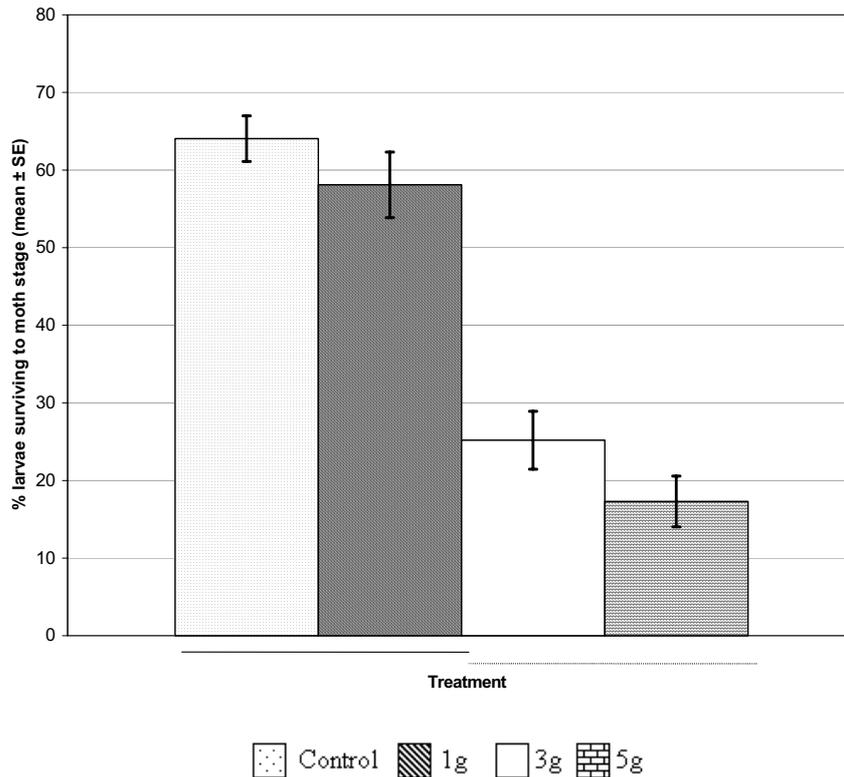
## Results

#### No choice test

When the overall proportion of dead larvae was compared for the different treatments, results showed that there were significant differences between the treatments ( $F_{6,35} = 48.58$ ,  $P < 0.001$ ). It is not the intention to compare the *A. indica* and the *M. azedarach* treatments as the doses are not comparable. Therefore we compared differences within the treatments.

#### Survival of larvae feeding on *M. azedarach*.

Results from Fisher's protected t-test showed that there were significant differences between the three doses. Survival of larvae was highest on the control treatment. Approximately 64% of the larvae feeding on the control treatment survived to the moth stage, followed by 58% of the larvae feeding on the 1 g treatment. These differences were not significant ( $P = 0.252$ ). Approximately 25% of the larvae feeding on the 3 g survived to the moth stage and only 17% of the larvae feeding on the 5 g treatment survived to the moth stage, these differences were also not significant ( $P = 0.125$ ). However, survival on the control and the 1 g treatment was significantly higher ( $P < 0.001$ ) than survival on the 3 g and 5 g treatments (Figure 1).



**Figure 1. Percentage of larvae surviving to the moth stage after feeding on leaves treated with *Melia azedarach*. Treatments underlined are not significantly different.**

#### Survival of larvae feeding on *A. indica*

Results from Fisher's protected t-test showed that there were significant differences between the treatments and the control and also between the different doses. Survival of larvae feeding on the control treatment was significantly higher ( $P < 0.001$ ) than on the other treatments. Approximately 64% of the larvae feeding on the control survived to the moth stage. Approximately 17% of the larvae feeding on the medium treatment survived to the moth stage and 13% feeding on the low treatment made it to adulthood, these two treatments were not significantly different ( $P = 0.353$ ). Only 3% of the larvae feeding on the high treatment made it to the moth stage, this was significantly lower than all the other treatments (low  $P = 0.009$ , medium  $P = 0.002$ ) (Figure 2).

#### Average weight of the pupae found on different treatments

The statistical analysis indicated that there were significant differences ( $F_{6,178} = 6.48$ ,  $P < 0.001$ ) between the different treatments.

#### Weight of pupae found on *M. azedarach*

Results from Fisher's protected t-test showed that the larvae which had been feeding on the control treatment were significantly ( $P < 0.001$ ) heavier as pupae than those larvae, which had been feeding on the treated plants. The pupae that were found on the 1 g treatment were also significantly heavier than those found on the 5 g treatment ( $P = 0.015$ ). There were no significant differences between the pupal weights of those larvae that had been feeding on the 3 g or 1 g treatment ( $P = 0.183$ ), nor between those feeding on the 5 g and 3 g treatments ( $P = 0.273$ ) (Figure 3).

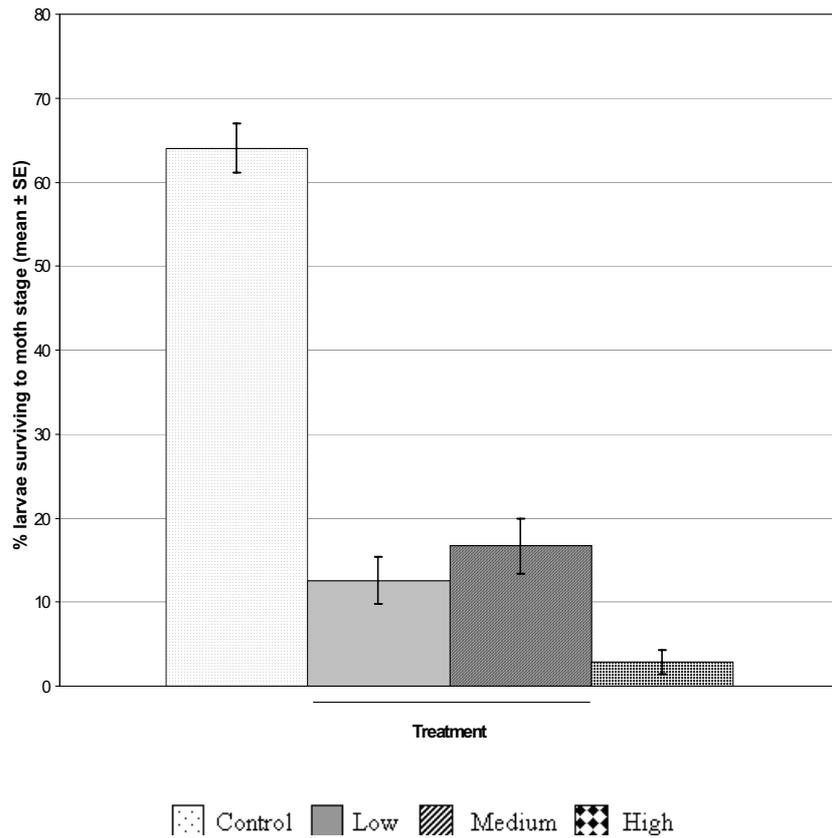


Figure 2. Percentage of larvae surviving to the moth stage after feeding on leaves treated with *Azadirachta indica*. Treatments underlined are not significantly different.

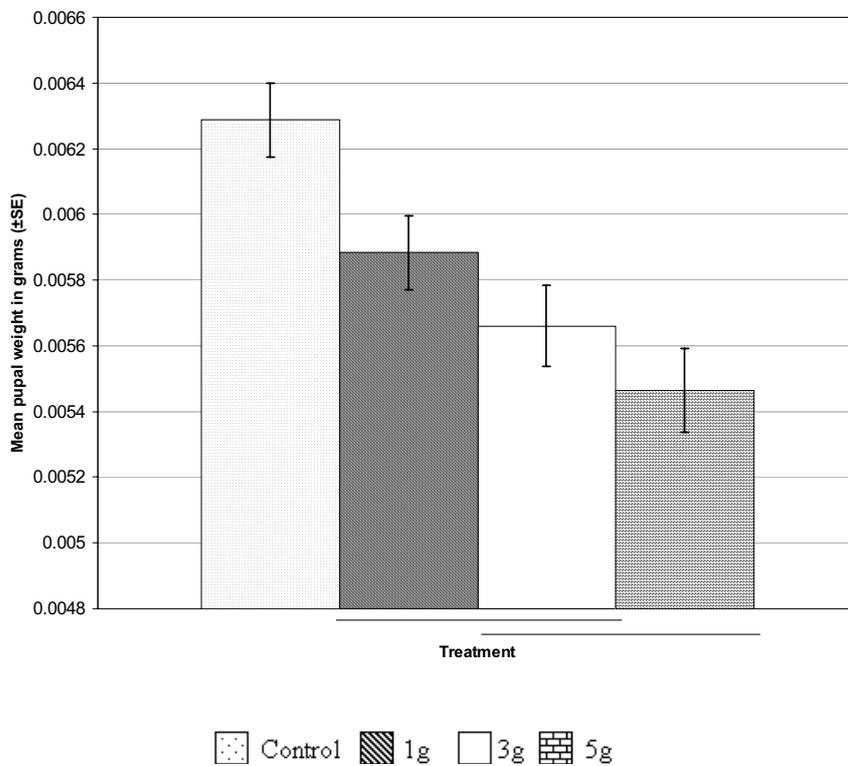
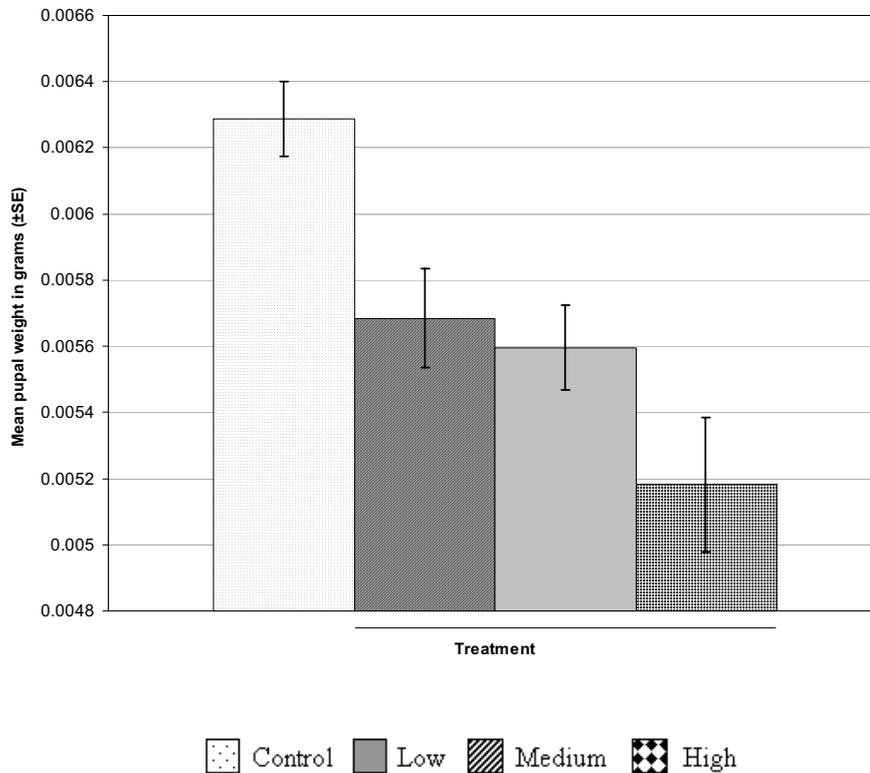


Figure 3. Average weight of pupae found on leaves treated with *Melia azedarach*. Treatments underlined are not significantly different.

### Weight of pupae found on *A. indica*

Results from Fisher's protected t-test showed that there were significant differences between the pupal weights of larvae that had been feeding on the different treatments. Those larvae that had been feeding on the control treatment were significantly ( $P < 0.001$ ) heavier as pupae than those that had been feeding on the treated plants (Figure 4).



**Figure 4. Average weight of pupae found on leaves treated with *Azadirachta indica*. Treatments underlined are not significantly different.**

### Choice tests

A one sample Chi-squared test was done comparing the number of times the larva was found on the treated side of the leaf with the untreated side of the leaf. The larvae spent significantly more time on the untreated side of the leaf if the plants had been treated with the extract (Figures 5 & 6). However, for the control leaf, which had been treated with the distilled water mixed with the liquid detergent, the differences between the number of times that the larvae spent on either the treated side or the untreated side of the leaf was not significant ( $\chi^2 = 0.251$ ,  $P = 0.6230$ ). On occasion, the larvae were found on the Petri dish, or died before the trial could be completed.

### Parasitoids

Results from the analysis of variance indicated that there were no significant differences between the survival of the parasitoids exposed to the different extracts ( $F_{6,383} = 1.14$ ,  $P = 0.341$  - *C. plutellae*;  $F_{6,290} = 0.44$ ,  $P = 0.852$  - *D. collaris*), and in fact they lived for a slightly longer period on the treated strips of filter paper.

Mortality was high at the beginning of the period, with a few individuals surviving for longer periods. Among *C. plutellae*, mortality had reached approximately 50% by the fifth day, with a maximum survival of 34 days for one individual. *Diadromus collaris* is a much longer-lived parasitoid. Once again the plant extracts did not appear to have any significantly negative impact on the parasitoid, with mortality reaching approximately 50% by the 18<sup>th</sup> day, with a maximum survival of 184 days for one individual.

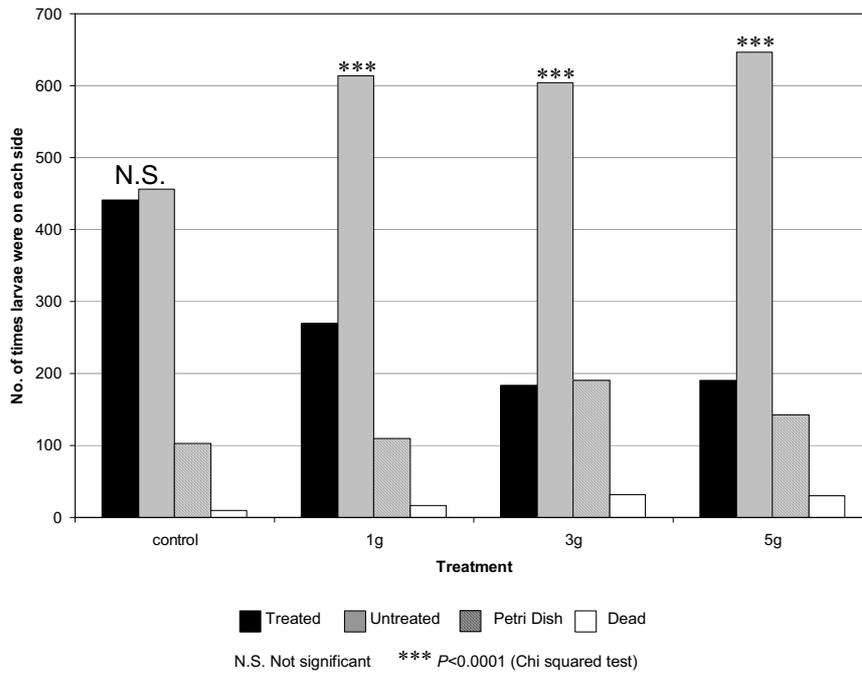


Figure 5. Number of times larvae were found on each side of leaves treated with *Melia azedarach*, or found on the Petri dish, or dead.

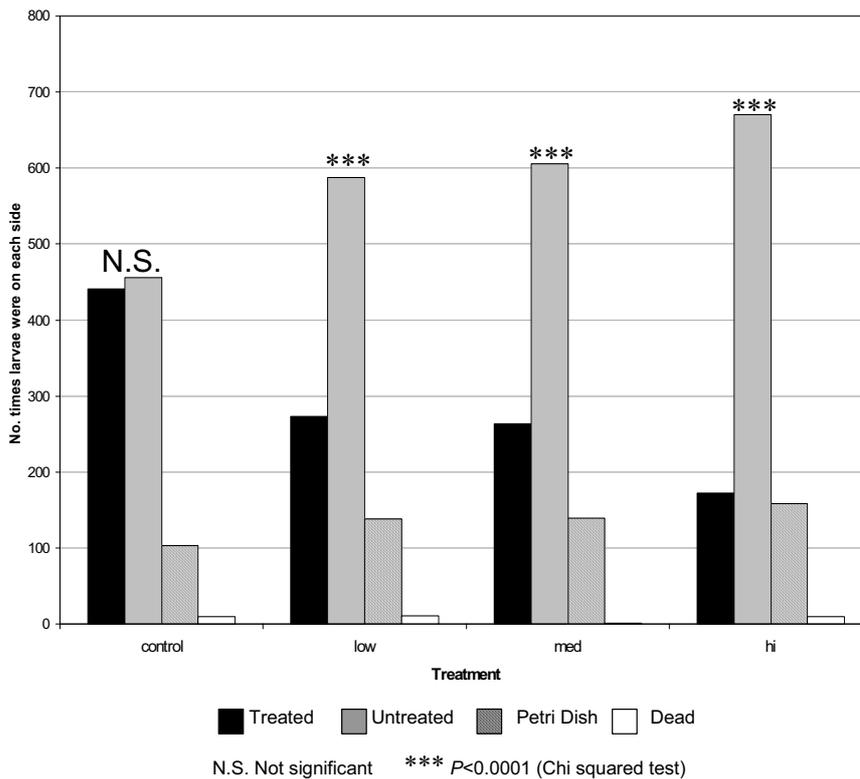


Figure 6. Number of times larvae were found on each side of leaves treated with *Azadirachta indica*, or found on the Petri dish, or dead.

## Discussion

Plants have evolved a variety of ways to defend themselves from invading organisms. From a phytocentric perspective, the chemical options available to a plant could simply be described as repel, deter or kill (Renwick 1996). Members of the plant family Cruciferae are chemically linked by the almost universal presence of glucosinolates, a class of sulfur-containing glycosides, also called mustard oil glycosides or thioglycosides. These compounds are considered the first line of defence of crucifers against insects and other organisms (Renwick 1996). However, some insects, such as *P. xylostella*, have adapted to this line of defence and are crucifer specialists that make use of the glucosinolates and their volatile hydrolysis products

to recognise or locate suitable host plants. The presence of secondary chemical substances such as glucosides and volatile mustard oils make crucifers a highly phagostimulant food substrate (Fagoonee 1981), causing increased feeding of the crucifer specialist.

In contrast, the complex tetranortriterpenoids found within plants from the Meliaceae family are thought to be feeding deterrents (Jacobson 1981). Deterrent chemicals play an important, if not major role, in host plant selection by phytophagous insects (Morgan 1981). In terms of secondary plant chemistry, the Meliaceae is best characterised by the production of limonoids, a group of modified triterpenes. The neem tree contains upwards of 100 different limonoids in its different tissues (Isman *et al.* 1996). Many of these are biologically active against insects as anti-feedants. There has been ample testimony, of a semi-scientific or folklore form, as to the insecticidal, repellent or deterrent qualities of the neem tree, *A. indica*, and the closely related syringa tree, *M. azedarach* (Morgan 1981). In this study we investigated the possibility of using extracts from these trees against the crucifer specialist *P. xylostella*.

Results indicated that neem and syringa extracts were effective against *P. xylostella*, significantly reducing the survival of larvae feeding on cabbage leaves treated with these extracts. When larvae were given a choice they preferred to remain on the untreated side of a leaf. Once the cabbage is treated with neem and syringa extracts, it no longer acts as a phagostimulant. The neem and syringa extracts appear to mask the inherent attractive property of the cabbage plant to the larvae. Similar results have been found for other crucifer specialists. *Crocidolomia binotalis* Zell. (Lepidoptera: Pyralidae) no longer fed on leaves that had been treated with neem extracts (Fagoonee 1981). Zhu (1991) investigated biological effects of syringa extracts on four species of lepidopteran cabbage pests, *Pieris brassicae* L., *Pieris rapae* L. (Lepidoptera: Pieridae), *P. xylostella* and *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae). At low concentrations, the extract caused a disturbance of metamorphosis of IV instars of *P. xylostella* and of various larval instars of the other three species at higher concentrations. *Pieris brassicae* and *P. xylostella* were more susceptible than the other two pests to these extracts. Zhang and Chiu (1983) tested extracts from seed kernels of syringa and found that a 2% extract gave an anti-feeding rate of 74% in choice tests and 76% in no-choice tests for *P. rapae*. When sprayed on the leaves of Chinese cabbage exposed to I instar larvae of *P. rapae*, the extract caused 75% mortality at a concentration of 5 000 ppm and 20% mortality at a concentration of 1 000 ppm. The authors consider the mortality to be due to both feeding inhibition leading to starvation and to stomach poisoning. The results from our current study appear to support this.

Biologically active substances show effects on target organisms but also side effects on non-targets. Consequently neem and syringa products being medium to broad spectrum biochemicals for pest control are not free of side effects on non-targets, but at the same time, these effects are as a rule relatively slight and therefore tolerable, especially in IPM (Schmutterer 1995). Parasitoids are in general less sensitive to neem products than are predators and sometimes even favoured by neem application, for example, when their hosts become more easily accessible for parasitism (Schmutterer 1995). In this study we did some initial trials looking at the direct impact of neem and syringa extracts on two parasitoid species, *C. plutellae* and *D. collaris*. Results indicated that these extracts do not have any direct negative impacts on these two species. However, some negative effects have been observed on growth of parasitoid larvae, weight of pupae and adults and longevity (McCloskey *et al.* 1993). In small species, negative influences have been recorded on emergence rate, walking and searching ability, longevity and fecundity (Feldhege & Schmutterer 1993). Further studies are currently underway to investigate these aspects within *C. plutellae* and *D. collaris*.

Repellents and attractants modify the behavioural response of insects. This is the basis for the principle of behavioural insect control, whereby a given species is either attracted to a bait, or pheromone; or repelled from a host plant by a repulsive agent (Fagoonee 1981). Any factor that selectively influences the production of either deterrent or stimulant could directly influence the direction in which the balance is tipped. We hope to use botanical pesticides to modify the behaviour of both the pest *P. xylostella*, and the parasitoids, *C. plutellae* and *D. collaris*. Results from this study are the first step in this direction, and indicate that syringa and neem extracts may play a role in altering the attractive properties of crucifer plants to *P. xylostella*. At the same time, these extracts appear to have no direct negative influence on *C. plutellae* and *D. collaris*, two important parasitoid species abundant in the field in South Africa. Further experiments are underway to investigate the impact that these extracts have on the semiochemical properties of cabbage plants and how these extracts may influence the behaviour of the pest and its parasitoid species.

Results from this study will help in understanding the tritrophic relationships that are important in the integrated pest management of *P. xylostella*. It is hoped that the results will be used to aid the small-scale

rural farmers in South Africa, and elsewhere, through the enhancement of biological control and a reduction in the use of chemical pesticides.

## Acknowledgements

Thanks go to the International Foundation of Science (IFS) for funding this project. To Liesl Morey who assisted with the statistical analysis. To Mary Erickson from Thermo Trilogy Corporation, USA who provided the Neemix 4.5<sup>®</sup> sample. To colleagues at work who provided numerous tips and hints for my presentation and manuscript.

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