Integrating biological control and botanical pesticides for management of *Plutella xylostella*

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INTEGRATING BIOLOGICAL CONTROL AND BOTANICAL PESTICIDES FOR MANAGEMENT OF *PLUTELLA XYLOSTELLA*

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ABSTRACT

A large number of different plant species have natural pesticidal properties, and man has made use of this since early times. By applying plant extracts to other susceptible plant species the defence of the susceptible plant is improved. This thesis focuses on the possibility of integrating botanical pesticides with biological control for management of the diamondback moth, *Plutella xylostella*, in South Africa.

Twenty-one species of primary parasitoids have been collected from *P. xylostella* in the field in South Africa. Biological control therefore provides a natural control technique. However, biological control alone is insufficient to provide adequate protection and requires integration with other control techniques. Plant products from the Meliaceae family have been widely used to control insect pests, particularly products from the neem tree, *Azadirachta indica*. The neem tree does not grow in South Africa but the closely related syringa tree *Melia azedarach* is a widespread invasive plant found throughout the country. In this thesis I make use of a commercial neem product, Neemix 4.5®, and aqueous leaf extracts derived from the syringa tree.

The neem- and syringa- derived botanical pesticides had adverse effects on the development, reproduction and survival of *P. xylostella*. These botanical pesticides also reduced feeding and oviposition, which are important factors in pest control. However, if a botanical pesticide is to be combined with biological control it must not hamper natural enemies. The neem- and syringa- derived botanical pesticides did not have a directly negative impact on the survival of *Cotesia plutellae* or *Diadromus collaris* two of the most abundant natural enemies found in South Africa. In a glasshouse, a significantly higher proportion of *P. xylostella* larvae were parasitised by *C. plutellae* on plants that been treated with the syringa extract than on control plants. Results from a choice test in a windtunnel showed that *C. plutellae* was attracted significantly more often to cabbage plants treated with the syringa extract than to the control plants. Headspace analysis revealed that treatment of cabbage with syringa extracts caused an increased emission of volatiles by the cabbage plants. This may explain the increased attraction of *C. plutellae* to plants that had been treated with the syringa extract.

It was important to verify results from the laboratory under more realistic conditions in the field. We did not find a difference in *P. xylostella* infestation levels between the treated and the control plants in the field. However, the damage on plants treated with the botanical pesticides was significantly lower. Therefore, it seems that reduced feeding by *P. xylostella* larvae was a more important factor in the reduction of damage than the actual population density. The proportion of *P. xylostella* larvae that had been parasitised was significantly higher on the treated plants than on the control plants. Direct observations showed that plants that had been treated with neem- and syringa- derived pesticides were still visited by parasitoids. Therefore these botanical pesticides do not appear to interfere with parasitoid foraging.

I assessed the possibility for introducing this control method to the rural farming community in South Africa. Syringa trees are invasive plants found throughout South Africa and therefore provide a free local resource for the botanical pesticide. Results indicated that the use of syringa extracts could be introduced. Water is the main factor limiting the introduction of this technique in more arid environments.

Results presented in this thesis indicate that biological control and the use of botanical pesticides derived from the neem and syringa trees can be integrated for the management of *P. xylostella*. However, mammalian toxicity and residual effects still require extensive investigation before any further recommendations can be made.

CHAPTER 1

GENERAL INTRODUCTION

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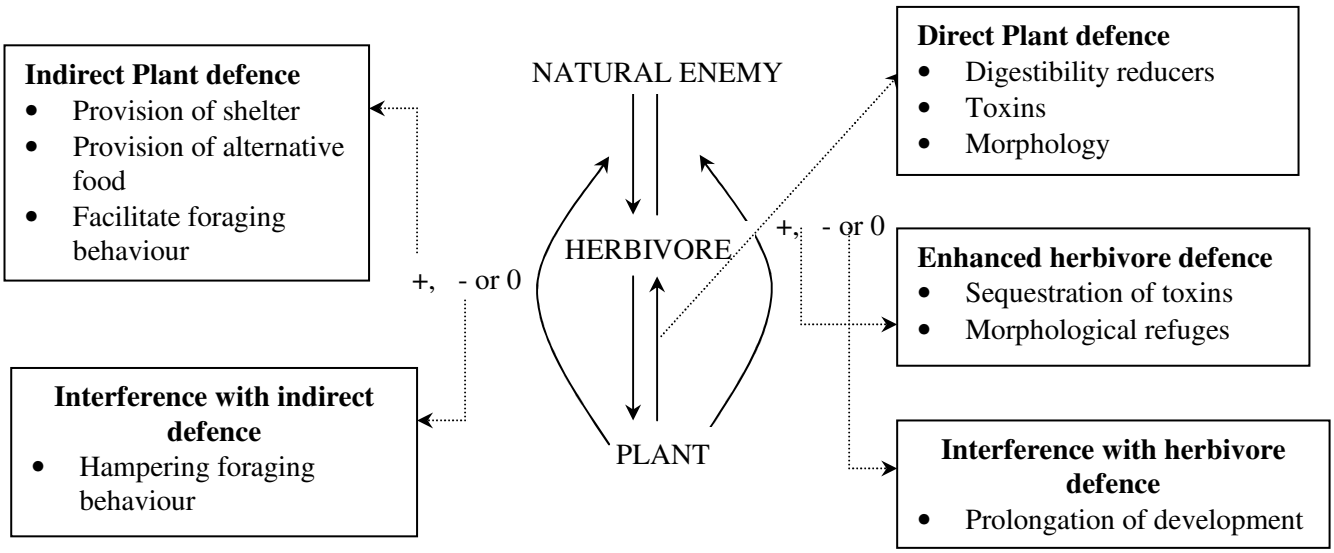
Insect - plant relations

Plant feeding insects make up over a quarter of the macroscopic organisms and the green plants on which they feed make up another quarter (Strong *et al*., 1984). Every living green plant has insect herbivores that attack it. Yet, despite this, plants are always green, so what controls the number of herbivores? There are two hypotheses: the first concerns bottom-up control, and states that plants defend themselves effectively against most herbivores and exhibit such diversity in defence mechanisms that herbivores are not able to master all defences; the second is top-down control, and states that predators suppress the herbivore populations to very low levels and have an overriding impact on herbivores, thereby releasing the plants from herbivore attack (Sabelis *et al*., 1999). In the 1970's ecological research concerning plant defence against herbivores focussed primarily on the first point, investigating interactions between two trophic levels, the plant and the herbivore (Price, 1986). In 1980 Price *et al*. put together a compelling argument, stating that development of the theory on insect-plant interactions could not progress realistically without consideration of the third trophic level. Consequently current ecological studies take a more comprehensive approach, considering the role of both plant defence and predator impact on plant-herbivore relations (Price *et al.,* 1984; Vet & Dicke, 1992; reviews in Olff *et al*., 1999).

Mechanisms of plant defence against herbivore attack.

Plants have direct (intrinsic) and indirect (extrinsic) defence mechanisms, which they employ against their herbivore attackers (Fig.1). Direct defence involves physical and chemical strategies. Physical strategies include plant structures that hinder the herbivore, for example, cuticle texture and thickness, glandular and non-glandular trichomes and thorns. Chemical strategies include toxins and secondary plant compounds that modify the quality of ingested food, intoxicate the herbivore or signal the plants' defended state leading to a discontinuation of herbivore attack (Sabelis *et al*, 1999; Dicke, 1999a; van Emden & Way, 1972). Indirect defence mechanisms bypass the second trophic level by promoting the effectiveness of members of the third, through the provisioning of alternative food sources and/or shelter/ domatia, or by the attraction of carnivores to the plant after herbivore infestation (Dicke, 1999a). There are links between intrinsic (host plant resistance) and extrinsic (biological control) defence mechanisms, and these links can be mediated by a variety of both physical and chemical plant factors (Fig. 1).

A



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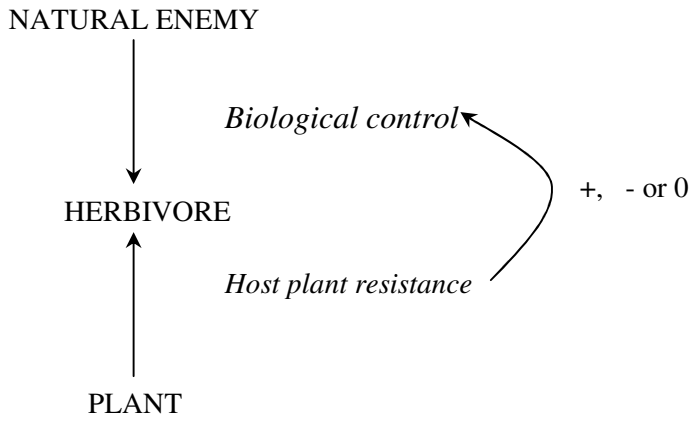

Figure 1. Impact of direct and indirect plant defence on herbivores and carnivores. Plant defence can have an impact on the natural enemy or the herbivore and can be positive (+) or negative (-) or have no effect at all (0) (From: Dicke, 1999a).

A Impact of direct and indirect plant defence in a tritrophic system.

B Biological control and host-plant resistance in a tritrophic context.

Tritrophic implications

There is growing evidence that carnivores play an important role in insect-plant interactions, and that plants influence carnivore behaviour (Nordlund *et al*, 1988; Dicke & Vet, 1999). Plant nutrients and resistance levels can influence growth rate and size of insect herbivores, which may in turn influence the responses of their natural enemies. Herbivores feeding on resistant varieties may be a little smaller than those on susceptible varieties (van Emden, 1991). This can have both a negative and postive impact on natural enemies.The smaller host size may result in smaller parasitoids with a reduced fecundity, or a sex ratio biased towards males (van Emden, 1991). However, the smaller insects on the more resistant varieties may be less able to defend themselves against attack from natural enemies; they are also more restless and dislodged from the plant more easily, and consequently become more susceptible to predation from ground dwelling predators (van Emden, 1991; Dicke, 1999a). Predators will also consume more prey until they are satiated if the prey are small (van Emden, 1991; Dicke, 1999a). In addition, the growth rate of herbivores on resistant plants is reduced and consequently their exposure to natural enemies is increased (van Emden, 1997). Resistant host plants can reduce the survivorship of herbivores and therefore the number available for attack by natural enemies. Plant nutrients and resistance levels can also influence the defence of herbivores against natural enemies. Some herbivores are able to sequester plant allelochemicals in their haemolymph and alter their suitability to natural enemies (Thomas & Waage, 1996), which may greatly reduce the extrinsic defence (biological control) provided by natural enemies.

The volatile chemicals released by plants in response to herbivory may influence the foraging activity of parasitoids and predators (Dicke & Vet, 1999). Natural enemies make use of these infochemicals to detect their herbivore hosts. The value of this chemical information depends on 1) its reliability in indicating the presence, identity, accessibility and suitability of the herbivore and 2) its detectability (Vet *et al*., 1991; Vet & Dicke, 1992). In this respect the information value of the stimuli from the herbivore and those from the host plant differ. Herbivore-derived information is more reliable, but harder to detect (Vet *et al*., 1991; Vet & Dicke, 1992) and chemical analysis showed that very few volatiles are generally detected from the herbivore itself or its faeces (e.g. Turlings, *et al*., 1991, Geervliet *et al*., 1997; Steinberg *et al*., 1993). In contrast, plants represent a much larger biomass and volatile information from the plant is easily detected, but it is less reliable in indicating herbivore presence (Vet & Dicke, 1992). This drives the evolution of indirect searching strategies, or the use of information not derived directly from the herbivore, but from other sources that predict its presence, for example the use of herbivore-induced plant volatiles (Vet & Dicke, 1992; Dicke 1999b).

Herbivore-induced plant volatiles are produced by plants in response to specific herbivore damage. These volatiles, which attract natural enemies, are both highly detectable and often highly specific (Vet, 1999; Dicke, 1999b). For example: attraction of the larval parasitoid *Cotesia marginiventris* to volatiles released by maize damaged by *Spodoptera* larvae (Turlings

et al. 1990), attraction of *C. rubecula* to cabbage infested by *Pieris rapae* (Agelopoulos & Keller, 1994a; Geervliet *et al*., 1997), attraction of *C. glomerata* to cabbage infested by *P. brassicae* (Steinberg *et al*. 1993), attraction of the predatory mite *Phytoseiulus persimilis* to spider mite induced volatiles produced by bean or cucumber plants (Dicke *et al*., 1990a). Herbivore-induced plant volatiles are more reliable for herbivore detection than signals released by the undamaged or mechanically damaged plant (Vet *et al*., 1991; Vet & Dicke, 1992, Vet, 1999; Dicke, 1999a).

Pest management

Despite the recognised importance of plants to the host selection behaviour of entomophagous insects, much research is still required on the interactions among plants, herbivores and entomophagous insects. The realisation that plants and carnivores interact and have an impact on the herbivore population dynamics should be taken into consideration when looking at pest management systems (Dicke, 1999a). Pest organisms place substantial constraints on crop production worldwide. Estimates put global losses to pests at about 30% of potential world food, fibre and feed production (NRI, 1992), with substantially higher proportions in developing countries (Thomas & Waage, 1996). Programmes of agricultural intensification have created new and/or greater pest problems in a number of ways (Thomas & Waage, 1996): monocultures increase plant apparancy to pests; genetically uniform, high yielding crop varieties provide improved conditions for pest colonisation; reduction in natural vegetation means that natural enemies need to move over greater distances to reach crop areas; overlapping seasons or continuous growing periods provide continuous resources for pests; accelerated movement of plant material results in the accelerated spread of pests; and the widespread reliance on chemical pesticides has led to resistance, recorded in over 500 insect species worldwide (Georghiou, 1990).

Integrated pest management

Biological control is the cornerstone of any sustainable pest management strategy and an essential component of integrated pest management. Biological control is defined as the "action of parasites, predators, and pathogens in maintaining another organism's (the pest) density at an average lower than would occur in their absence" (DeBach, 1964). Entomophagous insects (parasitoids and predators) are the cornerstone of biological control, acting as regulators of host/prey populations (DeBach, 1964; Price, 1984; van Lenteren & Woets, 1990). Therefore, the knowledge of the interactions of entomophagous insects with their hosts or prey is of great theoretical and practical value, and in the past many studies concentrated on these interactions. Biological control, however, also involves interactions between plants and organisms of the third trophic level and there is an increasing interest in these tritrophic aspects (Price *et al*, 1980; Barbosa & Letourneau, 1988; Turlings *et al*., 1990; van Lenteren & DePonti, 1990; Vet & Dicke, 1992; Turlings *et al*., 1995; Agrawal, 1998; Dicke & Vet 1999; Thaler, 1999), with many emerging research programmes.

Integrated pest management (IPM) attempts to integrate the available pest control methods to achieve a farmer's most effective, economical and sustainable combination for a particular local situation. Emphasis is placed on biological control, host plant resistance, cultural control and other non-polluting methods. Pesticides are used only when necessary, when they can be integrated with the other control methods, and only when cost-benefit analysis shows that their use is truly justifiable and acceptable alternatives are lacking (Lim *et al*. 1997).

Successful IPM programs produce many benefits, including: 1) lower production costs compared with conventional pest control strategies with a high input of synthetic pesticides, 2) reduced environmental pollution, particularly improvement of soil and water quality, 3) reduced farmer and consumer risks from pesticide poisoning and related hazards, and 4) ecological sustainability by conserving natural enemy species, biodiversity and genetic diversity (Lim *et al*. 1997). At a more general level the stability that IPM provides to agricultural production enhances political stability in countries where agriculture is a dominant sector of the economy. In rural areas it is important in developing local self-reliance through farmer empowerment (Lim *et al*. 1997). Therefore, IPM can achieve broad and long-lasting socio-economic benefits far beyond plant-protection activities. IPM is a sustainable system approach with many benefits. It can, and should, replace current conventional agriculture which relies solely on intensive agrochemical inputs that have caused many problems.

Making use of plant defence to protect crops

The plant kingdom is by far the most efficient 'factory' of chemical compounds, synthesising many products that are used in the defence against herbivores (Schoonhoven *et al*., 1998). The insecticidal secondary metabolites from one plant species can be applied to other plant species to provide protection for this second plant. By treating susceptible plants with botanical pesticides the susceptible plant will acquire an increased resistance. Extracts prepared from plants (botanical pesticides) 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; Boeke *et al*., 2001).

Most studies on botanical pesticides have centred on plants from the mahogany family, Meliaceae, and in particular on members from the genera *Azadirachta* and *Melia*, which are outstandingly effective against insects (Schmutterer, 1995). Limnoids, which are characteristic natural products of the plant families Meliaceae, Rutaceae and other Rutales, have marked biological activity against a variety of insects (Champagne, 1989). Compounds isolated from the neem tree, *Azadirachta indica* Juss. and the syringa tree *Melia azedarach* L. are the most conspicuous. Triterpenoids and tetranortriterpenoids are the main active ingredients found in these plants (Schmutterer, 1995). Azadirachtin, a tetranortriterpenoid isolated from the neem tree has attracted considerable attention during the past three decades because of its potent antifeedent and insect growth regulation properties. Two very potent insecticidal

tetranortriterpenoids have also been isolated from green syringa fruits, a novel meliacin called 1-cinnamoyl melianone and a new derivative of meliacarpin: 1-cinnamoyl-3,11-dihydroxymeliacarpin (Lee *et al*., 1991). The growth inhibition and antifeedent effects of these compounds compare favourably with azadirachtin. 1-Cinnamoyl melianone has also been isolated from syringa leaves (Ascher *et al*., 1995).

In evolutionary terms compounds with a high degree of toxicity may be counterproductive if they select more strongly for resistance in insect populations (Isman *et al*., 1996). The chemical pesticide industry has focussed on the use of acutely toxic chemicals, resulting in a rapid build up of pest resistance. The chemical defences of plants, shaped by 300 million years of evolutionary 'experience', are often more subtle in their actions on herbivores (Isman *et al*., 1996). However, natural products are not immune to herbivore resistance, although it has been suggested that extracts from plants, which contain numerous compounds, are more complex in comparison to synthetic pesticides and therefore delay the build up in resistance (Vollinger, 1995). Rice (1993) suggested that the combination of behavioural and physiological actions of botanical pesticides deters the development of resistance. This is a common phenomenon in natural ecosystems where herbivorous insects are controlled by plant allelochemicals. The use of botanical pesticides / natural plant products in agroecosystems is becoming more and more important as a means to protect crop produce and the environment from synthetic pesticide pollution (Prakash & Rao, 1997, Schmutterer, 1995). However, the impact of botanical pesticides on biological control may be advantageous or disadvantageous in terms of the pest management effect. Factors such as the mortality response of the pest to the botanical pesticide, the survival and fecundity of natural enemies in crop systems treated with botanical pesticides, and the attractiveness of plants treated with these botanical pesticides to parasitoids and predators, need to be considered. If these factors are not considered then the botanical pesticide could very well contribute to an increase in pest densities. It is essential to analyze plant-herbivore-natural enemy (tritrophic) interactions if botanical pesticides are to be combined with biological control.

The system to be studied

The pest

The diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae), presents one of the greatest threats to crucifer production in many parts of the world, sometimes causing more than 90% crop loss (Verkerk & Wright, 1996). Adult female moths lay their eggs on the underside of leaves. The larvae hatch and feed on the parenchyma, leaving the cuticle intact, but as the plant grows the cuticle tears resulting in a characteristic holey appearance on the surface of the leaf. There are four larval instars before pupation occurs, and at 25^{o}C the life cycle from egg to adult emergence takes approximately 24 days.

Plutella xylostella is regarded as oligophagous, with the larvae feeding specifically on members of the family Cruciferae (Thornsteinson, 1953). As a crucifer specialist, *P. xylostella*

has adapted to the unique secondary chemistry of this family, which makes crucifers toxic to most generalist feeders (Verkerk & Wright, 1996). The secondary chemistry of the Cruciferae is characterised by the presence of glucosinolates or hydrolysed derivatives (Feeny, 1977). Glucosinolates are a class of sulphur-containing glycosides also known as mustard oil glycosides or thioglycosides (Renwick, 1996). These compounds have been shown to adversely affect the growth and survival of generalist feeders (Renwick, 2002; Feeny, 1976). In contrast, specific glucosinolates (or derivatives) are powerful gustatory and oviposition stimulants to crucifer specialists (Fig. 2) (van Loon *et al*, 1992; Renwick & Radke, 1990; Renwick *et al*. 1992; McCloskey & Isman, 1993; Renwick, 2002). Insects from at least five orders are known to be attracted to volatiles of cruciferous plants (Pivnick *et al*. 1994). An important primary attractant compound for crucifer-feeding specialists and their parasitoids is allyl isothiocyanate (Renwick, 2002; Feeny *et al*., 1970; Read *et al*., 1970; Pivnick *et al*., 1994). Isothiocyanates are hydrolytic breakdown products of glucosinolates (Kjaer, 1961) released when the plant tissue is disrupted (Renwick, 1996; Pivnick *et al*., 1994) and the glucosinolates come into contact with myrosinase (Fig. 2). Isothiocyanates are present in 11 plant families with a predominant distribution in Brassicaceae (Kjaer, 1961).

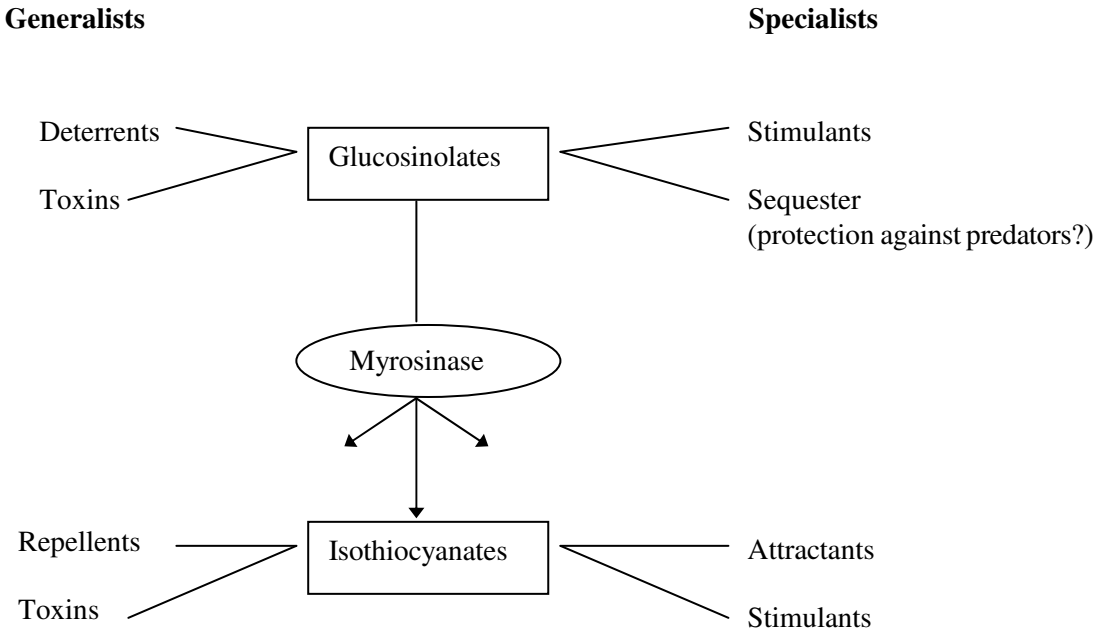

Figure 2. Comparative effects of glucosinolates and isothiocyanates on non-adapted (generalist) and adapted (specialist) insects (From: Renwick, 2002).

Brassica spp. are thought to be the preferred host for *P.xylostella* (Thornsteinson, 1953; Verkerk & Wright, 1996). Seven cultivated *Brassica* spp. have been described (*B. oleracea* (cabbage, cauliflower, broccoli)*, B. rapa* (turnip)*, B. pekinensis* (Chinese cabbage), *B. juncea* (Indian mustard)*, B. napus* (rapeseed)*, B. nigra* and *B. carinata*). All are thought to be native to the Eurasian continent (Siemonsma & Piluek, 1993). There are also wild crucifers that support *P. xylostella* (Kartosuwomdo & Sunjaya, 1991). Results from various studies indicate that *B. pekinensis*, *B. juncea*, *B. oleracea* var *botrytis* (cauliflower) and *B. rapa* are amongst *P. xylostella*'s most preferred host plants (Verkerk & Wright, 1996; Charleston & Kfir, 2000). *Brassica oleracea* var *capitata* (cabbage) is preferred as a host plant when compared with potential crucifer hosts from other genera, such as *Sinapis alba* (mustard) and *Raphanus sativus* (radish) (Verkerk & Wright, 1996). Plants previously recorded as hosts of *P. xylostella* are reviewed by Verkerk & Wright (1996).

Control of P. xylostella

Synthetic insecticides have dominated attempts to control *P. xylostella* for more than 40 years (Syed, 1992; Shelton *et al*, 1997). Compounds from virtually all classes of insecticide have been used, including organochlorines, organophosphates, carbamates, pyrethroids, avermectins and acylureas (Talekar & Griggs, 1986; Talekar, 1992). The widespread and intensive use of insecticides has led to several problems, including pesticide resistance, unacceptable levels of residue in vegetables, poisoning of farmers and labourers, reduction of natural enemies and rising costs in vegetable production (Jusoh *et al*, 1992). Previous research on chemical control has focused on measuring pesticide-induced effects on specific target insects, with little consideration of the impact on the third trophic level.

Integrated pest management has focussed on the use of selective insecticides combined with biological control. Since 1988 products based on *Bacillus thuringiensis* Berlinger (*Bt*) have been widely used (Verkerk & Wright, 1996; Ooi, 1992; Nel *et al*., 1999). However, over the last decade there has been increasing evidence for the development of resistance by *P. xylostella* to *Bt*-based products (Tabashnik *et al*, 1990; Syed, 1992; Shelton *et al*., 1993ab; Tabashnik, 1994). 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, rigorous studies on the biology and ecology of these parasitoids are relatively few, considering the important role they often play in regulating *P. xylostella* populations (Verkerk & Wright, 1996).

Botanical pesticides

Investigation into alternative control mechanisms for control of *P. xylostella* has led to the testing of plant extracts (Table 1). Of the 1800 plant species reported by Grainge *et al* (1984) to possess pest control properties, only 88 species have been reported to be active against *P. xylostella* (Morallo-Rejesus, 1986). The Meliaceae, Asteraceae, Fabaceae and Euphorbiaceae contain most of the insecticidal plant species reported. The botanical pesticides first widely

used against *P. xylostella*, pyrethrum and rotenone, were isolated from plant species belonging to Asteraceae and Fabaceae, respectively (Morallo-Rejesus, 1986). After World War II, synthetic pesticides, such as DDT, increased in popularity and the use of botanical pesticides rapidly declined. However, in recent years the problems associated with the use of synthetic pesticides has resulted in a renewed interest in the use of plant extracts to control *P. xylostella*.

Extracts from the neem tree, *Azardirachta indica* 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). In addition to this, *P. xylostella* appears to be susceptible to neem products for long periods of time. Vollinger (1987) reported that treated larvae were still equally susceptible to the extract up to 42 generations later. The closely related plant species *Melia azedarach* L. also has insecticidal properties and has been tested against a number of insect species including *P. xylostella* (Table 1). Fruit extracts from this plant have been shown to have a deterrent effect on the oviposition of adult moths (Chen *et al*., 1996a), and are also toxic to egg and larval stages and result in reduced pupal weight, adult emergence and longevity (Chen *et al*., 1996b). These botanical pesticides are also thought to be compatible with biological control as they have little or no deleterious impact on natural enemies (Tables 2 and 3).

The situation in South Africa

Diamondback moth was recorded as a pest on cabbage in South Africa as early as 1917 (Gunn, 1917). Since then there have been various studies reporting on the status of *P. xylostella* and it's parasitoids in South Africa (Ullyett, 1947; Dennill & Pretorious, 1995; Kfir, 1997; Kfir 2002; Smith, 2002). In 1947 Ullyett conducted a thorough investigation into *P. xylostella* in South Africa, and suggested integrated pest management as the best method to control the pest. However, many of his suggestions were ignored, and today control of crucifer pests is heavily dependent on insecticides with little consideration of natural enemies and biological control. Because of the widespread and indiscriminate use of pesticides local field populations of diamondback moth in South Africa have started showing signs of resistance to synthetic pyrethroids, organophosphates and carbamates (Sereda *et al*., 1997). Therefore, it is necessary to investigate alternative control measures if this pest is to be managed successfully.

In South Africa, cabbage *(B. oleracea* var *capitata*), and cauliflower (*B. oleracea* var *botrytis*) are the most popular brassica crops and approximately 21 000 ha of cabbage are planted annually. In South Africa diamondback moth feeds on crops with a comparatively low market value. However, cabbage is an important subsistence and staple diet crop in South Africa (Bell & McGeoch, 1996), and it is estimated that 80% of small-scale farmers that have access to water are growing cabbage as a subsistence crop. In South Africa there are a wide variety of parasitoids attacking *P. xylostella*. A survey carried out at the Plant Protection Research Institute in Pretoria yielded a total of twenty-one primary parasitoids, and twelve species of hyper-parasitoids (Kfir, 2003). From the primary parasitoids found in the survey, three species were egg-larval parasitoids, eight species were larval parasitoids, four species were larval-

pupal parasitoids, and six species were pupal parasitoids (Kfir, 2003). *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae) and *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) are the most abundant parasitoids in the field (D. Charleston, unpublished). *Diadromus collaris* is a solitary endoparasitoid of the pupal stage of *P. xylostella*, but until recently (Liu *et al.*, 2001; Wang & Liu, 2002; Lui *et al.*, 2002), knowledge on the biology and ecology of *D. collaris* was rare. *Cotesia plutellae* is a solitary endoparasitoid of larval *P. xylostella*. Aspects of the biology and life history of *Cotesia plutellae* have been reported by Velasco (1982), Talekar & Yang (1991) and Verkerk & Wright (1996). *Cotesia plutellae* attacks and can develop on all four larval instars of its host *P. xylostella*, although the second instar is most suitable for development (Talekar & Yang, 1991). It is generally reported to be host-specific, although it has been reported on other lepidopterous hosts, such as *Ocnogyna baetica* (rambur) (Arctiidae) in Spain (Lipa *et al*., 1993), and *Autographa gamma* (L.) (Noctuidae, Plusiinae) in Japan (Kaneko, 1993).

Botanical pesticides are not registered for use in South Africa. The neem tree, *Azadirachta indica*, does not grow in South Africa and therefore it is not possible to use this tree to make a botanical pesticide. However, the closely related syringa tree, *Melia azedarach*, is an abundant, invasive plant found throughout South Africa and therefore provides a good potential source for use in a botanical pesticide.

The Research Questions

Insects are among the chief pests of crops and stored products despite extensive and often environmentally hazardous control mechanisms. It is important to understand the factors governing the relationships between insects and plants that may help to unravel the causes of insect pest outbreaks. This knowledge is fundamental when trying to create environmentally safe control strategies. With the continued spread of resistance to *Bt*-based products by *P. xylostella* it is paramount that alternative strategies are developed. Such strategies are likely to be most successful if conducted from a multi-trophic perspective, with emphasis being placed on the development of multiple strategies that are compatible.

The negative impacts of synthetic pesticides and increasing pesticide resistance in *P. xylostella* have increased the interest in alternative control methods, with emphasis being placed on biological control, plant resistance, cultural control and other non-polluting methods (Lim *et al*., 1997). These methods are thought to have special importance to subsistence farming in developing countries. Many farmers in developing countries do not have the resources to buy and apply synthetic pesticides. Biological control through local natural enemies and botanical pesticides that can be easily prepared from trees within the area are free to the farmer. Therefore, these methods are well suited to low-input integrated pest management systems, provided the methods do not interfere with one another.

Integrated pest management relies, among others, on biological control and the judicious use of chemical control when necessary. Biological control in open agricultural systems is particularly suited to the relatively stable conditions found in many sub-tropical and tropical ecosystems and thus of potentially great value to many developing countries, such as South Africa. Few studies have been conducted on *P. xylostella* from a multi-trophic context (Beck & Cameron, 1990; Fox *et al.,* 1990; Talekar & Yang, 1991; Verkerk & Wright 1996; Verkerk & Wright, 1997), and very little of the research carried out in South Africa has focussed on these aspects. Work on tritrophic interactions has expanded rapidly in the past two decades (Price *et al*., 1980; Barbosa & Letourneau, 1988; Vet & Dicke, 1992; Wright & Verkerk, 1995; Thomas & Waage, 1996; Dicke, 1999ab; and see reviews in Olff *et al*., 1999). Evidence that certain host plants and cultivars are more attractive to particular key parasitoids and predators (Dicke *et al*., 1990a) should stimulate studies on olfactory and other host location cues used by the key parasitoids. Such research has been very limited in so far as it relates to *P. xylostella* despite its great economic significance as a pest.

Objectives

The main objective of the study is to investigate whether biological control and botanical pesticides are compatible in integrated pest management systems for the diamondback moth in South Africa. Aqueous leaf extracts from *Melia azedarach* and commercial extracts of *Azadirachta indica* will be investigated for their impact on *Plutella xylostella* and the two most abundant natural enemies, *Cotesia plutellae* and *Diadromus collaris*. Small-scale subsistence farmers generally cannot afford commercial synthetic insecticides. The use of botanical pesticides, which are inexpensive and easy to prepare, would provide a good alternative. The syringa tree, *Melia azedarch*, is an abundant invasive plant in South Africa and therefore provides a potential source for botanical pesticides. However, it is vital to investigate the interactions of all three trophic levels before considering the introduction of a botanical pesticide. In this thesis I investigate the tritrophic interactions that may be affected by treating cabbage with botanical pesticides made from *M. azedarach* and a commercial formulation prepared from *A. indica*.

Thesis outline

Question 1: Are botanical pesticides suitable for controlling *Plutella xylostella*?

Although much research has been conducted on the impact of neem extracts on the diamondback moth, very little is known about the impact of aqueous leaf extracts from the syringa tree on this pest. Chen *et al*. (1996ab) conducted an investigation using extracts from the fruit of the syringa tree, but I could not find any reference to the use of aqueous leaf extracts. In **chapter 2** we comparatively investigate the impact of neem and syringa derived botanical pesticides on the biology of the diamondback moth.

Glucosinolates are important stimulants to oviposition by *P. xylostella* (Renwick & Radke, 1990; Justus & Mitchell, 1996), and plant volatiles are thought to mediate host plant location

(Renwick, 2002). However, by treating a cabbage plant with a botanical pesticide the characteristics of the plant may be altered and this could have an impact on the resulting behaviour of the herbivore. In **chapter 3** we investigate whether treatment of cabbage plants with neem and syringa derived botanical pesticides influences the behaviour of the diamondback moth.

Question 2: Are botanical pesticides harmful to *Cotesia plutellae* and *Diadromus collaris*?
Cotesia plutellae and *Diadromus collaris* were chosen as test species as these are the two most abundant parasitoids found in the field in South Africa. The natural occurrence of these parasitoids in the field will be an advantage to the small scale rural farming community as supplementary releases of natural enemies would probably not be required. Despite the abundance of *C. plutellae* and *D. collaris*, the damage caused by *P. xylostella* is still significant, requiring an integrated pest management approach. If a botanical pesticide is to be introduced it must not have a negative effect on the natural enemies. In **chapter 4** we address the impact that commercial neem formulations and aqueous leaf extracts from the syringa tree have on the biology of these two natural enemies of the diamondback moth.

Question 3: How do the botanical pesticides affect the behaviour of natural enemies and do they alter the attractiveness of the cabbage plants to the parasitoids?
Parasitoids use chemical stimuli from their herbivorous hosts or their host's food plant during the host searching process (Vinson, 1976). The main components of the volatile blend released from cabbage are green leaf volatiles and terpenoids. Terpenoids are a large diverse group of compounds present in most plant species and are a major class among herbivore-induced volatiles that attract carnivores (reviewed by Dicke, 1994). Given the dramatic increase of green leaf volatiles in the headspace of damaged cabbage compared to undamaged plants, it is likely that these components play an important role in carnivore attraction, for example in the attraction of *Cotesia glomerata* to *Pieris*-infested or regurigitant-treated cabbage plants (Mattiacci *et al*, 1994). Smid *et al*. (2002) show which of these *Brassica* volatiles elicit an EAG response in *C. glomerata* and *C. rubecula* parasitoids. *Cotesia plutellae* also makes use of plant-derived stimuli during its in-flight searching behaviour (Potting *et al*., 1999). However, by treating susceptible plants with botanical pesticides it is possible that the volatile profile of the plant will be changed and therefore the response of the natural enemy will be altered. In **chapter 5** we investigate whether the treatment of cabbage plants with botanical pesticides alters the behavioural responses of *C. plutellae* and *D. collaris*. We also address the potential differences between the volatile profiles of treated and untreated cabbage plants.

Question 4: Are the botanical pesticides effective under field condtions?
Findings from laboratory trials have much more value if they can be verified under field conditions. In **chapter 6** we investigate the use of neem and syringa derived botanical pesticides in the field, looking at the implications for pest management. We compare the results from the laboratory with results under field conditions. We also assess the impacts that the botanical pesticides have on the behaviour of natural enemies and the adult diamondback moth in the field.

Question 5: What is the potential for *Melia azedarach* to be used by small-scale rural farmers in South Africa?
Ideally pest management systems should be simple, easy to apply and have little associated costs if they are to be employed successfully by resource-poor farming communities. Neem products are not available in South Africa and the tree does not grow in this country. However, syringa trees are invasive plants and therefore widely distributed throughout South Africa. If aqueous leaf extracts from syringa trees are to be used by the rural farmers in South Africa it is necessary to investigate the potential this tree has for the small-scale rural farmer. In **chapter 7** we report on visits to several rural areas in South Africa and address the possibilities of introducing this system to the small-scale rural farmers.

A summary and conclusion of the research is provided in **chapter 8**.

Table 1. Plants reported to have insecticidal properties against *P. xylostella* (extracted from Morallo-Rejesus, 1986).

Family / Sceintific name	Plant parts	Activity [a]	Reference
Annonaceae			
Annona reticulata	bark, fruit	I, AF, R	Grainge *et al*. 1984 Jacobson 1975
Annona squamosa	roots, fruit, oil	I, CP, SP, AF	Grainge *et al*. 1984 Jacobson 1958
Acanthaceae			
Fittonia argyroneura	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
F. verschaffeltii	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Apocynaceae			
Nerium oleandre	roots, bark, stem, leaves, flowers	I, AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Araliacea			
Hedera helix	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Aristolochiaceae			
Aristolochia elegans	Leaves	AF	Caasi, 1983
Aristolochia tagala	Leaves	AF	Caasi, 1983
Aroideae			
Philodendron sp.	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Asteraceae			
Ageratum conyzoides	Leaves	CP	Morallo-Rejesus, 1986
Blumiea balsamifera	Leaves	CP	Morallo-Rejesus, 1986
Chrysanthemum cinerariaefolium	whole plant, flowers	I, AF	Grainge *et al*. 1984
Matricaria matricarioidews	Flowers	I	Grainge *et al*. 1984 Jacobson 1958
Senecio cineraria	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Tithonia diversifolia	Leaves	CP	Carino & Morallo-Rejesus 1982 Grainge *et al*. 1984
Balsaminaceae			
Impatiens sultani	Leaves	I, AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Begoniaceae			
Begonia pearcei	Leaves	I	Grainge *et al*. 1984 Jacobson 1975
Buxaceae			
Buxus sempervirens	Leaves	AF, R	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960

Table 1 (cont.)

Caesalpiniaceae			
Caesalpinia pulcherrima	Flowers	CP	Morallo-Rejesus, 1986
Caryophyllaceae			
Dianthus sp.	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Celastraceae			
Euonymus japonicus	Leaves	R	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Tripterygium wilfordii	roots, tubers, bark	I, SP, AF	Swingle 1941 Jacobson 1958
Clusiaceae			
Mammea americana	roots, tubers, bark	I, CP, SP	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Columelliaceae			
Tagetes erecta	Roots	CP	Grainge *et al*. 1984 Morallo-Rejesus & Eroles 1978
T. patula	Roots	CP	Grainge *et al*. 1984 Morallo-Rejesus & Eroles 1978
Commelinaceae			
Tradescantia sp.	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Compositae			
Dahlia sp.	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Gynura sp.	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Convolvulaceae			
Ipomoea batatas	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Citrullus colocynthis	roots, tubers, leaves, fruit	I	Grainge *et al*. 1984
Cucumis sativus	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Ericaceae			
Azalea sp.	Leaves	AF	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960

Table 1 (cont.)

Euphorbiaceae			
Acalypha indica	leaves, bark	I	Grainge *et al*. 1984
Euphorbia lathyris	Leaves	I	Grainge *et al*. 1984 Jacobson 1975 Gupta & Thorsteinson 1960
E. splendens	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
E. poinsettiana	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Phyllantus acuminatus	roots, tubers	CP, SP	Grainge *et al* 1984 Jacobson 1958
Fabaeae			
Derris malaccensis	roots, tubers	I, SP, CP, R, AF	Grainge *et al* 1984
Derris philippinensis	Roots	CP	Maghanoy & Moarallo-Rejesus 1975
Pachyrhizus erosus	whole plants, fruits, sap, seeds	I, CP, SP, AF	Grainge *et al* 1984 Jacobson 1958
Piscidia acuminata	roots, tubers, leaves	I	Grainge et al 1984 Jacobson 1958
P. piseipula	roots, tubers, bark, leaves	I, CP, SP, AF	Grainge *et al* 1984 Jacobson 1958
Tephrosia vogelii	leaves, seeds	I, AF, R	Grainge *et al* 1984
Gentianaceae			
Exacum sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Geraniaceae			
Geranium sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Pelargonium sp.	leaves, stem, oil	AF, SP, AT, R	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Gesneriaceae			
Negelia hyacinthi	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Coleus sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Leguminoceae			
Calopogonium coerruleum	seeds, pods	I	Grainge *et al* 1984 Jacobson 1958

Table 1 (cont.)

Liliaceae			
Lilium longifiorum	Leaves	I, AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Hemerocallis dumortieri	Leaves	I, AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Tulipa sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Malvaceae			
Abutilon pictum	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Hibiscus syriacus	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Marantaceae			
Maranta bicolor	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Meliaceae			
Azadirachta indica	whole plant, bark, stem, leaves, fruit, seeds, oil	I, CP, SP, GI, AF, R	Grainge *et al* 1984 Schmutterer, 1997 Verkerk & Wright 1993 Prijono & Hassan 1993 Vollinger 1995
Melia azedarach	whole plant, bark, stem, leaves, fruit, seeds, oil	AF, GI, I, SP, R, CP	Chen *et al* 1996ab Steets 1975 Zhu 1991
Melianthaceae			
Melilotus officinalis	Leaves	R	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Onagraceae			
Fuchsia sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Oxalidaceae			
Oxalis deppei	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Passifloraceae			
Passiflora alata	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960

Table 1 (cont.)

Piperaceae			
Peperomia sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Piper nigrum	Seeds	CP	Grainge *et al* 1984 Javier 1981
Punicaceae			
Punica granatum	Leaves	I, AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Ranunculaceae			
Clematis sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Dalphinium chinensis	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Eranthis hyemalis	Bulbs	I	Grainge *et al* 1984 Jacobson 1958
Rhamnaceae			
Rhamnus crenata	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Rosaceae			
Rosa sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Rubiaceae			
Chinchona calisaya	roots, tubers, bark, wood	I	Grainge *et al* 1984 Jacobson 1958
Randia nilotica	roots, tubers	I	Grainge *et al* 1984 Jacobson 1958
Xeromphis spinosa	roots, tubers, fruit	I, AF, R	Grainge *et al* 1984 Jacobson 1958
Rutaceae			
Citrus aurantium	Leaves	I, AF, R	Grainge *et al* 1984 Jacobson 1975
Sapotaceae			
Medhuca latifolia	bark, stem, leaves	I	Grainge *et al* 1984
M. longifolia	Seeds	I	Grainge *et al* 1984
Saxifragaceae			
Heuchera sanguinea	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Hydrangea sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Simarolibaceae			
Balanites aegyptica	roots, fruits, seeds	I	Grainge *et al* 1984 Jacobson 1958

Table 1 (cont.)

Solanaceae			
Lycopercicum esculentum	whole plant, stem, fruits, leaves, flowers	I, AF, R,	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Petunia sp.	Leaves	I	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Solanaceae			
Solanum tuberosum	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Solanum sp.	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Theophrastaceae			
Jacquinia aristata	roots, fruits, leaves	I	Grainge *et al* 1984 Jacobson 1958
Urticaceae			
Ficus carica	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Pellionia pulchra	Leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Verbenaceae			
Lantana camara	flowers, leaves	AF	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960
Vitex negundo	leaves, stem, seeds, oil	I, GI, R	Grainge *et al* 1984
Vitaceae			
Cissus rbombifolia	stem, leaves	AF, SP	Grainge *et al* 1984 Jacobson 1975 Gupta & Thorsteinson 1960

[a] I = insecticidal, AF = antifeedant, R = repellent, CP = contact poison, SP = stomach poison, GI = growth inhibitor.

Table 2: Impact of botanical pesticides derived from *Azadirachta indica* on natural enemies: deleterious impact indicated by + , no impact indicated by 0, slight impact inidicated by ± (extracted from Schmutterer, 1995 pp 495 – 507 and Boeke, 2002).

PREDATOR / PARASITOID	PEST	IMPACT	REFERENCE
Pardosa pseudoannulata	Plant and leaf hoppers	0 0	Saxena *et al* 1984 Saxena 1987
Oxyopes javanus		±	Karim *et al* 1992
Chiracanthium mildei		0	Mansour *et al* 1987
Phytoseiulus persimilis	*Tetranychus cinnabarinus* *Tetranychus urticae*	0 ±	Mansour *et al* 1987 Sanguanpong 1992
Amblyseius	*Panonychus citri*	0	Chiu 1985
Doru taeniatum	*Spodoptera frugiperda*	0	Hellpap 1985
Metioche vittaticollis		0	Lamb & Saxena 1988
Tytthus parviceps	*Nephotettix virescens*	+	Krishnaiah & Kalode 1992
Perillus bioculatus	*Leptinotarsa decemlineata*	+	Hough-Goldstein & Keil 1991
Ectatomma ruidum	*Spodoptera frugiperda*	0	Hellpap 1985
Drino inconspicua	*Galleria mellonella*	±	Beitzen-Heineke & Hofmann 1992
Ganaspidium sp. *Disorygma sp.* *Opius sp.*	*Liriomyza sativae*	± ± ±	Serra 1992
Delphastus pusillus *Scymnus sp.* *Eretmocerus californics* *Lysiphlebus testaceipes* *Aphelinus asychis* *Encarsia formosa*	*Bemisia tabaci* *Aphis gossypii*	0 0 0 ± ± ±	Hoelmer *et al* 1990
Brinckochrysa scelestes		±	Yadav & Patel 1990
Telenomus remus	*Spodoptera litura*	±	Joshi *et al* 1982
Telenomus rowani	*Tryporyza incertulas*	0	Fernandez *et al* 1992
Trichogramma principium	*Plutella xylostella*	±	Klemm & Schmutterer 1993
Diadegma semiclausum	*Plutella xylostella*	0 0	Chandramohan & Nanjan 1990 Schneider & Madel 1991
Cotesia plutellae	*Plutella xylostella*	±	Loke *et al* 1992
Goniozus triangulifer	*Marasmia patnalis*	±	Lamb & Saxena 1988
Diadegma terebrans	*Ostrinia nubilalis*	±	McCloskey *et al*. 1993
Pediobius foveolatus	*Epilachna vigintioctopunctata*	±	Tewari & Moorthy 1985
Cotesia glomerata	*Pieris brassicae*	± ±	Schmutterer 1992 Osman & Bradley 1993
Tetrastichus howardi	*Chilo suppressalis*	+	Fernandez *et al* 1992
Diaeretiella rapae *Ephedrus cerasicola*	*Myzus persicae*	0	Schauer 1985
Encarsia formosa	*Trialeurodes vaporariorum*	±	Feldhege & Schmutterer 1993
Syrphid flies		+ +	Schauer 1985 Bidmon *et al* 1987
Chrysoperla carnea		±	Kaethner 1990

Table 2 (cont.)

PREDATOR / PARASITOID	PEST	IMPACT	REFERENCE
Paederus alfierii	*Spodoptera littoralis*	±	Saleem & Matter 1991
Coccinella undecimpunctata		±	
Chrysoperla carnea		±	
Platynus dorsalis		0	Förster 1991
Coccinella septempuctata	Aphids	0	Schmutterer 1981
		0	Kaethner 1990
	Melanaphis sacchari	0	Srivastava & Parmar 1985
	Myzus persicae	0	Eisenlohr *et al* 1992
Formica polyctena		±	Schmidt & Pesel 1987
Nesidiocoris	*Bemisia tabaci*	±	Serra 1992
Cyryorhinus lividipennis	Plant hoppers	±	Saxena *et al* 1984
		0	Fernandez *et al* 1992
Forficula auricularia	*Cereal aphids*	0	Schauer 1985
		±	Eisenlohr *et al* 1992
Uscana lariophaga	*Callosobruchus maculatus*	+	Boeke, 2002
Dinarmus basalis		±	Boeke, 2002

Table 3. Impact of Melia azedarach on natural enemies: no impact indicated by 0, slight impact indicated by ± (extracted from Schmutterer, 1995 p. 632).

PREDATOR/PARASITOID	PEST	IMPACT	REFERENCE
Cyrtorhinus lividipennis	Plant hoppers	±	Saxena *et al* 1984
Pardosa pseudoannulata		0	
Pediobius foveolatus	*Henosepilachna vigintioctopunctata*	±	Tewari & Moorthy 1985
Erigorgus femorata	*Thaumetopoea pityocampa*	0	Breuer & Devkota 1990
Phryxe caudata		0	
Phorocera grandis		0	
Laemostenus sp.		0	
Amblyseius newsami	*Panonychus citri*	0	Wei *et al* 1989
Coccinella undecimpunctata	*Aphis gossypii*	±	Matter *et al* 1993
Pardosa pseudoannulata	*Nilaparvata lugens*	0	Chiu 1985
Coccinella septempunctata	*Lipaphis erysimi*	0	Teotia & Tewari 1972

CHAPTER 2

IMPACT OF BOTANICAL PESTICIDES DERIVED FROM *MELIA AZEDARACH* AND *AZADIRACHTA INDICA* ON THE LIFE HISTORY OF THE DIAMONDBACK MOTH *PLUTELLA XYLOSTELLA*

CHAPTER 2

Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the life history of the diamondback moth, *Plutella xylostella*.

Abstract

This study was initiated to investigate the possibility of using botanical pesticides to control *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), a serious cosmopolitan pest of crucifer plants. Three different doses of aqueous leaf extracts were made from syringa trees, *Melia azedarach* L. (Meliaceae), and the impact of these extracts was tested on the life history parameters of *P. xylostella*. As a comparison three doses of a commercial product from the neem tree, A*zadirachta indica* Juss. (Meliaceae), i.e. Neemix 4.5®, were also tested. The extracts made from *M. azedarach* were most effective at the two highest doses, the lowest dose did not affect larval mortality, but it did reduce leaf consumption by the larvae, pupal weight, and fecundity of moths that had been feeding on treated cabbage plants. Extracts from *M. azedarach* appear to have both antifeedant and growth inhibition properties, and therefore provide a suitable alternative to neem which does not grow in South Africa. All of the neem treatments had a significant impact on all the life history parameters of *P. xylostella*. Results indicate that these botanical pesticides have good possibilities for control of *P. xylostella* in South Africa.

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), remains one of the most serious threats to crucifer production throughout the world, and was first reported as a pest in South Africa in 1917 (Gunn, 1917). In the past, attempts to control this pest have relied heavily on the use of non-selective synthetic pesticides (Roush, 1997), resulting in several problems including reduction of natural enemies in vegetable agroecosystems, unacceptable levels of pesticide residue, poisoning of farmers and labourers, escalating costs of production and pesticide resistance (Jusoh, *et al*., 1992). *Plutella xylostella* has shown a formidable capacity to evolve resistance to pesticides (Roush, 1997), and has become notorious for being the first pest to evolve resistance to DDT and more recently to *Bacillus thuringiensis* (*Bt*) (Tabashnik, 1994). Despite the abundance of natural enemies in South Africa, control has remained heavily dependent on synthetic pesticides (Sereda *et al.*, 1997; Charleston & Kfir, 2000) and as a consequence the pest has developed significant levels of resistance to most of the commercial insecticides in use in this country (Sereda *et al.*, 1997). Therefore, alternative control mechanisms are essential if this pest is to be managed effectively.

The plant kingdom is by far the most efficient producer of chemical compounds, synthesizing many products, which can partly be considered as weapons used in defence against the pests and diseases that challenge them. Insecticides of plant origin have been in use for a long time. For example, pyrethrum, obtained from the flower heads of *Chrysanthemum cinerariifolium* was already known during the time of the Persian king Darius the Great (521-486 B.C.) (Schmutterer, 1995). Approximately 1800 plant species have been reported to possess pest control properties (Grainge *et al.*, 1984), with 88 of these reported to be active against *P. xylostella* (Morallo-Rejesus, 1986). Azadirachtin, an active ingredient found in the neem tree, *Azadirachta indica* Juss. (Meliaceae), is a complex steroid-like tetranortriterpenoid (limonoid) that has been effectively used against more than 400 species of insects, including many key crop pests. This compound is considered as one of the most promising plant ingredients for IPM at the present time (Jacobson, 1989; Rembold, 1989; Schmutterer, 1990; Isman, 1999; Walter, 1999). The compound has a variety of properties including insecticidal activity, acting as a phago- and oviposition deterrent, an antifeedant, a growth retardant, a moulting inhibitor, and a sterilant as well as having antifungal, antiviral and antibacterial properties against pathogens (Prakash & Rao, 1986, 1997). Unlike their synthetic counterparts, botanical pesticides contain a cocktail of numerous chemical compounds, with complex physiological and behavioural actions (Rice, 1993). While *P. xylostella* may also eventually evolve resistance to plant extracts, investigations indicated that it was not able to develop resistance to *A. indica*, even after 40 – 60 generations of exposure to this botanical product (Völlinger, 1995).

Small-scale rural farmers in South Africa can grow cabbage as a subsistence and cash crop if they have access to water. Often these farmers do not have the financial means to purchase expensive synthetic chemical products and therefore rely on traditional control techniques, including inter-cropping and removal of insects by hand. The use of botanical pesticides is an attractive alternative to subsistence farmers in developing countries, as the products can be made using locally available tools and plants. Neem-based insecticides have been used in other parts of the world for the management of *P. xylostella* and other pests on cabbage (Leskovar & Boales, 1996; Perera *et al.*, 2000) and are generally effective with significant lethal and antifeedant effects accompanied by a significant reduction in food consumption (Liang, *et al.* 2003). Neem products are not registered for use in South Africa and the neem tree does not grow in this area. However, the closely related syringa tree, *Melia azedarach* L. (Meliaceae) is an abundant invasive plant found throughout the country. The leaves, seeds and fruits of the syringa tree are also well known for their insecticidal activity and have been used to control pests from most insect orders (Ascher *et al.*, 1995), including *P. xylostella* (Steets, 1975; Chen *et al.*, 1996b). This tree does not appear to contain azadirachtin (Lee *et al.*, 1991), but does contain two potent insecticidal tetranortriterpenoids, a novel meliacin and a new derivative of meliacarpin (Lee *et al.*, 1991).

Twenty-one species of parasitoids have been recorded attacking *P. xylostella* in the field in South Africa (Kfir, 2003), and this provides a good opportunity to develop an integrated pest management system for this pest. However the integration of botanical pesticides, in particular the use of extracts from *M. azedarach*, with biological control for *P. xylostella* has not been well studied. Despite the abundance of *M. azedarach* in South Africa, subsistence farmers in this country do not appear to be aware of the insecticidal properties of the tree and do not make use of extracts from this tree (see Chapter 7). This study looks at the impact of botanical pesticides on *P. xylostella*, and the possibility of using extracts from *M. azedarach* as an alternative control technique, with the vision of developing an integrated pest management system for diamondback moth in South Africa. Here we address the effects of syringa and neem on the life history of *P. xylostella*.

Materials and methods

The treatments

Syringa: *Melia azedarach* (hereafter referred to as syringa) leaves were collected from Rietondale in Pretoria, South Africa (28°15'S; 25°44'E). The leaves were collected from trees at a height of about 1.5 - 3.5 m at the beginning of the spring flush in September 1999, and placed in a glass house (30 ± 5°C) to dry; after which they were then crushed into a fine powder and stored in an airtight container until use. Three different doses of the extract were prepared by using different weights of leaf powder, 1 g (low), 3 g (medium) and 5 g (high). Each extract was made with 100 ml of distilled water. The water was

heated to 48°C, and the leaf powder was added to the water and shaken for approximately one minute. The extract was left in a refrigerator (± 4°C) 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 surfactant, without which the extract runs off the surface of plant leaves.

Neem: A commercial preparation of *Azadirachta indica,* Neemix 4.5® (hereafter referred to as neem), was provided by Thermo Trilogy Corporation, Columbia, USA. Three different doses were prepared: 10.7 µl (Low), 16 µl (Medium) and 32 µl (High) per 100 ml of distilled water. Liquid detergent was also added to these extracts.

Control: The control treatment consisted of 100 ml of distilled water mixed with three drops of liquid detergent.

Experimental plants and insects

Cabbage plants, *Brassica oleracea* var *capitata* L. (Cruciferae), were bought as seedlings and planted in black plastic bags in a glass house (30 ± 5°C). To protect the plants against insect damage they were placed within a tent-like construction made from fine netting (mesh size <1mm). The plants were fertilised when planted, and regularly watered.

Plutella xylostella were from a laboratory culture started in 1993. The laboratory culture was maintained on canola seedlings, *Brassica napus* L. (Cruciferae). For the present experiments first instar larvae were removed from the canola and placed on cabbage for 24 hours before being exposed to the experimental plants.

Insect rearing and all experiments were maintained in a controlled environment (24 ± 2°C; 65% r.h., L16:D8).

Bioassay trays

Leaf discs (30 mm x 35 mm) were cut from the cabbage plants and leaves were taken from plants of approximately the same age, (± five weeks after transplant). The leaf discs were placed in a randomised block design in a bioassay tray (440 mm x 210 mm). The bioassay tray had 32 cells (4 x 8 cells) and the leaf discs were cut to fit into these cells. Within each tray four blocks were used and each block had eight treatments. The treatments consisted of the three extracts prepared from syringa, the three extracts prepared from neem, and two control treatments. The leaves were dipped into the treatment and left to dry for approximately 60 minutes. Each cell in the bioassay tray had an agar (2%) base to prevent the leaves from dehydrating. The bioassay tray was covered with "Bio CV 4" vented covers to prevent the larvae from escaping.

Impact of botanical pesticides on larvae

First instar larvae were used in the experiment. One larva was exposed to the leaf disc in each cell. Six trays were placed randomly on the laboratory bench for each test and the test was replicated 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. Those larvae that survived were weighed at the pupal stage.

Assessment of leaf consumption

Twenty-four leaf discs were randomly selected from each treatment. Photocopies were made of the leaf discs after larval feeding. Two sets of leaf discs were copied from each treatment. The first set at the end of the first leaf change (to give an estimate of the feeding damage by first instar larvae) and the next set at the end of the final leaf change just before the larvae pupated (to give an estimate of feeding damage by the fourth instar larvae). These pictures clearly illustrated the surface area that was consumed by the larvae. The photocopies were scanned into the computer at 200 dpi (dots per inch) using the software Photoimpact SE ver. 3.02 and then converted to electronic Bitmap files with Adobe Photodelux Business Edition software. A freeware computer program, UTHSCSA Image Tool for Windows ver. 3.00 (The University of Texas Health Science Centre in San Antonio) was used to calculate the surface area consumed by the larvae, by counting the number of black and white pixels in the grayscale binary image. This was then converted back into amount of damage in square millimeters.

Impact on development period

Development from the egg stage onwards was investigated. To obtain the eggs, *P. xylostella* moths were exposed to untreated canola seedlings for 48 hours and left to oviposit. Eggs were subsequently removed from the canola seedlings using a paintbrush and distilled water and placed in the bioassay tray on cabbage leaf discs that had been treated with the botanical pesticide or with the control. One egg was placed on each leaf disc in each cell. After a pilot study had been conducted only three treatments were chosen: the control, the medium syringa treatment and the low neem treatment. Eight leaf discs from each of these treatments were placed in a randomised block design in the bioassay tray. Three trays were used and randomly placed on the laboratory bench. Each day the bioassay tray was checked and the larval stage and mortality was recorded. The leaf discs were replaced every second day.

To investigate whether the treatments were also effective at older instar stages, larvae were left to feed on untreated plants until they reached the third instar and then they were exposed to treated plants for the remaining duration of their development. For this experiment all the treatments were investigated. Four leaf discs from each treatment were placed in a randomised block design into the bioassay tray. Four trays were used and

randomly placed on the laboratory bench. The procedure was the same as that explained above.

Impact on fecundity of second generation

Seven cages (900 mm *(l)*, 450 mm *(w)*, 450 mm *(h)*) were used, one for each treatment (six plant extracts and one control). One gram of *Plutella xylostella* pupae were collected (±195 pupae) and placed into each cage. Once moths started to emerge they were exposed to four plants with a particular treatment. The moths were allowed to lay eggs for three days and the eggs were left to develop. Any larvae that survived and emerged as moths were exposed to cabbage plants. A mated female moth was placed into a 9 ml glass vial with a strip of cabbage. A hole was punched into the top of the lid of the vial, and a cotton stopper was dipped in 1% sugar solution and placed into the hole to provide the moth with a food source. The cotton stopper and leaf strip were replaced every second day and the number of eggs laid by each moth was counted to assess the impact that the treatment had on adult moth fecundity. The experiment continued for four months or until 35 females had emerged from the treatment/ control. Thirty-five females emerged from the control and the lowest syringa treatment, but as a consequence of the high mortality, this was less than 35 females for the other treatments.

Statistical analysis

Data were analysed using the statistical program GenStat (GenStat for Windows, 2000).

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 a generalised linear model (GLM) with binomial distribution and a logit link function, with treatment as a predicting factor (Crawley, 1993). Fisher's protected *t*-test of least significant differences (LSD) was applied to separate the mean proportions at the 5% level of significance (Snedecor & Cochran, 1980).

Differences in the developmental period of larvae that had been feeding on treated plants were analysed using analysis of variance (ANOVA). For the development of the third instar larvae the data were not normally distributed and a square-root transformation was carried out before the ANOVA.

An analysis of variance was carried out over the treatments for the pupal weights, as well as for the leaf area consumed by the larvae. For the leaf consumption by *P. xylostella* first instars the data were not normally distributed and a log transformation was carried out. For the other data the assumptions of normality and homogeneity of variances were met. Fisher's protected LSD was applied to separate the treatment means at the 5% level of significance.

Differences in the fecundity of moths were analysed using ANOVA, and pair-wise differences were found using Fisher's protected LSD. However, homogeneity of variances could not be controlled, due to the uneven number of observations. Therefore Fisher's protected LSD test was made more conservative by separating treatment means at the 1% level of significance instead of the 5% level.

Results

Mortality of larvae feeding on cabbage treated with botanical pesticides.
There were significant differences in the survival of *P. xylostella* larvae feeding on cabbage treated with botanical pesticides (GLM: $P < 0.001$).

Overall mortality - Syringa: Survival 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 low dose. These differences were not significant ($P = 0.25$). Approximately 25 and 17% of the larvae feeding on the medium and high doses respectively survived to the moth stage. These differences were also not significant ($P = 0.13$). However, survival on the control and the lowest dose was significantly higher than survival on the medium and the high dose ($P < 0.001$) (Fig. 1).

Overall mortality - Neem: Survival on the control treatment was significantly higher than on all the neem treatments ($P < 0.001$). Approximately 13 and 17% of the larvae feeding on the low and medium dose neem treatments respectively, survived to the moth stage. The effects of these two treatments were not significantly different ($P = 0.35$). Only 3% of the larvae feeding on the highest dose survived to the moth stage, which was significantly lower than at all the other doses (low: $P = 0.009$; medium: $P = 0.002$) (Fig. 1).

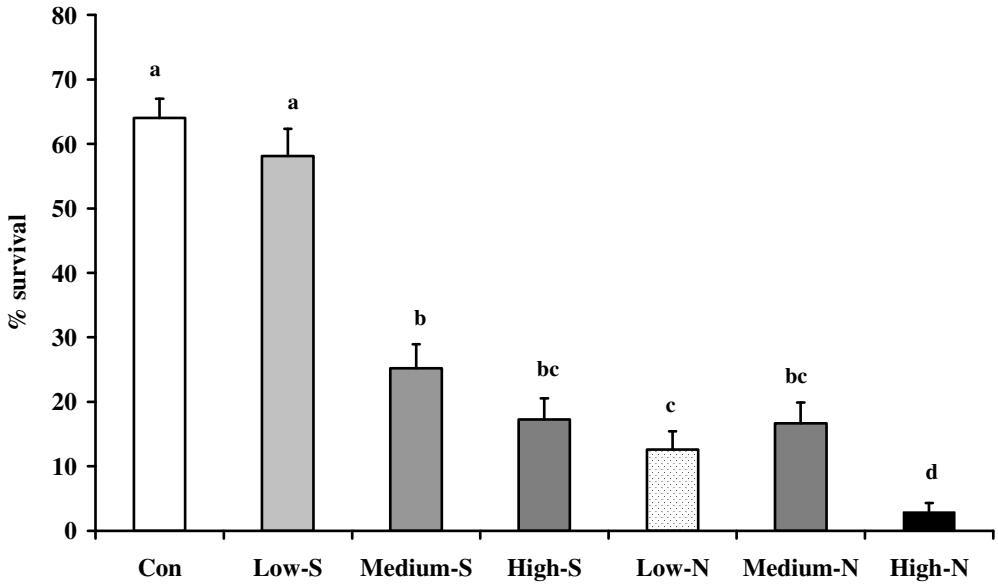

Figure 1. Percentage (± SE) *P. xylostella* larvae surviving on cabbage leaf discs treated with different doses of botanical pesticide: Low-S, Medium-S and High-S are three doses from the syringa tree; Low -N, Medium-N and High-N are three doses from the neem tree. For information on the doses see materials and methods. Each bar represents 6 replicate tests with 24 larvae per treatment and 48 larvae per control. Bars with the same letter are not significantly different (GLM: $\alpha = 0.05$).

Average weight of pupae on cabbage treated with botanical pesticides.

There were significant differences between pupal weights on the different treatments (ANOVA: $F_{0.05(6,178)} = 6.48$; $P<0.001$).

Syringa: Pupae collected from control cabbage plants were significantly heavier than those from the plants treated with syringa extracts (low: P = 0.012; medium: P = 0.000; high: P = 0.000). The pupae that were found on the low dose were also significantly heavier than those found on the high dose (P = 0.015). There were no significant differences between the pupal weights on the medium or low doses (P = 0.18), nor between those feeding on the high and the medium doses (P = 0.27) (Fig. 2).

Neem: Pupae collected from control cabbage plants were significantly heavier than pupae from the plants treated with neem (low: P = 0.000; medium: P = 0.001; high: P = 0.000), but there were no significant differences between the treatments (P >0.05) (Fig. 2).

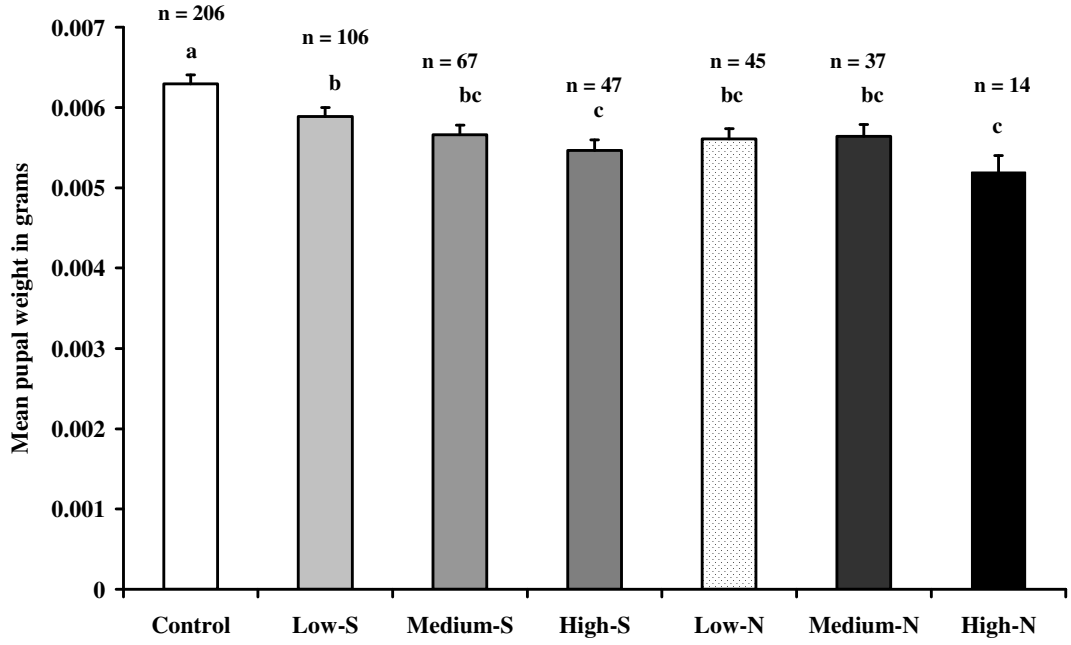

Figure 2: Mean (± SE) pupal weight of *P. xylostella* that have been feeding on cabbage leaf discs treated with different doses of botanical pesticide: Low-S, Medium-S and High-S are three doses from the syringa tree; Low -N, Medium-N and High-N are three doses from the neem tree. For information on the doses see materials and methods. Bars with the same letter are not significantly different (ANOVA; plus Fisher's LSD: $\alpha = 0.05$).

Developmental period

Pilot study: An initial pilot study was carried out using 50 *P. xylostella* eggs per treatment. Only one of the eggs made it to the moth stage on the high dose of the syringa treatment, and on both the medium and high dose of the neem treatments. Therefore, only three treatments were selected for the final trial looking at development time, i.e. the low dose of neem, the medium dose of syringa and the control.

Development from egg: Development from the egg stage was significantly slower on cabbage plants treated with the neem and syringa extracts (Fig. 3a). Larvae feeding on treated plants took significantly longer to reach the second instar stage (ANOVA: $F_{0.05(2,47)} = 12.40$; $P<0.001$), the third instar stage (ANOVA: $F_{0.05(2,31)} = 6.43$; $P = 0.005$), the fourth instar stage (ANOVA: $F_{0.05(2,28)} = 8.87$; $P = 0.001$) and the pupal stage (ANOVA: $F_{0.05(2,25)} = 11.26$; $P<0.001$). Only two larvae from 24 eggs that had been feeding on cabbages treated with the syringa extract made it to adulthood, while none that had been feeding on the neem treatment survived. In contrast, 19 out of the initial 24 eggs survived to the moth stage in the control treatments (Fig. 3b).

Survival and development from third instar: When *P. xylostella* larvae were only exposed to the botanical pesticides at the third instar stage, development was significantly prolonged even at this late stage. Larvae took significantly longer to reach the fourth instar (ANOVA: $F_{0.05(6,101)} = 2.19$; P = 0.05) and the pupal stage (ANOVA: $F_{0.05(6,83)} = 2.42$; P = 0.033) (Fig 3c). Third instar *P. xylostella* larvae took significantly longer to reach the fourth instar stage compared to the control plants when feeding on the highest dose of the syringa extract (P = 0.05) and when feeding on all the neem treatments (low: P = 0.01; medium: P = 0.032; high: P = 0.007). Development of *P. xylostella* larvae to the pupal stage was prolonged compared to the control on the medium (P = 0.043) and high (P = 0.005) neem doses (Fig. 3c). Survival was also significantly reduced (ANOVA: $F_{0.05(6,18)} = 8.53$; P<0.001). The highest dose of syringa significantly reduced survival (P<0.05), and all of the neem treatments significantly reduced survival, even at the lowest dose (P<0.05) (Fig. 3d).

Leaf consumption

When the surface area consumed was compared, results from the initial leaf change (damage by first instar *P. xylostella*) and the final leaf change (damage by fourth instar *P. xylostella*) were kept separately. After the first leaf change there were significant differences between the treatments (ANOVA: $F_{0.05(6,161)} = 5.84$; P < 0.001). The amount of leaf eaten was significantly higher for the control than for any of the other treatments (P < 0.05) (Fig 4a). After the final leaf change, there were also significant differences between the treatments (ANOVA: $F_{0.05(6,161)} = 18.73$; P < 0.001), but there were no significant differences between the lowest syringa dose and the control (P > 0.05) (Fig. 4b). However, significantly more leaf surface area had been consumed in the control than in any of the other treatments (P < 0.05) (Fig 4b).

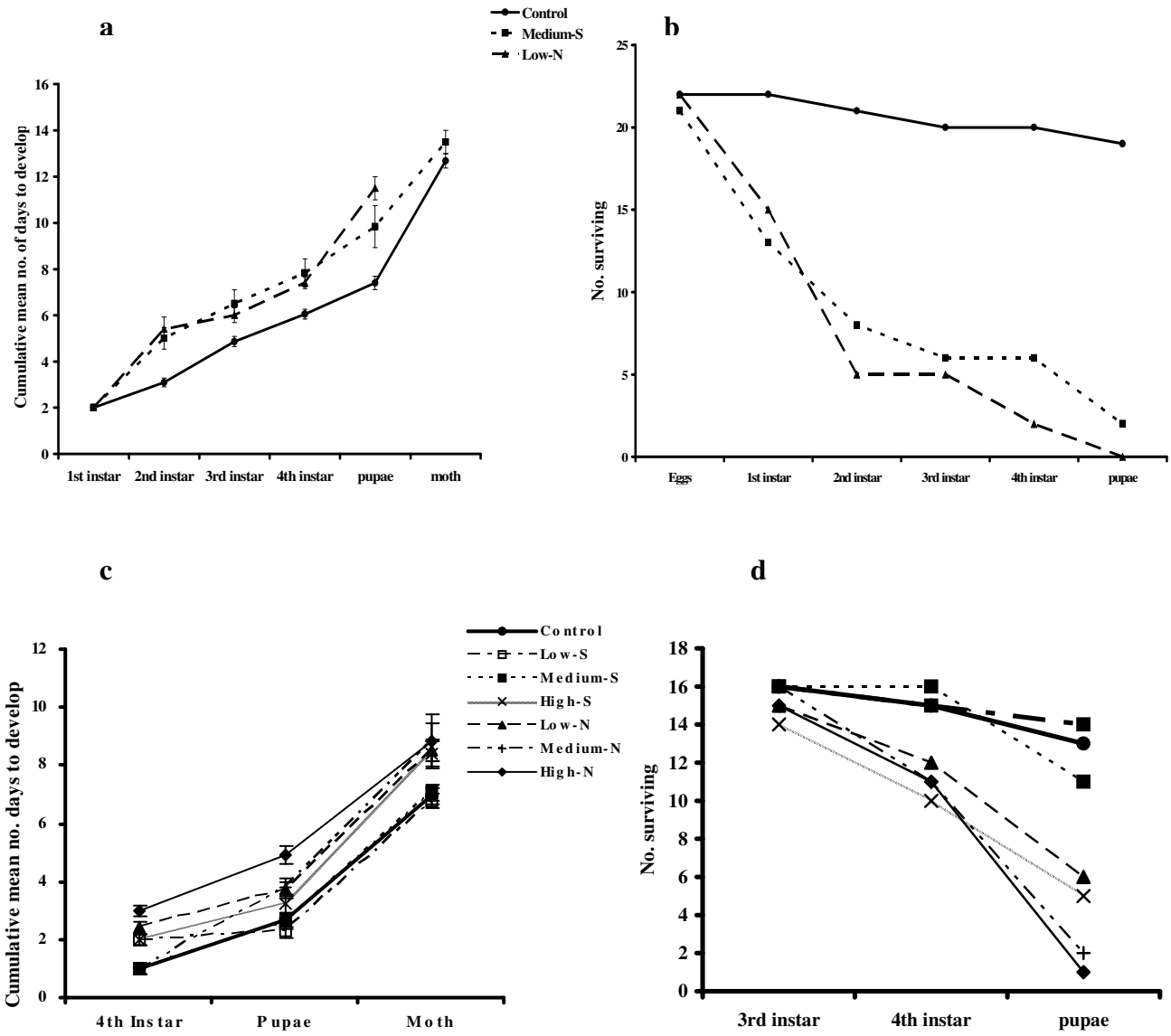

Figure 3. Development and survival of *P. xylostella* when exposed to different doses of botanical pesticides. Low-S, Medium-S and High-S are doses from the syringa tree and Low-N, Medium-N and High-N are doses from the neem tree. For information on the doses see materials and methods.

- **a.** Cumulative mean (± SE) number of days it takes *P. xylostella* to develop into different instars if eggs are put on cabbage leaf discs treated with different botanical pesticides. The experiment started with 24 eggs per treatment.
- **b.** Number of *P. xylostella* surviving at different instars if eggs are put on cabbage leaf discs treated with different botanical pesticides. The experiment started with 24 eggs per treatment.
- **c.** Cumulative mean (± SE) number of days it takes *P. xylostella* to develop if 3rd instar larvae are exposed to cabbage leaf discs treated with different doses of botanical pesticide. The experiment started with 16 larvae per treatment.
- **d.** Number of *P. xylostella* surviving at different instars if 3rd instar larvae are put on cabbage leaf discs treated with different botanical pesticides. The experiment started with 16 larvae per treatment.

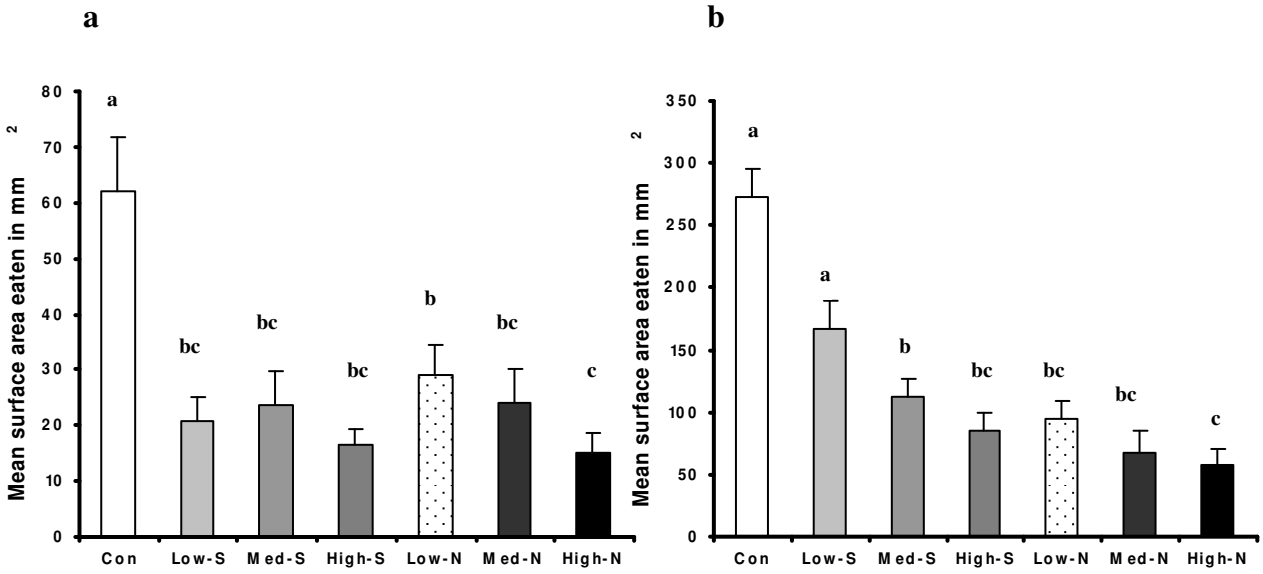

Figure 4. Mean (± SE) leaf surface area (in mm^2) eaten by *P. xylostella*. Cabbage leaf discs were treated with different doses of botanical pesticide: Low-S, Medium-S and High-S are the three doses from the syringa tree; Low -N, Medium-N and High-N are three doses from the neem tree. For information on the doses see materials and methods. Bars with the same letter are not significantly different (ANOVA; plus Fisher's LSD: $\alpha = 0.05$).

a. Mean (± SE) leaf surface area (in mm^2) eaten by first instar *P. xylostella* after the first leaf change (n = 24).

b. Mean (± SE) leaf surface area (in mm^2) eaten by fourth instar *P. xylostella* after the final leaf change (n = 24).

Average fecundity of moths

Mortality of larvae feeding on treated cabbage is very high (see above). As soon as 35 female moths had completed development, the trial for that treatment stopped. This was only successful for the control and the lowest dose of the syringa treatment. The trial was continued for four months in an attempt to obtain 35 female moths from the other treatments. In a four-month period only 13 and 15 larvae on the medium and high dose syringa treatments respectively survived to become female moths; and 25, 14 and 2 larvae on the low, medium and high dose neem treatments respectively survived to become female moths. Results from this trial were analysed, but due to the large differences in the numbers of moths and the heterogeneous variances these results should be viewed with caution. In order to be more conservative, means were only separated at the 1% level of significance. Female moths reared on the control treatment were found to lay significantly more eggs than female moths that had been reared on treated plants (Fig. 5), but other differences were not found.

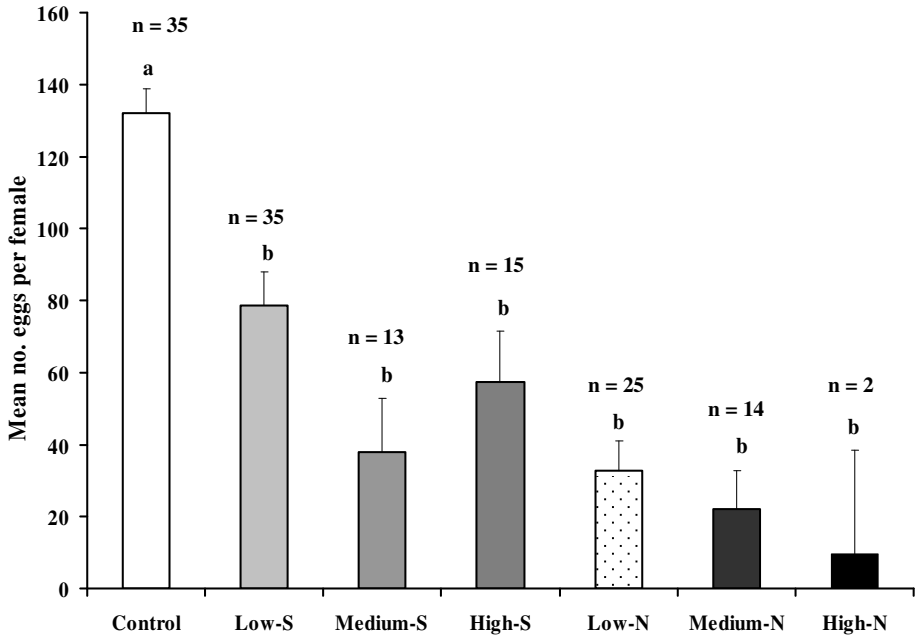

Figure 5. Mean (± SE) number of eggs oviposited by *P. xylostella* moths that had been reared on treated cabbage plants since emergence. Cabbage plants were treated with different doses of botanical pesticide: Low-S, Medium-S and High-S are the three doses from syringa; Low -N, Medium-N and High-N are the three doses from neem. For information on the doses see materials and methods Bars with the same letter are not significantly different (ANOVA; plus Fisher's LSD: $\alpha = 0.01$).

Discussion

The botanical pesticides that we tested had a significantly negative impact on larval survival of *P. xylostella*. Extracts from syringa were only effective at the higher doses, but all the doses from the neem treatment had a negative impact on survival. Neem is well known to have significant impacts on the mortality of *P. xylostella* (Verkerk & Wright, 1993; Schmutterer, 1992c; Isman, 1995; Perera *et al.*, 2000; Liang *et al.*, 2003). High larval mortality has also been reported for *P. xylostella* exposed to methanolic leaf extracts of syringa (Steets, 1975), and ethanolic fruit extracts from syringa have been reported to cause over 90% mortality (Chen *et al.*, 1996b). To our knowledge this is the first study that uses aqueous syringa leaf extracts and they were also found to have a significant impact on larval survival, especially at the higher concentrations.

Plutella xylostella appeared to be affected by the botanical pesticides at most stages of development. Larvae feeding on cabbage treated with the syringa extract were affected at the second and third instar stage, while all the developmental stages except the fourth instar of *P. xylostella* larvae feeding on cabbage treated with the neem extract were affected. Larval development time was also significantly prolonged by the botanical pesticides, even when larvae were only exposed to the treatments at the third instar. When

exposed at this stage the botanical pesticides appeared to prevent moulting of the insects and many died before they could pupate. Pupal mortality was high, with many adults failing to emerge. Some of the adults that did emerge from pupae that had been exposed to the botanical extracts were deformed, with shorter wings or wings that failed to expand properly. Moulting disruption at the time of pupation is perhaps the most dramatic physiological effect of neem that has been observed in Lepidoptera, with larvae failing to initiate the larval-pupal moult (Schmutterer, 1995; Prijono & Hassan, 1993). Larvae are most susceptible to this if they are treated during the final instar (Koul & Isman, 1991), and even larvae that are treated in the penultimate instar may complete development to the final instar, but then fail to pupate normally (Jagannadh & Nair, 1992; Prijono & Hassan, 1993). The slower rate of development and failure to moult has been previously reported for *P. xylostella* treated with neem extracts (Schmutterer, 1990; Isman, 1995; Verkerk & Wright, 1993; Liang *et al.*, 2003), as well as for other insects (Isman *et al.*, 1990; Prijono & Hassan, 1993; Breuer *et al.*, 1999). Results from our study confirm that in addition to neem treatments, aqueous leaf extracts from the syringa tree also have a severe impact on the ability of *P. xylostella* to moult.

The resulting pupal weight of *P. xylostella* was found to be significantly lower on the treated cabbage plants than on the control plants, which could be explained by reduced feeding. Results from the surface area consumed showed that significantly less leaf surface was consumed by the larvae on the cabbage plants that had been treated with the botanical pesticides than on the control cabbage plants. Reduced feeding has been reported for *P. xylostella* exposed to cabbage treated with different neem preparations in Sri Lanka (Perera *et al.*, 2000), and for *P. xylostella* feeding on Chinese kale treated with fruit extracts from syringa (Chen *et al.*, 1996b). In the study by Chen *et al*. (1996b) the syringa fruit extracts caused high mortality at all doses tested. However, at low doses the feeding activity was not reduced, and feeding activity was not reduced on the first day when larvae were exposed to the treated plants. This led the authors to conclude that the extract was effective as a growth inhibitor rather than an antifeedant, although they did find reduced feeding activity at higher doses of the extract. However, in our study, the low dose of the syringa extract did not cause significant mortality, but it did cause a significant reduction in food consumption, right from the first day, which indicates that this extract has antifeedant properties. In our study we made use of an aqueous leaf extract, while Chen *et al*. (1996b) made use of the fruit extracted with diethyl ether and diluted with acetone, which may have caused some of the different results. In our study we also found evidence of growth inhibition, with prolonged larval development, which may be due to the antifeedant effects, but even when *P. xylostella* was only exposed to the treatments at the third instar, we found that many of the larvae failed to complete development. Therefore the aqueous syringa leaf extracts used in our study appear to have both antifeedant and growth inhibition effects.

The botanical pesticides used in this study significantly reduced fecundity of *P. xylostella*. Völlinger (1987) observed that if *P. xylostella* larvae were fed on cabbage treated with neem there was a substantial reduction in fecundity of females. Zhu (1991) and Chen *et al.* (1996b) have also reported that treatment of *P. xylostella* with sub-lethal doses of methanolic leaf extracts or fruit extracts from the syringa tree significantly reduced female fecundity. Results from this study indicate that continuous feeding by *P. xylostella* on cabbage treated with aqueous leaf extracts from syringa also has a negative impact on the resulting fecundity.

This study showed that Neemix 4.5® and aqueous leaf extracts from the syringa tree result in a reduced fecundity, pupal weight and adult emergence and perhaps more importantly reduced the feeding damage by larval stages of *P. xylostella*. Syringa extracts therefore provide an additional alternative control technique for management of *P. xylostella* in South Africa, where neem products are not registered for use.

Treatment of cabbage plants with botanical pesticides may also result in negative behavioural responses by the herbivore called antixenosis. Further investigation of the impact of these botanical pesticides on the behaviour of *P. xylostella* and the biology and behaviour of its natural enemies are important aspects, which are currently under investigation (Chapters 3-6).

Acknowledgements

This research was carried out through a grant awarded under the IFS/KNAW Carolina MacGillavry PhD Fellowship Programme. The ARC-PPRI provided the working space and facilities. Staff of the ARC-PPRI insectary provided the insects used in the experiments. Liesl Morey and Edith van Den Berg from the ARC-Biometry unit provided valuable statistical advice.

CHAPTER 3

BEHAVIOURAL RESPONSES OF DIAMONDBACK MOTH TO BOTANICAL PESTICIDES DERIVED FROM *MELIA AZEDARACH* AND *AZADIRACHTA INDICA*

CHAPTER 3

Behavioural responses of diamondback moth to botanical pesticides derived from *Melia azedarach* and *Azadirachta indica*.

Abstract

The impact of three different doses of botanical pesticide derived from the syringa tree, *Melia azedarach* L. (Meliaceae) and the neem tree, *Azadirachta indica* Juss. (Meliaceae) was tested on the behaviour of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Both botanical pesticides had a significant impact on larval behaviour. At higher doses the extracts showed feeding deterrent activity, with larvae preferring the untreated sides of cabbage leaves and consuming less of the treated half of cabbage leaves. The botanical pesticides had less of an effect on the oviposition behaviour of *P. xylostella* moths. In laboratory two-choice trials significantly fewer eggs were oviposited on the cabbage plants treated with the medium dose of syringa than on the control plants, but there were no other differences. However, in the glasshouse, where moths were given a choice between all treatments and control plants, and could move about freely, significantly fewer eggs were oviposited on the plants that had been treated with syringa extracts. Therefore, the syringa extracts appear to have a repellent effect. In contrast, when exposed to the neem extracts the moths did not discriminate between control plants and treated plants, ovipositing an equal number of eggs on both. Behavioural observation of first choices indicated that, despite the lower number of eggs oviposited on cabbage treated with syringa extracts, the moths chose cabbage treated with the highest dose of syringa more often than they chose control cabbage plants. This behaviour did not change whether the plants were damaged or undamaged. When moths were exposed to cabbage treated with neem extracts they chose the medium dose more often than they chose the control, but they did not show this preference if the cabbage plants were damaged. Oviposition and feeding deterrent properties are important factors in pest control, and results from this study indicate that botanical pesticides have the potential to be incorporated into control programs for *P. xylostella* in South Africa.

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is an oligophagous species, that feeds on plant species in the family Cruciferae (Thorsteinson, 1953), which include economically important crops such as cabbage, cauliflower, broccoli, canola and Brussels sprouts. As such it is a world-wide pest, costing over US$ 1 billion to control annually (Talekar & Shelton, 1993). In the past, control of *P. xylostella* relied heavily on the use of synthetic chemical pesticides, which has resulted in the development of resistance to all modern synthetic pesticides that have been used intensively for any prolonged length of time (Talekar & Shelton, 1993). The widespread and indiscriminate use of insecticides in South Africa has resulted in resistance of *P. xylostella* to synthetic pyrethroids, organophosphates and carbamates (Sereda *et al*., 1997). Currently *Bacillus thuringiensis* (*Bt*) var. *aizawai* and var. *kurstaki* products are recommended for control of *P. xylostella* in South Africa (Nel *et al*., 1999). However, in other parts of the world *P. xylostella* has already developed resistance to *Bt* products (Tabashnik, 1994). Therefore the development of alternative control methods is essential if this pest is to be managed successfully.

Plants are a virtually inexhaustible source of structurally diverse biologically active substances, and approximately 1800 plants have been reported to possess insecticidal properties (Grainge *et al.*, 1984). The complex combination of behavioural and physiological actions contained in these plant compounds makes it difficult for insects to evolve resistance to them (Rice, 1993). Insecticides of plant origin have been in use for a long time. The plant families Asteraceae, Fabaceae, Euphorbiaceae and Meliaceae contain most of the insecticidal plant species (Morallo-Rejesus, 1986; Schmutterer, 1995). Products from the neem tree, *Azadirachta indica* Juss. (Meliaceae), and the closely related syringa tree, *Melia azedarach* L. (Meliaceae), have been found to be extremely effective against insect pests (Schmutterer, 1995), and it has been shown that *P. xylostella* was unable to evolve resistance to *A. indica* even after 40 – 60 generations of exposure to extracts from this tree (Völlinger, 1995).

Botanical pesticides have particular importance to developing countries as they can be made using local tools and trees. In South Africa approximately 80% of the small-scale rural farmers that have access to water are growing cabbage as a subsistance and cash crop. The neem tree is widely distributed in Asia, Africa, the Americas, Australia and the South Pacific islands (Schmutterer, 1995), but it is not found in South Africa. However, the closely related syringa tree is an invasive plant, and therefore commonly found throughout the country.

Botanical pesticides can influence the behaviour and development of the herbivorous insects that search for or use the plant for their reproduction. Host specificity in

oligophagous herbivore insects is due to gravid females showing discrimination in their choice of plant on which to oviposit, and the larvae only accepting the leaves of a few closely related species of plants as food (Thorsteinson, 1953). Crucifer plants contain glucosinolates, which provide chemical defence against generalist herbivore attack (Fahey *et al*., 2001), although specialists such as *P. xylostella*, *Pieris* spp. and *Phyllotreta cruciferae* use these glucosinolates as feeding and oviposition stimulants (Thorsteinson, 1953; van Loon *et al*., 2002; Siemens & Mitchell-Olds, 1996; Fahey *et al*., 2001). However, when botanical pesticides are applied to crucifers, they have been shown to alter the feeding and oviposition preferences of these specialists (Schoonhoven & Luo, 1994; Hough-Goldstein & Hahn, 1992; Javer *et al*., 1987; Dover, 1985; Tabashnik, 1985). In a previous paper we reported that treatment of cabbage plants with a neem formulation or with aqueous leaf extracts from the syringa tree severely disrupted the life history (i.e. survival, development and fecundity) of *P. xylostella* (Chapter 2). In the present study we examine the impact of these botanical pesticides on the feeding and oviposition behaviour of *P. xylostella*.

Materials and methods

The botanical pesticides

Syringa: *Melia azedarach* (hereafter referred to as syringa) leaves were collected from Rietondale in Pretoria, South Africa (28°15'S; 25°44'E). The leaves were collected from trees at a height of about 1.5 - 3.5m at the beginning of the spring flush in September 1999 and placed in a glass house (30 ± 5°C) to dry; after which they were crushed into a fine powder and stored in an airtight container until use. Three different doses of the extract were prepared by using different weights of leaf powder, 1 g (Low), 3 g (Medium) and 5 g (High). Each extract was made with 100 ml of distilled water. The water was heated to 48°C, and the leaf powder was added to the water and shaken for approximately one minute. The extract was left in a refrigerator (± 4°C) 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 surfactant, without which the extract runs off the surface of the leaf.

Neem: A commercial formulation of *Azadirachta indica,* Neemix 4.5® (hereafter referred to as neem), was provided by Thermo Trilogy Corporation, Columbia, USA. Three different doses were prepared: 10.7 µl (Low), 16 µl (Medium) and 32 µl (High) per 100 ml of distilled water. Three drops of liquid detergent was also added to these extracts.

Control: The control treatment consisted of 100 ml of distilled water mixed with three drops of liquid detergent.

Experimental plants and insects

Cabbage plants, *Brassica oleracea* var *capitata* L. (Cruciferae), were bought as seedlings and planted in black plastic bags in a glass house (30 ± 5°C), within a tent-like construction made from fine netting (mesh size <1 mm) to protect the plants from insect damage. The plants were fertilised when planted, and regularly watered.

Plutella xylostella were from a laboratory culture started in 1993. The laboratory culture was maintained on canola seedlings, *Brassica napus* L. (Cruciferae). Twenty-four hours prior to the experiments first instar larvae were removed from the canola and placed on cabbage.

Insect rearing and all experiments were maintained in a controlled environment (24 ± 2°C; 65% r.h., L16:D8).

Larval choice

Entire cabbage leaves of approximately the same size (± 6 cm diameter) and the same age (± five weeks after transplant) were used. One half of the leaf (using the main vein as a reference) was treated with plant extract or the control 'treatment' and one half was left untreated. The treated half was marked with a felt tip pen, dipped into the treatment and left to dry for approximately 60 minutes. Seven treatments were used. The three doses from syringa, the three doses prepared from neem, and one control treatment. The 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 experiment was repeated six times. One first instar larva was placed in the Petri dish, the dish was sealed with an elastic band and the position of the larva was recorded every hour, for a period of 10 hours.

Each time the larva was observed on one half of the leaf it could not be on the other half of the leaf. The data were therefore not independent and were analysed as paired comparisons (Sokal & Rohlf, 1995). Each larva was categorised as having been observed more often on the treated or the control side of the cabbage leaf. Within treatments a sign test was used to determine differences in the number of times the larva was observed on the treated or untreated side of the leaf (Sokal & Rohlf, 1995).

Assessment of leaf consumption by P. xylostella *larvae*

Leaf consumption by the larvae on either side of the leaf was estimated after 72 hours. Photocopies were made of the leaves these pictures clearly illustrated the feeding holes made by the larvae. The photocopies were scanned into the computer at 200 dpi (dots per inch) using the software Photoimpact SE ver. 3.02 and then converted to electronic Bitmap files with Adobe Photodelux Business Edition software. A freeware computer program, UTHSCSA Image Tool for Windows ver. 3.00 (The University of Texas Health Science

Centre in San Antinio) was used to calculate the surface area consumed by the larvae on either side of the leaf, by counting the number of black and white pixels in the greyscale binary image. This was then converted back into amount of damage in square millimeters.
Analysis of variance was used to compare the area of the leaf surface that was consumed by the larvae. This experiment was designed as a split plot design, repeated ten times. Treatments were divided between leaves using different concentrations (low, medium and high), while the two sub-plot treatments (treated and untreated) were randomly allocated to one half of the leaf. The assumptions of normality and homogeneity of variances were met. Treatment means were separated using Fisher's protected *t*-test of least significant differences (LSD) at the 5% level (Snedecor & Cochran, 1980).

Oviposition choice – cage trial
Six cages (900 mm *(l)*, 450 mm *(w)*, 450 mm *(h)*) were used in the experiment to compare the six doses of neem and syringa extracts against a control. Two cabbage plants were placed into each cage on opposite sides. One of the plants was treated with the botanical pesticide, while the other plant was treated with the control. The treatments were applied using a small hand-held spray bottle. The plants were sprayed until run-off (approximately 100 ml per plant) and then left to dry for 60 minutes before being placed in a cage. The cages were arranged randomly on the laboratory bench.

One gram of fresh pupae (± 195 pupae) was collected from the laboratory culture and placed into a separate cage. Approximately 48 hours after the first moths had started to emerge 30 female *P. xylostella* moths were collected and five moths were placed in each of the six cages for 20 hours. After this the cabbage plants were removed from the cage and the number of eggs oviposited on each plant was counted. The test was repeated six times. The numbers of eggs were counted separately for the top of the leaves, the bottom of the leaves, the main stem of the plant and the plastic bag that the plant was planted in. The data were analysed as paired comparisons using Wilcoxon's signed-ranks test for two groups (Sokal & Rohlf, 1995).

Ovipositon choice - glasshouse
An additional experiment was set up in a glass house (30 ± 5^{o}C) in order to provide *P. xylostella* moths with a larger area for flight and searching. Female moths were given the opportunity to freely choose between all treatments and control plants. Plants were placed in a randomized block design with four plants in each block and four blocks for each treatment. All seven treatments were used in the same experiment, creating a total of 112 plants. The plants were sprayed until run-off (approximately 100 ml per plant) using a medium sized hand-held spray bottle and then left to dry for 60 minutes. The plants were placed within a tent-like construction (5 m (*l*), 4 m (*w*), 2 m (*h*)) enclosed with fine nylon netting (mesh size: <1 mm). Approximately 48 hours after emergence 40 mated female moths were released into the tent and left for 48 hours. After this the number of eggs

oviposited on each plant was counted. Counts were taken for the top of the leaves, the bottom of the leaves and the bag in which the cabbage was planted. The experiment was repeated six times.

Data were analysed using the statistical program GenStat (GenStat for Windows, 2000) and Analysis of Variance (ANOVA). The assumptions of normality and homogeneity of variances were met. Treatment means were separated using Fisher's protected LSD at the 5% level of significance (Snedecor & Cochran, 1980).

Oviposition choice - observation

While the number of eggs oviposited on each plant provides an idea of the choices made by the moths, it is perhaps more accurate to observe the first choice that moths make when they are faced with alternatives. Two plants were placed into a cage (900 mm *(l)*, 450 mm *(w)*, 450 mm *(h)*). The plants were 450 mm apart. Treatments were applied using a small hand-held spray bottle; one plant was sprayed with 100 ml of the botanical pesticide and one was sprayed with 100 ml of the control. Approximately 48 hours after emergence a naïve mated female moth was released between the two plants and observed until she made her first choice. The plants were moved to opposite sides after every 5th observation. New plants were used after 20 observations had been made. A total of 60 female moths were observed for each treatment.

The response of moths to plants that had been damaged by *P. xylostella* larvae was also investigated. Treatments were applied using a small hand-held spray bottle. Each plant was sprayed with either 100 ml of the treatment or 100 ml of the control and infested with 15 third instar *P. xylostella* larvae for 24 hours. The larvae were then removed and the experiment was carried out as explained above.

Plutella xylostella moth choices between treated and control plants were analysed using binomial probability functions to assess a difference from a 50-50 distribution over the two treatments. Significance indicates preference for one of the two treatments (i.e. treated or control). To investigate whether there were any differences in the responses of the female moths to damaged or undamaged plants a R x C Chi-square test was carried out for each botanical pesticide (Sokal & Rohlf, 1995).

Results

Choice of P. xylostella *larvae when faced with cabbage plants treated with botanical pesticides*

There were significant differences in the larval behaviour between the control and the different doses of the extract. The larvae were observed more frequently on the untreated side of the leaf if the leaf had been treated with the botanical pesticides (Sign test: P <

0.01) (Fig. 1). However, for the control leaf, which had been treated with the distilled water mixed with the liquid detergent, the differences between the frequency of observations of the larvae on either the treated side or the untreated side of the leaf was not significant (Sign test: $P > 0.1$).

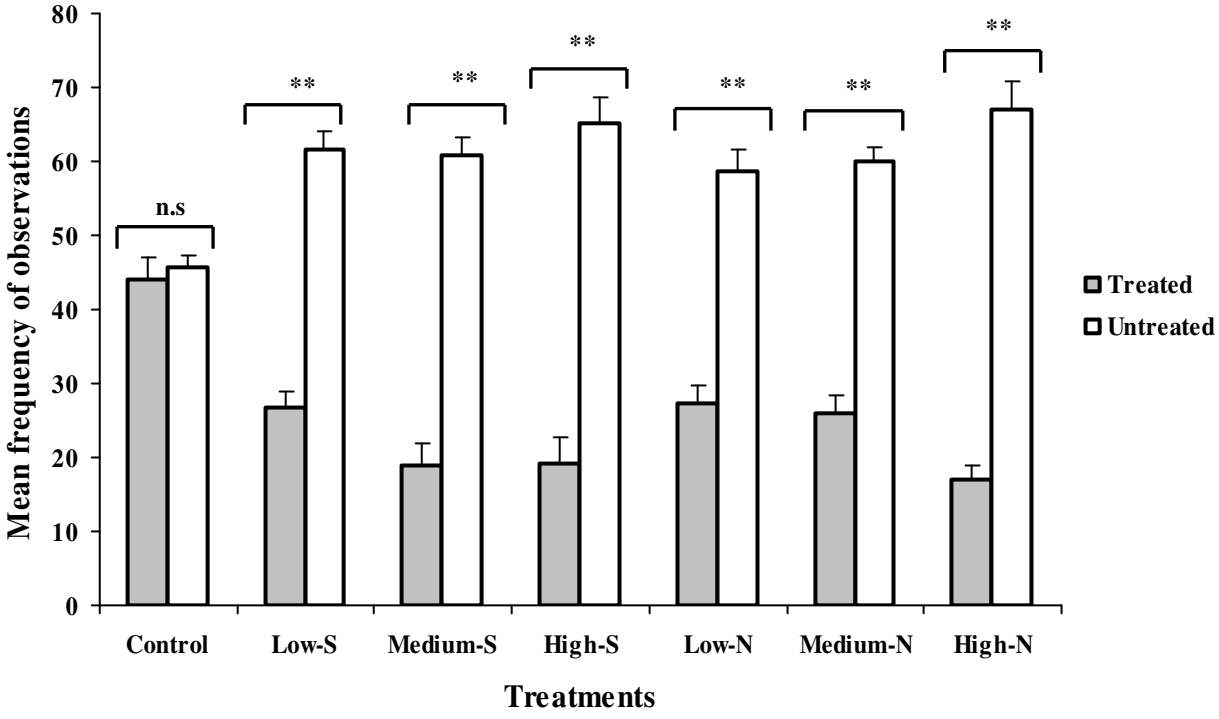

Figure 1. Mean frequency (± SE) that *P. xylostella* larva was observed on either the treated or untreated half of a cabbage leaf (n = 60). One half of the cabbage leaf was treated with the botanical pesticide or the control and the other half was untreated. Different doses of botanical pesticides were used: Low-S, Medium-S and High-S are the three doses from syringa; Low -N, Medium-N and High-N are the three doses from neem. For information on doses see materials and methods. Those treatments with significant differences between the frequency of observations on the treated side and the untreated side of the leaf are indicated with ** (Sign test: $\alpha < 0.01$).

Leaf consumption by P. xylostella *larvae*

There were no significant differences between the treatments (ANOVA: $F_{0.05(6,63)} = 1.58$; $P = 0.167$). However, there were significant differences between the amount of leaf surface consumed on the treated and untreated side of the leaf (ANOVA: $F_{0.05(1,63)} = 45.19$; $P < 0.001$).

Syringa: There were significant differences between the amount of surface area that the larvae consumed on the treated and untreated side of the leaf (Fig. 2). In all cases larvae consumed less leaf surface on the treated side of the leaf, and these differences were

statistically significant for the lowest and highest syringa treatment ($P < 0.05$) (Fig. 2), but not for the medium dose ($P > 0.05$).

Neem: There were significant differences between the amount of damage on the treated and the untreated side of the leaf (Fig. 2). The surface area consumed on the untreated side of the leaf was significantly higher than on the treated side for all the doses ($P < 0.05$) (Fig. 2).

Control: For larvae feeding on the cabbage leaf treated with the control there were no significant differences between the amount consumed on the treated and untreated sides of the leaf ($P > 0.05$) (Fig. 2).

We conclude that the botanical pesticides have antifeedant properties.

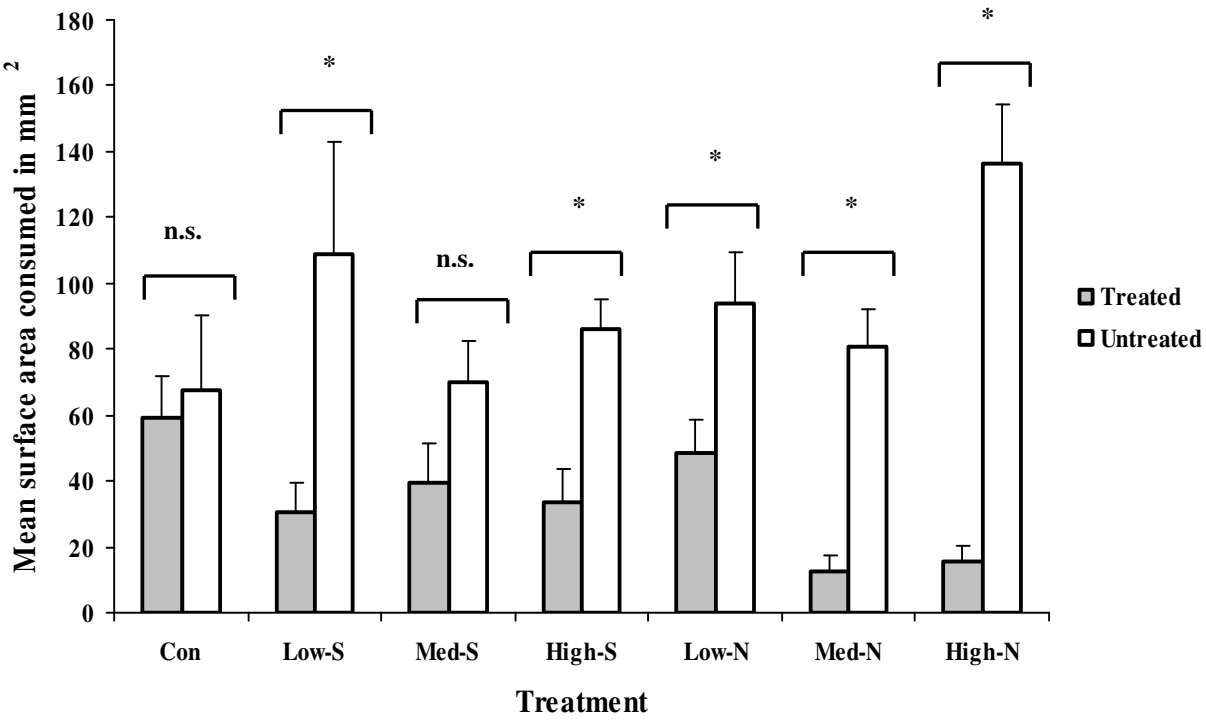

Figure 2. Mean (± SE) leaf surface area eaten by first instar *P. xylostella* during 72 hours. One half of a cabbage leaf was treated with the botanical pesticide or the control and the other half was untreated. Different doses of botanical pesticides were used: Low-S, Medium-S and High-S are the three doses from syringa; Low -N, Medium-N and High-N are the three doses from neem. For information on doses see materials and methods. Those treatments with significant differences between the amount eaten on the treated and untreated side of the leaf are indicated with * (ANOVA; plus Fisher's LSD: $\alpha = 0.05$).

Oviposition choices of P. xylostella *when faced with cabbage plants treated with botanical pesticides.*

Laboratory trials

Syringa: The numbers of eggs oviposited on different parts of the cabbage plant or on the bag that the cabbage was planted in were counted. Most eggs were oviposited on the upper surface of the leaves (Fig. 3a). The number of eggs oviposited on the bags was low, but this number increased with increasing extract concentration. The higher the concentration, the more eggs laid on the bag for both the control plant and the treated plant (Fig 3a.). Comparisons were made between the cabbage plant treated with the botanical pesticide and the cabbage plant treated with the control. The only differences found were those between the control cabbage plant and the cabbage plant sprayed with the medium dose of the syringa extract, with significantly more eggs being oviposited on the control plant than on the treated cabbage plant (Wilcoxon's signed-ranks: $P = 0.05$) (Fig. 3a). These differences were found for the number of eggs oviposited on the bottom surface of the cabbage leaves (Wilcoxon's signed ranks: $P = 0.05$) and the stem of the cabbage plant (Wilcoxon's signed ranks: $P = 0.05$) (Fig 3a).

Neem: Although more eggs were oviposited on the control plants than on the plants treated with the extract none of these differences were found to be significant (Wilcoxon's signed ranks: $P > 0.05$) (Fig. 3b).

Glasshouse trials

There were significant differences between the number of eggs oviposited on different treatments (ANOVA: $F_{0.05(6,90)} = 15.18$; $P < 0.001$). Significantly fewer eggs were oviposited on the plants treated with the syringa extracts ($P < 0.05$) (Fig. 4). There were no significant differences between the number of eggs laid on the control plant and the plants treated with the neem formulations ($P > 0.05$).

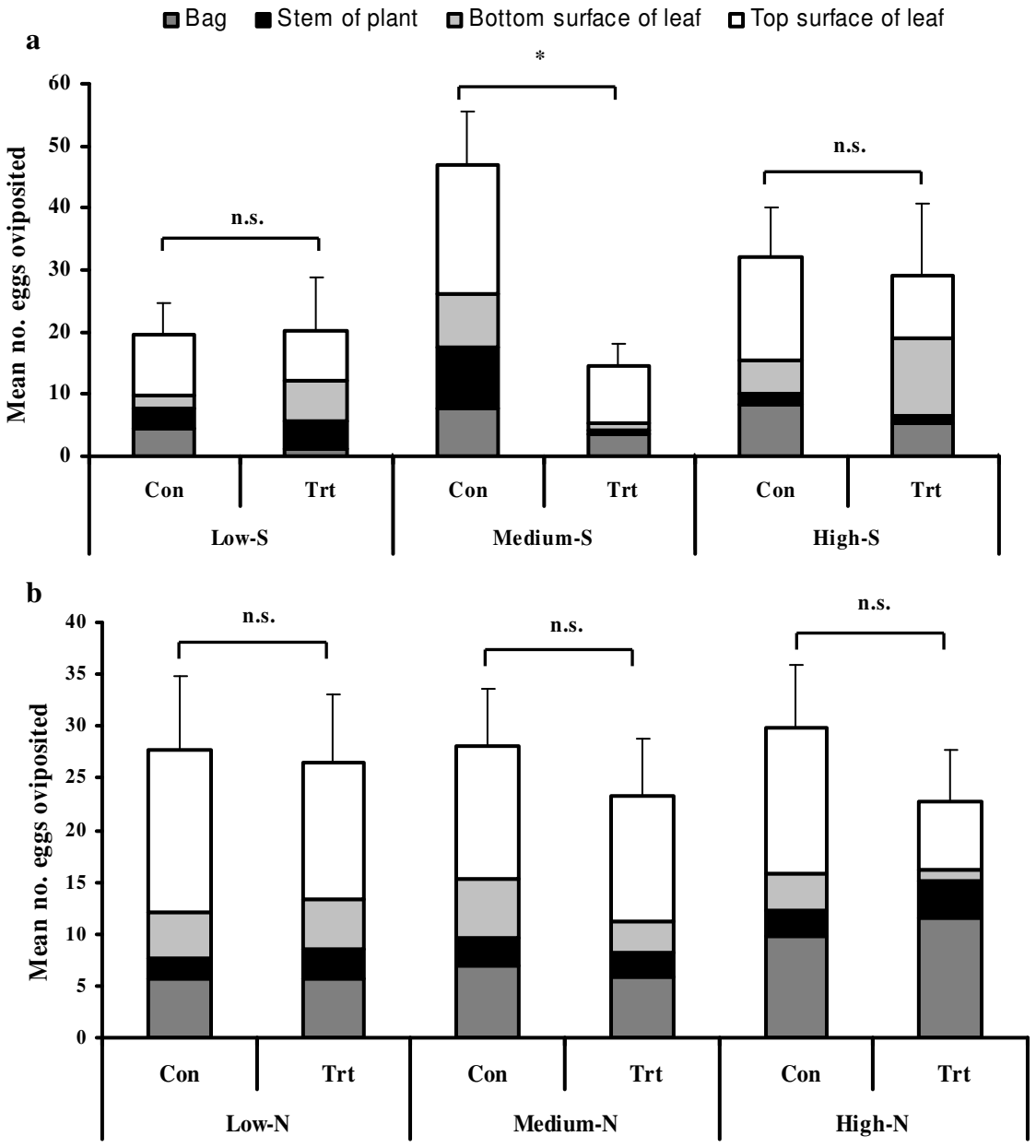

Figure 3. Mean (±SE) number of eggs oviposited by *P. xylostella* female moths on different parts of cabbage plants. Moths were given a choice between plants sprayed with the control (Con) and plants sprayed with the botanical pesticide (Trt). For information on doses see materials and methods. Significant differences are indicated with * (Wilcoxon's signed-ranks: $\alpha = 0.05$).

- **a.** The plants were treated with different doses of an aqueous leaf extract from the syringa tree: Low-S, Medium-S and High-S.
- **b.** The plants were treated with different doses of a neem solution, Neemix 4.5®: Low-N, Medium-N and High-N.

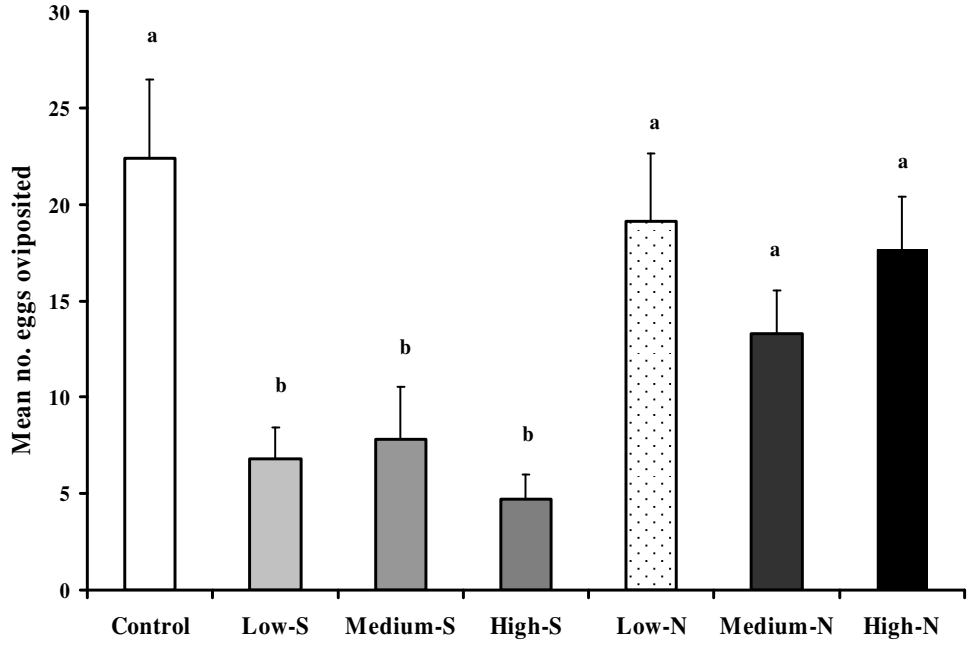

Figure 4. Mean (±SE) number of eggs oviposited by *P. xylostella* female moths (n = 40) on cabbage plants treated with different doses of botanical pesticides in a glasshouse: Low-S, Medium-S and High-S are the three doses from syringa; Low-N, Medium-N and High-N are the three doses from neem. For information on doses see materials and methods. Bars with the same letter are not significantly different (ANOVA; plus Fisher's LSD: $\alpha = 0.05$).

Behaviour

The choices of female *P. xylostella* moths remained the same irrespective of whether the cabbage plants were damaged by *P. xylostella* larvae or undamaged (χ^2 Syringa: = 1.01; df = 5; P = 0.96; χ^2 Neem: = 4.7; df = 5; P = 0.45) (Figs 5a & 5b).

Undamaged plants: The number of times that the female moths chose the treated plant first compared to the control plant was found to be significantly different for the highest dose of syringa (P = 0.029), and the medium dose of the neem (P = 0.007), whereby significantly more moths preferred to fly to the treated plant than the control plant (Fig. 5a). None of the other doses of the two botanical pesticides influenced the first choice of the moth.

Damaged plants: Significantly more moths chose the cabbage plants treated with the highest syringa concentration over the control plant (P = 0.014). The preference of the female moth for the medium neem treatment over the control was not found in the damaged plants (Fig. 5b).

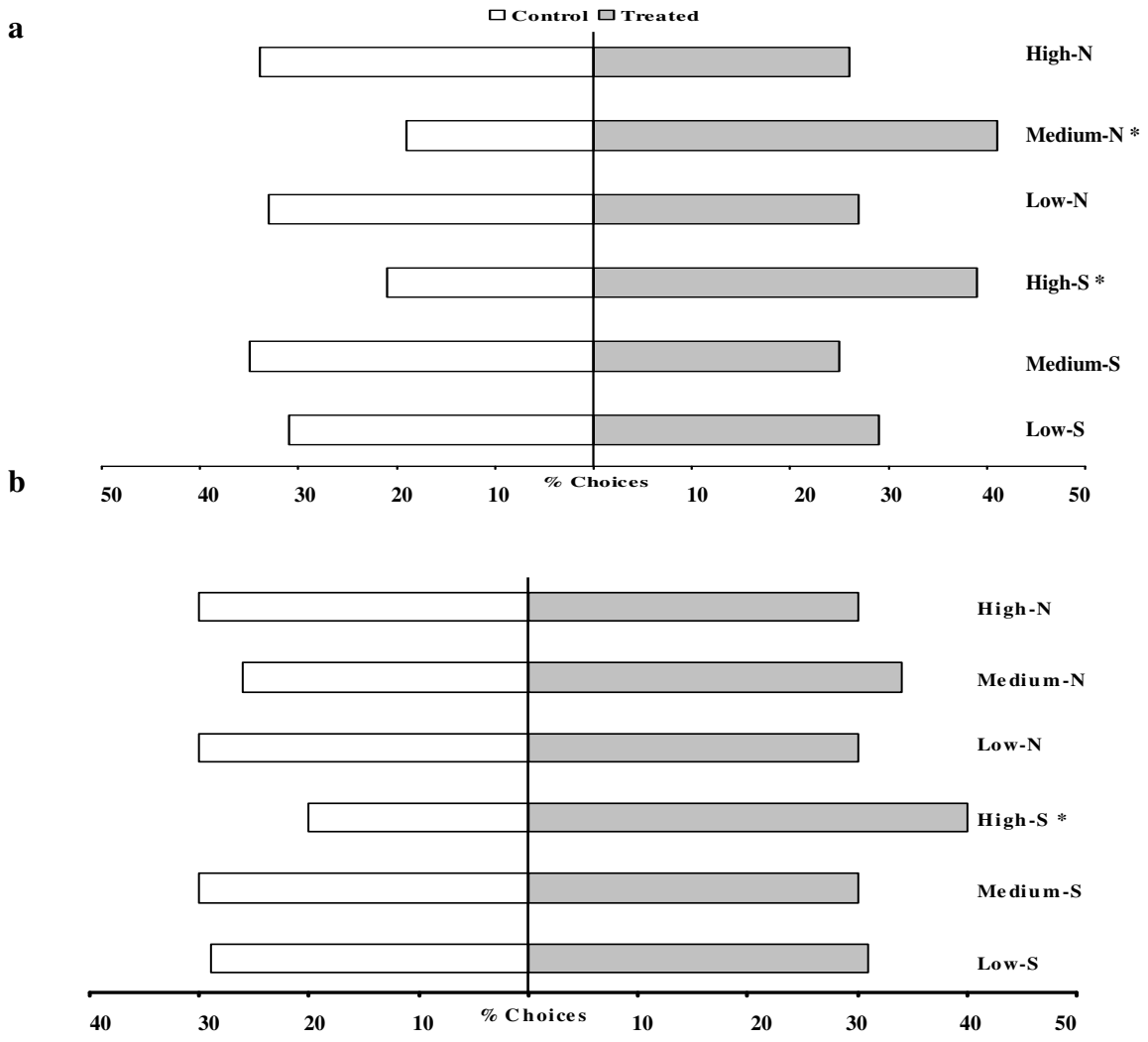

Figure 5. First choice of *P. xylostella* moths (n = 60) when exposed to cabbage plants that have been treated with different doses of botanical pesticides: Low-S, Medium-S and High-S are the three doses from syringa; Low-N, Medium-N and High-N are the three doses from neem. For information on doses see materials and methods. Those treatments followed with * are significantly different (Binomial test: $P < 0.05$).

a. Undamaged cabbage plants

b. Damaged cabbage plants

Discussion

The close association of crucifer specialists, such as *P. xylostella*, with host plants that contain glucosinolates has developed into a type of dependence on these chemicals (Renwick, 2002). The feeding responses of *P. xylostella* larvae are strongly stimulated by glucosinolates (Thorsteinson, 1953), with flavonoids further improving feeding preference, leading to the conclusion that *P. xylostella* larvae use a combination of feeding stimulants

which allows for a high degree of discrimination (van Loon *et al*., 2002). Results from this study show that *P. xylostella* larvae were able to detect the syringa extracts and the neem formulation. At high doses these botanical pesticides had a significant impact on the behaviour of the larvae. Larvae significantly preferred to be on the untreated side of a cabbage leaf if the leaf had been partially treated with botanical pesticides. In addition to this, significantly less leaf material was consumed on the treated side of the cabbage leaf.

Botanical pesticides derived from Meliaceous plants are known to affect other crucifer specialists as well. Azadirachtin is the main biologically active compound found in neem-based insecticides, and electrophysiological studies have shown that azadirachtin may act on chemoreceptors (Schoonhoven, 1982), affecting both gustatory and olfactory responses (Fagonee, 1980). The deterrent neuron of *Pieris brassicae* has a very low threshold of approximately of 10^{-7} M for azadirachtin, and this low threshold may reflect the potential bioactivity of the compound (Chapman, 2003). The triterpenoid toosendanin, a secondary compound from the tree *Melia toosendan* Sieb. & Zucc. (Meliaceae), was found to have similar effects, acting as a powerful feeding deterrent to *P. brassicae* caterpillars, even at concentrations as low as 1µM (Schoonhoven & Luo, 1994).

Host-plant finding by adult insects in crucifers may depend on the emanation of plant volatiles, and plant acceptance may depend on glucosinolates that are perceived upon contact with the plant (Renwick, 2002). For example, stimulatory and deterrent information perceived by tarsal chemoreceptors have been shown to be employed by *Pieris* butterflies (van Loon, 1996). Ovipositing *Pieris* butterflies depend on glucosinolates on the leaf surface to recognise suitable sites for their progeny to feed (van Loon *et al*., 1992; Renwick *et al*., 1992), although some crucifer genera also contain cardenolides that are potent oviposition deterrents to *Pieris* butterflies (van Loon & Schoonhoven, 1999). Previous work on *P. xylostella* has strongly suggested that host plant recognition for oviposition by this insect is also dependent on glucosinolates (Reed *et al*., 1989). However, a number of plant extracts have been shown to reduce oviposition by *P. xylostella*, for example aqueous extracts from tansy, *Tanacetum vulgare* L. (Hough-Goldstein & Hahn, 1992), alcoholic extracts of hyssop, rosemary, sage, thyme and white clover as well as essential oils from sage and thyme (Dover, 1985), and secondary plant compounds such as coumarin and rutin (Tabashnik, 1985). Ethanolic fruit extracts from the syringa tree have also been shown to have a deterrent effect on oviposition by *P. xylostella* (Chen *et al*., 1996a). The aqueous leaf extracts from the syringa tree used in our study also acted as an oviposition deterrent to *P. xylostella*, with fewer eggs being oviposited on cabbage plants treated with the syringa extract. Results from this study suggest that the effects of oviposition stimuli in host plants are partially reduced by the syringa extract.

Neem has been demonstrated to reduce oviposition in a number of different insect species, including the dried-fruit beetle, *Carpophilus hemipterous*, the brown plant hopper, *Nilaparvata lugens*, and the melon fly, *Dacus curcurbitae* (Jacobsen *et al*., 1978; Saxena *et al*., 1981; Singh & Srivastava, 1983). However, the oviposition response of *P. xylostella* to crucifers treated with neem extracts and formulations has been mixed. Chandramohan and Nanjan (1992) found that a neem oil product reduced oviposition, but Klemm and Schmutterer (1993) found a clear oviposition preference by *P. xylostella* for neem-treated cabbage. In yet another trial the number of eggs oviposited by *P. xylostella* on cabbage leaves treated with three different neem-based insecticides was not significantly different from the number of eggs oviposited on cabbages treated with water (Liang *et al*., 2003). The present study supports the results from Liang *et al*. (2003), since *P. xylostella* did not show major differential oviposition on cabbage plants treated with neem compared to control cabbage plants.

The spread of insect pests is strongly influenced by the female's choice of plant parts for oviposition and other oviposition characteristics. Therefore oviposition deterrence may be of importance to insect pest management by protecting plants from insects before any feeding damage occurs. However, if females still oviposit on low quality plants, then reduced feeding by larval stages reduces damage, which also has positive implications for pest management programs. The neem formulation used in this study did reduce feeding by *P. xylostella* larvae, but it did not have a strong effect on oviposition. Neem products are not registered for use in South Africa, and the tree is not found in the country, therefore possibilities of using this botanical pesticide in South Africa are limited. However, results from this study indicate that aqueous leaf extracts from the syringa tree also have significant impacts on the behavioural responses of *P. xylostella*. Oviposition appears to be deterred with females ovipositing fewer eggs on plants that had been treated with syringa extracts, and in addition larval feeding is also reduced. Therefore, the syringa extracts used in this study may provide the small-scale rural farmers in South Africa with an additional pest control strategy.

Results from this and a previous study (Chapter 2) indicate that extracts prepared from the syringa tree and the neem formulation Neemix 4.5® have negative impacts on the life history and behaviour of *P. xylostella*, a major pest species of crucifer crops. However, to achieve successful results with these botanical pesticides in small-scale farming systems we need to explore how this control method affects the natural control of this herbivore by its major natural enemies such as parasitic wasps. In a separate study we assess the impact of these botanical pesticides on the biology and behaviour of the two most abundant parasitoid species found in the field in South Africa (Chapters 4 & 5).

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CHAPTER 4

IMPACT OF BOTANICAL PESTICIDES DERIVED FROM *MELIA AZEDARACH* AND *AZADIRACHTA INDICA* ON THE BIOLOGY OF TWO PARASITOID SPECIES OF THE DIAMONDBACK MOTH

CHAPTER 4

Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of the diamondback moth.

Abstract

The effect of two botanical pesticides was tested on two species of parasitoids, *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) and *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae). Aqueous leaf extracts from the syringa tree, *Melia azedarach* L. (Meliaceae) and commercial formulations from the neem tree, *Azadirachta indica* Juss. (Meliaceae), Neemix 4.5® were investigated in the laboratory and in a glasshouse. No direct negative effect was recorded on the longevity of the parasitoid species. However, hind tibia length was found to be significantly shorter in male *C. plutellae* that emerged from *Plutella xylostella* that had been exposed to syringa extracts. Whether this negatively affects the fitness of male *C. plutellae* remains unknown. The impact of the botanical extracts on the fitness of *D. collaris* could not be investigated because the pesticides resulted in a high mortality of *P. xylostella* hosts. In the glasshouse a significantly higher proportion of *P. xylostella* were parasitized by *C. plutellae* on plants treated with botanical pesticides than on the control plants. However, there were no significant differences between the treatments for the proportion of *P. xylostella* parasitized by *D. collaris*. Results indicate that these botanical pesticides have the potential to be combined with biological control programs for *P. xylostella*.

Introduction

Pest organisms place substantial constraints on crop production worldwide. Estimates put global losses to pests at about 30% of potential world food, fibre and feed production (NRI 1992), with substantially higher proportions in developing countries (Thomas & Waage, 1996). The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the main pests of crucifer crops throughout the world and can cause up to 90% crop loss (Talekar & Shelton, 1993). The indiscriminate use of synthetic insecticides has resulted in the evolution of resistance of this pest to most classes of insecticides (Roush, 1997), including formulations of *Bacillus thuringiensis* (*Bt*) (Tabashnik, 1994). Largely because of the negative impact of synthetic pesticides and the increasing difficulty encountered in controlling *P. xylostella* populations, much effort has been devoted to finding alternative control measures for this pest.

Biological control is one alternative to the use of chemical pesticides in controlling insect pests. It is widely recognised as a major component of management strategies for *P. xylostella*, particularly where control with chemicals has failed. Over 50 egg, larval and pupal parasitoids have been associated with *P. xylostella* in the literature. Rigorous studies on the biology and ecology of these parasitoids are relatively few, considering the important role they often play in regulating *P. xylostella* populations (Verkerk & Wright, 1996). However, as a sole method of management 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 so that a synergistic outcome is obtained. For example, if pesticides are used, they must be harmless to the key biological control agents in the target ecosystem. Knowledge about the activity of the pesticide on the pest as well as on the non-target insects such as their natural enemies is a necessity.

Botanical pesticides have been shown to have little impact on natural enemies (Schmutterer, 1995, 1997) and therefore they have the potential to be used in combination with biological control in the development of an integrated pest management system. The use of plant extracts in agroecosystems is now emerging as one of the prime means to protect crop produce and the environment from synthetic pesticide pollution (Prakash & Rao, 1997, Schmutterer, 1995). In general, botanical pesticides have low mammalian toxicity, they have less impact on non-target organisms and they are easily available and less expensive than their synthetic counterparts (Prakash & Rao, 1997, Schmutterer, 1995). Botanical pesticides from plants within the Meliaceae family, in particular the neem tree, *Azadirachta indica* Juss. and the syringa tree, *Melia azedarach* L. have been shown to be particularly promising (Schmutterer, 1995; Isman, 1999).

In South Africa there is a wide variety of parasitoids attacking *P. xylostella*. A survey carried out at the plant protection research institute in Pretoria yielded a total of twenty-

one primary parasitoids, and twelve species of hyperparasitoids (Kfir, 2003). The parasitoids found most commonly in the field around Pretoria are *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) and *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae). *Cotesia plutellae* is a solitary endoparasitoid of larval *P. xylostella* and aspects of its biology and life history are well reported (Velasco, 1982; Talekar & Yang, 1991; Verkerk & Wright, 1996; Kawaguchi & Tanaka, 1999; Schuler *et al*., 1999; Shi *et al.*, 2002; Schuler *et al*., 2003). *Cotesia plutellae* attacks and can develop in all four larval instars of its host *P. xylostella*, although the second and third instars are most suitable for development (Talekar & Yang, 1991; Kawaguchi & Tanaka, 1999; Shi *et al.*, 2002). *Diadromus collaris* is a solitary endoparasitoid that attacks the pupal stage of *P. xylostella*, but until recently (Liu *et al.*, 2001; Wang & Liu, 2002; Lui *et al.*, 2002), knowledge on the biology and ecology of *D. collaris* was scarce.

Investigations into the impact of botanical pesticides on natural enemies of *P. xylostella* have mainly focussed on products from the neem tree. Most of these studies have shown this botanical pesticide to be relatively benign to natural enemies. In fact, in one study neem extracts actually enhanced parasitism by the ichneumonid *Diadegma semiclausum* (Verkerk & Wright, 1994). Longevity and foraging behaviour of *Diadegma mollipla* were not affected by exposure to neem (Akol *et al.*, 2002), and fecundity and activity of female *Diadegma semiclausum* emerging from *P. xylostella* treated with neem were not reduced (Schneider & Madel, 1991). Neem preparations did not have a significant effect on cocoon formation by *C. plutellae*, but they were detrimental to the parasitoid in terms of adult wasp emergence (Perera *et al.*, 2000). The neem tree does not grow in South Africa, but the closely related species, *M. azedarach* commonly known as the syringa tree, is an abundant, invasive plant in South Africa. Investigations on the impact that aqueous leaf extracts derived from the syringa tree may have on the natural enemies of *P. xylostella* are unknown.

Many farmers in developing countries do not have the resources to buy and apply pesticides. Biological control in the form of local natural enemies and botanical pesticides that can be easily prepared from locally abundant trees are free to the farmer. Therefore these methods are well suited to low-input integrated pest management systems, provided the methods are compatible. If botanical pesticides are to be used in concert with biological control they must not have negative effects on natural enemies. In this study we address the impact of aqueous leaf extracts prepared from syringa and commercial formulations from the neem tree on the two most abundant natural enemies of *P. xylostella* in South Africa, *C. plutellae* and *D. collaris*. We consider both the direct and indirect effects of these botanical pesticides on the two parasitoid species.

Materials and methods

The botanical pesticides

Syringa: Melia azedarach (hereafter referred to as syringa) leaves were collected from Rietondale in Pretoria, South Africa (28°15'S; 25°44'E). Leaves were collected from trees at a height of about 1.5 - 3.5 m at the start of spring flush, in September 1999, and placed in a glass house (30 ± 5°C) to dry; after which they were crushed into a fine powder and stored in an airtight container until use. Three different doses of the extract were prepared, by using three different weights of leaf powder, i.e. 1 g (low), 3 g (medium) and 5 g (high). Each extract was made with 100 ml of distilled water. The water was heated to 48°C and the leaf powder was added to the water and shaken for approximately one minute. The extract was left in a refrigerator (± 4°C) 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 surfactant, without which the extract runs off the surface of the leaf.

Neem: A commercial preparation of *Azadirachta indica,* Neemix 4.5® (hereafter referred to as neem) was provided by Thermo Trilogy Corporation, Columbia, USA. Three different doses were prepared: 10.7 µl (low), 16 µl (medium) and 32 µl (high) per 100 ml of distilled water. Three drops of liquid detergent were also added to the final solutions.

Control: The control treatment used consisted of 100 ml of distilled water mixed with three drops of liquid detergent.

Experimental plants and insects

Cabbage plants, *Brassica oleracea* var *capitata* L. (Cruciferae), were bought as seedlings and planted in black plastic bags in a glass house (30 ± 5°C), the plants were fertilised when planted, and regularly watered. To protect the plants against insect damage, they were placed inside a tent-like construction composed of fine netting (Mesh size: < 1 mm).

Plutella xylostella were from a culture started in 1993. The laboratory culture was maintained on canola seedlings, *Brassica napus* L. (Cruciferae). *Cotesia plutellae* and *Diadromus collaris* were from laboratory cultures started in 1993. Parasitoids were kept communally in glass cages (38 x 27 x 28 cm) and exposed to *P. xlostella* larvae on canola seedlings three times per week. *Cotesia plutellae* were exposed to second instar *P. xylostella*. After exposure to *C. plutellae* the *P. xylostella* larvae were maintained on cabbage leaves in shallow plastic rearing containers until parasitoid / moth emergence. In order to obtain fresh pupae for *D. collaris* they were exposed to late fourth instar hosts, which pupated within one day. After exposure to *D. collaris* the pupal stages of *P. xylostella* were collected and placed into plastic honey jars until parasitoid / moth emergence. The parasitoids were maintained on a diet of honey and water.

Insect rearing and all laboratory experiments were maintained in a controlled environment (24 ± 2°C; 65 ± 5% r.h., L16: D8).

Direct impact of botanical pesticides on parasitoid species

All six treatments and the control were used. A single parasitoid was placed in a test tube (75 mm (*l*), 23 mm (*diameter*)) with some honey and a strip of filter paper (60 x 10 mm), which had been dipped in one of the treatments. The filter paper 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.*

Data were analysed using the statistical program GenStat (GenStat for Windows, 2000). An unbalanced Analysis of Variance (ANOVA) was used to analyse the direct impact of the extracts on parasitoid mortality. Data were not normally distributed and a logarithmic transformation was used (Sokal & Rohlf, 1995).

Indirect impact of botanical pesticides on parasitoid species

Plutella xylostella larvae were reared on cabbage plants that had been treated with botanical pesticides or the control and then exposed to parasitoids to assess the effects on parasitoid biology. Two treatments and the control were used for this trial. Mortality of *P. xylostella* was found to be high on the higher doses of the extracts (Chapter 2). Consequently these higher concentrations were not suitable for combination with biological control due to high host mortality. We therefore decided to use only one dose from each of the botanical pesticides for the present experiment. The medium syringa treatment and the low neem treatment were found to be the most suitable (see Chapter 2) and were tested together with the control for their indirect impact on the parasitoid species. The treatments were sprayed onto cabbage plants using a small hand-held spray bottle (approximately 100 ml per plant) and the cabbage plants were left to dry for 60 minutes before being used in the experiments.

Three cages (90 *(l)* x 45 *(w)* x 45 cm *(h)*) were used. *Plutella xylostella* pupae were collected and 1 g of fresh pupae (± 195 pupae) was placed into each cage. Once moths started to emerge they were exposed to four plants of a certain treatment: the control, the medium dose of syringa or the low dose of neem. The moths were allowed to lay eggs for two days and the eggs were left to develop until second instar larvae (test with *C. plutellae*) or until pupae (test with *D. collaris*). Due to high mortality of *P. xylostella*, even at the low doses used, there were insufficient larvae that survived to the pupal stage, and the experiment for the indirect impact of the botanical pesticide could not be completed for the pupal parasitoid *D. collaris*.

A streak of honey was placed into a glass vial (75 mm (*l*), 23 mm (*diameter*)) and after emergence a single female parasitoid was placed in the vial with a male for 24 hours to ensure mating. The treated second instar *P. xylostella* larvae were placed on treated cabbage plants and then exposed to *C. plutellae*, after which these larvae were then reared until emergence of parasitoid offspring. The number and the sex of the parasitoids emerging from the larvae were recorded. The number of days until emergence was recorded, and since it is possible to see cocoon formation in larvae parasitised by *C. plutellae*, the number of days until cocoon formation was also recorded.

The number of days to cocoon formation and emergence were analysed using non-parametric Kaplan-Meier survival curves (Kleinbaum, 1996) with the statistical package SPSS. The data for the time until emergence was right censored (Kleinbaum, 1996) and the log-rank test was used to compare the survival curves from the three treatments (Kleinbaum, 1996). Where significant differences were found the Bonferroni method was used to limit the experimentwise error rates (Sokal & Rohlf, 1995). Parasitoid sex ratios were also compared between treatments using an R x C Chi-square test, and the sex ratio within each treatment was compared using binomial probability functions to assess whether it differed from a 50-50 distribution between the two sexes (Sokal & Rohlf, 1995).

Parasitoids were placed in a deep freezer (± -20^{o}C) as soon as they emerged. To provide an indirect measure of the fitness, the length of the hind tibia was measured for each individual using a Leica MZ 125 dissecting microscope and the Leica IM 1000 Version 1.20 computer program. Data were analysed using an unbalanced ANOVA, and treatment means were separated at the 5% level of significance using Fisher's protected *t*-test of least significant differences (LSD) (Snedecor & Cochran, 1980).

Parasitoid choice in a glasshouse

This experiment was carried out in a glasshouse (30 ± 5^{o}C). Two treatments and the control were used. The treatments consisted of the medium dose of syringa, and the low dose of neem. The treatments were applied to cabbage plants using a small hand-held spray bottle (approximately 100 ml per plant) and the cabbage plants were left to dry for 60 minutes. After this the cabbage plants were placed into a tent (5 x 4 x 2 m) constructed from fine nylon netting (mesh size: <1 mm). The plants were then artificially infested with *P. xylostella* larvae from the laboratory culture. Twelve larvae were placed onto each cabbage plant. The larvae were left to feed on the cabbage plants for 24 hours prior to parasitoid release. A randomized block design was used with four plants in each block, and eight blocks per treatment.

Freshly emerged female parasitoids were placed into cages with males for 24 hours to ensure mating. Female parasitoids were then collected in glass vials with 20 females per vial. A total of six vials were collected. Each vial was opened and left between the blocks.

The parasitoids were left in the tent for 48 hours, after which the *P. xylostella* larvae / pupae were collected. This trial was repeated twice for *Cotesia plutellae* and four times for *Diadromus collaris*.

For the experiment with *C. plutellae*, 12 second instar *P. xylostella* larvae were collected from the laboratory and placed onto each cabbage plant in the tent. After termination of the parasitoid exposure the larvae were allowed to feed for one more day, after which they were preserved in 70% alcohol and dissected as soon as possible to determine parasitism. Dissection is an accurate method to determine parasitism ratios as all larvae are investigated. If larvae are left to develop, some may die and it is not possible to determine whether mortality is natural, a result of parasitoid attack, or a result of the treatment.

Diadromus collaris attacks fresh pupae (Wang & Liu, 2002) and for this parasitoid, 12 late fourth instar larvae of *P. xylostella* were collected from the laboratory and placed onto each cabbage plant in the tent. After parasitoid exposure the pupae were taken back to the laboratory and left to complete development in order to determine parasitism ratios. Dead pupae were discarded, as it was not possible to determine the cause of mortality. Dissection of pupae to find the parasitoid egg was not possible. We therefore repeated this trial four times to obtain more accurate estimates of parasitism ratios.

Statistical analysis was carried out using the statistical program GenStat (GenStat for Windows, 2000). Proportions usually follow a binomial distribution, therefore differences between parasitism levels for the different treatments were tested using the generalised linear model (GLM) with binomial distribution and a Logit link function with treatment and replicate as predicting factors (Dobson, 1983). Treatment means were separated at the 5% level of significance using Fisher's protected LSD.

Results

Direct impact of extracts on parasitoid mortality

Cotesia plutellae: There were no significant differences between the survival of parasitoids exposed to the different treatments (ANOVA: $F_{0.05(6,377)} = 1.41$; $P = 0.21$). Mortality was high at the beginning of the period, with a few individuals surviving for longer periods. Mortality of the males and females was significantly different (ANOVA: $F_{0.05(1,377)} = 13.28$; $P<0.001$) (Fig. 1a, 1b). Females lived longer than males, but the treatment did not influence this (ANOVA: $F_{0.05(6,377)} = 0.33$; $P = 0.92$). A maximum survival of 34 days was recorded for one female exposed to the highest dose of neem (Fig 1a). Maximum survival of 29 days was recorded for one male exposed to the highest dose of syringa (Fig. 1b).

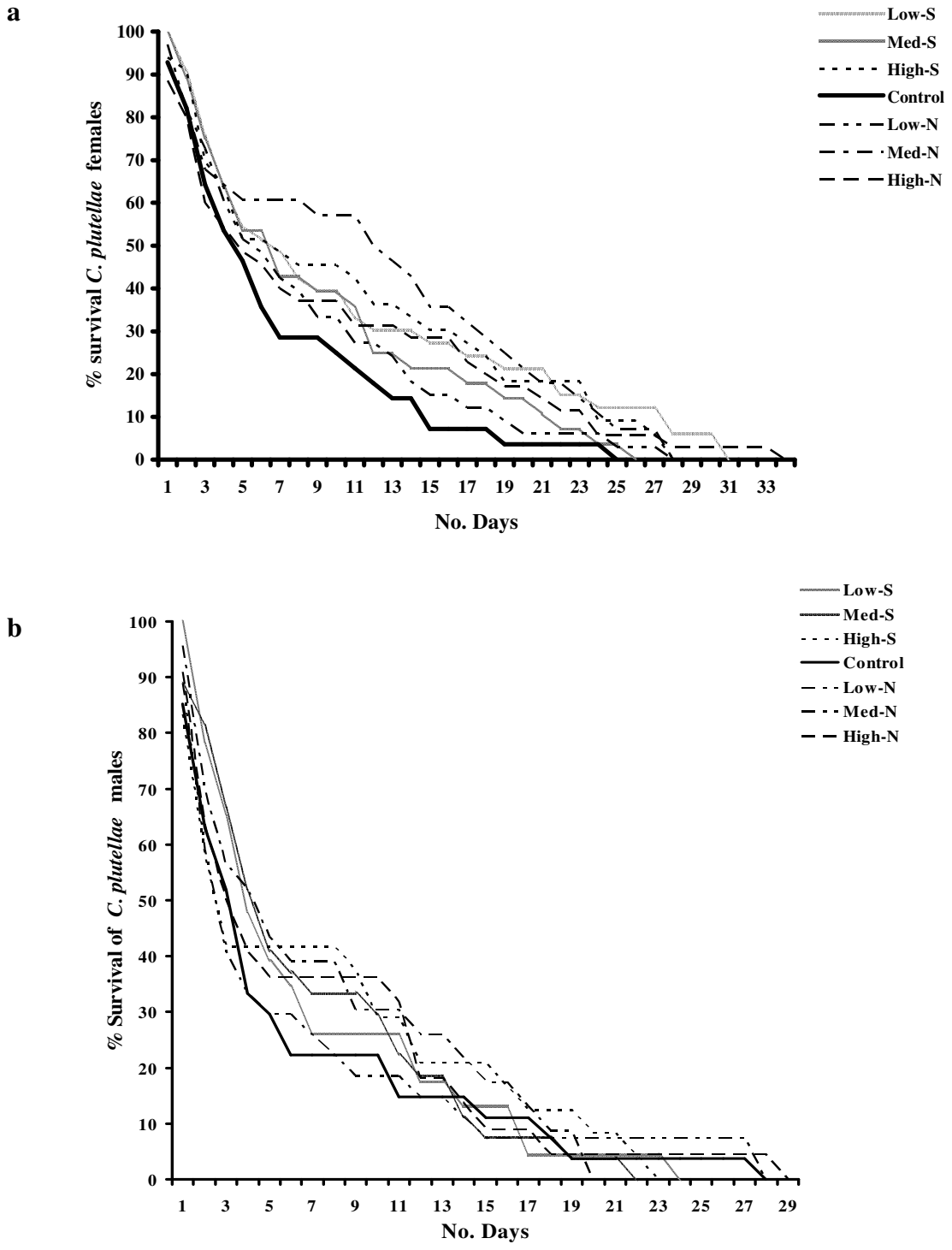

Figure 1. Survival of *Cotesia plutellae* parasitoids exposed to botanical extracts. Parasitoids were exposed to three doses from syringa: Low-S, Med-S and High-S; and to three doses from neem: Low-N, Med-N and High-N. For information on doses see materials and methods.

a. *Cotesia plutellae* females

b. *Cotesia plutellae* males

Diadromus collaris: This is a much longer-lived parasitoid. There were no significant differences between the survival of parasitoids exposed to the different treatments (ANOVA: $F_{0.05(6,289)} = 0.73$; $P = 0.51$). Also, for this species females live for a significantly longer period than males ($F_{0.05(1,289)} = 86.13$; $P<0.001$) (Fig 2a, 2b), but once again, the treatments did not have any impact on this ($F_{0.05(6,289)} = 0.73$; $P = 0.62$). One female survived for a maximum of 184 days, while the maximum survival was only 90 days for one male (Fig 2b).

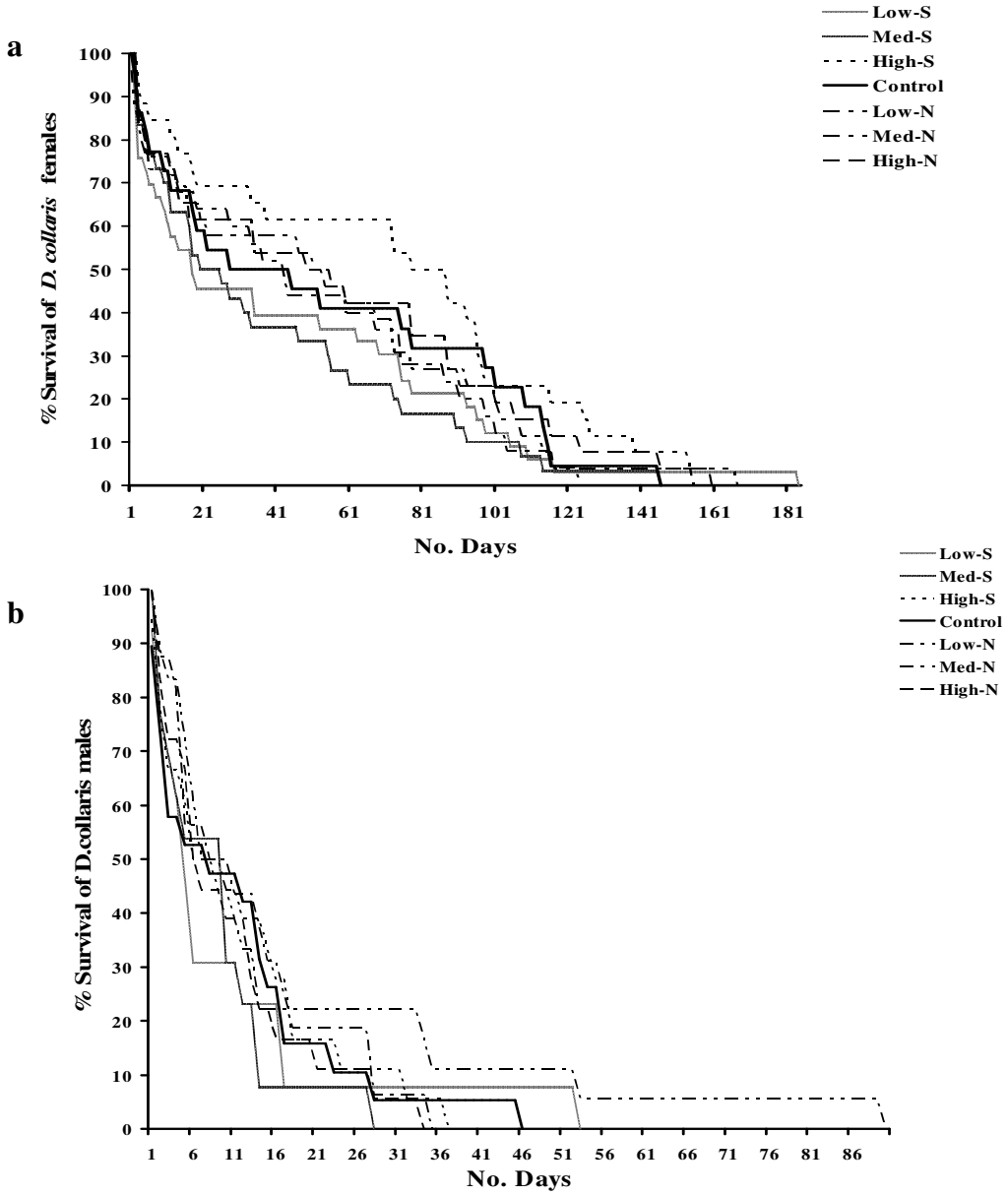

Figure 2. Survival of *Diadromus collaris* parasitoids exposed to botanical extracts. Parasitoids were exposed to three doses from syringa: Low-S, Med-S and High-S; and to three doses from neem: Low-N, Med-N and High-N. For information on doses see materials and methods.

a. *Diadromus collaris* females

b. *Diadromus collaris* males

Indirect impact of extracts on Cotesia plutellae

Hind tibia length: When the tibia length data of the two sexes were compared it was found that females in this species have significantly longer tibia than males (ANOVA: $F_{0.05(1,257)} = 13.65$; $P < 0.001$) (Fig. 3). Therefore each sex was analysed separately.

Development in hosts on the plants sprayed with the different treatments did have an effect on the hind tibia length of male parasitoids (Fig. 3) and there were significant differences between the treatments (ANOVA: $F_{0.05(2,167)} = 3.06$; $P = 0.05$). The tibia length of male *C. plutellae* was significantly shorter if they had been exposed to *P. xylostella* larvae that had been feeding on plants treated with syringa ($P = 0.018$) (Fig. 3). The neem treatment did not affect male tibia length ($P = 0.33$) and there were no significant differences between the neem and syringa treatments for male tibia length ($P = 0.096$) (Fig. 3).

The different treatments did not have a significant impact on the tibia length of female *C. plutellae* parasitoids (ANOVA: $F_{0.05(2,90)} = 1.38$; $P = 0.26$) (Fig. 3).

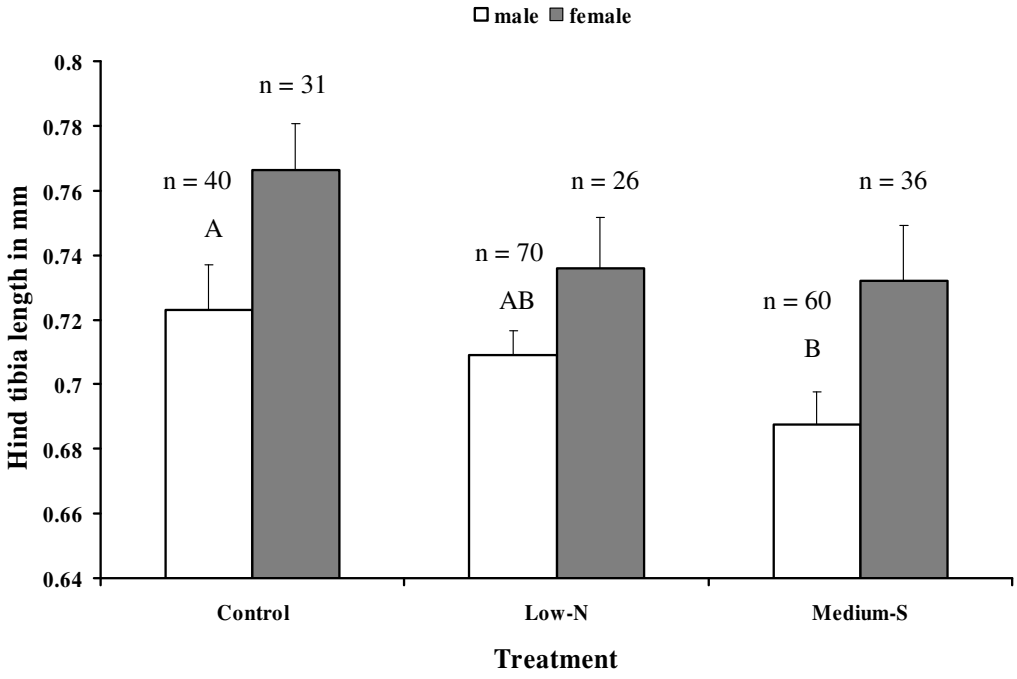

Figure 3. Mean (± SE) hind tibia length (in mm) of *Cotesia plutellae* that emerged from *Plutella xylostella* larvae that been reared on cabbage plants treated with the control or the botanical pesticides, neem (low-N) or syringa (medium-S). For information on doses see materials and methods. There were no significant differences between the tibia lengths of females exposed to the different treatments. For males those treatments with significant differences in tibia length are indicated by different letters (ANOVA; plus Fisher's LSD: $\alpha = 0.05$).

Days until cocoon formation and emergence: There were significant differences between the treatments for the days until cocoon formation (Log rank test = 7.70; df = 2; P = 0.021) (Fig. 4). Cocoon formation by *C. plutellae* was significantly slower in *P. xylostella* larvae that had been feeding on plants treated with the botanical pesticides than in the control (Log rank test: control vs neem = 5.83, P = 0.016; α = 0.05/2 = 0.025; control vs syringa = 6.26, P = 0.012; α 0.05/2 = 0.025), but there were no significant differences between the two botanical pesticides (Log rank test = 0.05, P = 0.83; α = 0.05). The number of days until parasitoid emergence was similar for all the treatments (Log rank test = 1.06; df = 2; P = 0.59).

Sex Ratio: The treatments did not have a significant impact on the resulting sex ratio of the offspring (χ^2 = 5.21; df = 2; P = 0.072). Within the treatments significantly more males emerged than females (binomial test: Syringa: P = 0.019; Neem: P = 0.000), but this difference was not found for the control (P = 0.34) (Fig. 5).

Parasitoid choice in a glasshouse: There were significant differences between the treatments for the percentage parasitism by *C. plutellae* (GLM: P<0.001) (Fig. 6). Significantly higher proportions of host larvae were parasitised on the treated plants than on the control (P<0.001), with the highest proportion parasitised on plants that been treated with syringa (66.9%), followed by neem (56.6%) and finally by the control (25.2%). The proportion of *P. xylostella* parasitised on plants treated with syringa was also significantly higher than the proportion parasitised on plants treated with neem (P = 0.039) (Fig. 6).

Diadromus collaris parasitised 25.1% of *P. xylostella* pupae on the control cabbage plants, 24.5% on the cabbage plants treated with syringa and 22.4% on the cabbage plants treated with neem. There were no significant differences between the treatments for the proportions of *P. xylostella* parasitised by *D. collaris* (GLM: P = 0.30).

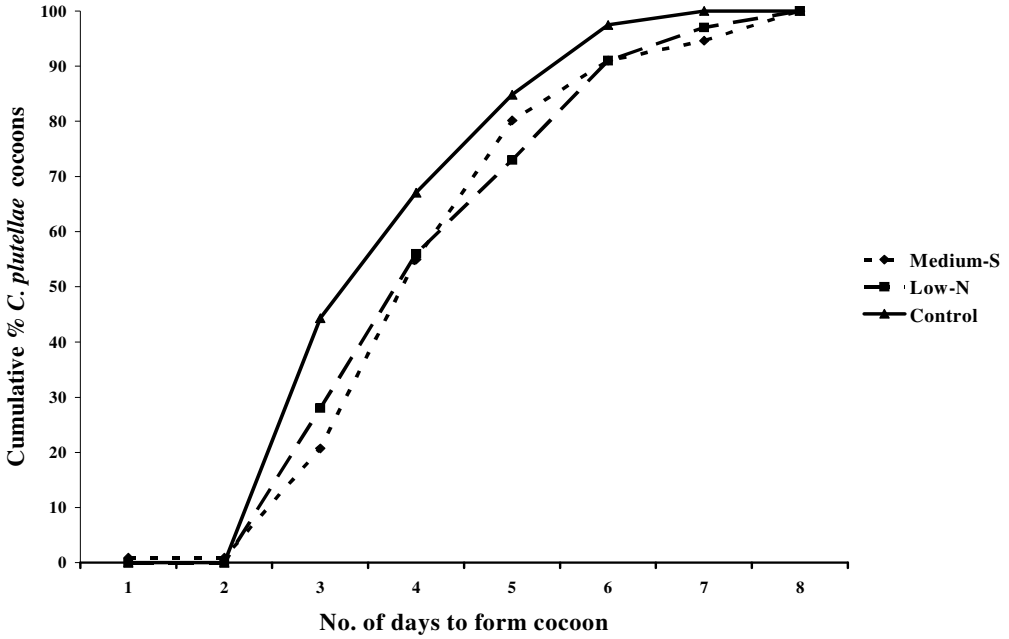

Figure 4. Cumulative percentage of cocoons formed on different days by *C. plutlellae* developing in *P. xylostella* larvae that had been either reared on cabbage plants treated with the control, with neem (low-N) or with syringa (med-S). For information on doses see materials and methods.

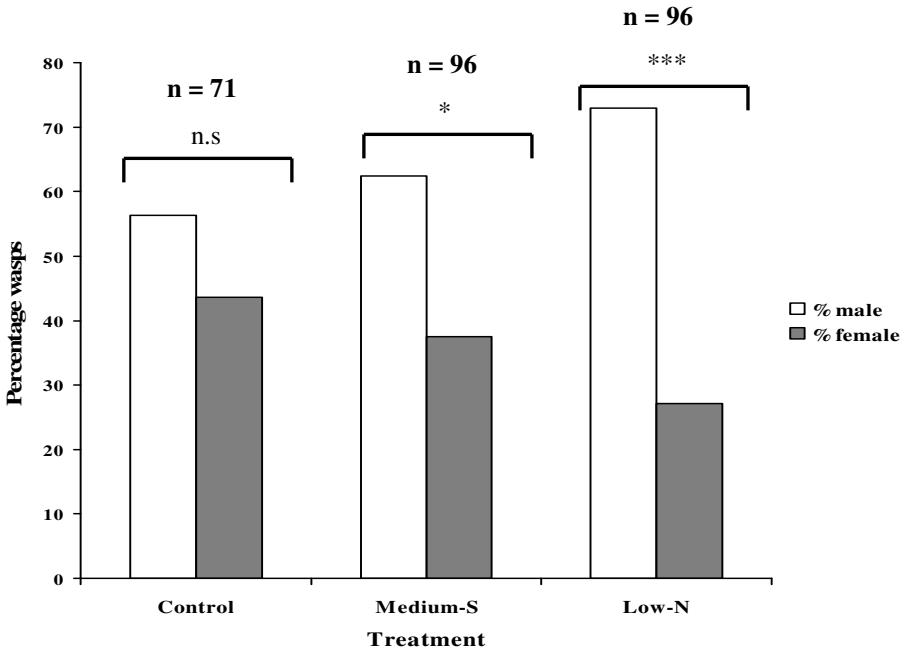

Figure 5. Resulting sex ratio of *Cotesia plutellae* that emerged from *Plutella xylostella* larvae that been reared on cabbage plants treated with the control or with the botanical pesticides, neem (low-N) or syringa (medium-S). For information on doses see materials and methods. (Binomial test: For medium-S: P = 0.019* for low-N: P = 0.000***).

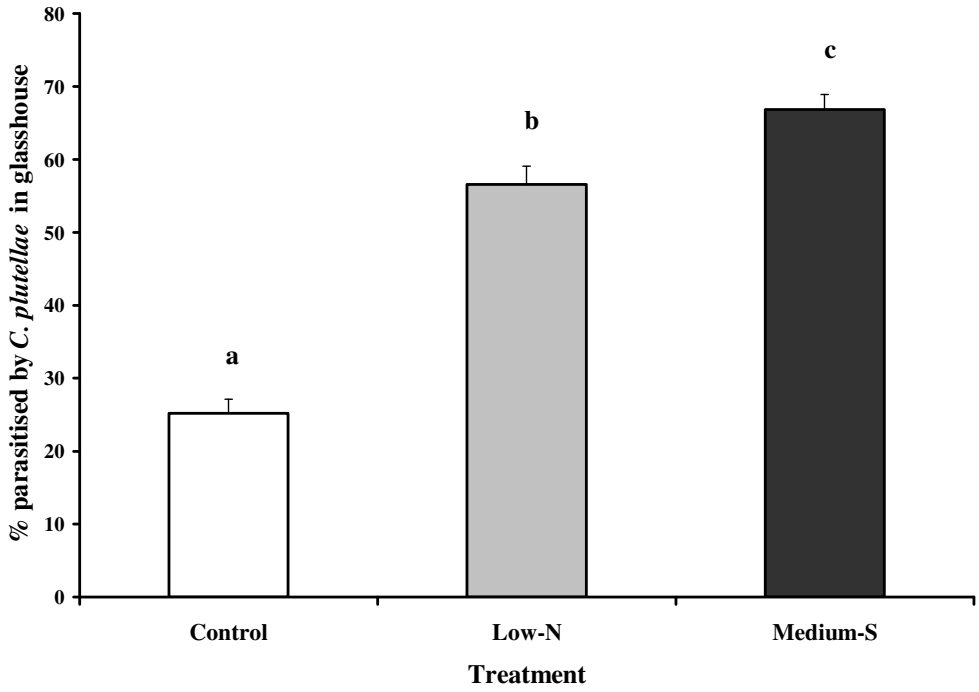

Figure 6. Mean (± SE) percentage of *Plutella xylostella* larvae parasitised by *Cotesia plutellae* in a glasshouse. Each bar represents eight replicates with 16 plants per replicate. *P. xylostella* were feeding on cabbage plants treated with botanical pesticides from neem (low-N) or syringa (medium-S). For information on doses see materials and methods. Treatments with the same letters are not significantly different (GLM; plus Fisher's LSD: $\alpha = 0.05$).

Discussion

The syringa extract and the neem formulation used in this experiment did not have a direct negative impact on the mean longevity (number of days lived) for either *C. plutellae* or *D. collaris*. Similar results were found by Schneider & Madel (1991) for adult *Diadegma semiclausum* exposed to aqueous neem seed extracts. Within the insect body, azadirachtin is rapidly excreted, and the little that remains mainly affects hormonal titres, which are more critical for developing insects than for adult insects (Rembold *et al.*, 1984). We could not find any information concerning the impact of *M. azedarach* on longevity of parasitoids that attack *P. xylostella*. Results from this study are the first to indicate that aqueous leaf extracts from *M. azedarach* do not affect the longevity of *C. plutellae* or *D. collaris*, two of the most abundant parasitoids of *P. xylostella* in South Africa.

It is usually assumed that aspects of parasitoid life history (e.g. growth, survival and sex ratio) respond directly to host properties such as body size (Godfray, 1994; Visser, 1994). A variety of studies have shown that host size influences parasitoid size and survival, and that parasitoid size is positively correlated with other fitness factors such as fecundity, mating capacity and longevity (Harvey *et al.*, 2000). Size is therefore assumed to be one of

the most important factors influencing parasitoid fitness, and this is especially true for female parasitoids (Godfray, 1994; Visser, 1994). Size has also been positively correlated with the field performance of parasitoids (Kazmer & Luck, 1995). Hind tibia length is often taken as an indirect measure of parasitoid size (Kazmer & Luck, 1995; West *et al.*, 1996). In our study the hind tibia length was found to be significantly shorter in male *C. plutellae* that emerged from *P. xylostella* larvae that had been feeding on cabbage plants treated with extracts from syringa compared to those that had been feeding on control cabbage plants. We have shown that *P. xylostella* feeding on cabbage plants treated with botanical pesticides are smaller than those that have been feeding on control plants (Chapter 2), which may explain the reduced size of the male parasitoid, although this does not explain why the female parasitoids were not similarly affected. It remains unknown if the smaller size of the male parasitoid is correlated to a reduced fitness in males. The most direct measure of male fitness is their mating ability (Roitberg *et al*., 2001). The fecundity and hence fitness of female wasps is usually strongly correlated with size, but the ability of males to inseminate females is less dependent on size (Godfray, 1994). Hence, the advantages of being large are probably relatively less for males than for females (Godfray, 1994). Jones (1982) found that the largest females of the braconid *Heterospilis prosopoidis* were able to lay 21 times more eggs than the smallest individuals. However, the difference between male insemination capacity in large and small wasps was only threefold (Jones, 1982).

The sex ratio of *C. plutellae* exposed to *P. xylostella* larvae that had been feeding on cabbage plants treated with the botanical pesticides was biased towards males. Parasitoid-host interactions are affected by sex ratios because only females forage for hosts (functional response), and the sexual allocation of their progeny directly affects the numerical response (Fox *et al.*, 1990). Parasitoid sex ratios are determined by a number of factors, both intrinsic (e.g. sex loci, haplo-diploidy, inherited endosymbiotic micro-organisms) and extrinsic (e.g. competition for resources and mates, the physical environment and the host size) (Fox *et al.*, 1990; Godfray, 1994). It is possible that there is differential mortality of male and female parasitoid larvae developing in *P. xylostella* larvae that have been exposed to these botanical pesticides, but we could not test this aspect. In 1913 Chewyreuv (cited in Godfray, 1994) made the first observation that host size influences parasitoid sex ratios, and since then this pattern has been found in a number of parasitoid species (Charnov *et al.*, 1981; King, 1989). The smaller size of *P. xylostella* larvae found on cabbage plants treated with botanical pesticides (Chapter 2) might explain the male biased sex ratio. However, Kawaguchi & Tanaka (1999) found no evidence to support that *C. plutellae* females regulated the sex ratio according to the host stage or size. It has been argued that size-dependent sex allocation is most likely to be found in species that attack non-growing host stages, or those that prevent their hosts from continued growth (idiobionts). Species in which the host continues to develop (koinobionts, such as *C. plutellae*) are less likely to show size-dependent sex allocation by the ovipositing

female, as it would be difficult for the parasitoid to estimate the final size of the host at time of oviposition (Waage, 1982). King (1989) tested this by collecting data from the literature and found that 17 out of 20 idiobionts showed size-dependent sex ratios, but only 5 out of 12 koinobionts did so, providing support for Waage's theory.

Sex ratios may also be influenced directly by the host's food-plant (Fox *et al.*, 1996). Sex ratios of *Diadegma insulare* changed with nutritional quality of the host's food-plants: more female wasps emerged from *P. xylostella* larvae on well-fertilised plants than on poorly fertilised plants (Fox *et al.*, 1990). This occurred even when wasps were offered identical 'neutral' host larvae on each type of host plant, indicating that this parasitoid can respond directly to the host plant (Fox *et al.*, 1996). In our study *P. xylostella* larvae were offered to *C. plutellae* on cabbage plants that had been treated with the botanical pesticides and this may have had a direct influence on the sex allocation response of *C. plutellae*.

Developing parasitoids have to maximise both their size and development rate in order to increase their chances of surviving to adulthood (Hemerik & Harvey, 1999). Destructive feeding occurs when the parasitoid larva switches from feeding on host haemolymph and begins attacking other tissues (Sequeira & Mackauer, 1992). The point at which a parasitoid larva begins destructive feeding strongly influences the final size and development time of the adult parasitoid (Hemerik & Harvey, 1999). When feeding on a small or suboptimal host, early destructive feeding may reduce the development time, but also leads to a reduced adult size. Alternatively, allowing the host to grow larger increases the parasitoid size at the potential cost of extending the development time (Hemerik & Harvey, 1999). We have shown that *P. xylostella* developing on plants treated with botanical pesticides have a prolonged development (Chapter 2). The development time of *C. plutellae* would therefore be significantly prolonged if it were to wait for the larger host size. Results from this study do show that the time until cocoon formation of *C. plutellae* is significantly longer if they have been feeding on *P. xylostella* that have been exposed to botanical pesticides, compared to the control. This may indicate that there is some delay in development time of *C. plutellae* feeding on larvae that have been exposed to the botanical pesticides, which would ensure that *P. xylostella* had increased sufficiently in size.

In the glasshouse, a significantly higher proportion of *P. xylostella* larvae were parasitized by *C. plutellae* on the cabbage plants that been sprayed with the botanical pesticides than on the control. Verkerk & Wright (1994) found similar effects in semi-field trials: cabbage plants that had been treated with neem seed extracts had significantly higher proportions of parasitoid cocoons; they suggest that *P. xylostella* larvae feeding on the treated plants have a reduced ability to encapsulate the developing parasitoids (Verkerk & Wright, 1994). In our study we did not observe this enhanced parasitism for the pupal parasitoid *D. collaris*. However, for this experiment, *P. xylostella* larvae were only exposed to the treated plants at the late fourth instar stage, just before pupation. At this stage the larvae do not feed very

much and it is possible that any detrimental effects of the extracts on the immunity of *P. xylostella* may have been too little and too late to have had any positive influence on the development of *D. collaris*.

Previous studies have shown that neem products did not have a detrimental effect on cocoon formation by *C. plutellae* (Perera *et al.*, 2000), and in some cases actually enhanced parasitism by *C. plutellae* (Verkerk & Wright, 1994). Results from our study also confirm that Neemix 4.5® did not have a detrimental effect on the survival and behaviour of *C. plutellae*. The neem formulation was also shown to be harmless to the longevity of the pupal parasitoid *D. collaris*, but indirect effects on fitness on this species could not be measured due to the high mortality of the host. However, under glasshouse conditions the levels of parasitism on treated plants did not differ to those on control plants, indicating that treatment with neem did not interfere with the foraging ability of this parasitoid. Neem products are not registered for use in South Africa and the tree does not grow in this country. Therefore one of the most important findings of this study is that syringa extracts can provide an important alternative to neem. Syringa extracts seem to be suitable for combination with biological control, as these extracts did not have a detrimental effect on longevity or behaviour of *C. plutellae* or *D. collaris*, two of the most abundant parasitoid species found in the field in South Africa.

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CHAPTER 5

IMPACT OF BOTANICAL PESTICIDES DERIVED FROM *MELIA AZEDARACH* AND *AZADIRACHTA INDICA* ON THE BEHAVIOUR OF TWO PARASITOID SPECIES OF THE DIAMONDBACK MOTH

CHAPTER 5

Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the behaviour of two parasitoid species of the diamondback moth.

Abstract

Foraging herbivores and carnivores make use of information from plants and plant volatiles can influence carnivore behaviour. In this study aqueous leaf extracts from the syringa tree, *Melia azedarach* L. (Meliaceae) and commercial formulations from the neem tree, *Azadirachta indica* Juss. (Meliaceae), Neemix 4.5®, were investigated for their impact on the behaviour of *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) and *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae) in a windtunnel. *Cotesia plutellae* was only attracted to plants in a windtunnel after an oviposition experience. Female *C. plutellae* did not distinguish between cabbage plants treated with neem and control plants. However, females always significantly preferred cabbage plants that had been treated with syringa extracts to control plants. The syringa extract on filter paper did not attract *C. plutellae*. This suggests that an interaction between the plant and the botanical pesticide enhances natural enemy activity. *Diadromus collaris* was not attracted to cabbage plants in a windtunnel and did not distinguish between damaged and undamaged cabbage plants. Volatile headspace analysis revealed a total of 49 compounds that could be detected in control cabbage plants and cabbage plants that had been treated with the syringa extract. Cabbage plants that had been treated with the syringa extract emitted significantly larger amounts of volatiles. The increased amounts emitted from cabbage plants treated with syringa extract are not derived from the syringa extract. Therefore, the syringa extract seems to induce the emission of cabbage volatiles. To our knowledge, this is the first example of a botanical pesticide inducing an increased plant volatile emission. This interesting phenomenon is likely to explain the significant preference of *C. plutellae* parasitoids for cabbage plants that have been treated with syringa extracts.

Introduction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is a major pest of crucifer crops, and is found throughout the world (Talekar & Shelton, 1993). Control failures caused by widespread insecticide resistance in this pest have stimulated interest in integrated pest management. Natural enemies are recognised as important components in *P. xylostella* management strategies, particularly where control with chemicals has failed. However, biological control is seldom sufficient and needs to be integrated with other strategies to obtain a successful outcome. Botanical pesticides provide an important method of control, which is thought to be compatible with biological control, as these compounds tend to be relatively harmless to parasitoids and predators (Schmutterer, 1995, 1997; Chapter 4). In South Africa twenty-one species of parasitoids have been associated with *P. xylostella* (Kfir, 2003), providing a rich abundant source for biological control. Botanical pesticides have not been registered for use in this country. However the syringa tree, *Melia azedarach* L. (Meliaceae) is an abundant invasive plant and is found throughout the country. This tree has been shown to have numerous insecticidal properties (Ascher *et al.*, 1995), and therefore may provide the small-scale rural farmer with an additional alternative control tactic. However, before these products can be recommended for use it is important to investigate the impact that these extracts have on the natural enemy fauna. Botanical pesticides act as repellents against a number of pest species, but very little information is available concerning their effect on the behavioural responses of natural enemies (Akol *et al.*, 2003).

It is well-known that plants can influence carnivore behaviour (Dicke *et al.*, 1990b; Turlings *et al*., 1990; Steinberg *et al.*, 1993; Dicke & Vet, 1999; Vet, 1999; Dicke, 1999a). Plants that are attacked by herbivores emit volatile cues that can be used by natural enemies of the herbivore to find their hosts (Vet & Dicke, 1992; Dicke, 1999a; Dicke & Vet, 1999). The plant volatiles produced in response to herbivore damage may contain information on the identity of the herbivore and therefore these herbivore-induced plant volatiles may be both well-detectable and reliable indicators of herbivore presence and identity (Vet *et al.*, 1991; Vet & Dicke, 1992). Identification of the volatile compounds emitted by infested plants have shown that feeding by herbivores can induce plants to release a blend of volatiles different from those released by intact or mechanically damaged plants (Dicke *et al.*, 1990b; Turlings *et al.*, 1991; Dicke & Takabayashi, 1991). These volatile blends can be either quantitatively (i.e. changed ratios of the same compounds) or qualitatively (i.e. release of novel compounds) different from the blend emitted by intact plants or mechanically damaged plants (Dicke & Vet, 1999), determining the signal to noise ratio of the plant volatiles and thus their reliability as indicators of herbviore presence to carnivores. When the volatile blend from herbivore-damaged plants contains novel compounds naïve insects can make a distinction between plants damaged by different herbivore species and also between undamaged/ mechanically damaged plants

(Vet, 1999). For example the parasitoid *Aphidius ervi* can distinguish between broad beans that have been infested with its host, the pea aphid, *Acyrthosiphon pisum* and broad beans infested by the non-host black bean aphid, *Aphis fabae* (Powell *et al*., 1998). When the volatile blends from herbivore-damaged plants only differ quantitatively from the volatile blends of undamaged/ mechanically damaged plants the parasitoids need to learn to make the distinction between these plants (Vet, 1999). Associative learning can lead to a temporary specialisation in generalist parasitoids but some variation in volatile blends is essential for this (Geervliet *et al*., 1997).

The main components of the volatile blend released from cabbage, are green leaf volatiles and terpenoids (Mattiacci *et al*., 1994). Terpenoids are a major class among herbivore-induced synomones that attract carnivores (reviewed by Dicke, 1994). Terpenoids are released in analogous amounts in both herbivore- and mechanically- damaged plants as well as in undamaged plants (Mattiacci *et al*., 1994). Green leaf volatiles are constantly emitted by plants during ageing or when injury occurs (Visser & Ave, 1978). However, there is also a dramatic increase of green leaf volatiles in the headspace of damaged cabbage plants compared to undamaged plants, and it is likely that these components play a role in carnivore attraction (Mattiacci *et al*., 1994; van Poecke, 2002). For example in the attraction of *Cotesia glomerata* and *Cotesia rubecula* to herbivore-damaged plants compared to undamaged plants (Steinberg *et al*., 1993; Geervliet *et al*., 1994; Agelopoulos & Keller, 1994a,b,c). Mechanically damaged cabbage plants also produce volatiles, but these volatiles do not have any qualitative differences compared to the volatiles released when the plant is damaged by herbivores (e.g. *Pieris* caterpillars (Mattiacci *et al*, 1994; Geervliet *et al*, 1997)). However, there are quantitative differences in the emission of volatiles from mechanically damaged and herbivore-damaged cabbage plants (Mattiacci *et al*., 1994). Herbivore damage results in the attraction of *C. glomerata* to plants damaged by *Pieris brassicae* in comparison to plants that have been artificially damaged (Steinberg *et al*, 1993; Mattiacci *et al*, 1994). *Cotesia glomerata* and *C. rubecula* have been shown to perceive a wide range of at least 20 odour compounds from the blend produced by herbivore damaged cabbage plants (Smid *et al*., 2002). Naïve *C. glomerata* and *C. rubecula* were not able to discriminate between plants which had been infested with different herbivore species, not even between plants infested by host and non-host species (Geervliet *et al*., 1997; Agelopoulos & Keller, 1994a). But *C. glomerata* was able to learn to discriminate after ovipositon experience through a process of associative learning (Geervliet *et al*., 1998).

Cotesia plutellae (Kurdjumov) (Hymenoptera: Braconidae) can distinguish between damaged and undamaged oilseed rape (*Brassica napus*) plants, but it cannot distinguish between artificial damage and herbivore damage (Potting *et al.*, 1999). Chemical analysis of the volatiles released by plants that had been damaged by *Pieris rapae* and *Plutella xylostella* indicated that there were many quantitative differences in the compounds

produced by damage from these two herbivores (Geervliet, 1997; Agelopoulos & Keller, 1994b), and slight qualitative differences (Agelopoulos & Keller 1994b). By treating plants with botanical pesticides the volatile profiles may be changed, which may have an impact on the responses by natural enemies. We are not aware of any studies that have investigated behaviour modifying effects of botanical pesticides.

Cotesia plutellae and *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae) are the two parasitoid species most commonly found in the field around Pretoria, South Africa. We have shown that aqueous leaf extracts from syringa tree, *Melia azedarach*, and the neem product, Neemix 4.5®, taken from the neem tree, *Azadirachta indica* Juss. (Meliaceae), do not have a direct impact on the survival of these parasitoids (Chapter 4) and that the parasitoids are still able to find their hosts. In fact, in both greenhouse and field experiments, *C. plutellae* parasitise a greater proportion of *P. xylostella* larvae on cabbage plants treated with syringa extracts than they do on control plants (Chapters 4 & 6). However, plant allelochemicals ingested by herbivores can be transferred to the next trophic level. Behaviourally parasitoids might increase their ability to detect or respond negatively to plant allelochemicals that adversely affect the survival of their offspring and avoid ovipositing in hosts that have ingested these plant allelochemicals (Kester & Barbosa, 1991). In this study we investigate the impact that these botanical pesticides have on the volatile profile of cabbage plants and how this may influence the resulting behaviour of *C. plutellae* and *D. collaris*.

Materials and Methods

Experimental plants and insects

Cabbage plants, *Brassica oleracea* var *capitata* L. (Cruciferae), were bought as seedlings and planted in black plastic bags and left in a glass house (30 ± 5°C) to grow. The plants were fertilised when planted, and regularly watered. To prevent insect damage the plants were placed inside a tent-like construction composed of fine nylon netting (Mesh size: <1 mm).

Plutella xylostella were from a culture originally collected near Pretoria (28°15'S; 25°44'E) and Brits (25°38'S; 27°47'E), South Africa in 1993. The laboratory culture was maintained on canola seedlings, *Brassica napus* L. (Cruciferae). The two parasitoid species most common in the field near Pretoria, South Africa were *Cotesia plutellae* and *Diadromus collaris*. Laboratory cultures of these parasitoids were established in 1993. Each parasitoid species was kept communally in glass cages (38 x 27 x 28 cm) and exposed to *P. xylostella* larvae on canola seedlings three times per week. *Cotesia plutellae* were exposed to second instar *P. xylostella*. After exposure to *C. plutellae* the *P. xylostella* larvae were maintained on cabbage leaves in shallow plastic rearing containers until cocoon formation. In order to obtain fresh pupae for *D. collaris* they were exposed to late

fourth instar hosts, which pupated within one day. After exposure to *D. collaris* the pupal stages of *P. xylostella* were collected. Cocoons of *C. plutellae* or *P. xylostella* pupae parasitized with *D. collaris* were placed into clean cages and emergence of wasps took place in clean cages without any plant or host material. The parasitoids were maintained on a diet of honey and water. Parasitoids used in the bioassays were 2-6 day old mated females.

All insect rearing was carried out in a controlled environment (24 ± 2°C; 65 ± 5% r.h., L16:D8).

The botanical pesticides

Syringa: Melia azedarach (hereafter referred to as syringa) leaves were collected from Rietondale in Pretoria, South Africa (28°15'S; 25°44'E). Leaves were collected from trees at a height of about 1.5 - 3.5m at the start of spring flush, in September 2002, placed in a glass house (30 ± 5°C) to dry; after which they were crushed into a fine powder and stored in an airtight container until use. The extract was made with 100 ml of distilled water. The water was heated to 48°C, and 5 g of leaf powder was added to the water and the mixture was shaken for approximately one minute. The extract was left in a refrigerator (± 4°C) 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 surfactant, without which the extract runs off the surface of the leaf.

Neem: A commercial preparation of *Azadirachta indica,* Neemix 4.5® (hereafter referred to as neem) was provided by Thermo Trilogy Corporation, Columbia, USA. A dose of 32 µl per 100 ml of distilled water was used. Three drops of liquid detergent were added to the final solution.

Control: The control treatment used consisted of 100 ml of distilled water mixed with three drops of liquid detergent.

The windtunnel design

A windtunnel was set up within a tent-like construction (3.5 x 3 x 2 m). The windtunnel was placed in a room with a controlled temperature of 24 ± 2°C. All windows were covered with black paper, which prevented any daylight from entering the room. Illumination was provided by rows of lights in the roof of the tent, simulating daylight (2300 Lux). Two table fans were placed at the end of a table; a sheet of gauze material (mesh size: 3mm) was placed in front of the fans to reduce the wind speed and to provide a more laminar airflow. The wind speed at the release point was approximately 0.133 m/s. The cabbage plants were placed on a table (1800 mm (l) x 900 mm (w)) (Fig. 1). The table was covered with white plastic sheeting to facilitate cleaning, and thick (47 mm) black strips of tape were placed at 300 mm intervals to provide a contrast for the flying insects.

To contain the insects within the experimental arena and to create diffuse lighting white cotton sheeting was placed across two poles 1120 mm above the table and left to hang over the sides, enclosing the arena. An equilateral triangle (450 mm) was marked out on the table and cabbage plants of the same age (± 5 weeks after transplant) were placed in groups of four on either side of the base of the triangle. The plants were placed in a square around the mark, with 200 mm between the centers of each cabbage plant. One group of cabbage plants was treated with the botanical pesticide and the other group was treated with the control. The plants were changed to opposite sides of the triangle after every five observations.

Behavioural recordings

Parasitoids were released from a platform at a 200 mm height, at the apex of the equilateral triangle (Fig. 1). A female parasitoid was faced with a choice between treated and control cabbage plants and was observed to see which plant she landed on first. 'Response' was recorded when the female left the release platform and landed on one of the plants. For each treatment 60 responding females were observed. The number of females that did not respond was also recorded, these numbers varied per treatment (see results section). 'No-response' occurred when females failed to leave the platform after 5 minutes, or if the female landed on any surface other than a cabbage plant.

Choices between treated and control plants were analysed using binomial probability functions to assess a difference from a 50-50 distribution between the treatment and control. Significance indicates preference for one of the two treatments (botanical extract or control).

Previous experience

In our study we found that *C. plutellae* did not respond in the windtunnel without an oviposition experience. Potting *et al.* (1999) have shown that an oviposition experience significantly increased the response of *C. plutellae* to volatiles. Therefore, each *C. plutellae* female was exposed to a cabbage leaf with feeding *P. xylostella* larvae. Each female was allowed to oviposit 2 – 3 times before being removed and placed into a glass vial with streaks of honey for food. Approximately 1 –2 hours later she was released in the windtunnel and observed.

Diadromus collaris was also given an experience before it was released in the windtunnel. In this case the female was exposed to a cabbage leaf that had been damaged by *P. xylostella* larvae, which had subsequently pupated on the leaf just before exposure to *D. collaris*. The female was left to explore the leaf for 3 minutes after which she was removed and placed in a glass vial with streaks of honey for food, and released in the windtunnel 24 hours later.

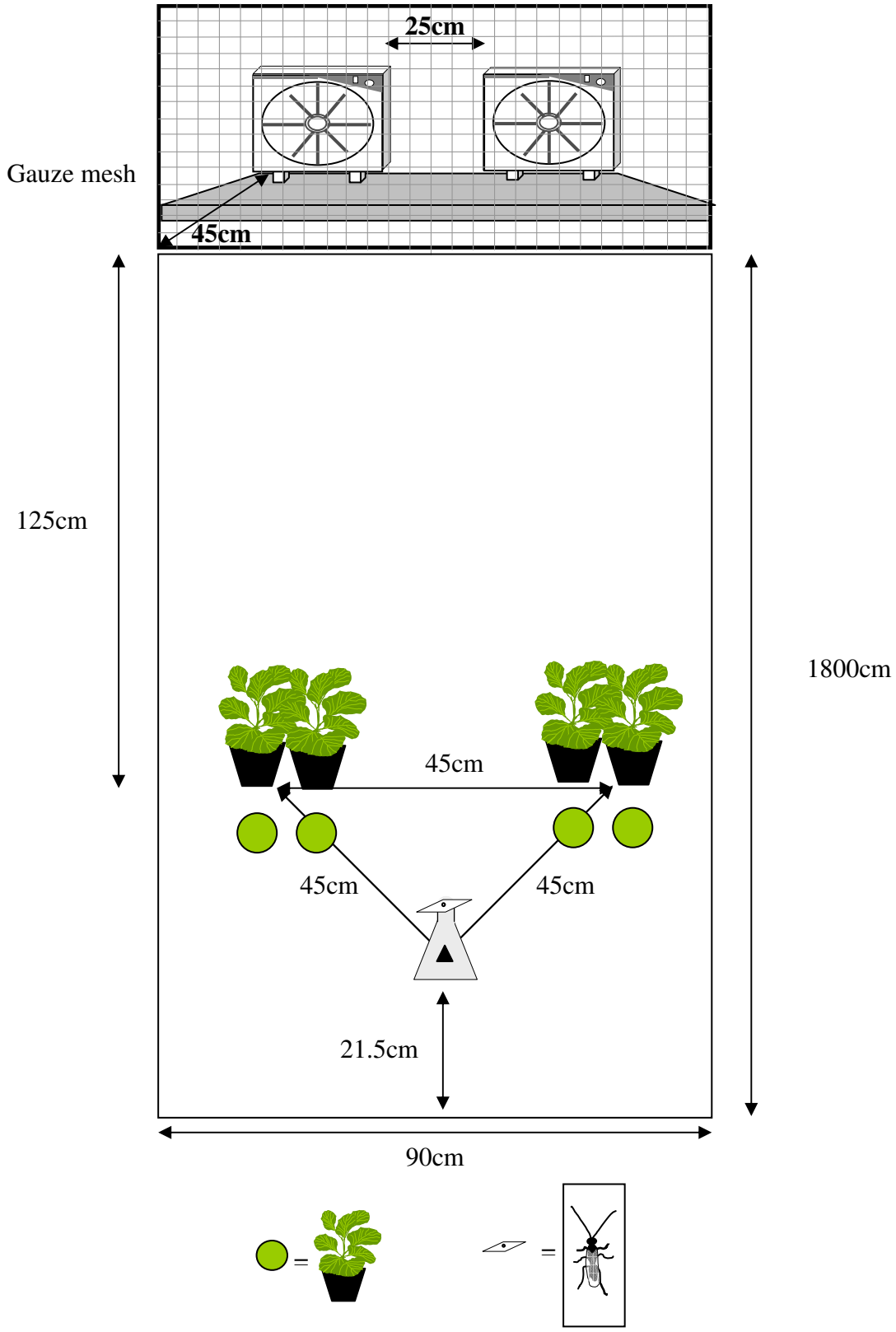

Figure 1: Diagram showing the design of the windtunnel

Experiments

Plant-host complex

Cabbage plants were first sprayed with either the botanical pesticide or the control (approximately 100 ml per plant) and then infested with 15 *P. xylostella* larvae (second instar - for test with *C. plutellae* or late fourth instar - for test with *D. collaris*). The larvae were left to feed on the cabbage plants for 24 hours, after which the infested plants were placed in the wind tunnel and the observations began. To compensate for any differences between the cabbage plants themselves, the groups of plants were replaced three times during the experiment.

Effect of experience: This trial was also used to investigate whether previous experience on cabbage treated with the plant extracts had an influence on the subsequent behaviour of the parasitoid. Each species was given experience with *P. xylostella* larvae (for *C. plutellae*) or pupae (for *D. collaris*) on either treated or untreated cabbage leaves and the subsequent behaviour was compared. Data were analysed using a Chi-square test.

Response of pupal parasitoid: Because *D. collaris* showed a low responsiveness in the windtunnel (< 50% responded) we carried out an additional experiment to investigate whether this species actually responded to volatiles released from damaged cabbage plants. For this experiment, one group of cabbage plants was undamaged and one group of plants was damaged by 15 late fourth instar *P. xylostella* larvae, which were left to feed and pupate. Approximately 48 hours later the pupae were removed from the cabbage plants and these plants were exposed to *D. collaris* in the windtunnel, with the undamaged plants for comparison. The groups of plants were replaced three times during the experiment. Again less than 50% *D. collaris* females responded to the plants and therefore no further experiments were carried out with this species.

Different syringa doses: Further experiments were carried out with *C. plutellae* using two lower doses of the syringa extract. The lower doses were made with 1 g and 3 g of leaf powder and 100 ml of distilled water and prepared as described above (Plant-host complex).

Damaged plants without hosts

To investigate whether the presence of the host by itself resulted in an attraction of *C. plutellae*, the experiment was repeated as described above (Plant-host complex). However, *P. xylostella* larvae were allowed to feed for 24 hours and then removed from the plant before the plants were exposed to *C. plutellae*.

Equally damaged plants without hosts

Schuler *et al.* (1999, 2003) have shown that the amount of damage is an important factor influencing the response of *C. plutellae*. Since the botanical pesticides reduce the feeding of *P. xylostella* larvae (Chapters 2 & 3) the experiment was repeated to compensate for any possible differences in the amount of damage. To create cabbage plants that had an approximately equal amount of damage, the cabbage was first infested with 15 second instar *P. xylostella* larvae. The larvae were left to feed on the plants for 24 hours, after which the larvae were removed from the cabbage plants. The plants were then sprayed with either the treatment or the control. The groups of plants were replaced twice during the experiment.

Undamaged plants and filter paper

To investigate whether the botanical pesticide has an influence on behaviour, irrespective of the presence of damage, undamaged plants were also compared. All three doses of the syringa extract were tested: 1 g, 3 g, and 5 g, and only the one dose of neem. Finally a test was done to investigate whether the syringa extract alone influenced behaviour. For this test filter paper was dipped into either the 5 g syringa treatment or into the control and parasitoid behaviour was observed.

Analysis of plant volatiles

In order to investigate the differences in volatile profile of cabbage plants that had been treated with syringa extracts and control plants, headspace analysis was carried out. The 5 g syringa extract was made as described above, and 100 ml was applied to a clean undamaged cabbage plant with a small hand-held sprayer. Plants that served as controls were sprayed with 100 ml of distilled water mixed with liquid detergent. The cabbage plants were left to dry for 60 minutes before being placed into 30 litre collecting jars.

Pressurised air was filtered over silica gel, molecular sieves (8-12 mesh beads, 4A pore width, Sigma), activated charcoal and a disposable Tenax-containing tube (90 mg Tenax TA) before entering the collecting jar. The air-inlet, air-outlet, filters and sampling jars were connected with 0.8 cm diameter Teflon tubing. Prior to the experiments and between each sample the system was purged with purified air overnight at a flow rate of 500 ml / minute.

The cabbage plants were carefully removed from the pots, taking care not to disturb the root system; then the entire soil and root system was covered in aluminum foil. The plants were placed individually into the collecting jars, which were covered with a glass lid. High frequency fluorescent lights (30 – 35 µMol photons / m^2 / sec) were placed 15 cm above the collection jars. The system with the plants was purged for one hour at 500 ml / minute, after which a Tenax (90 mg Tenax TA) trap was connected to the air outlet in the lid of the collecting jar. Volatiles were trapped at a rate of 175 ml / minute by pulling the air through

the trap using a vacuum. Volatiles were collected for 4.5 hours, after which the Tenax traps were refrigerated (± 4°C) until they could be analysed. Four samples were collected for each treatment (5 g syringa and control). After the second sample, and again after the final sample, two blank samples were taken from the empty collecting jars to ensure that volatiles always present in filtered / clean air were not considered in the final analysis.

Samples of volatiles were also taken from the syringa extract mixed with liquid detergent. For this sample, 25 ml of extract was placed into a glass Petri dish and placed on top of an erlenmeyer flask to provide sampling at approximately the same height as the cabbage plant. The Petri dishes were placed into the collecting jars and the system was purged for one hour. After one hour the airflow was reduced to 225 ml / minute. A Tenax TA trap was used to collect the volatiles, and air was pulled through the trap at 175 ml / minute. Volatiles were collected for 1 hour, after which the Tenax traps were refrigerated (± 4°C) until they could be analysed. Four samples were collected from the syringa extract.

Volatiles were released from the Tenax traps with a thermodesorption coldtrap set-up (Markes, UK) by heating at 200 °C for 10 min, with a He-flow of 30 ml/min. The desorbed volatiles were collected in the cold trap at –10 °C. Volatiles were injected in splitless mode into the RTX-5Silms column (Restec, 30 m x 0.32 mm ID, 0.33 µm film thickness) by heating of the coldtrap to 270 °C. After an initial column temperature of 40 °C for 2 min, the temperature was raised to 95 °C at 3 °C/min, then to 165 °C at 2 °C/min, and subsequently to 250 °C at 15 °C/min. The column was directly coupled to the ion source of a Finnigan quadrupole mass spectrometer, which was operating in the 70 eV EI ionisation mode and scanning from mass 33 to 300 at 3 scans/sec. Compounds were identified by comparison of mass spectra with those in the NIST 98 and Wiley 7th edition spectral libraries and by checking the retention indices.

For each compound the mean peak area was calculated and each compound was classified as being emitted in larger amounts by control or syringa treated cabbage plants, based on mean peak area. A sign test was used to determine whether the number of compounds that were emitted in larger amounts by control or syringa treated cabbage plants differed from a 50-50 distribution over the two treatments (Sokal & Rohlf, 1995).

Results

Plant-host complex

In this experiment cabbage plants were sprayed with the botanical pesticides or control and then infested with *P. xylostella*. Parasitoids were exposed to the entire plant-host complex. *Cotesia plutellae* did not show a preference for the treated or control plant when the plants were treated with the neem extract (P = 0.093) (Fig. 2). However, they did show a significant preference for plants that had been treated with the syringa extract (P = 0.000).

Diadromus collaris did not show a preference for the control or the treated cabbage plant for either of the botanical pesticides (neem: P = 0.37; syringa: P = 0.36).

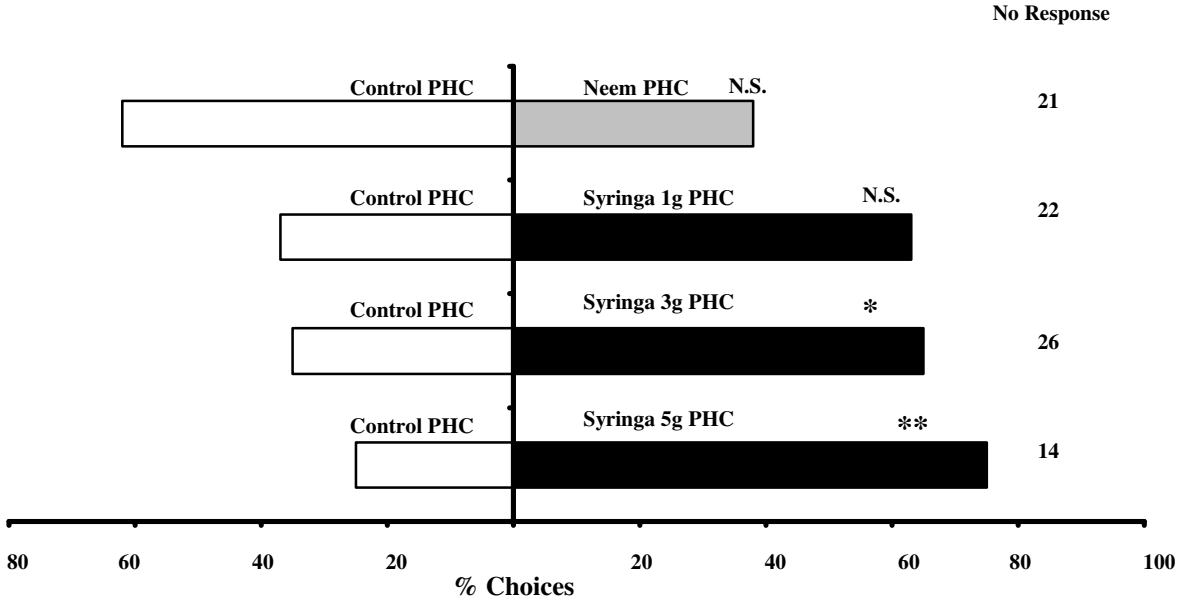

Figure 2. Effect of syringa extracts and neem on the response of female *C. plutellae* to cabbage plants infested with *P. xylostella*. Each plant was sprayed with the control or the botanical pesticide and then infested with *P. xylostella*. Parasitoids were exposed to the entire plant-host complex (PHC). Percentage indicates total number of landings on target per treatment group (no. responding wasps = 60). Significant differences are indicated in the graph (Binomial test: N.S. P > 0.05; * P < 0.05; *** P < 0.001). Number of females not responding is indicated on the right of the figure.

Effect of experience: The previous experience of female *C. plutellae* with treated or control cabbage plants did not make a difference to the response (Neem: $\chi^2 = 0.93$; df = 2; P = 0.64; Syringa: $\chi^2 = 1.679$; df = 2; P = 0.44) (Fig. 3a, 3b). Similar results were found for *D. collaris*, the previous experience of the female with treated or control cabbage plants did not influence the subsequent behaviour (Neem: $\chi^2 = 0.031$; df = 2; P = 0.97; Syringa: $\chi^2 = 0.304$; df = 2; P = 0.86) (Fig. 4a, 4b).

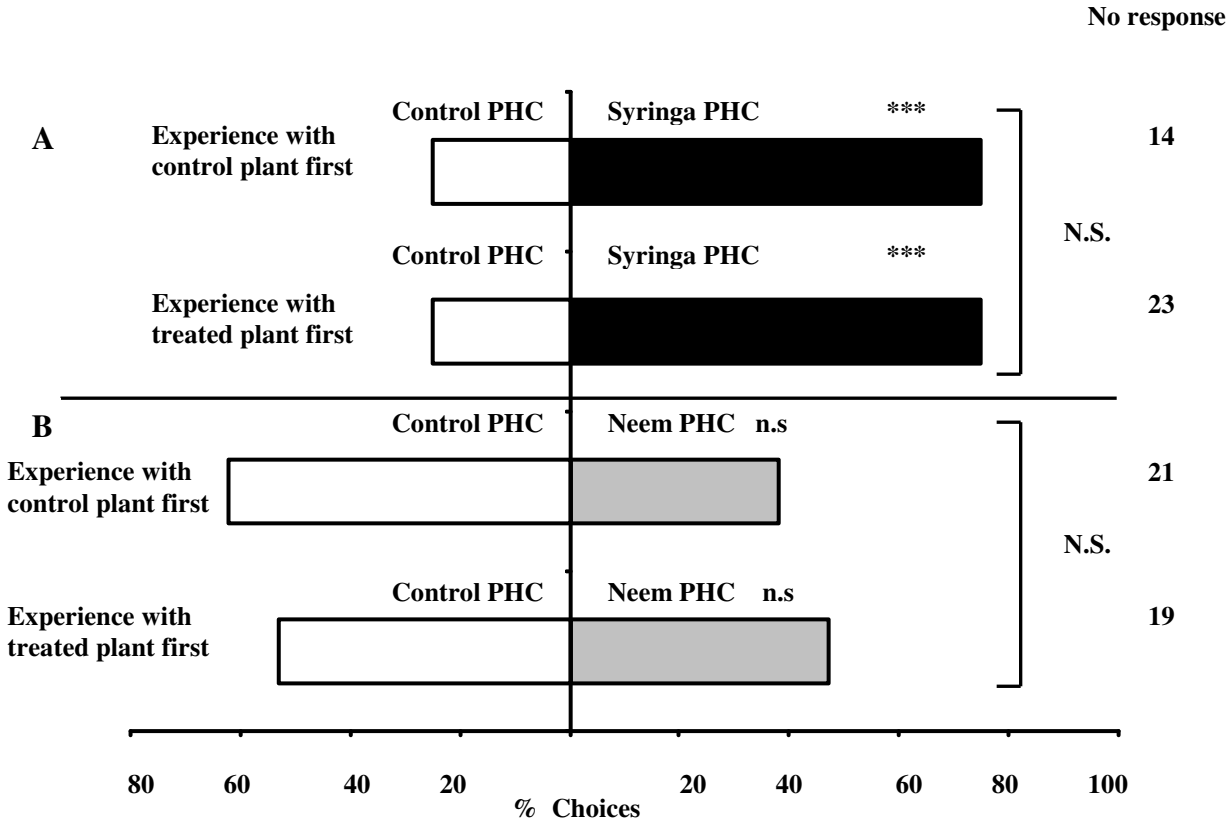

Figure 3. Effect of previous experience on the response of female *C. plutellae* to cabbage plants infested with *P. xylostella*. A = response to plants treated with syringa extract; B = response to plants treated with neem. Each plant was sprayed with the control or the botanical pesticide and then infested with *P. xylostella*. Parasitoids were exposed to the entire plant-host complex (PHC). Percentage indicates total number of landings on target per treatment group (no. responding wasps = 60). Significant differences are indicated in the graph (χ^2: N.S. $P > 0.5$; Binomial test: ns $P > 0.05$; *** $P < 0.001$). Number of females not responding is indicated on the right of the figure.

Response of pupal parasitoid: *Diadromus collaris* did not respond very well in the wind tunnel, which is clear from the large number of no responses (± 50%; Fig. 4a, 4b). When damaged and undamaged cabbage plants were compared, females did not distinguish between them (P = 1.0), and less than 50% responded (Fig. 4c). We therefore abandoned this species for the remaining trials.

Different syringa doses: When lower doses of the syringa extract were tested. *Cotesia plutellae* still showed a clear preference for the 3 g syringa dose (P = 0.029). Differences were just not significant at the lowest 1 g dose (P = 0.052) (Fig. 2).

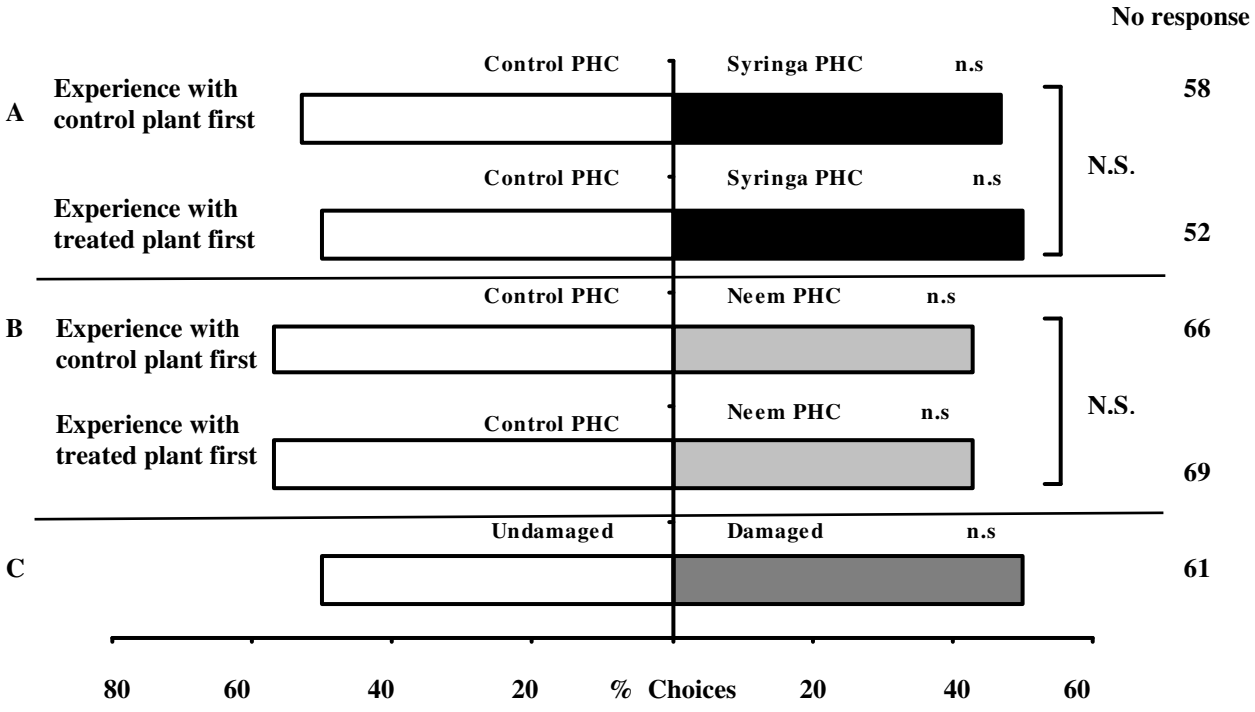

Figure 4. Effect of previous experience on the response of female *D. collaris* to cabbage plants infested with *P. xylostella*. A = response to plants treated with syringa extract; B = response to plants treated with neem. Each plant was sprayed with the control or the botanical pesticide and then infested with *P. xylostella*. Parasitoids were exposed to the entire plant-host complex (PHC). C = damaged versus undamaged cabbage plants, these plants were not sprayed with any treatment. Percentage indicates total number of landings on target per treatment group (no. responding wasps = 60). Significant differences are indicated in the graph (χ^2: N.S. $P > 0.5$; Binomial test: ns $P > 0.05$). Number of females not responding is indicated on the right of the figure.

Damaged plants without hosts

Plants were sprayed with the botanical pesticide or the control and damaged by *P. xylostella*, but before exposure to *C. plutellae* the *P. xylostella* larvae were removed. The removal of the hosts did not alter the response of the parasitoid. Again, *C. plutellae* did not show a preference for the treated or control plants if the plants had been sprayed with the neem extract ($P = 0.52$), but they did show a significant preference for the plants that had been treated with the syringa extract ($P = 0.000$) (Fig. 5a).

Equally damaged plants without hosts

To create equally damaged plants, the plants were infested with *P. xylostella* larvae for 24 hours, the larvae were removed and then the plants were sprayed with the botanical pesticide or the control. *Cotesia plutellae* did not show a preference for the treated or the control plant when the plants had been treated with the neem extract ($P = 0.7$), but they

again showed a significant preference for plants that had been treated with syringa extract (P = 0.000) (Fig. 5b).

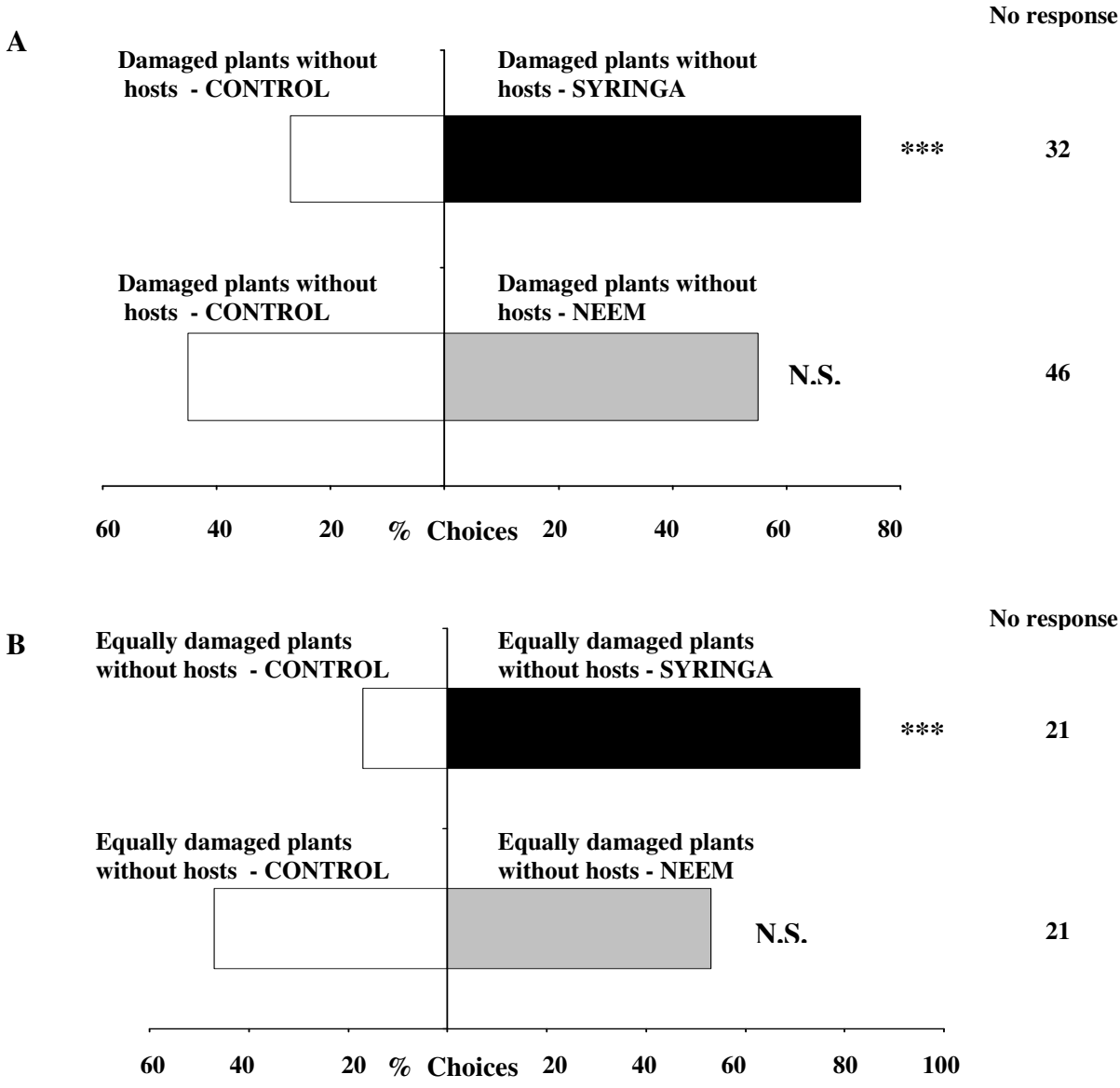

Figure 5. Effect of syringa and neem on the response of female *C. plutellae* to treated cabbage plants. Percentage indicates total number of landings on target per treatment group (no. responding wasps = 60). Significant differences are indicated in the graph (Binomial test: N.S. P > 0.05; *** P < 0.001). Number of females not responding is indicated on the right of the figure.

- **a.** Cabbage plants had previously been infested with *P. xylostella* hosts. Each plant was sprayed with the control or the botanical pesticide and then infested with *P. xylostella*. The *P. xylostella* hosts were removed just before the cabbage plants were exposed to the parasitoids.
- **b.** Cabbage plants were equally damaged. Each plant was infested with *P. xylostella* larvae for 24 hours, the larvae were removed and then the plants were sprayed with the botanical pesticide or the control.

Undamaged plants and filter paper

In this experiment *C. plutellae* was exposed to undamaged cabbage plants that had been sprayed with the botanical pesticides or the control; or to filter paper that had been dipped in the treatments. Despite the lack of damage, *C. plutellae* still showed a significant preference for plants that had been treated with the syringa extract, except when the lowest 1 g dose was used (1 g dose: P = 0.092; 3 g dose: P = 0.027; 5g dose: P = 0.000). Again there were no differences between plants that were treated with the neem solution (P = 0.52) (Fig. 6). The number of females responding to undamaged plants was less than the number responding to caterpillar-damaged plants.

The attraction of the parasitoid to the syringa extract was lost when filter paper was used instead of cabbage plants (P = 0.70) (Fig. 6).

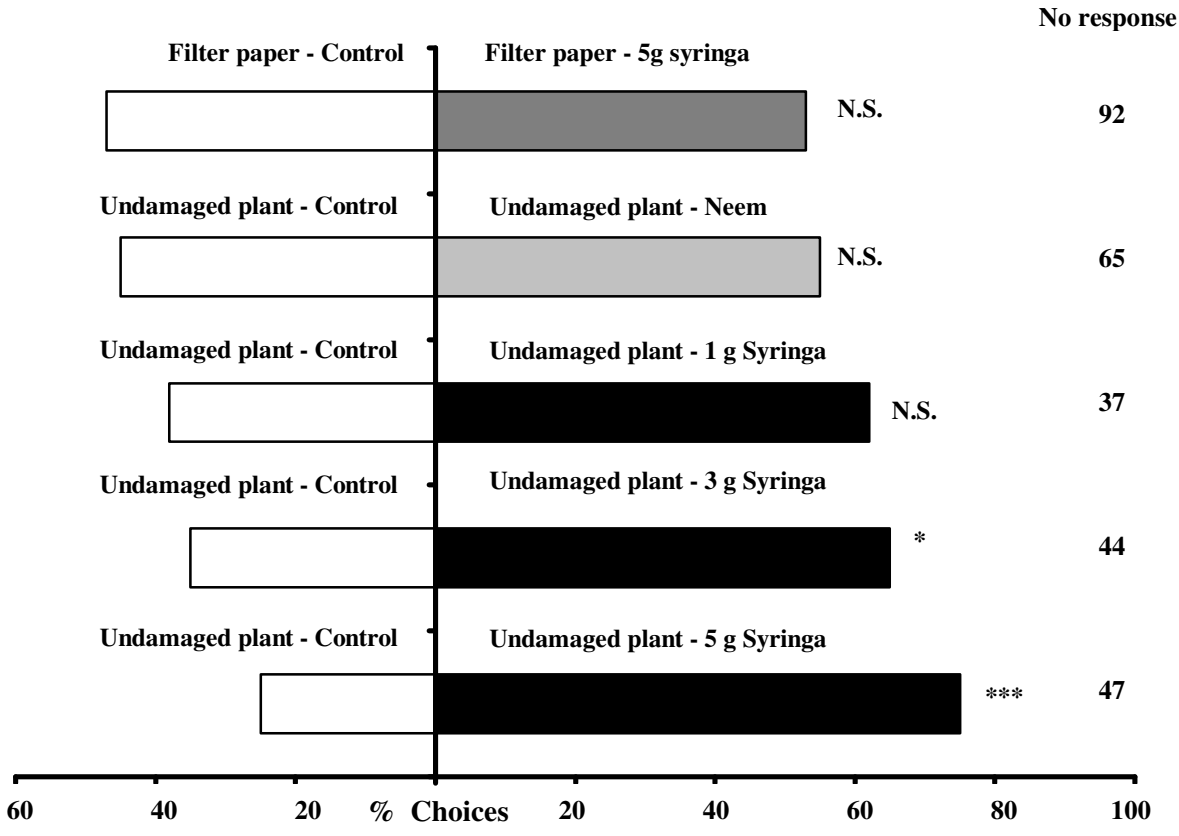

Figure 6. Effect of syringa and neem on the response of female *C. plutellae* to undamaged cabbage plants, or to filter paper dipped in the highest dose of the syringa treatment. Each plant was sprayed with the control or the botanical pesticide, but plants were not infested with *P. xylostella*. The filter paper was dipped into the control or the syringa extract. Percentage indicates total number of landings on target per treatment group (no. responding wasps = 60). Significant differences are indicated in the graph (Binomial test: N.S. P > 0.05; * P < 0.05; *** P < 0.001). Number of females not responding is indicated on the right of the figure.

Analysis of plant volatiles

Only compounds that were detected in 2 or more samples per treatment were included in the analysis. A total of 49 compounds were found in both control and treated plants (Table 1). Fifteen of these volatiles found in cabbage plants treated with the syringa extract were also present in the syringa extract (Table 1). Six additional compounds were also found in plants that had been treated with syringa extracts (Table 1). The syringa extract alone produced an additional 25 compounds that were not found in the control plants or in the cabbage plants that had been treated with the syringa extract (Table 2). The amounts of volatiles produced by plants that had been treated with the syringa extract were significantly higher than the amounts produced by control plants: of the 49 compounds, a total of 38 compounds were emitted in larger amounts (based on mean peak areas) by syringa treated plants and 11 were emitted in larger amounts by control plants (Sign test: $P < 0.001$).

Table 1. Mean (±SE) of GC peak area (aribtrary units) for compounds detected in headspace of control cabbage plants and cabbage plants treated with aqueous leaf extracts from *Melia azedarach*. n = no. of samples out of 4, in which the compound had been identified

No.	Cabbage volatiles	Control cabbage	n	Treated cabbage	n	Syringa extract	n
	Alcohols						
1	1-hexanol	8.30 ± 3.33	4	13.00 ± 1.15	3	69.00 ± 11.63	4
2	2-ethyl hexanol	624.00 ± 89.54	4	807.50 ± 3.50	2		
3	1-pentanol	4.25 ± 1.11	4	7.53 ± 0.32	4	100.75 ± 22.35	4
4	1-penten-3-ol	193.75 ± 69.28	4	339.25 ± 7.23	4	459.50 ± 47.06	4
5	3-hexen-1-ol	69.25 ± 29.68	4	113.50 ± 12.58	4	3.08 ± 0.46	4
	Aldehydes						
6	2-ethyl hexanal	52.75 ± 8.67	4	177.50 ± 66.19	4	364 ± 43.58	4
7	hexanal	9.25 ± 1.38	4	14.50 ± 1.55	4	343.50 ± 69.50	4
	Esters						
8	1-butanol-3methyl acetate	2.65 ± 1.05	2	2.38 ± 0.60	4		
9	2-penten-1ol acetate	47.10 ± 24.15	4	92.00 ± 16.05	4		
10	3-hexen-1-ol acetate	420.25 ± 116.57	4	608.75 ± 39.70	4	10.57 ± 2.69	3
11	3-hexen-1-ol-propanoate	1.13 ± 0.38	3	2.45 ± 0.75	2		
12	2-ethyl acetate	580.25 ± 86.18	4	733.20 ± 410.67	3		
13	acetic acid pentyl ester	2.95 ± 0.91	4	6.15 ± 0.85	2	11.4 ± 3.29	4
14	heptyl acetate	2.25 ± 0.67	4	7.03 ± 0.54	3		
15	methyl salicylate	2.40 ± 1.00	3	5.25 ± 2.21	4		
	Isothiocyanate						
16	methyl isothiocyanate	8.65 ± 2.25	4	8.33 ± 1.09	4		
	Ketones						
17	2-heptanone	3.75 ± 0.75	2	10.03 ± 1.23	4	36.25 ± 8.06	4
18	3-heptanone	15.75 ± 3.17	4	52.50 ± 10.84	4	311.75 ± 49.43	4
19	3-pentanone	101.75 ± 38.10	4	244.50 ± 32.85	4		

Table 1 (cont.)

No.	Cabbage volatiles	Control cabbage	n	Treated cabbage	n	Syringa extract	n
	Sulfides						
20	dimethyl disulfide	28.50 ± 12.51	4	42.75 ± 6.61	4		
21	dimethyl-trisulfide	4.05 ± 1.41	4	10.68 ± 2.90	4		
	Terpenoids						
22	1,8 cineole	167.00 ± 52.25	4	150.00 ± 30.82	4	1.03 ± 0.23	4
23	2-beta pinene	77.75 ± 22.40	4	57.75 ± 8.81	4		
24	alpha phellandrene	1.73 ± 0.32	3	18.35 ± 17.22	4		
25	alpha terpinolene	0.70 ± 0.15	3	15.63 ± 15.18	3		
26	alpha-pinene	201.00 ± 57.79	4	186.50 ± 37.38	4		
27	alpha-thujene	206.25 ± 63.60	4	185.75 ± 30.03	4		
28	beta myrcene	5.73 ± 1.07	3	33.28 ± 29.58	4		
29	gamma terpinene	295.50 ± 53.45	4	303.75 ± 41.35	4	5.25 ± 0.55	2
30	l-limonene	4.15 ± 0.55	2	3.23 ± 0.35	3		
31	mix alpha terpineol	507.00 ± 76.95	4	392.50 ± 111.45	4		
32	E beta ocimene	4.00 ± 1.56	4	7.98 ± 4.39	4		
33	alpha terpinene	2.15 ± 0.15	2	2.58 ± 0.32	4		
34	sabinene	2.60 ± 0.45	4	7.48 ± 3.58	4		
35	trans-4-thujanol	17.25 ± 5.57	4	13.57 ± 2.43	3		
36	Unknown terpenoid	106.00 ± 28.54	4	81.00 ± 11.60	4	4.93 ± 0.77	4
	Others						
37	Unknown 1	28.00 ± 1.00	3	44.67 ± 13.68	3		
38	Unknown 2	1.38 ± 0.25	4	2.25 ± 0.38	4		
39	Unknown 3	56.75 ± 23.78	4	105.75 ± 24.87	4		
40	Unknown 4	8.30 ± 0.85	3	27.25 ± 3.30	4		
41	Unknown 5	23.00 ± 8.74	3	40.67 ± 12.99	3	12.50 ± 0.50	2
42	Unknown 6	4.93 ± 1.07	3	6.70 ± 2.10	2		
43	Unknown 7	1.20 ± 0.20	2	1.03 ± 0.17	4		
44	Unknown 8	1.47 ± 0.26	3	5.77 ± 0.23	3		
45	Unknown 9	25.67 ± 15.27	2	32.70 ± 18.18	3		
46	Unknown 10	27.33 ± 11.26	3	29.63 ± 15.65	3		
47	Unknown 11	51.67 ± 9.60	3	481.33 ± 150.93	3	631.67 ± 65.17	3
48	Unknown 12	13.00 ± 4.56	4	28.00 ± 3.46	3		
49	Unknown 13	17.25 ± 8.02	4	31.67 ± 4.48	3		

	Compounds only found in cabbage plants treated with syringa		**n**
1	methyl pyrrole	18.40 ± 11.60	2
2	3-methyl butanal	9.00 ± 1.57	3
3	quinhydrone	6.83 ± 2.04	4
4	1-octen-3-ol	4.23 ± 0.69	3
5	benzoxazole	2.65 ± 0.75	2
6	trans carryophyllene	1.85 ± 0.55	2

Table 2. Mean (±SE) of GC peak areas (arbitrary units) for compounds detected in headspace of syringa extract only.
n = no. of samples out of 4, in which the compound had been identified

No.	Compounds only in syringa	Syringa			n
	Alcohols				
1	3-methyl-1-butanol	34.00	±	8.33	3
2	1-nonanol	11.13	±	2.58	4
	Aldehydes				
3	2-hexenal (E)	8.58	±	2.00	4
4	2-pentenal	124.75	±	31.68	4
5	E-2-hexenal	483.00	±	84.00	2
	Ketone				
6	6-methyl-5-hepten-2-one	184.50	±	41.05	4
	Others				
7	Unknown 1	18.15	±	4.66	4
8	Unknown 2	24.00	±	5.03	3
9	Unknown 3	116.25	±	27.16	4
10	Unknown 4	52.50	±	10.25	4
11	Unknown 5	132.75	±	10.35	4
12	Unknown 6	3.43	±	1.15	3
13	Unknown 7	11.00	±	1.00	3
14	Unknown 8	16.53	±	8.24	3
15	Unknown 9	8.47	±	3.50	3
16	Unknown 10	12.93	±	2.15	3
17	Unknown Terpenoid 1	23.50	±	5.24	4
18	Unknown 11	11.60	±	3.24	3
19	Unknown Terpenoid 2	4.60	±	3.70	3
20	Unknown 12	13.25	±	2.21	4
21	Unknown 13	19.33	±	0.67	3
22	Unknown 14	1.33	±	0.35	4
23	Unknown 15	311.50	±	57.12	4
24	Unknown 16	3.43	±	1.04	4
25	Unknown 17	4.98	±	1.27	4

Discussion

After an oviposition experience *C. plutellae* responded very well in the windtunnel. Females did not distinguish between cabbage plants that had been treated with the neem solution and cabbage plants that had been sprayed with the control. However, *C. plutellae* always preferred cabbage plants that had been sprayed with the syringa extract to the control cabbage plants, except at the lowest dose. *Cotesia plutellae* appears to detect and respond differently to volatiles from plants treated with these two botanical pesticides. Akol *et al.* (2003) have shown that *Diadegma mollipla* was also able to detect and distinguish between volatiles emitted by cabbage plants sprayed with two different neem formulations. In their experiment they found that a neem seed oil formulation had a negative effect on the foraging of *D. mollipla*, as females were significantly attracted to volatiles from control plants in comparison to those sprayed with the neem formulation. However, when *D. mollipla* had a choice between plants sprayed with a solution from a neem kernel cake powder and control plants they did not distinguish between the sprayed and control plants, and uninfested plants sprayed with this formulation were actually more attractive (Akol *et al.*, 2003). Boeke (2002) found that *Uscana lariophaga*, an egg parasitoid of the bruchid *Callosobruchus maculatus* was repelled by neem seed oils on cowpea beans, but that the larval parasitoid, *Dinarmus basalis* did not discriminate between control and neem treated beans. In our study the neem formulation, Neemix 4.5®, did not appear to have an adverse effect on foraging by *C. plutellae*.

Pre-flight experience can have a distinct effect on parasitoid behaviour (reviewed in Turlings *et al*., 1993 and Vet *et al.*, 1995). For example, the previous experience of the generalist parasitoid, *Cotesia glomerata*, can modify its preference for certain plant-host complexes through associative learning (Geervliet *et al*., 1998). In this experiment we gave female parasitoids a pre-oviposition experience on cabbage plants that had either been treated with botanical pesticides or with the control. However, we found that the responses of *C. plutellae* and *D. collaris* did not change with these changes in previous oviposition experience, and females with both experiences significantly preferred cabbage plants treated with the syringa extracts. Learning is thought to have a less important role for specialist parasitoids (Geervliet *et al*., 1998). Both *C. plutellae* and *D. collaris* are relatively specialised parasitoids attacking mainly *P. xylostella* and results from this study show no indication that these parasitoids acquired a response to syringa through the (common) process of associative learning.

The response of *C. plutellae* to herbivore-damaged plants without hosts remained the same, since females still showed a significant preference for the plants sprayed with the syringa extract. Therefore, this parasitoid responds to the volatiles from the herbivore-damaged plant, even if the host is not present, and the botanical pesticide did not alter this

response. Previous studies have also shown that herbivore-damaged plants are still attractive to *C. plutellae* even after removal of the hosts (Potting *et al.*, 1999).

Schuler *et al.* (1999, 2003) found that the amount of damage was an important factor influencing the flight responses of *C. plutellae*. In previous studies we have shown that *P. xylostella* feeds less on cabbage plants treated with botanical pesticides (Chapters 2 & 3). However, in this experiment we did not notice a change in the response of *C. plutellae* if the cabbage plants had been sprayed with the botanical pesticide first, or if the plants were first damaged by *P. xylostella* larvae and then sprayed with the botanical pesticide. It is possible that since larvae were only allowed to feed on the plants for 24 hours, the difference in feeding damage between the treated and control cabbages was insufficient to make any difference to the flight response of *C. plutellae*.

Diadromus collaris was not attracted to volatiles emitted by cabbage plants in the windtunnel, and did not distinguish between caterpillar-damaged and undamaged plants. *Diadromus collaris* is a pupal parasitoid, and whilst there is a wealth of knowledge available on the role of volatile cues used by parasitoids attacking larval stages of herbivores (Dicke & Vet, 1999), there is not much information about the chemical cues used by pupal parasitoids (Vet *et al.*, 1995). Some pupal parasitoids have been shown to respond to plant volatiles. The stemborer pupal parasitoid *Dentichasmias busseolae* for example, makes use of plant volatiles from maize and sorghum, and is particularly attracted to herbivore damaged plants (Gohole, 2003*).* For pupal parasitoids, as for all parasitoids, the possibilities of using direct host-derived cues is limited, and in addition larvae often pupate away from the site of damage, restricting the use of predictable indirect cues by pupal parasitoids (Vet *et al.*, 1995). The opportunity of using volatiles from larval feeding damage is limited to situations where the pupae stay in or on the plant, and larval and pupal stages more or less co- occur. *Plutella xylostella* do tend to pupate on the plant (although not directly near feeding damage) and, due to fast development and overlapping generations, larval and pupal stages are often found together in the field. Hence *D. collaris* could make use of plant volatiles to find its host. Electroantennogram (EAG) studies indicate that *D. collaris* does respond to cabbage volatiles (Lecomte & Pouzat, 1985), which further suggests that this parasitoid may make use of plant volatiles. In our studies *D. collaris* did not appear to use herbivore-induced plant volatiles since they did not differentiate between damaged and undamaged cabbage plants. Therefore, it is possible that *D. collaris* makes use of other strategies to find its host. *Diadromus collaris* is a large, long-lived specialist parasitoid attacking mainly *P. xylostella*. An investment in quality such as a large size, allowing for greater flight ability, and a long life may provide a solution to finding only a limited number of hosts if the parasitoid does not make use of volatile cues (Vet *et al.*, 1995). Since pupae are sessile they can be examined meticulously so as to optimise oviposition decisions on sex allocation; and host recognition characteristics such as odour, shape and colour can be learnt thereby increasing the

reliability of future host acceptance (Drost & Cardé, 1993). *Diadromus collaris* is abundant in the field and although it did not readily respond in the windtunnel, it is possible that the windtunnel set-up used in this experiment was not suited to the study of this parasitoid. The treatment of cabbage plants with botanical pesticides did not alter the response of *D. collaris*.

Cotesia plutellae was always significantly attracted to the cabbage plants treated with the syringa extract. Even undamaged cabbage plants treated with syringa extracts still significantly attracted the parasitoids. However, *C. plutellae* did not show a significant preference for filter paper dipped in the syringa extract. In our study headspace analysis yielded a total of 49 compounds from both undamaged control cabbage plants and undamaged cabbage plants that had been treated with syringa extract. The amount of volatiles was significantly higher in the plants treated with the syringa extract. Some of the volatiles found in cabbage plants treated with the syringa extract were also present in the syringa extract itself. However, many of the volatiles present in the syringa extract alone were not emitted, or were only emitted at low rates, from cabbage plants treated with the syringa extract. This indicates that the enhanced emission of the plant volatiles from cabbage plants treated with the extract is caused by an induction of these volatiles in cabbage rather than an evaporation of the syringa extract from the treated plants. To our knowledge this is the first example of a botanical pesticide causing an increased emission of plant volatiles, which is likely to explain the significant attraction of *C. plutellae* to plants that have been treated with the syringa extract.

Results from the present study show that treatment of cabbage with syringa extracts or Neemix 4.5® would not significantly impair the process of host habitat location by *C. plutellae* or *D. collaris*. In fact *C. plutellae* was always attracted to cabbage plants treated with the syringa extract, even if the plants were undamaged, which may indicate an interaction between a plant and a botanical pesticide that enhances natural enemy activity. This may have negative implications for biological control. If the parasitoids do not discriminate between infested and uninfested plants then they may waste time searching uninfested plants, which may result in a reduction of the parasitisation rate (Dicke *et al*., 1990b). Treatment of plants with the plant hormone jasmonic acid results in an increased emission of plant volatiles, and this results in an attraction of parasitoids and predators (Gols *et al*., 1999; Thaler, 1999). Yet, in field grown tomatoes, treatment with jasmonic acid resulted in higher parasitisation levels of *Spodoptera exigua* compared to control plots (Thaler, 1999). In our study the parasitoids were only given a short pre-oviposition experience with cabbage leaves that were infested with host insects, and were not given the opportunity to discriminate between damaged and undamaged plants. This is not representative for most foraging decisions in nature, where previous foraging experiences, both positive and negative, can be integrated (Dicke, 1999c). Under field conditions infested and uninfested plants are likely to be in close proximity and the parasitoid may

soon learn to discriminate. It is interesting to expand these observations to investigate the impact that these botanical pesticides have on parasitoid foraging behaviour in field situations (see Chapter 6).

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CHAPTER 6

IMPACT OF BOTANICAL PESTICIDES DERIVED FROM *MELIA AZEDARACH* AND *AZADIRACHTA INDICA* ON POPULATIONS OF *PLUTELLA XYLOSTELLA* AND ITS NATURAL ENEMIES: PREDICTING FIELD EFFECTS FROM LABORATORY DATA

CHAPTER 6

Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on populations of *Plutella xylostella* and its natural enemies: predicting field effects from laboratory data.

Abstract

The gap between results from ecological laboratory studies and what actually happens in the field can be large. Therefore, field experiments are essential to validate laboratory findings. In previous extensive laboratory trials we investigated the impact of aqueous leaf extracts from the syringa tree, *Melia azedarach* L. (Meliaceae) and commercial formulations from the neem tree, *Azadirachta indica* Juss. (Meliaceae), Neemix 4.5®, on the biology and behaviour of the diamondback moth *Plutella xylostella* and two of its most abundant natural enemies, *Cotesia plutellae* and *Diadromus collaris*. In the laboratory we demonstrated that these botanical pesticides had adverse effects on survival, fecundity, development, oviposition and feeding of *P. xylostella*, but no directly negative effects on the survival and foraging of the two aforementioned natural enemies was recorded. In the current field trials we verified the importance of these previous laboratory findings through field experiments. We treated cabbage plants in the field with the neem product and syringa extract and asssessed the infestation levels of *P. xylostella* and the parasitism rates by natural enemies. Infestation levels of *P. xylostella* were similar in the plots treated with the botanical pesticides and the control plots. However, the damage in the treated plots was significantly lower than in the control plots, indicating that reduced feeding by *P. xylostella* was a more important factor in the reduction of damage than the actual population density. The proportion of parasitoids found emerging from *P. xylostella* was also significantly higher in the treated plots than in the control plots and direct observations of foraging insects indicated that parasitoids were still visiting cabbage plants that had been treated with the botanical pesticides. In general, results from the field trial could be explained by the findings from the laboratory experiments and indicated that Neemix 4.5® and syringa extracts can be integrated with biological control for management of *P. xylostella* populations, by providing an interesting alternative to synthetic pesticides.

Introduction

Interactions involving parasitoids, their herbivore hosts and the plants on which the hosts are encountered are complex and dynamic. Natural enemies of phytophagous insects function and develop in these multitrophic systems (Price *et al.*, 1980, Vet & Dicke, 1992). Their behaviour and physiology, which determine their fitness or performance, are influenced by both the plant (first trophic level) and the phytophagous (herbivore) host (second trophic level) (Reed *et al.*, 1991). For example, parasitoids may use plant stimuli for locating their hosts but their survival and development may be adversely affected by the plant allelochemicals ingested by their hosts (Kester & Barbosa, 1991, Harvey *et al*., 2003). Plant toxins may be sequestered in the haemolymph or body tissues of resistant herbivores, thus providing them with some degree of protection from their natural enemy complex (Nishida, 2002). The realisation that plants and carnivores interact and can have an impact on herbivore population dynamics should be taken into consideration when looking at pest management systems (Dicke, 1999c). While much of the recent work in crop protection has been within a tritrophic context, this approach is almost entirely lacking with pesticides (Wright & Verkerk, 1995). *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is one of the most important pests of crucifer crops (Verkerk & Wright, 1996). Despite its economic importance, most studies on the management of *P. xylostella* have been ditrophic, focussing on the relationship between plants and herbivores or on the relationship between herbivores and natural enemies (Verkerk & Wright, 1996), with only a few studies looking at tritrophic aspects that may be affected by treatment with pesticides (Verkerk & Wright, 1994; Perera *et al.*, 2000; Akol *et al*., 2002).

In this study we look at the impact of botanical pesticides on populations of *P. xylostella* and their natural enemies in the field. Field experiments and observations are essential to validate conclusions from laboratory trials and to indicate whether the parameters measured in the laboratory explain the ecological processes taking place in the field (Geervliet *et al*., 2000). In previous extensive laboratory trials we investigated the impact of two botanical pesticides, a commercial formulation, Neemix 4.5®, from the neem tree, *Azadirachta indica* Juss. (Meliaceae), and aqueous leaf extracts from the syringa tree, *Melia azedarach* L. (Meliaceae) on populations of the diamondback moth, *P. xylostella*, and two of its natural enemies, *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) and *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae). Results from these studies showed: 1. The botanical pesticides reduced the survival and fecundity of *P. xylostella* and prolonged larval development (Chapter 2). 2. The botanical pesticides caused a reduction in *P. xylostella* feeding damage (Chapters 2 and 3). 3. The syringa extracts reduced ovipostion by the adult female moth (Chapter 3). 4. The botanical pesticides did not have a directly negative effect on the survival of *C. plutellae* and *D. collaris* (Chapter 4). 5. In a glasshouse the proportion of *P. xylostella* larvae parasitised by *C. plutellae* was significantly higher on the treated plants than on the control (Chapter 4).

6. In a windtunnel choice test *C. plutellae* showed a distinct preference for plants that had been treated with the syringa extracts compared to control plants (Chapter 5).

After this extensive laboratory investigation the question still remained as to how these botanical pesticides would perform under field conditions. The aim of this experimental field study was to investigate whether the botanical pesticides were effective in controlling *P. xylostella* populations, whether they had a negative impact on natural enemy performance in the field and whether our laboratory findings could be extrapolated to field findings. We treated cabbage plants in the field with the botanical pesticides and took note of the infestation levels of *P. xylostella* and other pest species. We studied parasitism of *P. xylostella* and how it varied with the treatment and we made direct observations of parasitoid foraging in the field. At the end of the experimental period we harvested the plants and collected information on the yield and damage to the cabbage plants. These methods enabled us to test some specific hypotheses that were based on our laboratory findings:

1. *Populations of P. xylostella will be reduced on plants treated with the botanical pesticides*. Laboratory findings suggest that if survival is reduced on plants treated with the botanical pesticides and if female moths are repelled from treated plants, then the *P. xylostella* population on the treated plants in the field will be reduced.
2. *Damage will be reduced on plants treated with botanical pesticides*. Results from laboratory trials suggest that the treated plants have anti-feedant effects. This factor together with the reduced pest population is expected to reduce the amount of damage to the plants and hence improve the yield.
3. *Parasitism rates will be higher on treated plants than on control plants*. Laboratory trials indicated that these botanical pesticides did not have a directly negative effect on the survival of *C. plutellae* or *D. collaris*. Results from a glasshouse trial indicated that parasitism by *C. plutellae* was higher on treated plants than on control plants and behavioural observations in a windtunnel indicated that syringa treated plants were in fact attractive to *C. plutellae*. Therefore, we expect that parasitism rates on treated plants in the field will be higher than parasitism rates on control plants. However, almost nothing is known of how parasitoids forage in the field, and present understanding of parasitoid foraging in nature is largely based on inference from field patterns of parasitism (Morrison & Strong, 1981). This method has limitations, since absence of parasitism in a patch does not necessarily indicate absence of foraging parasitoids (Chesson, 1982). Interpretation of field parasitism may be enhanced by concurrent independent observations of foraging parasitoids (Waage, 1983, Geervliet *et al*., 2000). We investigated this by making direct observations of parasitoid foraging in the field.

Materials and Methods

The treatments

Syringa: *Melia azedarach* (hereafter referred to as syringa) leaves were collected from Rietondale in Pretoria, South Africa (28°15'S; 25°44'E). Leaves were collected from trees at a height of about 1.5 - 3.5m at the start of spring flush, in September 2002 and placed in a glass house (30 ± 5°C) to dry, after which they were crushed into a fine powder and stored in an airtight container until use. The extract was made by mixing 3 g of leaf powder with 100 ml of distilled water. The water was heated to 48°C, and the leaf powder was added to the water and shaken for approximately one minute. Six liters of extract was made up. The extract was left in a refrigerator (± 4°C) overnight. The following morning the extract was filtered through a metal sieve and then through a fine muslin cloth. Five ml of liquid detergent was added to the final extract to act as a wetting agent, without which the extract runs off the surface of the leaf.

Neem: A commercial preparation of *Azadirachta indica,* Neemix 4.5® (hereafter referred to as neem), was provided by Thermo Trilogy Corporation, Columbia, USA. A dose of 10.7 µl per 100 ml of distilled water was used. Six liters was made up, and 5 ml of liquid detergent was also added to the final product.

Results from laboratory trials indicated that these doses were effective against *P. xylostella* populations and did not have a negative impact on *C. plutellae* or *D. collaris* (Chapters 2, 3, 4 and 5).

Control: The control treatment used consisted of distilled water mixed with 5 ml liquid detergent. Six liters of this treatment was also made up.

Field design

A field trial was set up at Boschkop (25° 47,73' S; 28° 26,60'E) near Pretoria, South Africa. Approximately 2300 cabbage seedlings, *Brassica oleracea* var *capitata* L. (Cruciferae), were planted in the field on August the 20th 2002. The cabbages were planted in beds with three rows in each bed, the distance between the rows and between plants within the row was approximately 40 cm. The distance between beds was approximately 60 cm. A total of seven beds were planted. The field was divided into a 3 x 3 "Latin Square" design with three treatments in three blocks. Plastic pegs were used to mark out treatment plots (3.4 m x 15.2 m). Each treatment plot consisted of approximately 260 cabbage plants. Treatments and control were directly next to each other, but the outer two rows of each plot were not considered during scouting and harvest.

The entire plot was sprayed with the appropriate treatment twice per week using a large garden pressure sprayer. Six litres of each treatment was made and 2 l was then applied to

each plot. Each week ten plants were randomly chosen in each treatment plot, ignoring the outer two rows of the plot, resulting in 30 plants being scouted per treatment, per week. Each plant was marked with a tie-on plastic label, to ensure that each plant was only scouted once. This plant was scouted and all insects on the plant were recorded. Second, third and fourth instar *P. xylostella* were collected, pupae and parasitoid cocoons were also collected and taken back to the laboratory and left to develop in order to determine parasitoid diversity and parasitism levels within the different treatments. The experiment continued for the entire spring season and plants were scouted for 13 weeks, from 10 September 2002 until 3 December 2002.

Behaviour in the field

The foraging behaviour of parasitoids and *P. xylostella* moths was observed in a field plot next to the plot used for scouting. This field was also divided into a 3 x 3 "Latin Square" design with the same three treatments and three blocks. Plastic pegs were used to mark out treatment plots. However, the treatment was not applied to the plants, until the day before the experiment. The day before the experiment ten plants were randomly chosen in each treatment plot, ignoring the outer two rows of the plot, making a total of 30 plants per treatment. Each plant was marked with a peg and then sprayed with the appropriate treatment. One litre of the botanical pesticide or control was applied to the 30 plants. The treatment was left to dry for approximately one hour. The plants were checked to ensure that they were clean from field infestation of *P. xylostella*. Then each plant was artificially infested with 12 second instar *P. xylostella* and 12 fourth instar *P. xylostella* from a laboratory culture. This was intended to attract both larval and pupal parasitoid species. The larvae were left to feed on the plants for 24 hours and observations of these infested plants were done the following morning.

Two observers walked around the field in opposite directions recording with binoculars the number of parasitoids and moths on each of the treated plants. Each plant was observed for one minute. One wasp or moth observation was taken to be the sighting of one wasp or moth on a particular plant during the one minute observation. In order to prevent disturbance of foraging wasps, observers kept to a path that was 4 m from the edge of the field. After the observer had moved from one side of the field to the opposite side, the trial was repeated again with each observer moving in the opposite direction. The experiment was replicated five times. At the end of the replicate the treated plants were removed from the field, the larvae collected and taken back to the laboratory and reared until emergence to determine parasitoid diversity and parasitism ratios. On three of the occasions 50% of the larvae that were collected were dissected, to provide a more accurate estimate of parasitism.

Statistical Analysis

Data collected from scouting were analysed using the statistical program GenStat (GenStat for Windows, 2000). Results were analysed using Analysis of Variance (ANOVA). The infestation levels of *P. xylostella*, other herbivores and predators on the different treatments were compared. Data were not normally distributed and were transformed before analysis, using a logarithmic transformation (Sokal & Rohlf, 1995).

The proportion of parasitoids emerging from *P. xylostella* was also compared. Proportional data follow a binomial distribution and analysis was carried out using a generalised linear model (GLM) for binomial data with a Logit link function and treatment as a predicting factor (Crawley, 1993).

Observational data were not normally distributed and were transformed using a logarithmic transformation before analysis with analysis of variance (ANOVA). Proportions of *P. xylostella* parasitised were analysed using a generalised linear model (GLM) with binomial distribution and a Logit link function, with treatment as a predicting factor (Crawley, 1993).

Harvesting of plants

At the end of the season the cabbages were harvested. Each cabbage was rated for insect damage. For the outer leaves and the inner leaves the damage was rated from 0 to 2. This damage rating was based on a qualitative assessment of damage, with 0 being little to no damage (less than 10% of the leaf showing feeding damage), 1 being some damage (11 - 30% of the leaf showing feeding damage) and 2 being maximum damage (feeding damage greater than 30%). The head of the cabbage is the most important part as this is sent to the market by the farmer, therefore damage to the head was divided into smaller portions and was rated from 0 to 4, with 0 being no damage and 4 making the head unmarketable. Damage rating of 0 (no feeding damage), damage rating of 1 (feeding damage between 1 and 10%), damage rating of 2 (feeding damage between 11 and 20%), damage rating of 3 (feeding damage between 21 and 30%), damage rating of 4 (feeding damage greater than 30%). Each cabbage was also weighed on a digital scale (Digi DI-20 S.A. 1237) operating on batteries. If cabbages are attacked early in the season and the growing tip of the plant is damaged then the plant develops split heads. Such cabbages are also unmarketable. The number of cabbages with split heads and cabbages that had not developed heads by the time of the harvest were also counted. Data were analysed using the statistical program GenStat (GenStat for Windows, 2000). The number of unmarketable heads and the final weights were compared and analysed using analysis of variance (ANOVA). The damage ratings were in categories and the data were analysed using a Chi - square test (Sokal & Rohlf, 1995).

Results

Eighteen herbivore species were found, belonging to four orders and 12 families. Abundance of all the herbivores found within the different treatments is reported in Table 1.

Infestation levels of P. xylostella.

Infestation levels were high in both the treatments and the control. However, infestation levels did not differ between the different treatments and the control (ANOVA: $F_{0.05(2,26)} = 2.22$; $P = 0.129$). Infestation levels were higher on the control plots at the beginning of the season (weeks 4 and 5), but in weeks 9 and 10 the infestation levels were higher on the treated plots than on the control (Fig. 1). Infestation reached a peak around the end of October (weeks 8 & 9).

Infestation levels of other herbivores

Several other herbivore species were found in the field (Table 1), the most common of which were aphids, in particular *Brevicoryne brassicae* (L.). A separate analysis of variance was conducted for the aphids, but there were no significant differences between the treatments and the control (ANOVA: $F_{0.05(2,26)} = 0.68$; $P = 0.52$). The remaining herbivore species were pooled and analysed, but again the treatments did not have an impact on the infestation levels (ANOVA: $F_{0.05(2,26)} = 0.54$; $P = 0.59$).

Parasitoids of P. xylostella and their abundance in different treatments

A wide variety of parasitoids was associated with *P. xylostella* (Table 2). The most abundant species found in the field were *Cotesia plutellae* and *Diadromus collaris*. *Cotesia plutellae* attacks the larval stages of *P. xylostella*, preferring the second and third larval instar, although it can attack all stages, while *D. collaris* is a pupal parasitoid.

Pteromalus sp. was the most abundant hyper-parasitoid, followed by *Mesochorus* sp.. Both these species were found emerging from *C. plutellae* cocoons. *Oomyzus sokolowskii* is a facultative hyper-parasitoid, but in this trial it was only found emerging from the pupal stage of *P. xylostella*, and it was not very abundant.

Table 1. Herbivore species and total numbers found on *Brassica oleraceae* var *captiata* treated with different botanical extracts or with control solution. Data are summaries for 13 weeks of sampling.

HERBIVORE				TREATMENT		
Order	**Family**	**Species name**	**Status**	**Control**	**Neem**	**Syringa**
Hemiptera	Aphididae	*Brevicoryne brassicae* (L.)	Pest	1088	1057	1720
	Aphididae	*Myzus persicae* (Sulzer)	Pest	164	257	228
	Aphididae	*Lipaphis pseudobrassicae* (Davis)	Pest	19	34	42
	Aphididae	*Macrosiphum euphorbiae* (Thomas)	Pest	2	4	4
	Pentatomidae	*Bagrada hilaris* (Burmeister)	Pest	92	74	53
	Pentatomidae	*Nezara viridula* (L.)	Occasional pest	2		
	Lygaeidae	*Spilostethus pandurus elegans* (Wolff)				1
	Dictyopharidae	*Putala transvaalensis*				1
Coleptera	Coccinellidae	*Epilachna dregei* (Mulsant)	Occasional pest	2	4	3
	Chrysomelidae	*Phyllotreta mashonana* Jacoby	Occasional pest	1		
Lepidoptera	Plutellidae	*Plutella xylostella* (L.)	Pest	1553	1820	1943
	Noctuidae	*Helicoverpa armigera* (Hübner)	Pest	4	2	3
	Pyralidae	*Hellula undalis* (F.)	Pest		3	
	Pyralidae	Unidentified sp.			1	
	Pyralidae	Unidentified sp.			1	
	Noctuidae	Unidentified sp.		1		1
	Tortricidae	Unidentified sp.		1		
Thysanoptera	Thripidae	*Thrips tabaci* Lindeman	Pest	140	151	179
TOTAL				**3069**	**3048**	**4178**

Table 2. Predatory and parasitoid species and total numbers found on *Brassica oleraceae* var *capitata* treated with different botanical extracts or with control solution. Data are summaries for 13 weeks of sampling.

PREDATORS AND PARASITOIDS					TREATMENT		
Order	**Family**	**Species name**	**Status**	**Host**	**Control**	**Neem**	**Syringa**
Coleoptera	Coccinellidae	*Hippodamia variegata* (Goeze)	Predator	Aphids	20	15	29
Diptera	Syrphidae	Unidentified Sp.	Predator	Aphids	103	82	67
Hymenoptera	Braconidae	*Cotesia plutellae* (Kurdjumov)	Parasitoid	*P. xylostella*	272	438	543
	Braconidae	*Apanteles eriophyes* Nixon	Parasitoid	*P. xylostella*			3
	Braconidae Subfamily: Aphidiinae	Unidentified sp.	Parasitoid	Aphids	3	16	10
	Ichneumonidae	*Diadromus collaris* Gravenhorst	Parasitoid	*P. xylostella*	74	146	154
	Ichneumonidae	*Diadegma mollipla*	Parasitoid	*P. xylostella*	11	7	9
	Ichneumonidae	I*toplectis* sp.	Parasitoid	*P. xylostella*		3	2
	Ichneumonidae	*Mesochorus* sp.	Hyper-parasitoid	*C. plutellae*	3	8	16
	Ichneumonidae	Unidentified sp.	Parasitoid	*P. xylostella*		1	
	Eulophidae	*Oomyzus sokolowskii* (Kurdjumov)	Parasitoid	*P. xylostella*	2	2	1
	Chalcididae	*Hockeria* sp.	Hyper-parasitoid	*C. plutellae*		3	
	Chalcididae	*Brachymeria* sp.	Hyper-parasitoid	*C. plutellae*	2	1	4
	Pteromalidae	*Pteromalus* sp.	Hyper-parasitoid	*C. plutellae*	22	45	67
	Cynipidae Subfamily: Alloxystinae	*Alloxysta* sp.	Parasitoid	Aphids	6	1	4
Arachnida	Miturgidae	*Cheiracanthuim* sp.	Predator	Generalist	10	5	3
		Unidentified sp.	Predator	Generalist			1
TOTAL					**528**	**773**	**913**

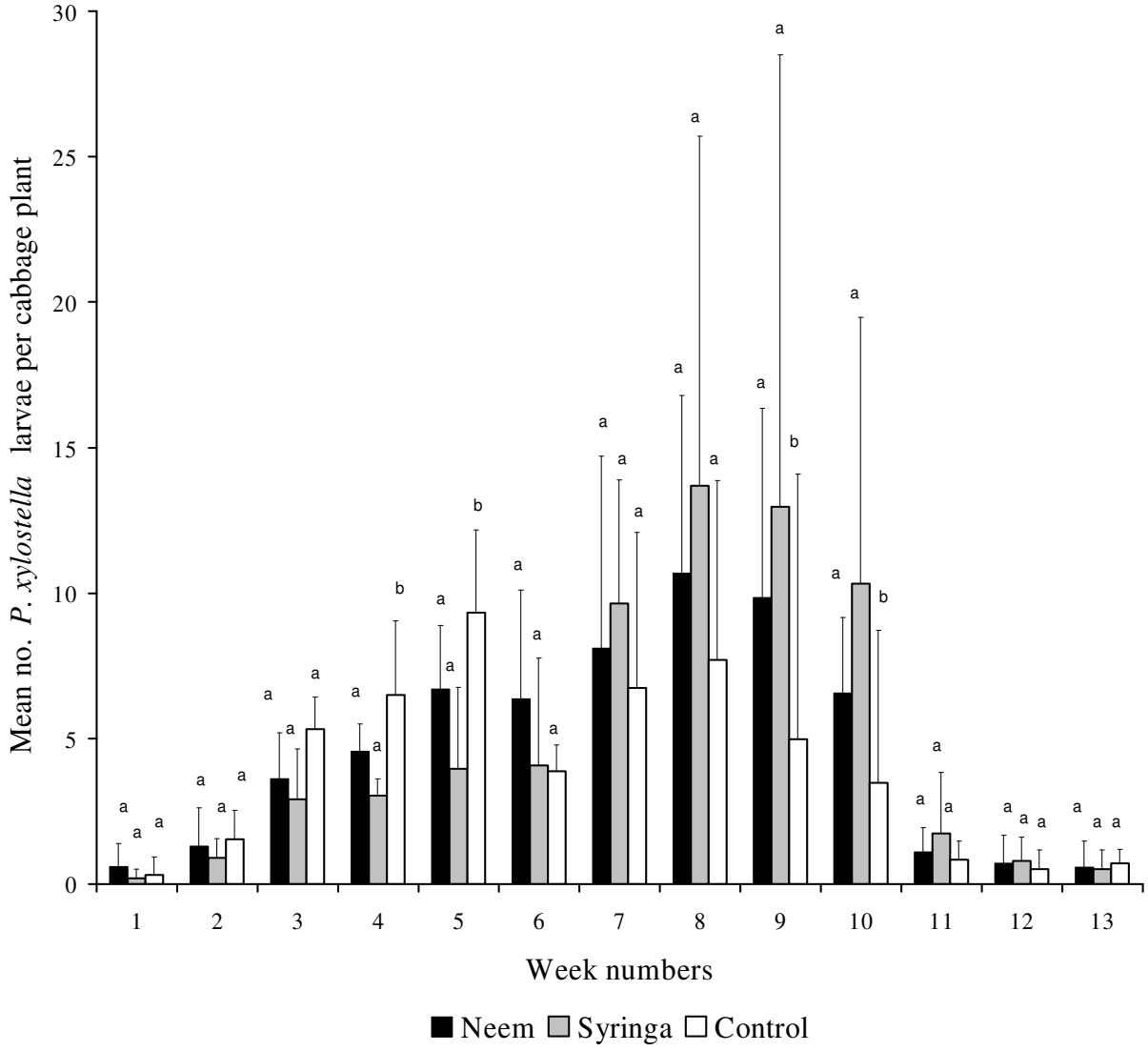

Figure 1. Mean (±SE) number of *P. xylostella* larvae found per cabbage plant treated with different botanical pesticides. Week numbers represent the sampling dates starting on 10 September and ending on 3 December 2002. Those bars within sampling dates with the same letter are not significantly different (ANOVA; plus Fisher's protected LSD: $\alpha = 0.05$).

During scouting, the cocoons of *C. plutellae* could be identified and the numbers found in the different treatments could be compared. There was a lag between the time *P. xylostella* was first observed and when cocoons were found on the plants. Cocoons only started appearing on the plants five weeks after sampling had begun. The number of cocoons was found to differ significantly between the different treatments (ANOVA: $F_{0.05(2,16)} = 29.34$; $P<0.001$), but these differences were also a function of the date (ANOVA: $F_{0.05(14,16)} = 3.74$; $P = 0.007$), therefore each date had to be investigated. For the final five dates of scouting the number of cocoons was found to be significantly higher in the treated plots than in the control plots (Fig. 2).

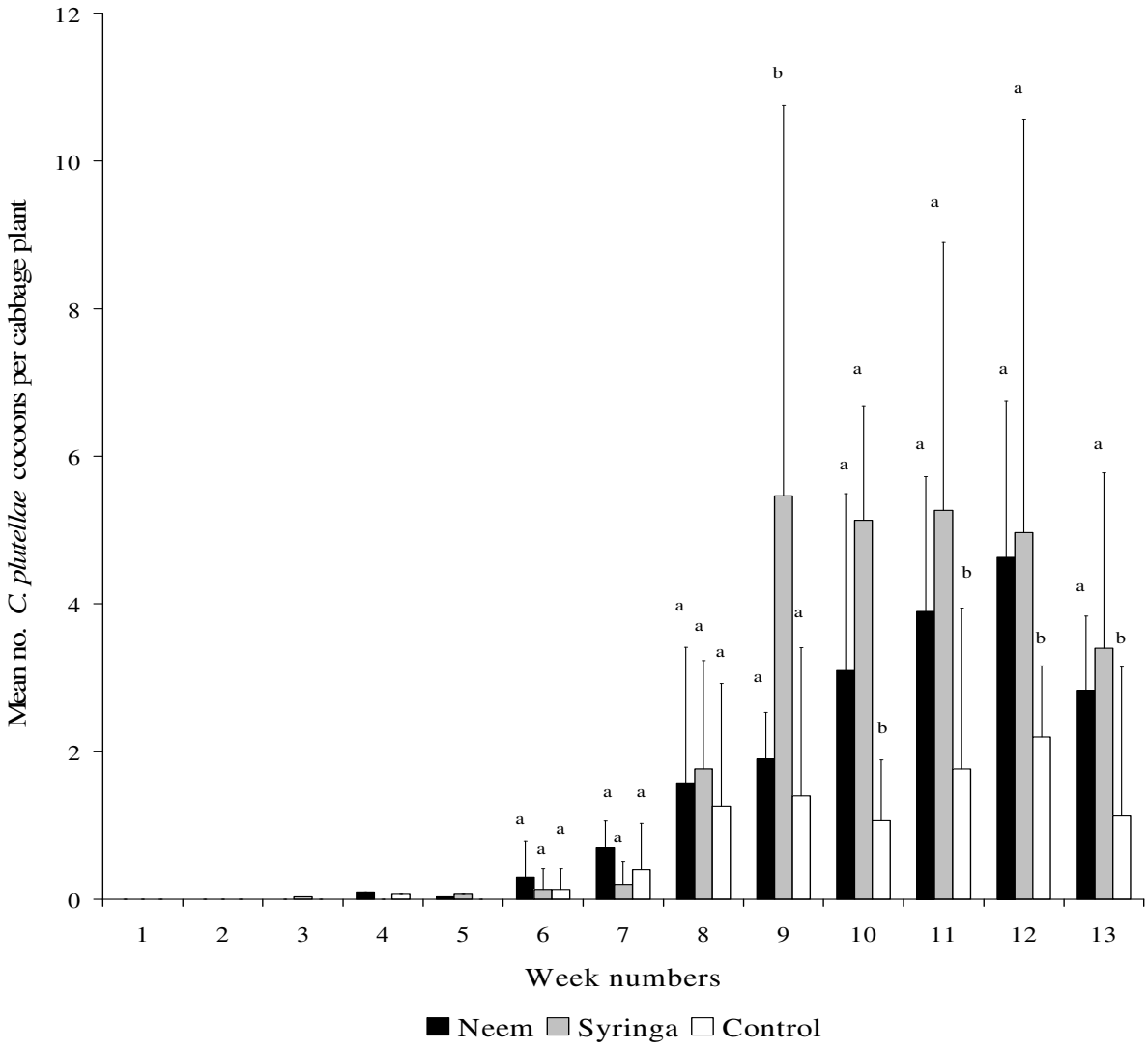

Figure 2. Mean (±SE) number of *C. plutellae* cocoons found per cabbage plant treated with different botanical pesticides. Week numbers represent the sampling dates starting on 10 September and ending on 3 December 2002. Those bars within sampling dates with the same letter are not significantly different (ANOVA; plus Fisher's protected LSD: $\alpha = 0.05$).

The larvae and pupae of *P. xylostella* were taken back to the laboratory and reared to estimate parasitoid diversity and abundance. The proportion of parasitoids emerging from the different treatments was found to be significantly different (GLM: $P<0.001$), with the greatest proportion of parasitoids emerging from the syringa treatment, followed by the neem treatment and finally the control treatment (Fig. 3).

Other predators and parasitoids

Most of the other predators and parasitoids that were found were those which feed on aphids. Predators that were found in relatively high numbers were the ladybird,

Hippodamia variegata and a syrphid fly (Table 2). Generalist predators, in particular the arachnids, were not found to be very abundant. Scouting of plants however, is not a very accurate method to determine arachnid abundance as many are able to escape while the plant is being scouted. Therefore this group is expected to be under-represented in the sample. The numbers of other predators and parasitoids were pooled, but no significant differences were found between the different treatments (ANOVA: $F_{0.05(2,26)} = 1.36$; P = 0.27).

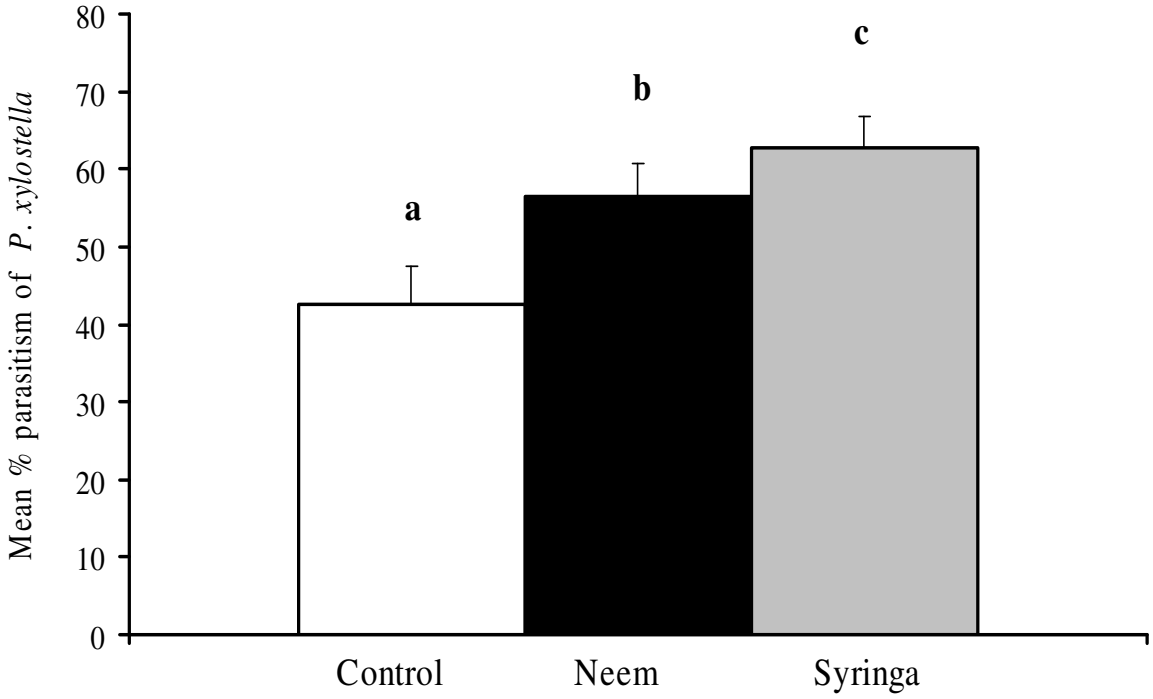

Figure 3. Mean (±SE) percentage parasitism found in *P. xylostella* exposed to cabbage plants treated with different botanical pesticides. All treatments are significantly different (GLM: $\alpha = 0.05$).

Harvest data

The total number of heads harvested from each treatment plot excluding the outer two rows was approximately 180, making a total of approximately 540 cabbages per treatment or control. The number of unmarketable heads (split and unformed heads) was found to be significantly higher in the control plots than in the plots that had been treated with the botanical pesticides (ANOVA: $F_{0.05(2,2)} = 31.00$; P = 0.031) (Fig. 4).

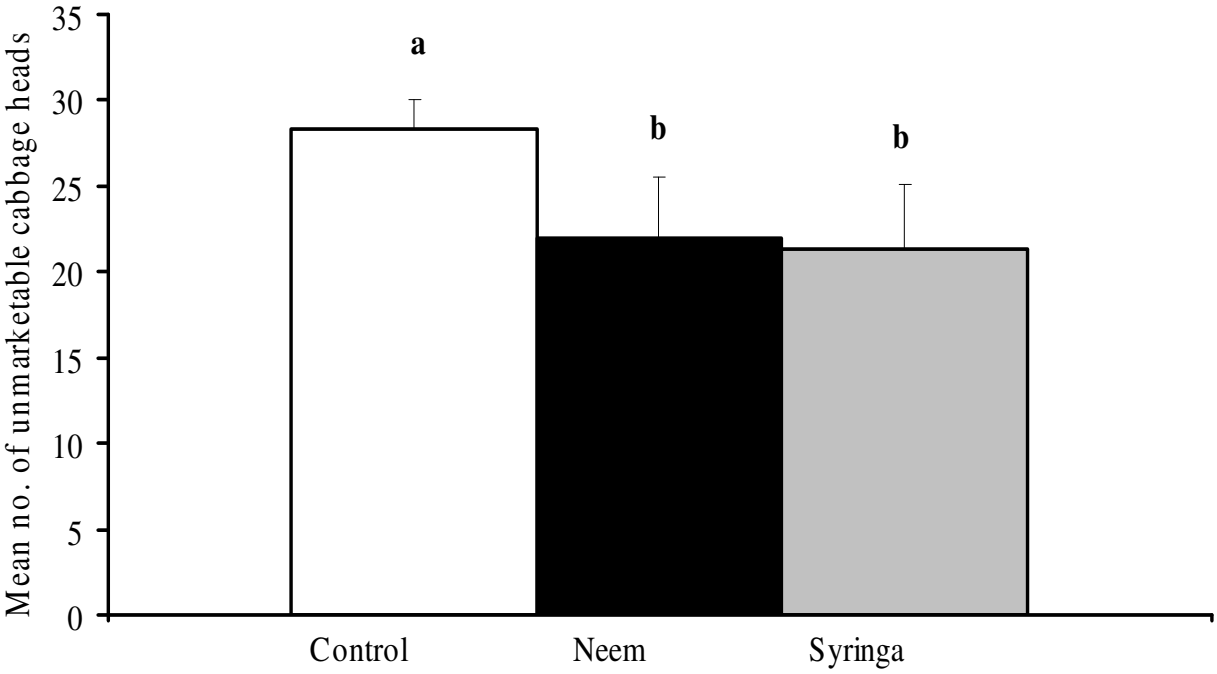

Figure 4. Mean (±SE) number of unmarketable cabbage heads per replicate experiment found at harvest in plots that had been treated with different botanical pesticides. Treatments followed by the same letter are not significantly different (ANOVA; plus Fisher's protected LSD: $\alpha = 0.05$).

A damage category of 8 was the highest possible score, which meant that insect damage to leaves and the head of the cabbage were at a maximum. When the damage categories were compared, the frequencies were found to be significantly different (χ^2 value = 60.3; df = 12; P<0.0001) (Fig. 5). The damage was significantly lower in treated plots than in control plots, with higher numbers of cabbages falling into lower damage categories (1 – 4) than in the control plots where more cabbages fell into higher damage categories (5 – 8). There were no significant differences between the neem and the syringa treatment (χ^2 value = 14.19; df = 6; P=0.05; $\alpha = 0.017$). The damage was significantly lower in the plots treated with neem than in the control plots (χ^2 value = 24.42; df = 6; P<0.001; $\alpha = 0.017$) and also significantly lower in the syringa plots than in the control plots (χ^2 value = 55.51; df = 6; P<0.001; $\alpha = 0.017$).

All the cabbages that were harvested were weighed (approximately 540 per treatment / control). However the weights of the harvested cabbage heads did not differ between the treated plots and the control plots (ANOVA: $F_{0.05(2,2)} = 5.38$; P = 0.16).

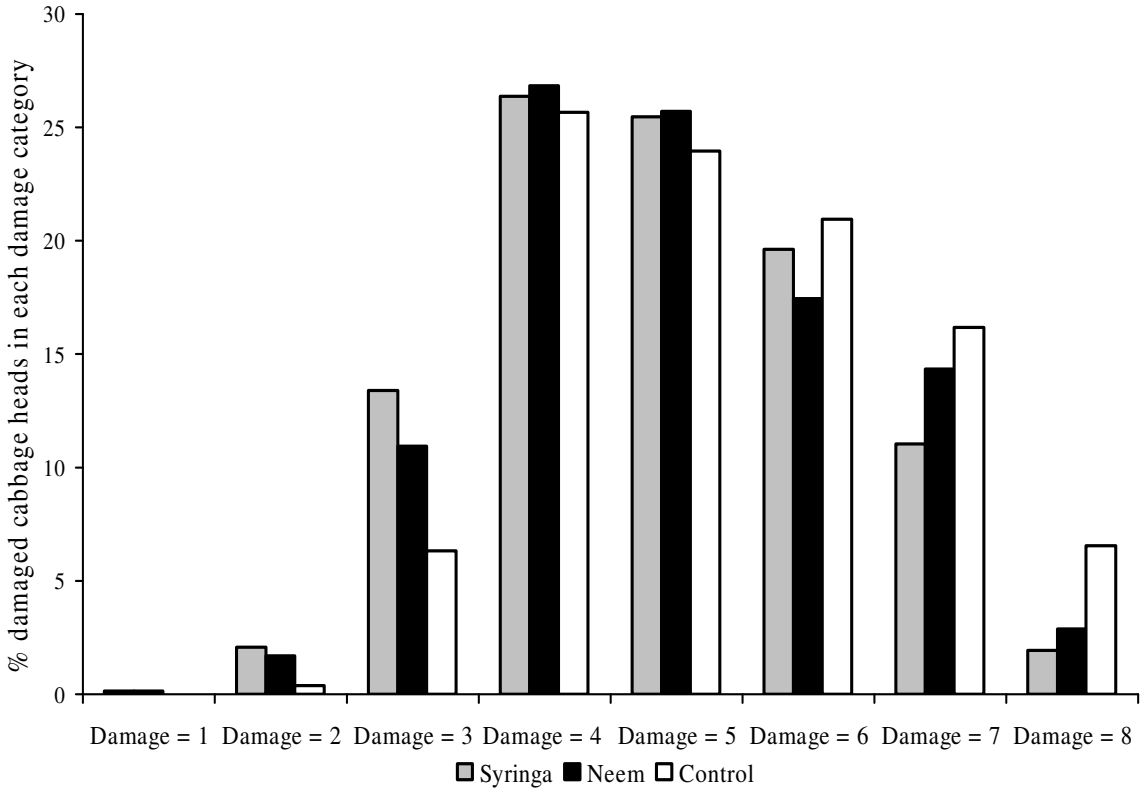

Figure 5. Percentage of damaged cabbage heads falling into particular damage categories: Damage = 1 is lowest category and Damage = 8 is highest category. Cabbages were harvested from plots that had been sprayed with different botanical pesticides. There are significant differences between each of the treatments and control (Chi – Square: $P < 0.05$).

Observations

The numbers of insects observed visiting the different treatments did not differ. There were no significant differences for the number of adult *P. xylostella* (ANOVA: $F_{0.05(2,10)} = 1.81$; $P = 0.21$), nor for the number of parasitoids (ANOVA: $F_{0.05(2,10)} = 0.85$; $P = 0.46$) and nor for the number of predators (ANOVA: $F_{0.05(2,10)} = 1.38$; $P = 0.30$) observed in the different treatments.

However a significantly higher proportion of parasitoids emerged from the *P. xylostella* larvae that were collected from treated plants compared to the control plants (GLM: $P<0.001$) (Fig. 6a). For those samples that were dissected, the differences between the treatments was only just significant at the 5% level (GLM: $P = 0.05$). The proportions parasitised were higher on the treated plants, but these differences were only significant at the 10% level (neem vs control $P = 0.082$; syringa vs control $P = 0.10$) (Fig. 6b). The proportion of parasitoids found emerging after rearing was much lower than the proportion

of parasitoids found during dissections. It is possible that a high proportion of parasitoid larvae died within their *P. xylostella* host before emerging. Another possibility is that despite the random selection of the 50% of *P. xylostella* larvae used in the dissections, this 50% may have still had a higher proportion of parasitoids than the remaining 50%. However, the difference in parasitism was almost equal for all the treatments (± 50%), therefore the botanical pesticides do not appear to have a differential effect on the mortality of the parasitoid larvae.

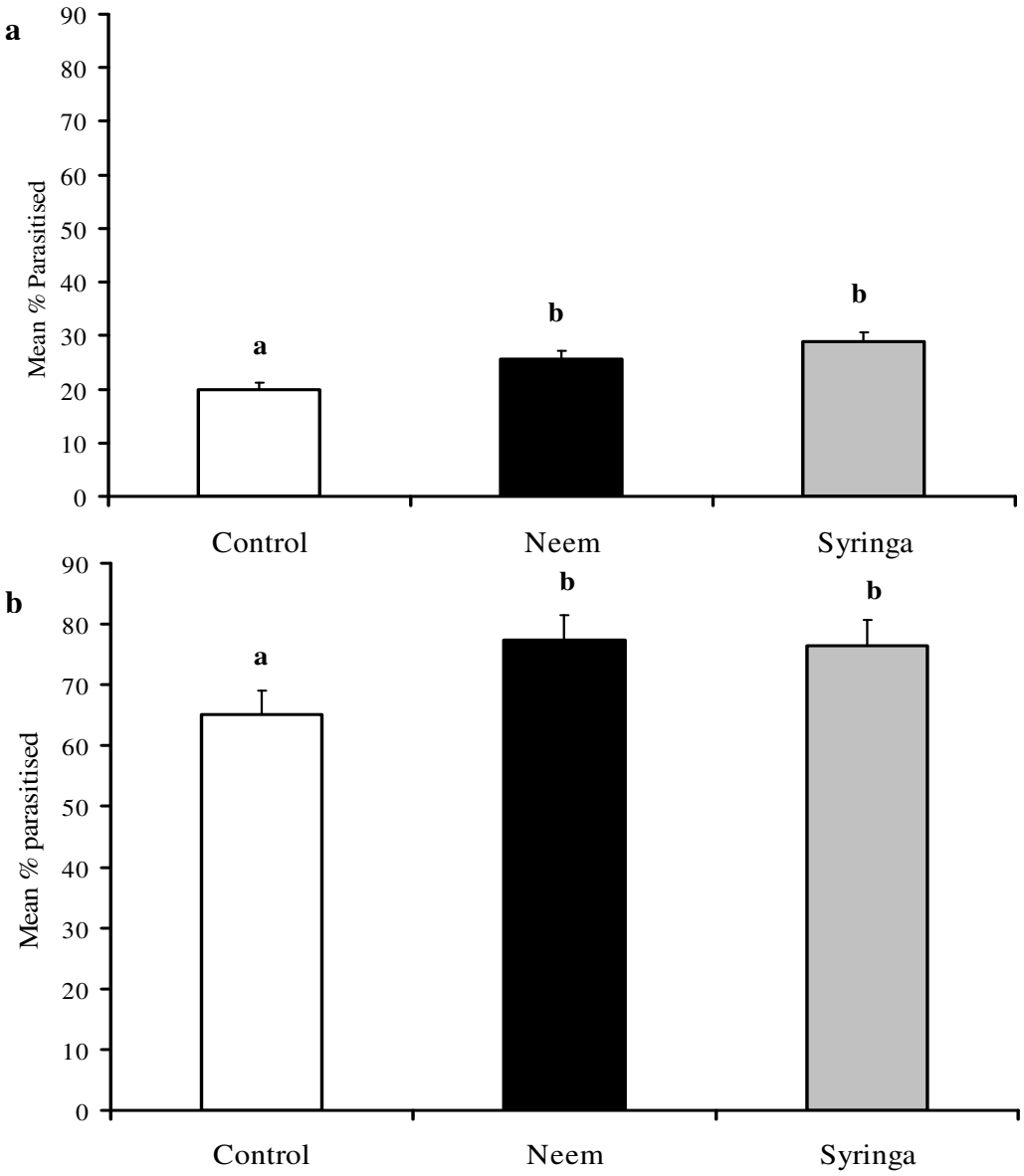

Figure 6. Mean (±SE) percentage of parasitised *P. xylostella* Cabbage plants were treated with different botanical pesticides and then artificially infested with *P. xylostella* and left in the field for three days. Treatments followed by the same letter are not significantly different (GLM: $\alpha = 0.05$).

a. Percentage parasitoids emerging after observation experiments.

b. Percentage parasitoids found during dissection after observation experiments.

Discussion

Results from our earlier laboratory experiments could be used to explain several of the observations that we found in this field trial.

Reduced insect damage in treated plots.
Despite equal population levels of *P. xylostella* in treated and control plots the resulting damage was significantly lower on cabbage plants from the treated plots than in the control plots. This confirms previous laboratory trials (Chapters 2 & 3) where we showed that feeding damage by *P. xylostella* larvae was significantly lower on cabbage plants treated with these botanical pesticides. Other field experiments conducted in crucifer systems using neem products show similar results, with damage to cabbage heads being significantly lower in plots treated with neem products (Saucke *et al.*, 2000; Verkerk & Wright, 1994; and see Schmutterer, 1992c for examples in Togo, the Philippines and Latin America). Although there was less damage in the treated plots, the final weight of the cabbage heads in treated plots did not differ from the weight of cabbages in the control plots. Goudegnon *et al.* (2000) also found that neem extracts reduced damage by *P. xylostella* but that there were no significant differences between cabbage weights in the control and neem treated plots. They speculate that this could be due to heterogeneity of plants or the presence of other pests in the cabbage plots such as the pyralid *Hellula undalis* that fed on the terminal buds of seedlings and produced split heads. In our experiment the main pests found in cabbage were *P. xylostella*, various aphid species, the pentatomid *Bagrada hilaris* and *Thrips tabaci* (see Table 1). In many cases the total populations of these insects were in fact higher on treated plants than on control plants (e.g. *B. brassicae* on syringa vs control), which may have contributed to the resulting similarity in cabbage weight. The higher numbers of pest populations found on the treated cabbage were all due to an increased number of these insects in treated plots later in the season. Damage to the terminal buds of cabbage plants early in the season can cause the plant to develop split heads. The higher infestation of pests on control plants at the start of the season may have resulted in the higher number of unmarketable cabbages with split heads in the control plots.

Reduced populations of P. xylostella *on plants treated with botanical pesticides*.
The laboratory trials suggested that we would find lower populations of *P. xylostella* on the treated plants. However, there were no significant differences between infestation levels of *P. xylostella* populations in the control plots and the plots that had been treated with the botanical pesticides. In laboratory trials the plants are all carefully sprayed to ensure that all parts of the plants are treated, it is not possible to obtain such perfect coverage in the field, and this may provide some refuge for the pest species. It is also possible that the doses of botanical pesticides used in this field experiment were insufficient to reduce the *P. xylostella* populations to the low levels that were expected.

However, the reduced amount of damage found in the treated plots would suggest, that even during periods of high infestation, the reduced feeding by *P. xylostella* larvae on plants treated with botanical pesticides is a more important factor than the actual population density in reducing the damage and improving the yield.

Higher proportions of parasitoids in treated plots

The proportion of parasitised *P. xylostella* was found to be significantly higher in the plots treated with botanical pesticides, with the greatest proportions emerging from *P. xylostella* collected in the plots treated with the syringa extract. Direct observations of parasitoid foraging indicated that parasitoids were still visiting cabbage plants that had been treated with botanical pesticides. The numbers of parasitoids observed visiting the cabbage plants did not differ between control plots and the plots that had been treated with the botanical pesticide, but the proportion of parasitoids emerging from *P. xylostella* after the observations was significantly higher in the treated plots than in the control plots. We found similar results in our studies conducted in a glasshouse (Chapter 4), with significantly higher proportions of *C. plutellae* emerging from *P. xylostella* feeding on cabbages treated with these botanical pesticides. This shows how changes in the first trophic level (e.g. by spraying cabbage plants with botanical pesticides) can influence the third trophic level. Verkerk and Wright (1994) also found that low (sub-lethal) doses of botanical extracts from neem enhanced parasitism success by *Diadegma semiclausum* in the laboratory. They suggested that the increased success of this parasitoid was due to stress-induced impairment of *P. xylostella* immune system. An additional explanation may be that the development of *P. xylostella* is significantly prolonged on plants treated with these botanical pesticides (Chapter 3), which means that *P. xylostella* are available to natural enemies on treated plants for longer periods, which may also enhance parasitism levels. This supports the hypothesis that plant conditions that lower the growth rate of herbivores increase the duration of their availability to natural enemies and hence the probability of mortality (Feeny, 1976). Kaule & Wright (2002) also found increased parasitism on aphid populations feeding on resistant cabbage varieties. Observations in a windtunnel have also shown that *C. plutellae* is significantly more attracted to cabbage plants treated with syringa extracts than to control plants (Chapter 5), which may also explain the higher levels of parasitism observed in this treatment in the field.

The present study provides evidence of a beneficial interaction between botanical pesticides and biological control in the field. In control plots the amount of damage to plants was significantly higher than in the treated plots. In the treated plots, an increased percentage of parasitism in combination with the use of the botanical pesticides was an important factor in reducing the final damage to cabbage plants. These field results confirm results from the laboratory and show that Neemix 4.5® and aqueous leaf extracts from the syringa tree offer good alternative control techniques that could be successfully incorporated with biological control for the management of *P. xylostella*.

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CHAPTER 7

POSSIBILITIES FOR INTRODUCING A BOTANICAL PESTICIDE DERIVED FROM *MELIA AZEDARACH* INTO RURAL FARMING COMMUNITIES IN SOUTH AFRICA

CHAPTER 7

Possibilities for introducing a botanical pesticide derived from *Melia azedarach* into rural farming communities in South Africa.

Abstract

Small-scale agriculture is practised in most parts of Africa. Farmers practising this type of agriculture often cannot afford expensive chemical control and rely on traditional farming and control techniques, as well as naturally occuring biological control. The use of a botanical pesticide that can be made using local tools and trees provides an additional free control technique, which could be combined with biological control, provided the methods are compatible. We have visited four rural villages in the Limpopo province of South Africa and investigated the possibility of introducing aqueous leaf extracts prepared from the syringa tree, *Melia azedarach*, for control of vegetable pests. Results indicate that there are good opportunities for introduction of this technique in areas where rainfall is high, or where villagers have access to water. However, mammalian toxicity testing is vital before the technique can be introduced.

Introduction

Over 815 million people worldwide are estimated as undernourished (Williamson, 2002), and numbers are growing despite the pledges from heads of state at the 1996 World Food Summit to halve the number of hungry people by 2015 (FAO, 2001a). The highest proportion (34%) of undernourished people live in sub-Saharan Africa, the only place where food production per capita has actually declined since the 1970's (Williamson, 2002). Most farmers in sub-Saharan Africa are resource poor in terms of access to natural resources, credit, information and external inputs (van Huis & Meerman, 1997). About 80% of these farmers practise mostly subsistence agriculture on poor soils with uncertain rainfall. These farmers rely on low-input traditional farming and control techniques. Since the beginnings of agriculture, farmers have developed a very wide range of farming practices that contribute either directly or indirectly to pest management, e.g. sanitation, seed selection, rotation, weeding, multiple cropping, tillage, fire, flooding and natural pesticides (Lenné, 2000). From 1900 onwards, and especially in industrialised countries, pest control was increasingly based on chemical pesticides, which led to a number of problems, including elimination of natural enemies and poisoning of farmers and labourers, pesticide resistance and pest resurgence (Jusoh *et al*., 1992). Alternative control techniques were required. This led to the adoption of integrated pest management (IPM), where a number of control techniques were combined, and pesticides were only used as a last resort.

Small-scale agriculture is practiced in most parts of Africa, and usually plots are smaller than two hectares. Legumes, roots, tubers, grains, vegetables and oil crops are often cultivated in home gardens (FAO, 2001b). The extent of these crops is largely influenced by availability of a reliable water source. Agriculture contributes about 3.4% of the total value of the South African economy, although it employs approximately 13% of the economically active population (van Dyk, 2000). In addition, many rural communities have their own form of small-scale agriculture, which provides food for home use. As in other African countries, the most important factor limiting agricultural production in South Africa is the availability of water. Only 10% of the country has an annual precipitation of more than 750 mm (Anonymous, 1989). Integrated pest management (IPM) has a long and mostly successful history in South Africa and is currently practised in many crops throughout the country. Current political climate emphasizes social upliftment and the support of small-scale rural farming communities (Charleston *et al*., 2003). In light of this, IPM has many benefits to offer, by reducing the reliance on chemical pesticides, which many small-scale farmers cannot afford and which are hazardous to their health. Food security is also vital in a country where it is estimated that 16 million people are living in poverty (Simbi, 2001). Agricultural research has a high poverty elimination pay-off (Hossain, 1998) and improved pest management is an essential part of a holistic approach

to crop improvement, substantially contributing to poverty elimination, enhanced livelihood security and reduced environmental degradation (Lenné, 2000).

Ideally, for resource-poor farming, pest management should require no extra cash input from the farmer, as exemplified by naturally occuring biological control. However, biological control is seldom sufficient and often needs to be combined with other control techniques to obtain a successful outcome. Pesticides made from plants (botanical pesticides) also have a number of advantages for the small-scale farmer, provided the plant extracts do not interfere with biological control. During the last 30 years intensive research has identified the plant family Meliaceae as one of the most promising sources of compounds with insect-control properties, and in particular plants from the genera *Azadirachta* and *Melia* (Schmutterer, 1995). Azadirachtin, a complex tetranortriterpenoid obtained from the seed kernels of the neem tree, *Azadirachta indica* Juss. (Meliaceae), has proved to be one of the most promising plant ingredients for IPM. This compound has been reported to have very low toxicity to biological control agents and also to warm-blooded organisms, including man (Schmutterer, 1995; 1997). At high doses it has been shown to have negative impacts on mammalian reproduction, although at the low doses often used it is not considered harmful (Boeke *et al*., 2003b). Unfortunately the neem tree does not grow in South Africa and therefore is not an option for the small-scale farmer. However, the closely related syringa tree, *Melia azedarach* L. (Meliaceae), is an exotic invasive plant and is therefore commonly found throughout the country, especially in disturbed areas. Phytochemical studies (reviewed by Lee *et al*., 1991) emphasize the close similarity between secondary metabolites of *M. azedarach* and *A. indica*, although *M. azedarach* does not contain azadirachtin. Instead, it contains two other potent insecticidal tetranortriterpenoids, a novel meliacin and a new derivative of meliacarpin (Lee *et al*., 1991).

In previous studies (Chapters 1-6) we have shown that extracts made from *M. azedarach* can be used to control *Plutella xylostella* (Lepidoptera: Plutellidae), an important pest of crucifer crops. These extracts reduce the amount of damage to cabbage and appear to be compatible with biological control. When small-scale rural farmers have access to water they often plant small gardens near their homes, and cabbage is often included in these gardens. In this study we investigated the possibilities of farmers making use of simple aqueous leaf extracts made from *M. azedarach* (hereafter referred to as syringa) to control *P. xylostella* in cabbage.

Materials and methods

Botanical pesticides

If a botanical pesticide is to be introduced into a small-scale rural farming community the plants must grow in the area, the extraction techniques must be simple and any other materials required must be easily available. We used a simple hot water extraction technique that does not require any specialised equipment or materials. Leaves were collected from syringa trees and dried in the sun. Once dry, the leaves were removed from the main branches and stems and crushed into a fine powder, and then stored in a container in a cool dark place. When the extract was required, dried powdered leaves were added to hot water and mixed well. The leaves were left soaking overnight in a cool place. The next morning the extract was filtered through a cloth, a few drops of liquid detergent were added and the extract was applied to plants using a small hand-held spray bottle (see Chapters 1-6 for recommended doses). The dried leaves can be stored for long periods of time until they are required.

Participatory Rural appraisal (PRA)

During August and September of 2000 a Participatory Rural Appraisal (PRA) survey was carried out in a number of villages in the Limpopo province of South Africa (Figure 1). Results from the PRA are summarized in each of the village reports below. All tables and historical information were extracted from the PRA report compiled by von Maltitz *et al*. (2000).

During the PRA a number of interviews were conducted and a scoring system was set up. Villagers were asked to list the income sources per household and the crops grown in their village, and then to score the items in the lists according to the relative importance of that item. The groups were given 100 stones, which they distributed between the items, the more important the item the more stones placed next to that item.

Villages visited

During May 2003 we visited four villages in the Limpopo province of South Africa (Figure 1) to investigate the possibilities for introducing the use of plant extracts made from syringa trees to these local communities. These villages covered a variety of altitudes and climatic conditions, from areas with good consistant rainfall to more arid areas where rainfall is limited and sporadic. The villages were chosen from three districts within the province ensuring that we covered a good cross section of the rural farming community.

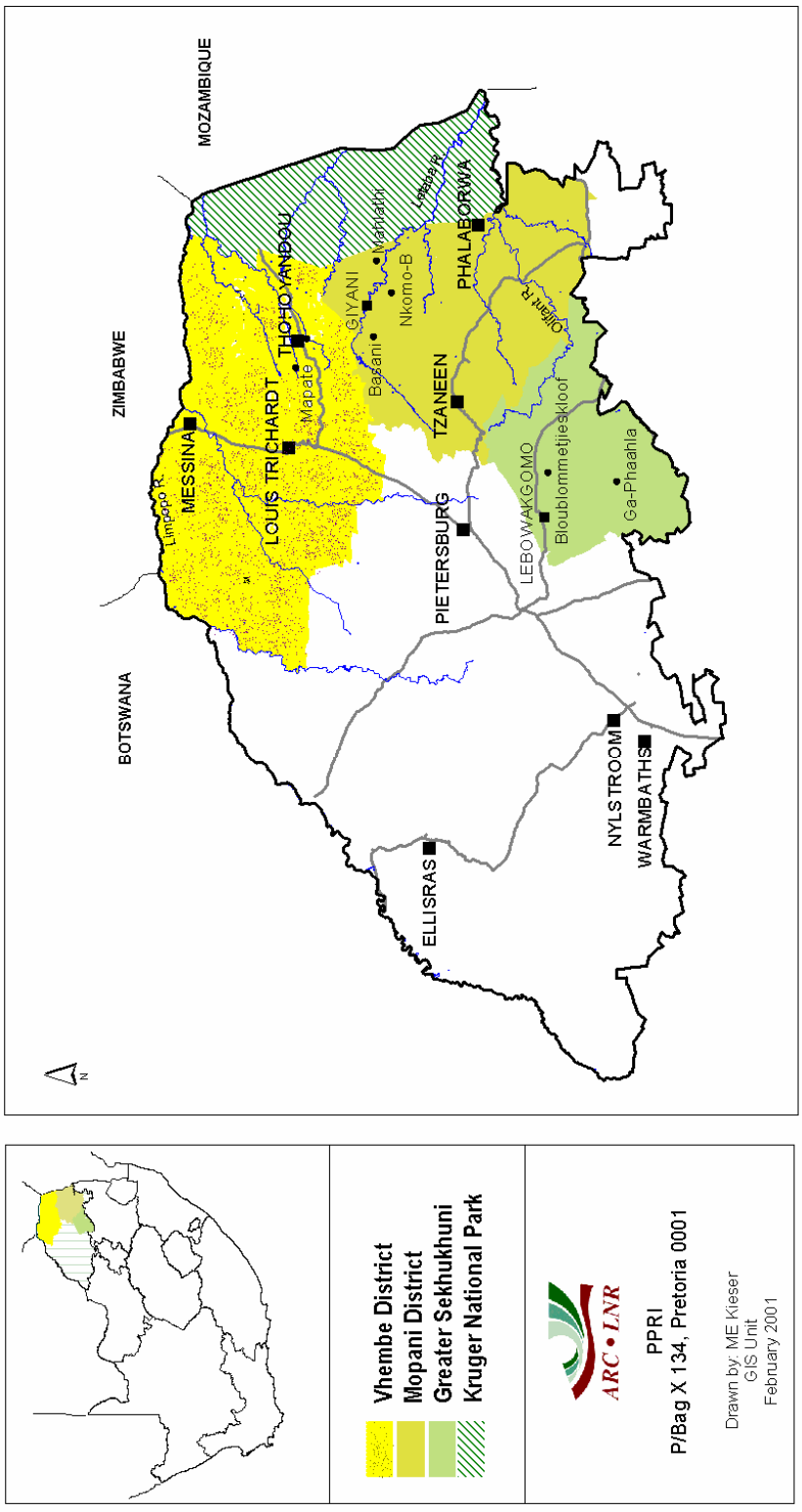

Figure 1. Map showing the Limpopo province of the Republic of South Africa

Mapate
Location: Vhembe district (22° 59,05' S; 30° 21,85' E)
Elevation: 650 – 700 m above sea level
Annual rainfall: 900 – 1050 mm

Mapate originated in 1921 with less than 100 households living scattered in the area. Mapate is nestled in the folds of the Soutpansberg mountain range, and has a sub-tropical climate. It is estimated that, at present, Mapate consists of over a thousand households, of which only 30 have registered with the agricultural extension office as being full-time crop farmers (von Maltitz *et al*., 2000). Housing styles in this village are mainly the more traditional huts, constructed from mud bricks and wooden poles, with grass thatch on the roof (Photograph 1). Rainfall is abundant and a number of people practice a form of home gardening (Photograph 2).

Nkomo - B
Location: Mopani district (23° 24,967'S; 30° 47,133'E)
Elevation: 450 m above sea level
Annual rainfall: 500 – 600 mm

Previously people had lived scattered throughout the area, but were formally relocated into Nkomo in 1968 by the government. This village has a community garden where vegetables are grown. Home gardens are rare and rainfall is limited.

Bloublommetjieskloof
Location: Greater Sekhukhuni district (24° 18,66'S; 29° 46,17'E)
Elevation: 750 – 800 m above sea level
Annual rainfall: 300 – 500 mm

This area consists of three villages situated along the Olifants River in the northwestern corner of the district. Most crops are grown along the banks of the river. This area is very arid and home gardens are restricted to a few plants and some trees planted for shade.

Ga – Phaahla
Location: Greater Sekhukhuni district (24° 41,56'S; 29° 44,14'E)
Elevation: 1150 m above sea level
Annual rainfall: 500 – 650 mm

Farm owners are those who were born between 1922 and 1960. Land sizes range between one and two hectares. Some men have vegetable garden projects along the river; vegetables are sold in the town and the towns nearby. From 1959 there was a change in the building style, houses were built with brick and corrugated iron (Photograph 3). The

people migrating to the cities brought back these new building ideas. The area lacks the grass that is used in thatching and these new materials made building much easier. Home gardens are more prominent in this village, although not as abundant as those in Mapate.

Results

Mapate

Results from the PRA survey (von Maltitz *et al*., 2000) provided a list of the sources of income, and the types of crops grown in this village.

A listing of the income sources per household was done with a combined group of 15 men and 18 women. However, the scoring was carried out separately in each group (Table 1). Agriculture was seen as one of the main sources of income. Cash crops (including cabbage) were rated as important sources of income (Table 1).

The list of crops grown in the village was done with a combined group of 19 women and 12 men. But the scoring exercise was carried out separately in each group. Once separated into the two groups the men decided to add chillies to the list (Table 2).

Two brassica crops are grown in this village, cabbage and a type of Indian mustard (*Brassica juncea*). The leaves of the Indian mustard are harvested and cooked before eating. Leaves are continually harvested with the plant remaining in the field, once the plant starts to sprout the leaves are no longer harvested and the plant is left to produce seed which can be planted again (Photograph 4).

Women ranked Indian mustard (leaves) as their main crop followed by maize and pumpkin (leaves) (Table 2). Men ranked maize as the main crop, followed by pumpkin (leaves) and the vegetable cash crops (Table 2). Sugar cane and fruit were also important crops, although villagers found it difficult to rank these long-term crops, and compare them to the other crops, which were harvested in the same season as planting. Vegetables were planted throughout the year, particularly in the rainy season (March – September).

Mapate has the highest rainfall of the four villages visited and during our visit to this village we saw several gardens with Indian mustard (mutshani). We also met one "commercial farmer" who was planting cabbage on a larger scale (approximately 15000 heads of cabbage, which he had planted in five different areas in small plots). These fields were closer to the main road and outside Mapate itself. The main insect problem the farmer complained of was aphids. A closer inspection of one of his fields showed quite heavy infestation with *Brevicoryne brassicae* (L.). Yet, we did notice that a large number of these had been parasitised and had formed mummies. He treated his cabbages with cypermethrin, a pesticide registered for use on cabbages for the control of *Plutella*

xylostella and *Helicoverpa armigera*. However, the farmer believed that this chemical killed aphids. He also believed that the "white aphids" (mummies) were formed as a result of his spraying this pesticide and that the "white ones were dead". He did not complain of any problems with *P. xylostella*. Inspection of his field did not yield any *P. xylostella* individuals although there was evidence of some infestation, with the presence of feeding damage and some cabbages with split heads (Photograph 5). We also found some dead *C. plutellae*. Recent sprays with cypermethrin may have accounted for both the absence of *P. xylostella* and the dead *C. plutellae* individuals.

Syringa trees were not found in the village itself, but they were extremely abundant along the main roadsides near to the villages, where they were found growing in thickets. The "commercial farmer" had a thicket growing along the edge of the field that we inspected (Photograph 6). People in the village did not know anything about the tree. However, the "commercial farmer" did know about the tree, although he did not know of any use for it.

In Mapate, where rainfall is abundant and the majority of the people practice a form of home gardening, the possibilities of introducing a botanical pesticide are good. Although the syringa tree was not present in the village, there are opportiunities for entrepreneurial individuals to collect the leaves from the trees along the roadside, dry them and sell them to other villagers.

Table 1. Relative importance of income sources in Mapate

Listing of income sources was done with a group of 15 men and 18 women, but the scoring was done separately in each group. Each group was given 100 stones, which were placed according to importance into the various categories.

	Relative importance (score from 100)		
Income source	**Men**	**Women**	**Description**
Farming (vhulumi)	26	47	Subsistence & cash crop
General worker (vhashumi)	28	4	Employed by others
Pension (mundende)	20	9	
Self employed (vhadishumaho)	19	36	Self employed
Traditional healers (Vhumanga)	7	4	
TOTAL	**100**	**100**	

Photograph 1. Housing style in Mapate

Photograph 2. Home gardens in Mapate

Photograph 3. Housing style in Ga-Phaahla

Photograph 4. *Brassica juncea* left to produce seed for replanting.

Photograph 5. Cabbages with split heads, caused by damage to growing points early in the season

Photograph 6. Syringa thickets next to commercial farmer's cabbage field.

Table 2. Relative importance of the crops grown in Mapate
The list of crops was set up by a group of 12 men and 19 women, but the scoring was done separately in each group. Each group was given 100 stones, which were placed according to importance into the various categories.

		Relative importance (score from 100)		
Crop	**Responsibility**	**Men**	**Women**	**Comments**
Maize (mavhele)	Both	25	19	Staple food
Indian mustard (mutshani)	Women	5	22	
Pumpkin (thanga)	Women	14	19	
Cabbage (khavhishi)	Both	11	9	Cash crop
Tomatoes (matamatisi)	Both	13	6	Cash crop
Groundnuts (nduhu)	Both	6	11	
Onion (nyala)	Both	11	4	Cash crop
Swiss chard (spinach)	Both	8	5	Cash crop
Sweet potato (murambo)	Women	2	2	Barter crop
Nightshade (muxwe)	Women	1	3	
Chilies (phiri phiri)	Men	4		Cash crop
TOTAL		**100**	**100**	

Nkomo - B
Results from the PRA survey (von Maltitz *et al*., 2000) provided a list of the sources of income, and the types of crops grown in this village.

A listing of income sources was done with a combined group of 7 men and 21 women. However, the scoring was carried out separately in each group (Table 3). Both men and women ranked farming as the main source of income (Table 3). Informal markets are used to sell vegetables within the community, with the women typically selling from house to house, and the men selling from the back of pick-up trucks (von Maltitz *et al*., 2000).

The list of the crops grown in Nkomo-B was compiled by a combined group of 7 men and 21 women and the scoring exercise was carried out together (Table 4). Maize was the main crop, used both as green maize, which is cooked and used at home or sold, and grain, which was used for home consumption (Table 4). Yields were often sufficient to support a household for a year. Both men and women were responsible for the production of these crops, but harvesting was the responsibility of the women.

Rainfall is lower in this village than in Mapate and the growth of vegetables and home gardens is restricted by a lack of water. Rainfall is highest between September and

December. We did not notice any cabbage at the time of our visit to this village and syringa trees also appeared to be absent. Therefore, the prospects for introducing the use of syringa as a botanical pesticide to this village are limited, as the closest syringa trees are found along the main tar road, about 20 km away.

Table 3. Relative importance of income sources in Nkomo-B

Listing of income sources was done with a group of 7 men and 21 women, but the scoring was done separately in each group. Each group was given 100 stones, which were placed according to importance into the various categories.

		Relative Importance (score from 100)	
Income source	**Responsibility**	**Men**	**Women**
Farming (crops)	Both	31	26
Farming (livestock)	Both	12	15
Remittances	Both	9	9
Informal markets	Both	7	2
Pension	Both	7	13
Roof thatching	Both	5	0
Firewood	Both	4	8
Hand Craft	Women	0	18
Brick making	Both	4	0
Brick laying	Both	7	9
Welding	Men	6	0
Fence making and installation	Both	8	0
Selling used clothes	Both	0	0
TOTAL		**100**	**100**

Table 4. Relative importance of crops grown in Nkomo-B
Listing of income sources was done with a group of 7 men and 21 women. Both groups did the scoring together. The group was given 100 stones, which were placed according to importance into the various categories.

	Relative importance (score from 100)
Crop	**Men and Women**
Maize	24
Groundnuts	10
Njugo beans	9
Water melon	7
Spinach	7
Cabbage	6
Cowpea	6
Pumpkin	6
Okra	6
Tomatoes	6
Butternut	4
Marangha	2
Sweet potatoes	2
Mango	2
Sweet Sorghum	2
Paw-paw	1
Millet	0
Baby marrow	0
TOTAL	**100**

Bloublommetjieskloof
Results from the PRA survey (von Maltitz *et al*., 2000) provided a list of the sources of income, and the types of crops grown in this village.

A listing of income sources was done with a combined group of 9 men and 18 women. However, the scoring was carried out separately in each group (Table 5). In this village farming was not considered as one of the main sources of income. Pensions and salaries from employment were ranked as higher sources of income than farming (Table 5). This is a reflection of the arid habitat in this village. Water is severely restricted and comes mainly from the nearby river and some boreholes in the village.

The list of the crops grown in Bloublommetjieskloof was compiled by a combined group of 6 men and 15 women. The men did the scoring first and the women agreed with their scores (Table 6). Maize and sorghum were rated as the main crops (Table 6). These crops were only sold for cash in emergency situations, as they were mainly grown for home use. Vegetables grown along the riverbank did include some cabbage, but quantities were very small.

During our visit to this village we did not see any home gardens, some households had a few plants mainly fruit trees in their "gardens", but no vegetables. Syringa is found in small numbers in this area, and one household had planted a tree in their garden to provide shade. The owner of this household did not know of any other use for the tree. The possibilities for introducing syringa extracts to this village for the control of agricultural pests on vegetables are limited, mainly by the arid habitat.

Table 5. Relative importance of income sources in Bloublommetjieskloof

Listing of income sources was done with a group of 9 men and 18 women, but the scoring was done separately in each group. Each group was given 100 stones, which were placed according to importance into the various categories.

		Realtive importance (score from 100)	
Income source	**Responsibility**	**Men**	**Women**
Pension (pentshene)	Both	15	27
Mine workers	Men	15	15
Government workers	Both	15	15
Livestock (dipholofolo)	Both	13	9
Farming (temo)	Both	11	8
"Spaza" shops	Both	2	8
Traditional doctors (dingaka)	Men	6	3
Sale of sand (sanda)	Both	5	2
Sale of chickens (dikgogo)	Both	3	3
Taxi and bus drivers	Men	6	1
Compensation (marele) from asbestos mine	Both	3	2
Collect and sell firewood (dikgong)	Both	3	2
Dressmaking (goroka)	Women	3	1
Sale of cosmetics and household detergents	Women	0	2
Brick-making (ditene)	Women	0	1
Maintenance (divorce settlements)	Women	0	1
TOTAL		**100**	**100**

Table 6. Relative importance of crops grown in Bloublommetjieskloof
The list of crops was set up by a group of 6 men and 15 women. The men scored first and the women agreed with scores. The group was given 100 stones, which were placed according to importance into the various categories.

		Relative importance (score from 100)	
Crop	**Responsibility**	**Men and Women**	**Comments**
Maize (mafela)	Both	36	Subsistence (& cash crop)
Sorghum (mabele)	Both	32	Subsistence (& cash crop)
Beans (dinawa)	Both	11	Subsistence
Sweet-sorghum (ntsho)	Women	6	Subsistence & cash crop
Vegetables	Women	5	Subsistence & cash crop
Pumpkin (mafodi)	Women	4	Subsistence
Watermelon (legopu)	Women	4	Subsistence & cash crop
Fruit	Women	2	Fresh from the tree
TOTAL		**100**	

Ga-Phaahla
Results from the PRA survey (von Maltitz *et al*., 2000) provided a list of the sources of income, and the types of crops grown in this village.

A listing of income sources was done with a combined group of 6 men and 8 women. However, the scoring was carried out separately in each group (Table 7). Both men and women scored agriculture as their main source of income. Men also scored pension as a main source of income. Scores varied on the other aspects of income.

The list of the crops grown in Ga-Phaahla was compiled by a combined group of 6 men and 8 women. However, the scoring exercise was carried out separately in each group (Table 8). Both men and women were responsible for the crops. Sorghum and millet were the main crops grown (Table 8). They were used for home consumption, to brew beer and as a cash crop. Maize was used as green maize and mostly grown in home gardens. Water is restricted and other vegetables are only grown on a small scale. During our visit we did not notice any cabbage, although the agricultural extension officer did say that the villagers grow cabbage at other times of the year. Syringa, however, is abundant in the village. Most of the trees are planted for shade or used as a windbreak. The villagers did not know of any other uses for this tree.

Possibilities for introducing the use of syringa extracts to control pest problems in Ga-Phaahla are good. Syringa is abundant and vegetables are grown in the area. PRA survey

results also indicated that one of the main problems villagers complained about was insect pests (von Maltitz *et al*., 2000).

Table 7. Relative importance of income sources in Ga-Phaala

Listing of income sources was done with a group of 6 men and 8 women, but the scoring was done separately in each group. Each group was given 100 stones, which were placed according to importance into the various categories.

		Relative importance (score from 100)	
Income source	**Responsibility**	**Men**	**Women**
Agriculture	Both	30	25
Hand crafts	Both		10
Selling of chickens	Both		
Beer brewing	Women		10
Making mud walls	Both		7
Brick making	Both	10	20
Fruit and vegetable selling	Both	10	
Fencing	Men		
Pension	Both	30	8
Brick laying	Both	20	
Seasonal labour	Women		20
TOTAL		**100**	**100**

Table 8. Relative importance of crops grown in Ga-Phaala
The list of crops was set up by a group of 6 men and 8 women, but the scoring was done separately in each group. Each group was given 100 stones, which were placed according to importance into the various categories.

	Relative Importance (score from 100)	
Crop	**Men**	**Women**
Sorghum	28	20
Millet	28	20
Maize	20	10
Cowpea	10	10
Beans	5	10
China peas	5	10
Njugo beans	2	10
Groundnuts	0	5
Watermelon	2	3
Sweet sorghum	0	1
Pumpkin	0	1
TOTAL	**100**	**100**

Discussion

The prospects for introducing the use of a botanical pesticide to rural communities are good. This field trip indicated that of the four villages surveyed two of them could make use of this technology. Water is the main factor restricting the introduction of this botanical extract. Crucifer crops are limited by water, and are only grown in areas with high rainfall, or good access to piped water. Most of these villages have problems with obtaining water for gardening. Many households rely on water being carried to the house or garden from taps or boreholes within the village, most do not have taps within their own household.

Syringa trees are classified as invasive plants and as such are abundant in most areas of South Africa. If the tree is not present in the village itself it is often abundant in surrounding areas, particularly in disturbed habitats such as roadsides. In the villages that we visited, the people plant these trees for shade or to use as a windbreak, they do not appear to have any cultural beliefs surrounding the trees, nor do the villagers appear to be aware of the insecticidal properties of the syringa tree. This means that additional participatory rural appraisals will need to be carried out and training and technology

transfer would have to be a large part of any project introducing this concept. If this is to be successful the farmers must be involved in the process from the outset.

Before extracts from syringa can be introduced to the rural communities a number of questions will need to be answered. Additional surveys need to be carried out in other parts of the country, to investigate whether the use of syringa extracts could be introduced into other rural areas. Experimentation should look at the possibility of using syringa extracts for controlling pests on other crops, and in particular on maize and sorghum crops, which are part of the staple diets of many rural communities. Perhaps one of the most important aspects that require additional experimentation before syringa extracts can be introduced is the query surrounding the mammalian toxicity of these extracts. The fruit and berries from the syringa tree are reported to be highly toxic to mammals (Schmutterer, 1987, 1992a). In children 6-8 fruits can cause nausea, spasms and choleric symptoms followed by death (Sinniah & Braskaran, 1981). Fortunately leaves from the syringa tree appear to be much less toxic, as they are used as fodder for goats in India (Ascher *et al*., 1995), and are occasionally fed to sheep and goats in India to free them from parasitic worms (Bhandari & Govil, 1978). Although the leaves are reported to be far less toxic it would be vital to investigate this aspect before introducing this technique as a pest management tool.

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CHAPTER 8

SUMMARY AND CONCLUSIONS

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Summary and conclusions

Plants have evolved over some 400 million years and have acquired effective defence mechanisms during evolution, which secure their survival in a hostile environment. Direct plant defence in the form of thick cuticular waxes, thorns and trichomes are easily identified. Less obvious, subtle, defence mechanisms are based on chemicals and secondary metabolites, which protect plants from attack by insects and other herbivorous animals. These secondary metabolites may not cause instantaneous mortality, but their effects are manifested by an adverse impact on normal biochemical and physiological functions (Prakash & Rao, 1997). A large number of different plant species contain natural pesticidal properties, and man has made use of some of these since early times. By applying plant extracts to other susceptible plant species the defence of the susceptible plant is improved, and the use of natural plant products in agroecosystems is emerging as one of the prime means to protect crop produce.

This thesis focusses on the possibility of integrating botanical pesticides with biological control for management of the diamondback moth, *Plutella xylostella* (L.) (Lepidopetra: Plutellidae) in South Africa. *Plutella xylostella* is a major pest of crucifer crops. Female moths lay their eggs on the underside of leaves, and when the larvae hatch they feed on the parenchyma causing substantial damage to the plant, and in some cases cause more than 90% crop loss. In South Africa crucifer crops are grown throughout the year and are a staple food crop for resource poor farming communities. Many of these farmers cannot afford expensive chemical pesticides and pest control is limited to traditional techniques such as intercropping and removal of insects by hand. Fortunately a wide variety of parasitoids are associated with *P. xylostella*, and twenty-one species of primary parasitoids have been collected from *P. xylostella* in the field in South Africa. Biological control therefore provides an additional natural control technique. However, biological control alone is insufficient to provide adequate protection and requires integration with other control techniques. The use of botanical pesticides is widely recognised in other parts of the world for control of *P. xylostella*, but as yet botanical pesticides have not been registered for use in South Africa. Plant products from the Meliaceae family have been widely used to control insect pests, particularly products from the neem tree, *Azadirachta indica* Juss. and the syringa tree, *Melia azedarach* L. The neem tree does not grow in South Africa but the syringa tree is a widespread invasive plant found throughout the country. This thesis investigates the use of a commercial neem product, Neemix 4.5® and aqueous leaf extracts derived from the syringa tree as possible botanical pesticides for integration with biological control in the management of *P. xylostella*.

The impact of neem and syringa extracts on the diamondback moth

The use of neem products for plant protection is well reported, with a number of books and reviews on the topic (Schmutterer, 1995, 1997; Isman *et al*., 1996; Parkash & Rao, 1997; Boeke, 2002). Therefore neem products provide a useful starting point for comparative research. There was, however, very little information concerning the impact of syringa products on *P. xylostella*, and no knowledge on the impact of aqueous leaf extracts from the syringa tree. In the first part of the thesis (chapters 2 and 3) I investigated the impact of Neemix 4.5® and aqueous leaf extracts derived from the syringa tree on the biology and behaviour of *P. xylostella*. The neem and syringa- derived botanical pesticides had adverse effects on the development, reproduction and survival of *P. xylostella*. Treatment with the neem product and the syringa extract resulted in reduced feeding by *P. xylostella* larvae. The syringa extract also had an oviposition repellent effect against female moths, with fewer eggs oviposited on plants that had been treated with the syringa extract compared to control plants. Reduced feeding and oviposition are important factors in pest control and indicate that neem products and syringa extracts have the potential to be incorporated into control programs for *P. xylostella*.

The impact of neem and syringa extracts on natural enemies of the diamondback moth

In general, botanical pesticides have been reported to be harmless to natural enemies (Schmutterer, 1995; Prakash & Rao, 1997). However, there are reports of studies in which botanical pesticides have had a negative impact on natural enemies (Schmutterer, 1995; Boeke *et al*., 2003a; and see Tables 2 and 3 in Chapter 1). If a botanical pesticide is to be combined with biological control it must not hamper natural enemies. In chapters 4 and 5 I investigated the impact of Neemix 4.5® and aqueous leaf extracts derived from the syringa tree on the two most abundant natural enemies of *P. xylostella* found in the field in South Africa, i.e. *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) and *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae). The neem- and syringa- derived botanical pesticides did not have a directly negative impact on the survival of *C. plutellae* and *D. collaris*. The hind tibia length of male *C. plutellae* developing on plants sprayed with the syringa extract was reduced, but it is not known whether this had a negative impact on the resulting fitness of the male parasitoid. In a glasshouse, a significantly higher proportion of *P. xylostella* larvae were parasitised by *C. plutellae* on plants that been treated with the syringa extract than on control plants. Results from a choice test in a windtunnel showed that *C. plutellae* was attracted significantly more often to cabbage plants treated with the syringa extract than to the control plants. Volatile headspace analysis revealed that treatment of cabbage with syringa extracts caused an increased emission of plant volatiles. This increased emission is not a factor of the syringa extract alone, but rather an induced response by the cabbage plant after treatment with the extract. This may explain the increased attraction of *C. plutellae* to plants that have been treated with the syringa extract.

Verifying laboratory results with field trials

Differences between results from laboratory trials and what actually happens in the field can be extensive. Therefore, it is important to verify laboratory data under more realistic conditions in the field. In chapter 6 I conducted a field trial and looked at the effects of the neem product and the syringa extract on herbivore populations, and in particular *P. xylostella*, and their natural enemies. Results from laboratory trials suggested that populations of *P. xylostella* would be lower on plants treated with these botanical pesticides. However, in the field we did not find a difference in infestation levels between the treated and the control plants. Although the pest density is as high on treated plants, as it is on control plants, the damage on plants treated with the botanical pesticides was significantly lower. Therefore, it seems that reduced feeding by *P. xylostella* larvae is a more important factor in the reduction of damage than the actual population density. The proportion of *P. xylostella* larvae that had been parasitised was significantly higher on the treated plants than on the control plants. Direct observations showed that plants that had been treated with neem- and syringa- derived pesticides were still visited by parasitoids. Therefore, these botanical pesticides do not appear to interfere with parasitoid foraging. This confirms results from the laboratory and indicates that there are good possibilities for integrating neem products or aqueous leaf extracts derived from the syringa tree with biological control for management of *P. xylostella*.

The use of syringa extracts by small scale rural farmers in South Africa

Resource-poor farmers often cannot afford synthetic chemical pestcides and rely instead on low-input traditional farming techniques to control insect pest problems. Results from the previous chapters in this thesis indicated that neem products and syringa extracts were compatible with biological control and therefore provided an additional control technique that could be employed by the small-scale rural farmer in South Africa. In chapter 7 I report on my visits to four rural villages in South Africa where I assessed the possibility for introducing this control method. Syringa trees are invasive plants found throughout South Africa and therefore provide a free local resource for the botanical pesticide. Results from this visit indicated that in two of the villages the use of syringa extracts might be introduced. Water is the main factor limiting the introduction of this technique in the two other villages. Due to the arid environment of these two villages crucifer crops were not grown, and in one of the villages syringa trees were also absent.

Future perspectives and a word of caution

Plant products are complex and contain variable mixtures of many chemical compounds with possible negative side effects on humans. Before any recommendations can be made for the introduction of aqueous leaf extracts derived from syringa trees a detailed description of their toxicity is required. The berries or fruit from the syringa tree have been reported to be highly toxic to mammals, especially pigs (Schmutterer, 1987, 1992a). In children 6-8 fruits can cause nausea, spasms and choleric symptoms followed by death

(Sinniah & Braskaran, 1981). Leaf extracts from the syringa tree have not been well investigated for their mammalian toxicity. However, leaves from the syringa tree appear to be much less toxic, as they are used as fodder for goats in India (Ascher *et al*., 1995). Another report by Bhandari and Govil (1978) states that syringa leaves are occasionally fed to sheep and goats in India to free them from worms parasitizing their digestive system. The toxicity of syringa fruits to warm-blooded animals is an obstacle to their use as an insecticide, and although the leaves are reported to be far less toxic it would be vital to investigate this aspect before introducing this technique as a pest management tool.

Neem products are known to be relatively safe to mammals although some negative effects on mammalian reproduction have been reported (Boeke *et al*., 2003b; Coghlan, 2003). Neem has been found to be toxic, but not if it is used at the recommended dose (Boeke *et al*., 2003). Neem products are in fact registered for use as insecticides in many countries (e.g. South and Southeast Asia, the USA, Australia, Kenya, the Netherlands, Spain, Sweden, Costa Rica, Nicaragua) (Immaraju, 1998). However registration of neem products has not taken place in South Africa. Components in neem products incapacitate pests by repelling them, stopping them from feeding or by upsetting their growth, and only indirectly killing them. These varying modes of action and the complex and synergistic mixture of ingredients raise barriers for pesticide registration. The products, which are available commercially in other countries, are often highly purified and contain only an exactly uniform amount of the active ingredient azadirachtin. These products are likely to be more acceptable for registration in South Africa. However, if they were to be registered for use in South Africa they would still be unaffordable for most of the small-scale rural farmers in this country. Therefore the use of aqueous leaf extracts from the syringa tree to control *P. xylostella* would provide an additional useful free resource for the small-scale rural farmer in South Africa.

Results presented in this thesis indicate that biological control and the use of botanical pesticides derived from the neem and syringa trees can be integrated for management of *P. xylostella*. However, mammalian toxicity and residual effects still require extensive investigation before any further recommendations can be made.

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NEDERLANDSE SAMENVATTING

Veel plantensoorten hebben van nature insecten-bestrijdende eigenschappen, en daar heeft de mens reeds lang gebruik van gemaakt. Door plantenextracten toe te passen op vatbare plantensoorten kan de verdediging van vatbare planten verhoogd worden. Dit proefschrift richt zich op de mogelijkheid om plantaardige bestrijdingsmiddelen in combinatie met biologische bestrijding te gebruiken om de koolmot, *Plutella xylostella* (L) (Lepidoptera: Plutellidae) in Zuid-Afrika te beheersen. *Plutella xylostella* is een belangrijke plaag op kruisbloemige gewassen waaraan ze veel schade toebrengt, in sommige gevallen leidt dat tot een verlies aan gewas van meer dan 90 %. In Zuid-Afrika worden kruisbloemige gewassen het gehele jaar door geteeld en ze worden als voedselgewas opgeslagen door boerengemeenschappen die weinig hulpbronnen tot hun beschikking hebben. Veel van deze boeren kunnen zich geen dure chemische bestrijdingsmiddelen veroorloven en het beheersen van plagen is beperkt tot de traditionele technieken. Gelukkig is een grote verscheidenheid aan parasitoiden geassocieerd met *P. xylostella*, en 21 soorten primaire parasitoiden zijn verzameld uit *P. xylostella* in het veld in Zuid-Afrika. Biologische bestrijding biedt dus een bepaalde mate van natuurlijke beheersing, maar die is niet voldoende om het gewas voldoende te beschermen. Daarom is integratie nodig van biologische bestrijding met andere technieken. Plantenproducten van de familie Meliaceae zijn veel toegepast om insectenplagen te beheersen, in het bijzonder producten van de neemboom, *Azadirachta indica* Juss. en de paternosterboom, *Melia azedarach* L. De neemboom groeit niet in Zuid-Afrika maar de paternosterboom is een wijd verspreide invasieve plant die door het gehele land gevonden wordt. Dit proefschrift onderzoekt het gebruik van een commercieel neem product, Neemix 4.5® en bladextracten op waterbasis van de paternosterboom, als mogelijke plantaardige bestrijdingsmiddelen die met biologische bestrijding geïntegreerd kunnen worden om *P. xylostella* plagen te beheersen.

In het eerste deel van het proefschrift (hoofdstuk 2 en 3) heb ik het effect onderzocht van Neemix 4.5®, en bladextracten op waterbasis van de paternosterboom op de biologie en het gedrag van *P. xylostella*. De plantaardige bestrijdingsmiddelen van de neem- en paternosterboom hadden een negatief effect op de ontwikkeling, reproductie en overleving van *P. xylostella*. Deze plantaardige bestrijdingsmiddelen verlaagden ook de vraat en eileg, twee belangrijke factoren in de beheersing van plagen. Om een plantaardig bestrijdingsmiddel te kunnen combineren met biologische bestrijding moet het middel de natuurlijke vijanden niet hinderen. In hoofdstuk 4 en 5 heb ik daarom het effect onderzocht van de plantaardige bestrijdingsmiddelen op de twee natuurlijke vijanden van *P. xylostella* die in het veld in Zuid-Afrika het meest voorkomen, namelijk *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) en *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae). De plantaardige bestrijdingsmiddelen van de neem- en paternosterboom hadden geen directe negatieve invloed op de overleving van *C. plutellae* en *D. collaris*. In de kas werd een significant groter deel van de *P. xylostella* larven geparasiteerd door *C. plutellae* op planten die met het paternosterboom extract behandeld waren dan op controle planten. Resultaten van een keuzetest in een windtunnel lieten zien dat *C. plutellae* significant aangetrokken werd door koolplanten die met het paternoster-extract behandeld waren.. Een analyse

van de vluchtige stoffen toonde aan dat een behandeling met paternosterboom-extract leidde tot een grotere afgifte van vluchtige stoffen door koolplanten. Deze verhoogde afgifte van vluchtige stoffen komt niet door het paternosterboom extract alleen, maar wordt veroorzaakt door een geïnduceerde respons in de koolplant na behandeling met het extract. Dit kan een verklaring zijn voor de verhoogde aantrekking van *C. plutellae* tot koolplanten behandeld met het paternosterboom-extract.

De data die in het laboratorium verzameld waren, zijn geverifieerd onder meer realistische omstandigheden in het veld. In hoofdstuk 6 heb ik een veldproef uitgevoerd en gekeken naar de effecten van het neemboom-product en het paternosterboom-extract op de populaties van herbivoren, in het bijzonder *P. xylostella*, en hun natuurlijke vijanden. In het veld vonden we geen verschil in de mate van aantasting van de behandelde en de controle planten. Hoewel de dichtheid van plaaginsecten even hoog was op de behandelde als op de controle planten, was de schade significant lager op de planten behandeld met plantaardige bestrijdingsmiddelen. Het lijkt daarom dat de verlaagde vraat van *P. xylostella* larven een belangrijkere factor in de verlaging van de schade is dan de eigenlijke populatie dichtheid. Directe waarnemingen lieten zien dat planten die behandeld waren met bestrijdingsmiddelen afkomstig van neem- en paternosterbomen nog steeds bezocht werden door parasitoiden. Het aandeel *P. xylostella* larven dat geparasiteerd was, was significant hoger op de behandelde dan op de controle planten. De plantaardige bestrijdingsmiddelen lijken dus niet te interfereren met het foerageren van parasitoiden. Dit bevestigt de resultaten uit het laboratorium en geeft aan dat er goede mogelijkheden zijn om neemproducten of extracten op waterbasis van de paternosterboom te integreren met biologische bestrijding voor de beheersing van *P. xylostella*.

In hoofdstuk 7 doe ik verslag van mijn bezoeken aan vier landelijke dorpen in Zuid-Afrika waar ik de mogelijkheid om deze bestrijdingsmethode te introduceren heb geëvalueerd. Paternosterbomen zijn invasieve planten in heel Zuid-Afrika en ze verschaffen daarom een gratis lokale bron voor het plantaardige bestrijdingsmiddel. Resultaten van mijn bezoeken gaven aan dat in twee van de dorpen het gebruik van paternosterboom extracten geïntroduceerd zou kunnen worden. Water is de belangrijkste beperkende factor voor de introductie van deze techniek in de twee andere dorpen. De droge omgeving van deze twee dorpen is de belangrijkste reden dat er geen kruisbloemige gewassen geteeld worden, en in één van de dorpen waren paternosterbomen ook afwezig.

De resultaten die in dit proefschrift gepresenteerd werden, geven aan dat biologische bestrijding en het gebruik van plantaardige bestrijdingsmiddelen afkomstig van neem- en paternosterbomen geïntegreerd kunnen worden voor de beheersing van *P. xylostella*. De toxiciteit voor zoogdieren en de effecten van residuen moeten echter nog onderzocht worden voordat een verdere aanbeveling gemaakt kan worden.

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CURRICULUM VITAE

Deidre Suzanne Charleston (neé Ingham) was born on 26 April, 1972 in Lusaka, Zambia. A few months later her parents moved to Malawi, where Deidre spent the first nine years of her life. Almost from the first day she opened her eyes she was surrounded by wildlife, as her father was a nature conservator, which ensured a constant fascination with and love for nature and the outdoors. In 1982 her family moved to South Africa. In 1990, she started her B.Sc. majoring in Zoology and Entomology. Continuing her studies she completed her B.Sc.$_{(hons.)}$ with a study on landscape fragmentation and its impact on invertebrates. For her M.Sc. she looked at applied aspects of insect control in forestry. In 1995 she started working at the Agricultural Research Council - Plant Protection Research Institute (ARC-PPRI), in Pretoria, South Africa. The main focus of her work was on the biological control of *Plutella xylostella*. At the end of 1999 she received the Carolina MacGillavry Ph.D. fellowship from the International Foundation of Science (IFS) and Royal Netherlands Academy of Arts and Sciences (KNAW) and started working on a Ph.D. project at the Laboratory of Entomology at Wageningen University. The practical work was carried out in South Africa at ARC-PPRI. Her studies are now complete and the result of her project lies before you.

LIST OF PUBLICATIONS

Ingham, D.S & M.J. Samways 1996. Application of fragmentation and variegation models to epigaeic invertebrates in South Africa. *Conservation Biology* 10: 1353-1358

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