Oviposition site preference of lacewings in maize ecosystems and the effect of Bt maize on *Chrysoperla pudica* (Neuroptera: Chrysopidae)

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Abstract

Resistance development and possible non-target effects have been of concern since the first deployment of genetically modified crops with insecticidal properties. It is especially at the third trophic level and with important predators such as lacewings (*Chrysoperla* spp.) (Neuroptera: Chrysopidae) where negative effects of *Cry* 1Ab protein could have adverse effects in agro-ecosystems. Monitoring of the effect of genetically modified Bt maize on non-target organisms is required by law in South-Africa. Neuroptera are excellent indicators of environmental and habitat transformation, and also include key species for signifying areas and faunas that require priority protection. Monitoring techniques, especially for insect eggs, are often labour intensive and time consuming. A study was conducted to determine the preferred oviposition site of *Chrysoperla* spp. on maize plants to facilitate time-effective searching for eggs of these beneficial insects. Furthermore we determined if the presence of aphids on plants influenced *Chrysoperla* spp. oviposition preference. Another study was conducted to evaluate the effect of indirect exposure of *C. pudica* to *Cry* 1Ab protein, through healthy Bt-maize feeding prey, on its biology. Daily flight activity patterns and the height at which chrysopid adults fly above the crop canopy were also determined, as well as the movement of adult *Chrysoperla* spp. between maize fields and adjacent headlands. A clear spatial oviposition pattern was observed on maize plants and oviposition was not random as reported in earlier studies. This data facilitates rapid monitoring of the presence of eggs in maize cropping systems and is also of use in general pest management. Choice-test data showed that females responded positively to host plants that were infested with aphids. Feeding studies in which *C. pudica* larvae were indirectly exposed to Bt-toxin at the 3rd trophic level, showed a limited effect of Bt-toxin on only a few of the parameters that were evaluated. The pupal period and percentage adult emergence of larvae exposed to an unusually high amount of Bt-toxin was significantly shorter and lower respectively than that of the control group. The overall result of this study, in which the possible effect of food quality (prey) was excluded, showed that *Cry* 1Ab protein had

an adverse affect only on certain fitness components during the life cycle of *C. pudica*. However, since this study represented a worst-case scenario where diverse prey was not available to *C. pudica*, negligible effects is expected under field conditions where prey is more diverse. It was determined that chrysopids was most active between 16:00 - 23:00 and that they fly largely between 0.5 m - 2.5 m above ground level. An attempt was also made to quantify migration between different vegetations types. This part was terminated because of bad weather conditions at several occasions when the experiment was attempted. Chrysopids were never present in grassland vegetation, but an adjacent lucerne field maintained a large population. As the maize crop developed chrysopid population numbers increased inside the field, presumably originating from the lucerne field.

Uittreksel

Weerstandsontwikkeling en moontlike nie-teikeneffekte is vanaf die eerste ontplooiing van geneties-gemodifiseerde (GM) gewasse met insekdodende eienskappe, as risiko uitgewys. Dit is veral op die derde trofiese vlak en met belangrike predatore soos goudogies (*Chrysoperla* spp.) (Neuroptera: Chrysopidae) waar negatiewe gevolge van *Cry* 1Ab proteïen nadelige gevolge kan hê in landbou-ekosisteme. Monitering van die effek van GM Bt-mielies op nie-teiken organismes word volgens wetgewing in Suid-Afrika vereis. Neuroptera is uitstekende indikatore van omgewing- en habitat-transformasie. Moniterings-tegnieke vir insekeiers is gewoonlik intensief en tydrowend. 'n Studie is gedoen om die eierleggings-posisie van *Chrysoperla* spesies op mielieplante te bepaal om sodoende tyd-effektiewe monitering te fasiliteer. Verder is bepaal dat die teenwoordigheid van plantluise op mielieplante die eierlegging-posisie van *Chrysoperla* spesies positief beïnvloed. 'n Studie is ook gedoen om die effek van indirekte blootstelling aan *Cry* 1Ab proteïen op die biologie van *C. pudica* te bepaal. Hierdie studie is gedoen deur Bt-weerstandbiedende gesonde stamboorderlarwes aan *C. pudica* larwes te voer. Daaglikse vlugaktiwiteitspatrone, asook die hoogte wat chrysopid volwassenes bokant gewasse vlieg is bepaal. Verder is die beweging van volwasse *Chrysoperla* spesies tussen 'n mielieland en die omliggende habitatte bepaal. 'n Duidelike eierleggings-patroon is op mielieplante waargeneem en daar is waargeneem dat eierlegging nie ewekansig is soos in vorige studies gerapporteer is nie. Hierdie data fasiliteer vinnige monitering vir die teenwoordigheid van eiers in mielielande en kan ook ‘n rol speel in algemene plaagbestuursbesluite. Keusetoetse het getoon dat chrysopid-wyfies positief reageer teenoor gasheerplante wat met plantluise besmet is. Voedingstudies waar *C. pudica* larwes blootgestel was aan die Bt-toksien het getoon dat die toksien slegs sekere parameters van chrysopid biologie beïnvloed het. Die papieperiode en die persentasie volwasse individue wat suksesvol uit kokonne verskyn het, was onderskeidelik aansienlik korter en laer vir larwes wat blootgestel was aan ‘n buitengewoon-hoë konsentrasie van die Bt-toksien. Hierdie studie, waarin die moontlike uitwerking van

voedselkwaliteit uitgesluit is, het getoon dat *Cry* 1Ab proteïen ‘n nadelige invloed het op slegs sekere aspekte van die lewenssiklus van *C. pudica*. Aangesien hierdie studie die ergste scenario verteenwoordig waar slegs een voedselbron vir *C. pudica* beskikbaar was, word weglaatbaar-klein effekte onder veldtoestande verwag waar prooi meer divers is. Daar is vasgestel dat chrysopids die meeste aktief was tussen 16:00-23:00 en dat hulle grootliks tussen 0.5 m – 2.5 m bo die grond oppervlak vlieg. 'n Poging is ook aangewend om migrasie tussen die verskillende habitat-tipes te kwantifiseer. Hierdie aspek van die studie is egter beëindig as gevolg van slegte weerstoestande wat voorgekom het tydens die studie. Chrysopids is nooit in monsters gekry wat in die grasveld geneem is nie, maar die aangrensende lusern het groot getalle chrysopids onderhou. Soos wat die mielie-aanplanting ontwikkel het, het chrysopidgetalle toegeneem, waarvan die individue vermoedelik afkomstig was van die naburige lusernland.

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Chapter 1

Introduction and literature overview

1.1 Bt maize in South Africa, history and effect

Maize is the most important crop in South-Africa and it is therefore important that maize production is done in a sustainable way. The major pests of maize are the stem borers *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) which can cause between 10-100% yield loss, depending on planting date (Kfir *et al*., 2002).

Because of the susceptibility of maize to insect pests, especially Lepidoptera, and the negative effects of continuous use of insecticides on ecosystems, genetically modified (GM) maize was developed to control certain insect pests. New developments in agricultural biotechnology are being used to increase the productivity of crops through reducing the cost of production by decreasing the need for pesticides.

The adoption of GM crops in world agriculture differs largely between countries. Eight countries plant biotech crops on more than 1 million hectares each and the rapid adoption across all continents provide a very broad and stable foundation for future global use of GM crops (James, 2009). Based on surface area South-Africa is ranked number eight in the world (Fig. 1.1), with a total GM crop area of 2.1 million hectares in 2008. This was a 30% increase over the 1.4 million hectares in 2006 (James, 2009). This very high adoption rate by farmers reflects the fact that GM crops have consistently performed well and delivered significant economic, environmental health and social benefits (James, 2009).

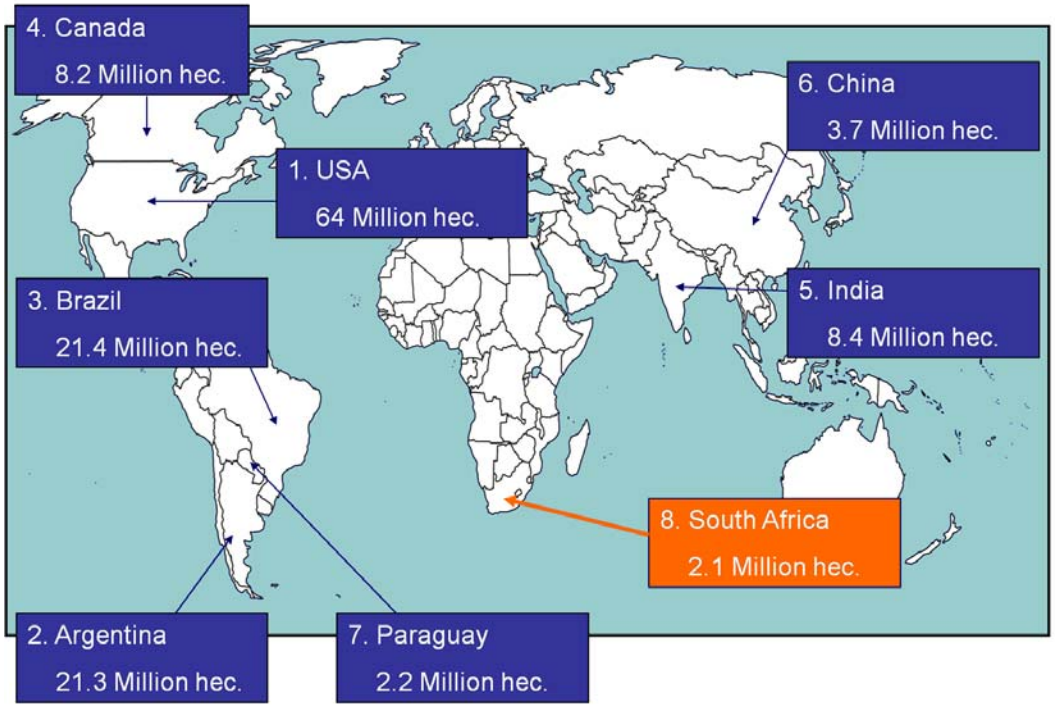

Fig.1.1 Global map of biotech crop countries and mega-countries in 2009 (James, 2009).

Bt maize was commercially released in South Africa during 1998 after testing of experimental Bt-events for the control of stem borers commenced in 1994 (Van Rensburg, 2007).

1.2 Resistance development

Since the first deployment of GM crops with insecticidal properties, there has been concern with regard to resistance development of target pests and possible non-target effects (Meeusen & Warren, 1989; Tabashnik, 1994; Gould, 1998; Dutton *et al*., 2002). Although Bt proteins are considered safe due to their selective mode of action, there are concerns due to the continuous expression of the insecticidal protein in most plant tissues throughout the growing season (Dutton *et al.*, 2002).

Prior to 1994 microbial preparations of the entomopathogenic bacterium *Bacillus thuringiensis* (Bt) applied as spray formulations, had been in use for decades without resistance development (Tabashnik, 1994). The only insect to eventually develop resistance to Bt applied as a biopesticide was the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Ferré & Rie, 2002). More than 62 million ha Bt crops were planted world wide between 1996 and 2002 and pest populations were considered to be under pronounced selection pressure to evolve resistance (Tabashnik *et al.,* 2003). The first report of field resistance by *B. fusca* to Bt maize was in 2006 in Christiana in the Northern Cape province in South Africa (Van Rensburg, 2007). Since then another report of field resistance was made in the Vaalharts irrigation scheme (Kruger *et al.,* 2009).

1.3 Advantages and disadvantages of GM maize

In assessing the advantages and disadvantages involved in the use of modern biotechnology, there are a series of issues to be addressed so that informed decisions may be made on the appropriateness of the use of this technology. These issues include risk assessment and risk management within an effective regulatory system, as well as the role of intellectual property management in rewarding local innovation and enabling access to technology developed by others (Cook, 1999).

The evaluation of the environmental impact of transgenic organisms often centres on the risks attached to them. This is justified, as this new large scale technology may have risks and unforeseen consequences. However, a number of arguments have suggested a positive environmental impact from large-scale production of transgenic plants (Wolfenbarger & Phifer, 2000).

1.3.1 Advantages of transgenic crops with insecticidal properties

GM crops are engineered to tolerate biotic stresses resulting in subsequent reduced losses. This may in turn result in reduced surface areas needed for

crop production and protection of the environment (Harris, 2010; Lövei, 2001; Persley & Siedow, 1999).

Planting of transgenic crops may result in decreased use of harmful wide spectrum herbicides and pesticides (Ismael *et al.,* 2001). GM crops can be considered environmentally friendly since the "pesticides" produced by the plant are not released into the air, soil and water (Wolfenbarger & Phifer, 2000; Csanad, 2010). However, recent studies found that *Cry* 1Ab proteins can enter the soil via pollen drift, plant residues after harvest, as well as root exudates and decomposition of dead plant material (Zwahlen *et al*., 2007). During plant growth *Cry* 1Ab protein is released by roots and persists in soil at least until the occurrence of the first frost (Saxena *et al*., 1999; Saxena & Stotzky, 2000; Saxena & Stotzky, 2001).

Meeusen and Warren (1989) indicated several advantages of using Bt endotoxin-producing crops for the control of lepidopterous pests. By growing Bt crops, control is no longer affected by the weather. The crop is protected even when field conditions do not allow spray equipment to enter into fields or the weather is too severe to allow aerial applications (Meeusen & Warren, 1989). Another advantage is the protection of plant parts such as roots, shaded lower leaves and new growth that emerges between applications and which are difficult to reach with insecticide sprays. The crop is also protected continuously in the field and scouting may no longer be needed since the endotoxin is produced inside plant tissues. Bt crops also do not require any specialized equipment and could therefore be effective on farms of all sizes (Meeusen & Warren, 1989).

1.3.2 Disadvantages of transgenic crops with insecticidal properties

Maize is wind pollinated and can be cross-pollinated with maize pollen from fields within several hundred meters. There is a strong possibility that cross-pollination can take place and gene escape from GM crop fields has been recognised as a potential significant hazard for many crops (Wolfenbarger & Phifer, 2000). Out-crossing and hybridisation with wild relatives is possible in

areas where close relatives occur. Cross pollination whereby pollen from GM crops spreads to non-GM crops in nearby fields, may allow the spread of traits such as herbicide-tolerance or insecticidal characteristics from GM plants to non-GM plants (Persley & Siedow, 1999). This is, however, not a possibility with maize in Africa since it is an exotic species of the continent.

Other potential ecological risks stem from the widespread use of GM maize and cotton insecticidal genes from *B. thuringiensis* (Bt gene). This may lead to the development of resistance to Bt in insect populations exposed to GM crops (Persley & Siedow, 1999). Although Meeusen and Warren (1989) stated that resistance to Bt toxin would not develop rapidly, this phenomenon became a reality and necessitated changes back to the use of wide spectrum insecticides only eight years after Bt maize was commercialized in South Africa (Van Rensburg, 2007).

There are also risks to non-target organisms and natural enemies. Insect-resistant Bt crops are aimed to reduce the densities of certain phytophagous pests. However, these pests also serve as prey for a range of natural enemies and if the prey is reduced, densities of the natural enemies may also be reduced (Lövei, 2001).

1.4 Effects of GM crops on non-target species

1.4.1 *The fate of Bt protein in the environment*: *Different levels of exposure*

The biodiversity of an agro-ecosystem is not only important for its intrinsic value, but also because it influences ecological functions that are vital for crop production in sustainable agricultural systems (Hilbeck *et al.,* 2006). Species assemblages in an agro-ecosystem fulfil a variety of ecosystem functions that may be harmed if changed (Dutton *et al.,* 2003). Guild rearrangements due to the elimination of target or non-target pests and subsequent changes in guild structure can lead to development of secondary pests. For this reason it is essential to assess the potential environmental risk that the release of GM crops may hold (Van Wyk *et al.,* 2007).

Resistant plants, whether produced through conventional breeding or biotechnology, can potentially affect natural enemies in many different ways and their interactions with these beneficial arthropods may be additive, antagonistic or synergistic for pest control (Schuler, 2004). Target pests can be directly affected if they are susceptible to the transgenic product (2nd trophic level). Natural enemies, however, may be affected indirectly via changes in prey quality, prey behaviour or plant activity. At population level, natural enemies can also be affected by reduction in prey availability and through changes in crop management practices (Schuler, 2004). The potential effects that insecticidal proteins may have on ton-target organisms depend on the level of exposure to *Cry* proteins, which is affected by the specific exposure pathway (Schuler, 2004).

Exposure pathways to Bt toxins produced by Bt maize plants are explained in more detail in Figure 1.2 (Romeis *et al.*, 2009):

Exposure pathway 1: Ingesting insecticidal protein by feeding directly on the Bt plant. This pathway depends on both the herbivores' mode of feeding and on the site and time of protein expression in the plant (Dutton *et al.,* 2003). Chewing herbivores and herbivores with piercing-sucking mouth parts are exposed to insecticidal protein (Dutton *et al.,* 2002). In contrast phloem-sap feeders such as aphids do not ingest insecticidal protein (Raps *et al.,* 2001) (discussed in 1.4.3).

Exposure pathway 2: This pathway is facilitated in the case of wind-pollinated plants such as maize. Pollen can expose non-target organisms to the insecticidal protein both within and beyond the crop borders.

Exposure pathway 3: In contrast to Bt crops, certain experimental plants expressing lectins are known to transport insecticidal proteins in the phloem. When sap-feeding Hemiptera feed on such plants the insecticidal proteins are likely to appear in their honeydew (Shi *et al.,* 1994; Kanrar *et al.,* 2002).

Exposure pathway 4: The major pathway of exposure to entomophagous arthropods is through their prey or host. Usually the prey or host is a herbivore that feeds on the GM plant, but may also be other entomophagous species. In either case exposure through prey or host organisms is highly variable and difficult to predict.

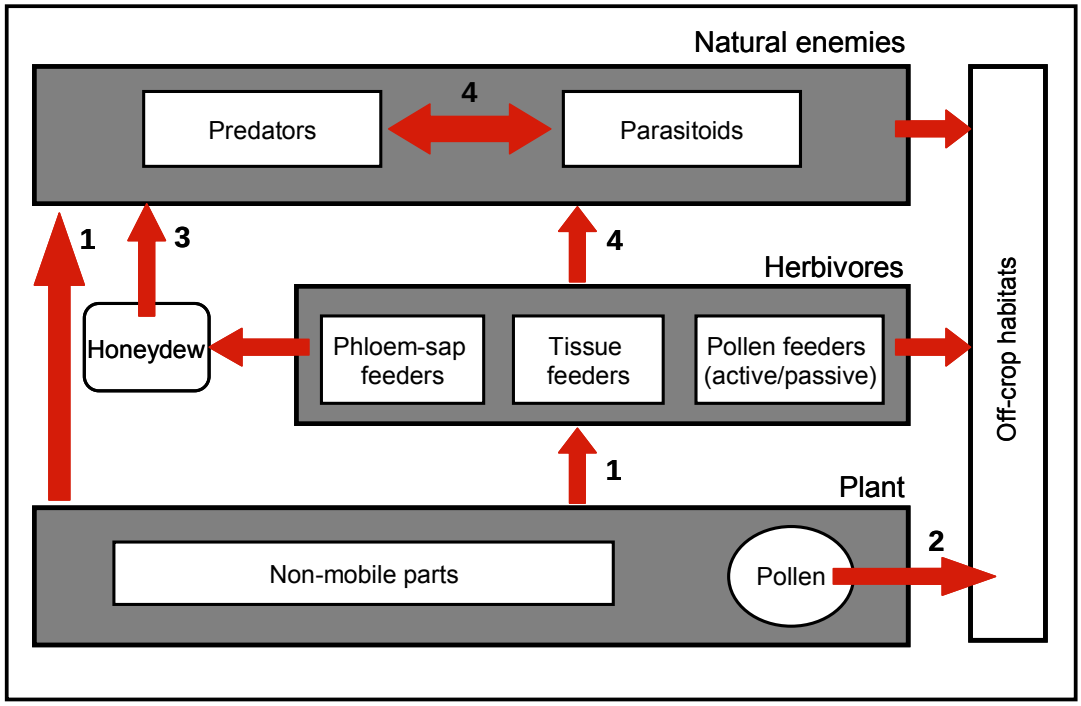

Fig.1.2 Exposure pathways through which non-target arthropods can be exposed to insecticidal proteins expressed by Bt plants (Romeis *et al*., 2009).

1.4.2 Non-target effects of GM crops

It is difficult to make generalizations about the non-target effects of the *B. thuringiensis* bacteria because of the large number of strains and toxins that have been isolated for insect control (Hellmich *et al.*, 2004). Crops that produce these toxins to control some key pests are planted on millions of hectares. The toxins are produced in Bt plants throughout the entire growing season. Target and non-target arthropods therefore have the opportunity to encounter Bt toxins on a continuous basis. This has raised the issue of whether

widespread adoption of Bt crops reduces arthropod abundance and diversity (Sisterson *et al*., 2004).

Many studies have been done on different non-target organisms, including mammals, birds, fish, and arthropods. Vertebrates are exposed to Bt proteins through direct consumption of Bt plant material or indirectly by consuming herbivores feeding on these plants. There is not much reason to expect toxicity to these organisms (Clark *et al.,* 2005). The normal mode of toxic action for the protein is very unlikely to occur in the vertebrate digestive system, and the protein has been used in direct testing with mammals (Siegel, 1987; McClintock, 1995) with no adverse effects reported.

One of the most important examples of the adverse effects that Bt could have on a non-target organism is that of the monarch butterfly, *Danaus plexippus* (L.) (Lepidoptera: Danaidae). Losey *et al*. (1999) exposed larvae of the monarch butterfly to leaves of tropical milkweed plant dusted with pollen from Bt maize. When compared to larvae that fed on leaves with no pollen or leaves with pollen from non-Bt maize, larvae consuming leaves treated with Bt maize pollen consumed less material, weighed less, and had higher mortality. It was subsequently suggested that maize pollen drifting onto the monarch's primary host plant, could pose a danger to monarch butterfly populations in areas of the United States where Bt maize is grown (Losey *et al.*, 1999).

1.4.3 Effect of Bt toxins on aphids

Maize is often infested by aphids such as *Rhopalosiphum* spp. (Homoptera: Aphididae). Aphids are important prey for beneficial insects such as Coccinellidae and Chrysopidae. Because aphids are obligatory phloem sap feeders, the question whether *Cry* 1Ab toxin is present in the phloem sap of Bt crop is of great ecological relevance (Raps *et al.,* 2001).

Raps *et al.* (2001) tested whether *Cry* 1Ab toxin is translocated into the phloem sap of Bt maize and whether it appears inside aphids and in their honeydew. No *Cry* 1Ab protein could be detected either in the phloem sap or in honeydew

of *R. padi* (L.). It was subsequently concluded that *R. padi* on Bt maize represent no hazard as a Bt-containing prey to the beneficial insects (Raps *et al*., 2001).

Head *et al.* (2001) observed that inside *R. maidis* (Fitch) feeding on diet solutions containing *Cry* 1Ab protein, the level of the protein was 250-500 times less than the original levels in the diet, whereas no *Cry* 1Ab was detected in aphids feeding directly on Bt maize. Lozzia *et al.* (1998) also did not find negative effects of exposure to *Cry* 1Ab protein on biological parameters of *R. padi.*

1.5 *Chrysoperla* spp. biology, importance as biocontrol agents and indicator species for *Cry* 1Ab protein toxicity testing

1.5.1 Biology of Chrysoperla spp.

Skaife (1979) described *Chrysoperla* as “the pretty little green insects with eyes of a yellow, metallic lustre”. These insects are commonly known as green lacewings or golden-eyes. They belong to the order Neuroptera and family Chrysopidae (Fig. 1.3A). Closely related families are the Coniopterygidae, commonly known as Dusty-winged lacewings (Fig. 1.3C) and Hemerobiidae, commonly known as Brown lacewings (Fig. 1.3B).

Fig. 1.3 A) Green lacewing (Chrysopidae) (Anon, 2010), B) Brown lacewing (Hemerobiidae) (Leung, 2005) and C) Dusty-winged lacewing (Coniopterygidae) (Anon, 2010). Eggs of chrysopids are unique to the family and are laid on stalks (Fig. 1.4). The advantages of the eggs being laid on stalks is that it minimizes the chance of being found by crawling predators. *Chrysoperla* larvae are also fiercely cannibalistic and by laying eggs on these stalks it reduces the possibility of the larvae coming into contact with eggs. Therefore they have a better chance of survival than if the eggs were simply laid on leaf surfaces (Skaife, 1979).

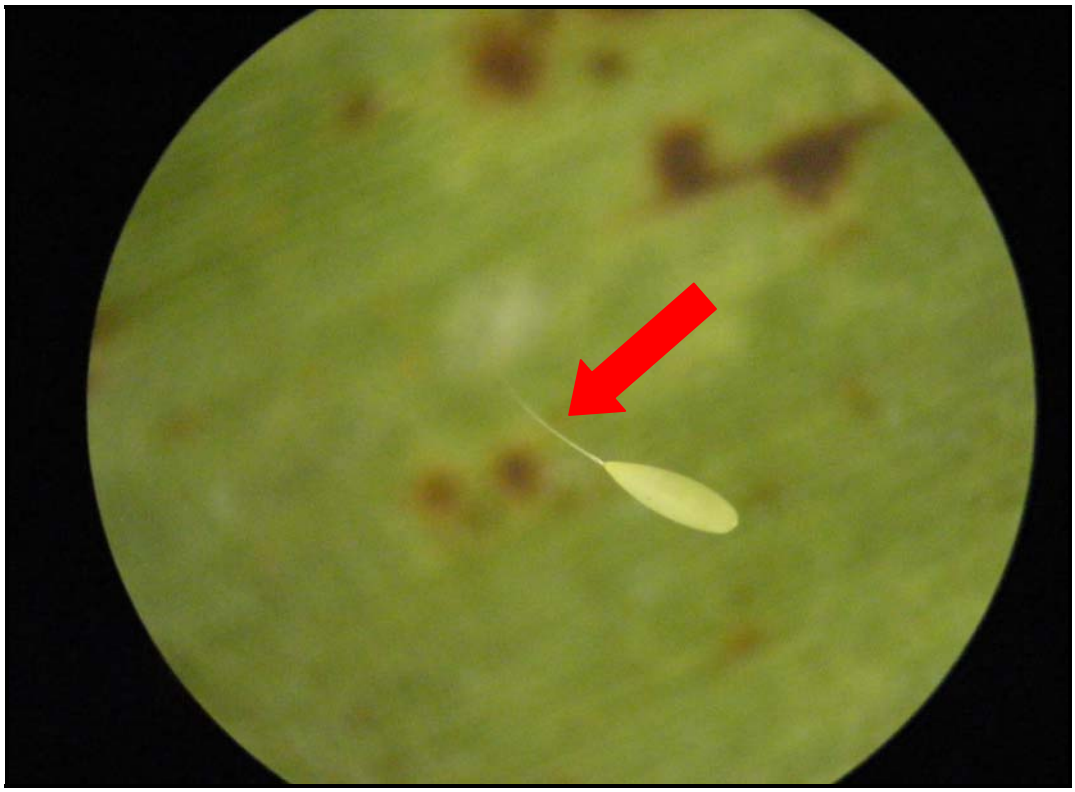

Fig. 1.4 An egg of *Chrysoperla pudica*, laid on a stalk indicated by the red arrow.

To lay the egg the female touches the leaf surface with the tip of her abdomen and ejects a drop of sticky fluid from glands that are associated with her ovaries. With a lift of the tip of her abdomen the fluid substance draws out in a thread which rapidly hardens in the air. On top of this thread she deposits the egg (Skaife, 1979). Eggs can be deposited in different patterns. They can either be laid as single eggs (Fig. 1.5), in batches (Fig. 1.6) or in clusters (Fig. 1.7).

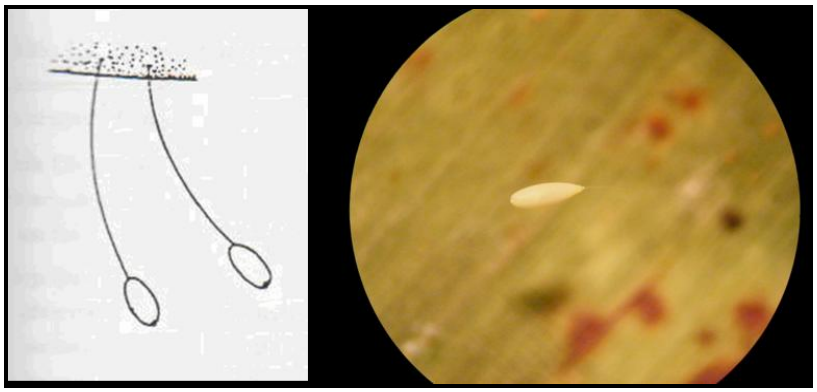

Fig. 1.5 Schematic (Monserrat *et al.,* 2007) and photo presentation of a single chrysopid egg.

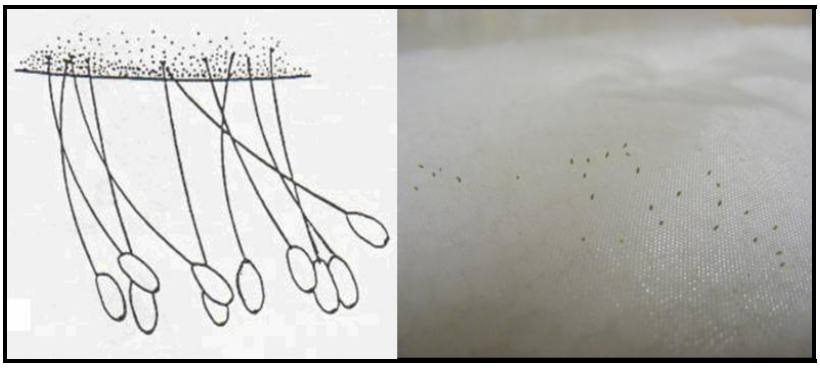

Fig. 1.6 Schematic (Monserrat *et al.,* 2007) and photo presentation of chrysopid eggs that were laid in batches.

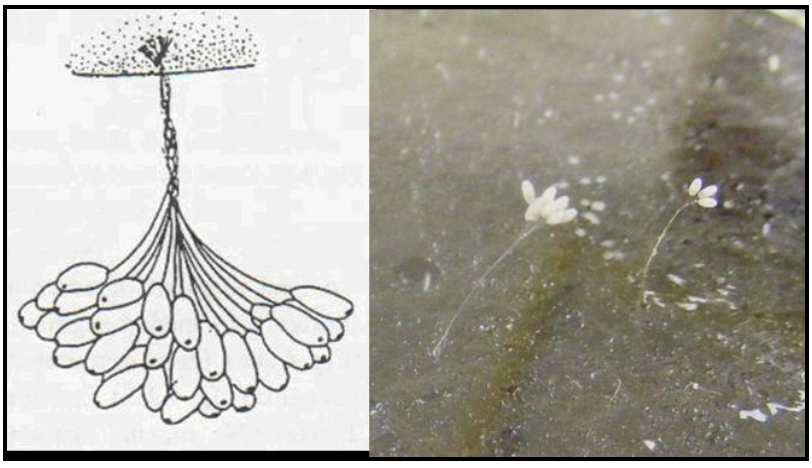

Fig. 1.7 Schematic (Monserrat *et al.,* 2007) and photo presentation of chrysopid eggs that were laid in clusters.

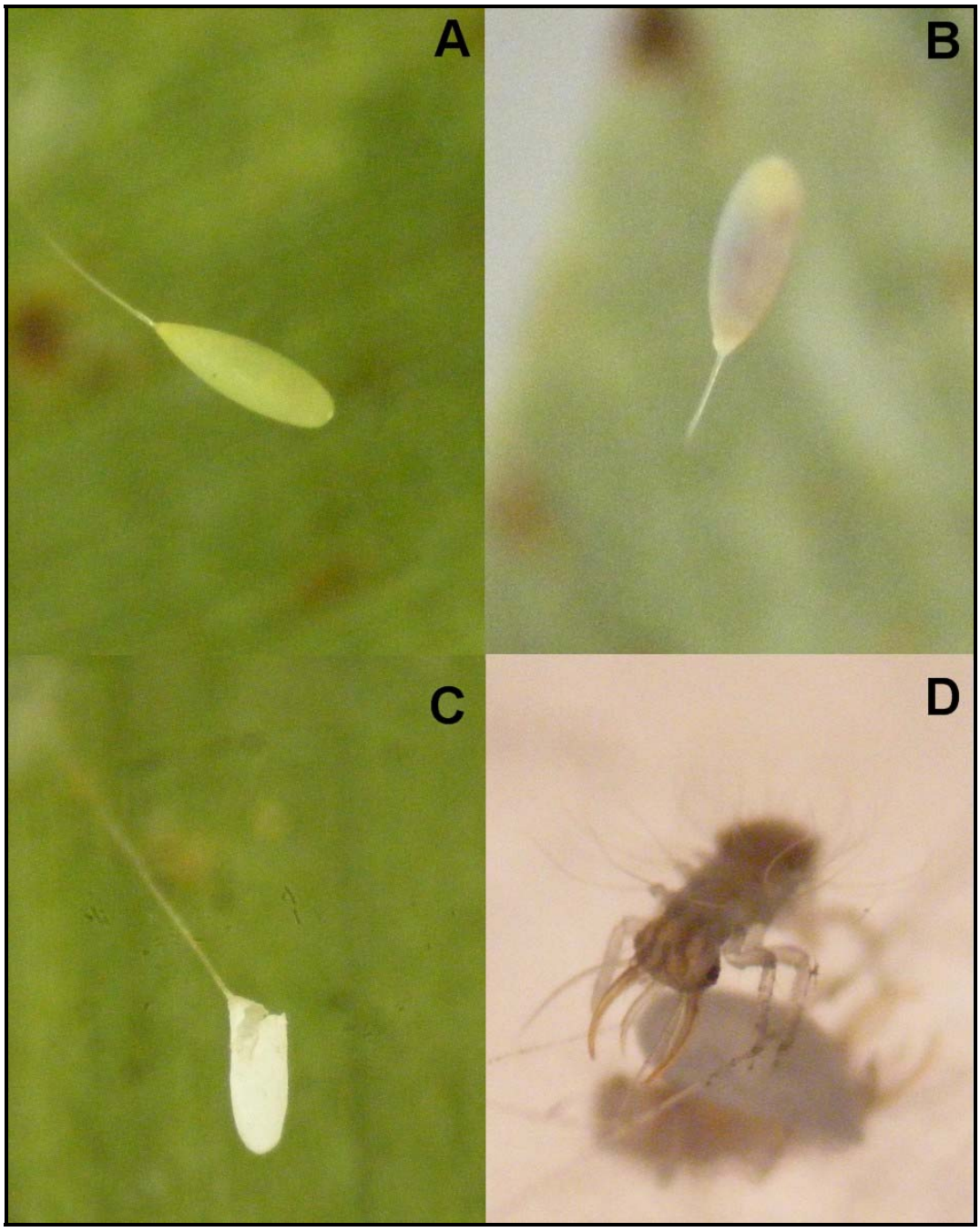

Fig. 1.8 Photo series displaying colour changes of an egg as it develops. A) young egg (2 days old); B) a 4-day old egg; C) empty egg shell; D) 1st instar nymph sitting on egg shell.

As eggs develop they change colour from green to beige and then light purple just before hatching (Fig. 1.8). After hatching the larva sit on the empty shell, resting for a few hours in order to complete the final embryonic phase which is the closing of its mouth. During this period the larvae is defenceless and if there is any disturbance during this period, the mouthparts can be under-developed which result in insufficient food uptake (Canard *et al*., 2007).

Larvae are active and can survive without food for approximately 24 hours after hatching. There are three larval instars. As larvae grow, food consumption also increases (Barnes, 1975). The fusi-form larvae can be smooth (Fig. 1.9) or have hairs on the dorsal surface (Fig. 1.10). In some species the larvae carry the remains of their prey on their back (Fig. 1.11). These larvae are called “trash-carriers”. It is believed that this behaviour helps in camouflage (Scholtz *et al.*, 1986).

Fig. 1.9 *Chrysoperla* larva with smooth dorsal side.

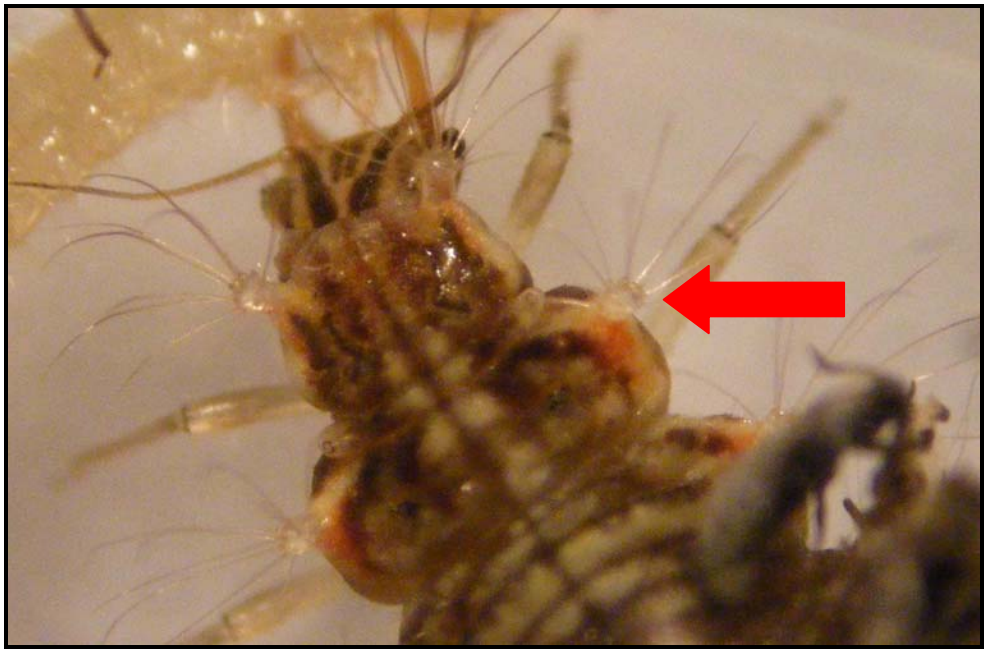

Fig. 1.10 *Chrysoperla* larva with hairs on dorsal side (indicated by red arrow).

Fig. 1.11 A trash-carrier *Chrysoperla* larva, with aphid cadavers on its back.

A cocoon (Fig. 1.12) is usually spun by the third-instar larva, after it has grown to maturity. The larva usually searches for a dark and dry place to pupate, but actual knowledge of pupation sites in the field is poor. To spin the cocoon the larva needs contact with anchoring points allowing good fixation of the external web (Canard, 2007). Spinning the cocoon is a complicated and long process which can last between 24 and 48 hours.

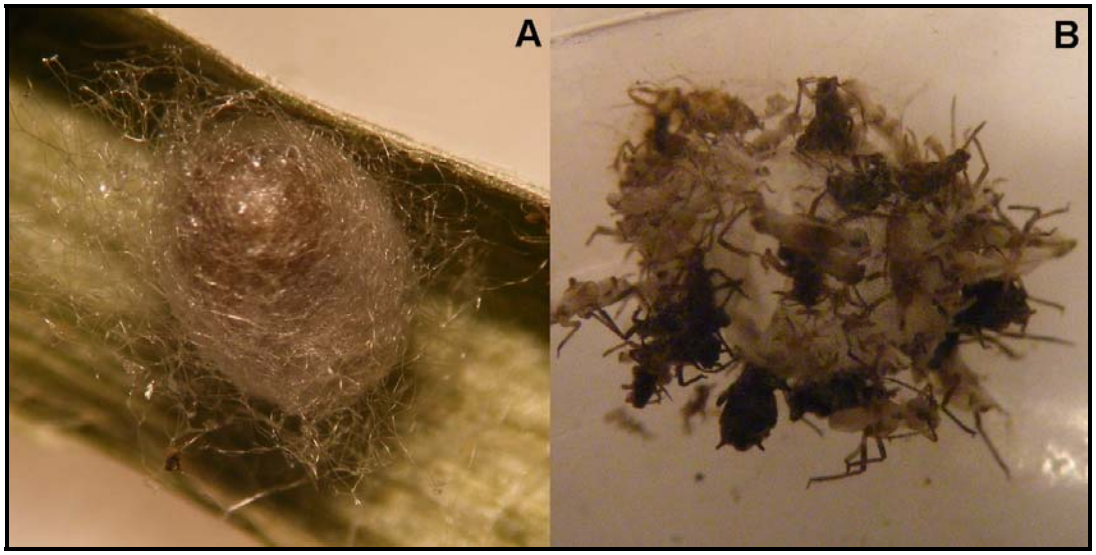

Fig. 1.12 Cocoon types of *Chrysoperla* spp. A) a cocoon with a smooth surface; B) a cocoon of a trash-carrier larva.

As the pupa develops it changes colour. At first it is white, but after a few days the pupa changes to a light-green colour. The adult emerges from the cocoon by exerting pressure on one side of the cocoon (where the cocoon is spun less thick at the extremities of the longer axis of the cocoon). This side tears and forms a lid where the adult crawls out (Barnes, 1975).

After emergence from the cocoon, the adult quickly finds a nearby vertical substrate to grasp unto before moulting (Canard, 2007). After moulding the adult expands its wings within approximately half an hour after emergence (Barnes, 1975). Adults (Fig. 1.13) feed on pollen, nectar and honeydew.

Fig. 1.13 An adult chrysopid (Anon, 2009).

During the period shortly after emergence, *Chrysoperla* individuals still have immature gonads and mating cannot occur. There is a pre-mating period during which the gonads mature and partners find each other. These time periods differ and change according to the specific species and environmental conditions (Canard, 2007).

1.5.2 The use of Chrysoperla spp. as biological control agents

Larvae of *Chrysoperla* are voracious active predators with a high effective searching capacity. Being a predator implies that the larvae prey on any species that is suitable to eat. *Chrysoperla* are also polyphagous, thus feeding on large or small soft-bodied arthropods. Among the prey consumed are pests of economic importance such as aphids, whitefly, mites and mealy bugs (Senior & McEwen, 2007). *Chrysoperla* spp. larvae also have a very high prey consumption rate. *Chrysoperla zastrowi* (Esben-Petersen) can consume an average of 488 aphids or 906 potato tuber moth eggs during their larval stage.

The capacity for food consumption increases as the larvae grow and by the 3rd larval stage up to 84 aphids or 200 potato tuber moth eggs can be consumed per day (Barnes, 1975).

These characteristics enable larvae of *Chrysoperla* species to be effective biological control agents (Senior & McEwen, 2007). This makes them a very important commercial consideration, since they may be used to control several arthropod pests. One of the first studies done on *Chrysoperla* spp. as biological control agent was that by Doutt and Hagen (1949) who found that mealy bug numbers were suppressed by *Chrysoperla* larvae. They also found the larvae of the specific species they used (*C. californica*) (Coquillett) were able to survive field sprays of insecticides. Thus, as early as 1949 successful integrated pest management (IPM) systems were already in use in which *Chrysoperla* played an important role.

There are three strategies in which *Chrysoperla* can be used for biological control. These are classical-, augmentation- and conservation biological control.

1.5.2.1 Classical biological control

This method is used to control pest species that were introduced into the crop environment from foreign areas. Enemies of the pest species from the pests country of origin are introduced into the country where it has become a pest and are mass reared and released in the crop environment to control the pest species. (Senior & McEwen, 2007). It is important that the newly introduced natural enemy does not cause more harm than the pest that it has to control, for example by feeding on beneficial insects rather than feeding on the insect pest species (Senior & McEwen, 2007).

Chrysoperla spp. are not commonly used in classical biocontrol because there is usually at least one species of *Chrysoperla* already present in the crop in the native area (Senior & McEwen, 2007). The family Chrysopidae is distributed all over South-Africa, where they are found in a variety of vegetation types (Picker

et al., 2004). There are 32 *Chrysoperla* spp. that occur in South Africa (Barnes, 1975).

It is, however, more practical and cost effective to enhance the numbers of an existing species than to introduce an exotic species. To enhance the numbers of the species augmentation and conservation biocontrol methods can be used.

1.5.2.2 Augmentative control

Augmentation can be done either by inoculative releases or inundative releases. Augmentation tends to increase the already existing natural enemy population densities to effectively control pest species.

Inoculative releases are used to introduce a small number of natural enemies early in the crop cycle with the expectation that they will reproduce in the crop environment and that their offspring will continue to provide pest control for an extended period of time (Senior & McEwen, 2007).

One of the disadvantages of inoculative releases is that if natural enemies do not have enough prey/food for them to be sustained early in the cropping season, the adults will not remain in the crop environment. Inoculative releases are very successful in glasshouse crops/protected crops, where *Chrysoperla* adults are unable to disperse. Larock and Ellington (1996) successfully used inoculative releases to control pecan aphids in an IPM program through the release of *C. rufilabris* (Burmeister).

Inundative releases are made when insufficient reproduction of the released natural enemies is likely to occur, and pest control will be achieved exclusively by the released individuals themselves. This method is used commonly where immediate control is necessary on short term crops and where the pest species will not support the development of the *Chrysoperla* spp. (Senior & McEwen, 2007). This method works through mass production of natural enemies (*Chrysoperla* spp.*)* and release (eggs or larvae) into cropping systems to control the pest. In contrast to that of inoculative releases which aim for the released individuals to reproduce, the released species remains in the crop

environment and reach equilibrium with the pest insect species. During inundative releases, a great number of individuals are released that quickly reduces pest species numbers by preying on them. It is therefore not likely that they will reproduce and that the population will reach an equilibrium.

The need for of mass producing *Chrysoperla* spp. became important once their value as predators of pest species was realized. Finney (1948) began the first studies to mass produce and distribute *C. carnea* (Stephens) and *C. california* (Burmeister) in the USA. Augmentative biological control is inextricably dependent upon the commercial natural enemy rearing industry, as well as the cost, availability and quantity of the natural enemies.

1.5.2.3 Conservation biological control

The aim of this strategy is to identify and rectify factors that adversely affect and suppress natural enemy reproduction, and to enhance agricultural fields as habitats for natural enemies (Senior & McEwen, 2007). Actions are therefore taken to increase and improve the existing natural enemy's living condition so that population numbers can increase. Some of the actions are: attractants such as food supplements, providing hibernation shelters, minimizing the use of pesticides and managing natural enemies of *Chrysoperla* spp. Itioka and Inoue (1996 cited by Senior & McEwen, 2007) described how *Chrysoperla* where not able to control mealy bugs in a crop were ants where present. Once the ants were removed the pest population decreased by 94%. Conservation biocontrol techniques do not depend on mass production of natural enemies, thus making it more affordable.

1.5.3 Effects of Bt-toxins on Chrysoperla spp. at a tri-trophic level

Neuroptera are excellent indicators of environmental and habitat transformation, and also include key species for signifying areas and faunas that require priority protection (Mansell, 2002). A few factors need to be investigated to analyse the tri-trophic effects that *Cry* 1Ab toxins may have on natural enemies such as *Chrysoperla* spp. Dutton *et al*. (2002) summarized and analysed these factors. Firstly, the range, suitability and importance of the

available prey in the field are important. Chrysopids feed on a large number of soft-bodied arthropods, including economically important pests such as aphids, mites, white flies and small Lepidoptera larvae (Senior & McEwen, 2007). Secondly, it is important to know if the prey and the predator ingests the Bt proteins. The Bt protein can be ingested either by feeding directly on the genetically modified plant (prey) or at the tri-trophic level by feeding on the contaminated prey (predator). Predators can also ingest Bt proteins directly by feeding on plant tissue, for example chrysopid adults feeding on Bt pollen. Aphids are the major prey for chrysopid species (Coderre, 1988). Finally it is important to establish whether the Bt protein affects the prey itself. In some cases it is believed that the Bt protein affects prey quality which in turn affects the predator because of poor prey quality (sick prey) and not because of the Bt protein (Dutton *et al.*, 2002; Hilbeck *et al.*, 1998a).

Dutton *et al.* (2002) did a series of experiments with *C. carnea* and their prey species to study the possible effects of indirect exposure to Bt protein produced by Bt maize. Firstly they examined the performance of the different herbivore prey species that are often consumed by chrysopid larvae. These prey species were aphids (*R. padi*), spider mites (*Tetranychus urticae*) (Koch) (Acari: Tetranychidae) and Lepidoptera larvae (*Spodoptera littoralis*) (Boisduval) (Lepidoptera: Noctuidae). For the aphids and mite species, no differences in performance were observed between individuals that were reared on Bt maize or non-Bt maize. However, in the case of the Lepidoptera larvae a high mortality rate and delay in development were observed in individuals that fed on Bt maize. Secondly, they quantified the ingestion of *Cry* 1Ab toxin by the prey (herbivores). The highest amount of *Cry* 1Ab protein was found in mites followed by the larvae and none in the aphid species. These prey herbivores were then fed to chrysopid larvae to determine the tri-trophic effect of Bt proteins. Results indicated that when chrysopids fed on mites, which ingested Bt protein, or aphids that did not ingest Bt protein no effect on survival, development or mass of the chrysopids was evident. In contrast to these observations it was observed that when chrysopid larvae fed on Bt-fed herbivorous larvae (which ingested Bt protein and performance of the prey itself was affected by Bt protein) mortality was significantly higher and development

was delayed compared to when consuming non-Bt maize fed larvae. Dutton *et al.* (2002) therefore concluded that a combined interaction of poor prey quality and *Cry* 1Ab toxin could be the reason for the adverse effects observed on predator species.

Hilbeck *et al.* (1998a) assessed the impact of Bt proteins (*Cry* 1Ab) on *C. carnea* larvae by only using *S. littoralis* as prey. They observed that chrysopids that were fed Bt-reared prey, had a higher mortality and prolonged development time compared to feedinf on prey that was reared on non-Bt maize. The study also concluded that the poor quality of the prey used in combination with the Bt proteins could have be the reason for the negative effects observed on *C. carnea* development.

Obrist *et al.* (2006) also investigated interactions between lacewings and *Cry* 1Ab protein in a study on the possible transformation of protein through the exposure pathway from the prey to predator. They investigated the uptake of *Cry* 1Ab toxin by larvae of *C. carnea* by means of immunological tests. Results showed that chrysopids feeding on toxin containing *S. littoralis* larvae or mites (*T. urticae*), consumed approximately 50% or 33% of the prey‘s *Cry* 1Ab toxin concentration respectively. It was therefore concluded that chrysopid larvae do ingest *Cry* 1Ab protein when feeding on these prey species. Negative effects on survival and development time of *S. littoralis* were only observed when chrysopids fed on larvae, even though the concentration of *Cry* 1Ab protein was higher in mites. These experiments concluded that chrysopid larvae were not affected by *Cry* 1Ab protein and that the negative effects observed in the treatment with *S. littoralis* as food source were due to the prey quality and not the presence of Bt protein (Obrist *et al.,* 2006).

None of the above mentioned studies could exclude the effect of sick prey due to *Cry* 1Ab protein on chrysopid development and the question still remained whether *Cry* 1Ab toxin affected the biology of chrysopid larvae. To test whether the observed negative effects were directly caused by the Bt toxin, high-dose toxicity bioassays can be done by providing an artificial diet, incorporating the *Cry* 1Ab protein.

Hilbeck *et al.* (1998b) developed a bioassay technique incorporating the *Cry* 1Ab protein into a liquid diet that was then encapsulated within small paraffin spheres, that was with or without the protein (artificial diet). *Chrysoperla carnea* larvae were provided with these spheres as food source. Groups that were fed *Cry* 1Ab protein spheres had higher larval mortalities than the control groups and required more time to complete larval development (Hilbeck *et al.,* 1998b). Results concluded that *Cry* 1Ab was toxic to *C. carnea* at 100 μg/ml of artificial *Cry* 1Ab encapsulated diet.

Similar studies on *C. carnea* were done by Lawo and Romeis (2007), Rodrigo-Simon *et al.* (2006) and Romeis *et al.* (2004). Romeis *et al.* (2004) concluded that transgenic maize expressing *Cry* 1Ab poses a negligible risk for this predator. Rodrigo-Simón *et al.* (2006) similarly concluded that the *Cry* toxins tested, even at concentrations higher than those expected in real-life situations, do not have detrimental effects on the green lacewing. The contradicting results produced by the above mentioned studies resulted in confusion on the effects of *Cry* 1Ab protein on *Chrysoperla* spp.

Resent studies conducted by Li *et al.* (2008) on pollen-feeding adult *C. carnea* indicated that *Cry* 1Ab protein in Bt-containing pollen did not affect adult chrysopid fitness even at concentrations exceeding the levels in pollen. A follow-up study was done to evaluate digestion of pollen by *C. carnea* (Li *et al.*, 2010) which concluded that even though the pollen grains were not fully digested, the insects were exposed to transgenic insecticidal proteins that were contained in the pollen (Li *et al.*, 2010). Some maize cultivars (Events 176 and TC1507) containing Bt protein, prolonged the adult stages of *C. plorabunda* (Fitch), compared to their non-Bt isolines (Mason *et al.,* 2008). They also observed that the mean number of eggs produced per female were significantly fewer for the group that was fed MON810 pollen compared to the non-Bt isoline (Mason *et al.,* 2008).

1.6 Oviposition site preference of *Chrysoperla* species

Limited and contradicting information exists on the oviposition patterns of *Chrysoperla* species on crops. Barnes (1975) observed that *C. zastrowi* appeared to be indiscriminate in their choice of oviposition sites in the field and that they did not have preferences for specific plant parts such as leaves or stems for oviposition. While some studies reported that females preferred to lay eggs near aphid infested areas on plants (Skaife, 1979; Petersen & Hunter, 2002; Kunkel & Cottrell, 2007). Oviposition was also recorded in locations where their major food source, aphids, was absent (Coderre, 1988; Duelli, 1984a).

The reasons for chrysopids to choose specific oviposition sites has long been discussed. Skaife (1979) observed that lacewing females generally choose the underside of leaves which is infested by aphids. Patel and Vyas (1985 cited by Szentkiralyi, 2007) reported that the majority of eggs of *C. carnea* in cotton were located on the leaves, with only a few deposited on fruits and branches. They also observed that more eggs were laid on the abaxial surfaces of leaves. On maize *C. oculata* (Say) females deposited their eggs on the six leaves below the ear, with no consideration of the availability of the aphids they prey on (Coderr, 1987). On the other hand Liao *et al.* (1984) found that *C. rufilabris* oviposition increased as aphid density increased in pecan orchards. In behavioural bioassays studying *C. carnea* orientation behaviour, Reddy (2002) found that volatiles from eggplant, okra and peppers were attractive to *C. Carnea,* but that tomato volatiles were not. Boo *et al.* (1998) observed that aphid sex pheromone components were attractive for *C. cognata.*

Although the presence of aphids seems to influence chrysopid oviposition behaviour, it is most likely not critical to the survival of *Chrysoperla* spp. *Chrysoperla* larvae are polyphagous and active predators with a high mobility and effective searching capacity (Senior & McEwen, 2007; Barnes, 1975). These larval characteristics make the importance to oviposit near aphid colonies less critical for survival immediately following egg hatching (Coderre, 1988). When species have generalized feeding habits and are mobile enough

to move to other plants or there are different requirements for juvenile and adult feeding, ovipositing females may show little discrimination among host plants (Thompson, 1988). In aphid infested locations, Hagen *et al.* (1976) indicated that any habitat with a high population density of aphids should be a suitable site for chrysopids to oviposit, since females are attracted to both the odour of honeydew (Hagen *et al.,* 1976) and aphid sex pheromones (Boo *et al.,* 1998).

Knowledge on oviposition and distribution of lacewings eggs on maize will contribute to development of scouting techniques and improved IPM strategies for pests.

1.7 Flight activity

Crop fields are temporary habitats and may be unsuitable for *Chrysoperla* habitation at certain times of the year or cropping cycle (Duelli, 2007). Lacewings must therefore be able to migrate between vegetation types to follow their prey as the crop and season change in order to survive. Lacewings are predominantly arboreal, but a few species are able to live in patchy and temporary environments. The Green lacewings are the most successful in field crops (Duelli, 2007).

Migration of lacewings is a movement process towards or away from the crop field, because of an increase or decrease in availability of food or due to unfavourable environmental conditions. For example, when a crop is harvested, the habitat is totally lost and food becomes scarce. For practical reasons, migration patterns are generally difficult to quantify (Dingle, 1996). Migrating insects use moving airstreams to travel hundreds of kilometres in a single flight. Duelli (1980) studied flight patterns of *C. carnea* and found that shortly after emergence adults performed adaptive dispersal flights in a downwind direction. The use of wind to migrate and move combined with the fact that lacewings are small and that flight activity is nocturnal, make it difficult to observe and study the migration and flight activity patterns.

Different flight behaviour strategies have been described for lacewings. These are: Migration flight to over-wintering sites after diapause induction in late summer and back into field crops in spring, pre-ovipository migration flights to find new habitats with aphid colonies, and continued nomadism throughout the reproductive period. The latter is done to spread the risk for the offspring in unpredictable, temporary and patchy habitats (Duelli, 2007).

Shortly after emergence the adults perform additive dispersal flights which are straight downwind flights mostly at elevations higher than 3 m above ground. Direction and ground speed are mainly dictated by the wind and the reaction to kairomones is slight or absent (Van Emden & Hagen, 1976). Only after these migration flights during the first two nights do females mate and after another two to four days oviposition takes place. Three to four days after emergence the flying adults start to show a strong reaction to the scent of certain tryptophan products (both honeydew and artificial food sprays contain tryptophan). These products induce lacewings to land (Van Emden & Hagen, 1976) and may also result in appetitive downwind flights. The difference in the appetitive downwind flight and the adaptive dispersal flight lies in the height above ground and in the types of stimuli that elicits landing responses. Both are downwind cruising flights, but the main appetitive flight activity takes place within a 2 m layer above crop level (Duelli, 1980). Field experiments in alfalfa fields with strips sprayed with an artificial food mixture imitating aphid honeydew have shown that the numbers of pre-reproductive females caught in sticky grids were the same upwind and downwind of the sprayed strips (Duelli, 1984a). The number of gravid females, however, was much lower in the traps downwind of the food sprays than upwind. The interpretation made by Duelli (1984b) was that gravid females reacted to food spray by landing, while newly emerged females continued their downwind migration flight.

Most insects performing migration flights after adult emergence become more sedentary as soon as they start their reproductive period. Many species, particularly those of agricultural importance, disperse throughout their reproductive period, depositing eggs in different fields. Reproductive females

were reported not to remain in a single crop field for more than two days, even though food sources were not limiting (Duelli, 1984b).

In Switzerland the weekly immigration and emigration rates of *C. lucasina* (Lacroix) were investigated using 7 m high sticky grid traps on sides of a 1 ha maize field (Duelli, 2007). Results indicated an average immigration of 1500 and a emigration of 1700 lacewings per hectare per night during the summer months of July and August. The presence of adults, larvae and pupae was determined weekly and showed an average of 3500 adults per hectare of maize. Assuming that all immigrating lacewings fly lower than 7 m and that they land in maize fields infested with aphids, it was concluded that adults remained in the field for an average of only two days. Migration behaviour of lacewings was described by Duelli (2007) as a continuous rolling downwind movement along the prevalent nightly wind direction, a kind of downwind nomadism (Duelli, 2007).

Information regarding migration between different crop species is, however, lacking from literature. This information will contribute to understanding of the role of wild habitats in lacewing ecology, as well as in development of pest management systems that take these important predator species into account.

1.8 Objectives of this study

1.8.1 General objective

The general aim of the project was to provide information that can be used during monitoring of *Chrysoperla* spp. in GM crops and to assess the potential impact of Bt-toxin on these important organisms in agro-ecosystems. Flight activity patterns, height of flying and inter-habitat movement was also studied.

1.8.2 Specific objectives

The specific objectives were to determine:

- ☆ if chrysopids have preferred oviposition sites on maize plants
- ☆ if the presence of aphids on maize plants has an influence on *Chrysoperla* spp. preference under laboratory conditions
- ☆ the effect of the Bt-toxin uptake on *Chrysoperla pudica* biology
- ☆ movement of adult *Chrysoperla* spp. between maize fields and surrounding headlands
- ☆ when during a 24-hour period do *Chrysoperla* spp. adults fly and how high above the crop canopy do they fly.

Results of this study are presented in the form of chapters with the following titles:

- ☆ Spatial distribution and abundance of *Chrysoperla* spp. (Neuroptera*:* Chrysopidae) eggs on maize.
- ☆ Effect of *Cry* 1Ab protein from Bt maize on the biology of *Chrysoperla pudica* (Neuroptera: Chrysopidae).
- ☆ Flight activity patterns and flight height of *Chrysoperla* spp. (Neuroptera*:* Chrysopidae) in lucerne fields.

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Chapter 2

Spatial distribution pattern of *Chrysoperla* spp. (Neuroptera*:* Chrysopidae) eggs on maize plants

2.1 Abstract

Monitoring the impact of genetically modified Bt maize on non-target organisms is required in South-Africa. Monitoring techniques, especially for insect eggs, are often labour intensive and time consuming. Since lacewings are important beneficial insects, this study was conducted to determine the preferred oviposition site of *Chrysoperla* spp. on maize plants, so as to facilitate time-effective searching for eggs. Furthermore, we determined whether if the presence of aphids on plants influenced *Chrysoperla* spp. preference. The preferred area of oviposition was determined by inspecting maize plants for *Chrysoperla* eggs and recording the exact position of eggs on leaves. Oviposition was not random and occurred near maize ears. In choice-tests, a positive response was elicited in both males and females to maize ears, while females responded significantly more towards aphid infested ears than males did. Data on spatial distribution of eggs facilitates rapid monitoring of the presence of eggs in maize cropping systems and is also of use in general pest management.

Key words: Bt maize, monitoring, non-target insects, predators, scouting

2.2 Introduction

Bt maize which produces an insecticidal *Cry* protein is planted in South Africa for control of lepidopterous stem borers of which *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is the most important (Van Wyk *et al*., 2007). Since the first deployment of genetically modified (GM) crops with insecticidal properties, there has been concern with regard to possible non-target effects (Meeusen & Warren, 1989; Gould, 1998). Post-release monitoring of the environmental impact of genetically modified Bt maize on non-target organisms in South-Africa is required by law (Biodiversity act, 2004, Act No. 10 of 2004).

Lacewings, *Chrysoperla* spp. (Neuroptera: Chrysopidae), is one of the potential non-target species that could be indirectly affected by feeding herbivorous prey that consumed Bt protein aimed at the target pest (Hilbeck *et al*., 2006). *Chrysoperla* spp. larvae are voracious predators with a high effective searching capacity and high prey consumption rate. These characteristics enable larvae of *Chrysoperla* species to be effective biological control agents (Senior & McEwen, 2007). Among the prey consumed are pests of economic importance such as aphids, whitefly, mites and mealy bugs (Senior & McEwen, 2007). *Chrysoperla zastrowi* (Esben-Petersen), for example, each consume an average of 488 aphids or 906 potato tuber moth eggs during its larval stage (Barnes, 1975).

The potential effect of Bt proteins ingested by the third trophic level, on beneficial non-target insects such as lacewings is of specific concern. If the Bt protein produced by Bt maize affects natural enemies such as green lacewings it may disrupt beneficial interactions in agro-ecosystems where these insects contribute to ecosystem services as predators (Van Wyk *et al*., 2007; Dutton *et al.*, 2002; Hilbeck *et al.*, 1998). Monitoring of chrysopid numbers in crop fields is complicated by the fact that adults are nocturnal (Dueli, 1986). Searching for chrysopids eggs is labour intensive and time consuming since it is also difficult to observe eggs on plants. Eggs are small and the egg laying position on maize plants is not known. It is therefore

difficult to assess the abundance of eggs in crop fields and to collect data that would be important the monitoring of these beneficial organisms. The need therefore exists to develop a scouting technique that enables time-effective and efficient search and finding of chrysopid eggs on maize plants.

Limited and contradicting information exists on the oviposition patterns of *Chrysoperla* spp. on crops. *Chrysoperla zastrowi* appeared to be indiscriminate in their choice of oviposition sites in the field and they do not have preferences for specific plant parts such as leaves or stems for oviposition (Barnes, 1975). While some studies reported that females preferred to lay eggs near aphid infested areas on plants (Skaife, 1979; Petersen & Hunter, 2002; Kunkel & Cottrell, 2007), oviposition in locations where their major food source, aphids, was absent, was also reported (Coderre *et al.,* 1987; Duelli, 1984).

The aims of this study were to determine if there was a pattern in the spatial distribution of *Chrysoperla* spp. eggs on maize plants and to determine if the presence of aphids on maize plants has any influence on lacewing preference under laboratory conditions.

2.3 Material and methods

2.3.1 Species identification

Sweep net samples were taken at each site to determine the species composition of *Chrysoperla* spp.

2.3.2 Spatial distribution of Chrysoperla eggs

Distribution of chrysopid eggs on plants was studied in two maize production areas in South Africa during the 2007/08 growing season. The Vaalharts irrigation scheme (27°50'S; 24°50'E), is located in the Northern Cape Province while Tshiombo (22°47'S; 030°27'E) is situated in the Limpopo

Province. The latter site is a low-input resource-poor farming system in which intercropping is common. The Vaalharts site is a high input irrigation scheme where no intercropping is done. All maize fields on which data were collected were surveyed approximately 2-3 weeks after flowering and maize ears were present on all plants.

Sixty maize plants on which *Chrysoperla* females oviposited were inspected at the Vaalharts irrigation scheme and 76 at the Tshiombo irrigation scheme. More plants were inspected at the latter site since the abundance of *Chrysoperla* eggs per plant was lower than at Vaalharts.

In order to record data, leaves of each maize plant were counted and numbered from the top of the plant towards the soil level. The number of the leaf that covered the maize ear was documented and in cases where there was more than one ear per plant, leaves of both ears were recorded. Each leaf of the plant was examined for eggs, starting at the top (flag leaf), downwards to the oldest (lowest) leaf. When an egg was observed it was recorded whether the egg was at the proximal or distal half of the leaf and whether it was on the adaxial or abaxial surface. Both hatched and un-hatched eggs were counted (Fig. 2.1).

Fig. 2.1 Inspecting maize plants for *Chrysoperla* eggs (Groblersdal, Mpumalanga Province, 2008).

2.3.3 Bioassays

A Y-tube olfactometer (Fig. 2.2) was used to investigate the response of *Chrysoperla* adults towards aphid-infested maize ears. The main olfactometer arm was 25 cm long and the length of arms was 20 cm with a diameter of 4.5 cm. Airflow was controlled with a flow meter at 2 ml per min. Bioassays were done in a dark room since *Chrysoperla* is nocturnal. Individuals were kept in a dark room six hours before assays were initiated.

Fig. 2.2 Y-tube olfactometer used in bioassays.

There were two pairs of treatments. Treatment one consisted of a maize ear without aphids and a blank treatment without a maize ear, while the second treatment was a maize ear infested by aphids and a maize ear without aphids. The test materials that were used were maize ears (non-Bt cultivar) with or without infestation of aphids, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae). There were between 500 and 700 aphids on each of the ears that were used in the assays. Fifteen different *C. pudica* individuals of each sex were evaluated in each treatment. The aphid-infested ears were collected in maize fields at the Agriculture Research Council (ARC) in Potchefstroom. Lacewings were provided from a rearing colony maintained at the North-West University.

One chrysopid adult was introduced into the Y-tube and observed for a period of five minutes. Individuals who did not respond or remained in the main arm of the olfactometer was excluded from the experiment. Response was considered positive if individuals moved 1 cm along the arm connected to one of the test chambers. The time to a positive response was also recorded. After

every third replicate the apparatus was rotated 180^0 to exclude directional stimuli and after every five runs the apparatus was cleaned and fresh plant material used.

2.3.3 Data analysis

Data on spatial distribution of eggs were expressed as proportion of total number of eggs per specific leaf, as well as per abaxial or adaxial leaf surface. Different bioassay treatments were compared in pairs using the method of Cox (1970) for binary data.

2.4 Results

2.4.1 Species identification

The majority of the lacewing complex collected at both the Vaalharts and Tshiombo sites were *Chrysoperla pudica*. Although eggs of different *Chrysoperla* spp. could not be distinguished under field conditions, we ascribed the results to *Chrysoperla pudica.*

2.4.2 Spatial distribution of Chrysoperla eggs

At the Vaalharts irrigation scheme a total of 275 eggs were found with a mean number of 4.58 eggs per plant. At the Tshiombo irrigation scheme a total of 112 eggs were found with a mean number of 1.47 eggs per plant.

At Vaalharts, 60% of maize ears were located in the axil of leaf number seven (Fig. 2.3a). At Tshiombo, the ear location on plants was more variable with 87% of the 132 ears recorded occurring in the axils of leaves number 6-8 (Fig. 2.3b).

At the Vaalharts site a clear pattern was observed with regard to vertical distribution of eggs on the plants relative to the position of maize ears.

Seventy three percent of eggs were laid on the leaf that covered the ear (leaf nr. 7) and the four leaves below that (Fig. 2.4a). The same tendency was observed at the Tshiombo irrigation scheme where 72% of the eggs were laid on leaf numbers 5-9, which is the area on the plant were the majority of ears occurred (Fig. 2.4b).

A definite pattern in the horizontal distribution of *Chrysoperla* eggs was also observed. Data indicated that 90% of eggs were laid on the proximal end of the leaf and only 10% on the distal end (Fig. 2.5). Eighty eight percent of eggs were laid on the adaxial leaf surface and only 12% on the abaxial surface (Fig. 2.5a). In the Tshiombo irrigation scheme 88% of eggs were found on the proximal end of the leaves and 12% on distal ends. Eggs were also distributed more evenly along the surface with 42% occurring on the adaxial and 58% on the abaxial surface of leaves (Fig. 2.5b).

2.4.3 Bioassays

When individuals were provided with the choice of aphid infested and uninfested maize ears, a different response was observed between males and females. There was a significant difference (P=0.04) between males and females in the time taken to respond towards different treatments, with males taking 3.7 (±SE 0.30) minutes compared to the 2.5 (±SE 0.44) minutes of females. The proportion of males and females making the same response was, however, similar with 66.7% of individuals responding positively towards the aphid-infested ear. The choice of treatments for both males and females did, however, not differ significantly (Table 1). When provided with a choice between a maize ear and a blank treatment, males took a mean of 2.7 (±SE 0.39) minutes to choose and females 2.5 (±SE 0.42) minutes, which was not significantly different (P=0.81). There was no significant difference in male response between the two treatments (P>0.05). However, the majority of females (73%) chose the chamber with the aphid infested ear.

2.5 Discussion

Since the number and position of maize ears on plants is largely genetically determined and therefore relatively constant, the position of eggs on plants is described relative to ear position.

The spatial distribution of *Chrysoperla* eggs at two diverse sites was not random and a clear spatial pattern of egg distribution in relation to maize ears emerged. The largest proportion of eggs was laid on leaves near maize ears. The preferred oviposition sites for chrysopids were therefore on leaves in the vicinity of maize ears. As a result, scouting of *Chrysoperla* can be done quicker since a reduced area on the plant can now be scouted with high reliability. It is no longer needed to inspect the whole plant, but only the ear-covering leaves and leaves near the ear, in search for *Chrysoperla* eggs

The reasons why chrysopids choose particular oviposition sites has long been discussed. Some species have been documented to lay eggs in locations without aphid colonies while some studies contradict these findings indicating that females choose to lay their eggs near aphid infested locations. Skaife (1979) observed that lacewing females generally choose the underside of leaves which is infested with aphids. Patel and Vyas (1985, cited by Szentkiralyi, 2007) reported that the overwhelming majority of *C. carnea* eggs in cotton were located on the leaves, with only a few deposited on fruits and branches. They also observed that more eggs were laid on the abaxial surface of leaves. On maize *C. oculata* (Say) females deposited their eggs on the six leaves below the ear, with no consideration of the availability of the aphids they prey on (Coderre *et al*., 1987). On the other hand Liao *et al.* (1984) found that *C. rufilabris* oviposition increased as aphid density increased in pecan orchards. In behavioural bioassays studying *C. carnea* orientation behaviour, Reddy (2002) found that volatiles from eggplant, okra and peppers were attractive to *C. carnea* but that tomato volatiles were not. Boo *et al.* (1998) observed that aphid sex pheromone components were attractive for *C. cognata* (McLachlan).

Although the presence of aphids seems to influence chrysopid oviposition behaviour, it is most likely not critical to the survival of lacewings since their larvae are polyphagous and active predators with a high effective searching capacity (Senior & McEwen, 2007; Barnes, 1975). These larval characteristics make the importance to oviposit near aphid colonies less critical for survival following oviposition (Coderre *et al.,* 1987). When a species have generalized feeding habits they are mobile enough to move to other plants or there are different requirements for juvenile and adult feeding, ovipositing females may show little discrimination among host plants (Thompson, 1988). Any habitat with a high population density of aphids should, however, be a suitable site for chrysopids to oviposit since females are attracted to both the smell of honeydew (Hagen *et al*., 1976) and aphid sex pheromones (Boo *et al*., 1998).

The tendency observed in this study was that chrysopid females lay their eggs in the region near maize ears. Choice-test data showed that females responded positively to host plants that were infested with aphids.

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Table 1. Response of *Chrysoperla pudica* adults to maize and aphid infested maize ears.

Bioassay with aphid infested ear and non-aphid infested ear treatments		
Sex	**aphid-infested ear**	**uninfested ear**
Male	10a	5a
Female	10a	5a
Bioassay with aphid infested ear and a blank treatment		
Sex	**aphid-infested ear**	**blank treatment**
Male	6a	9a
Female	11a	4b

Means within columns of each group followed by the same letter do not differ significantly at p=0.05 according to the test for binary data (Cox, 1970).

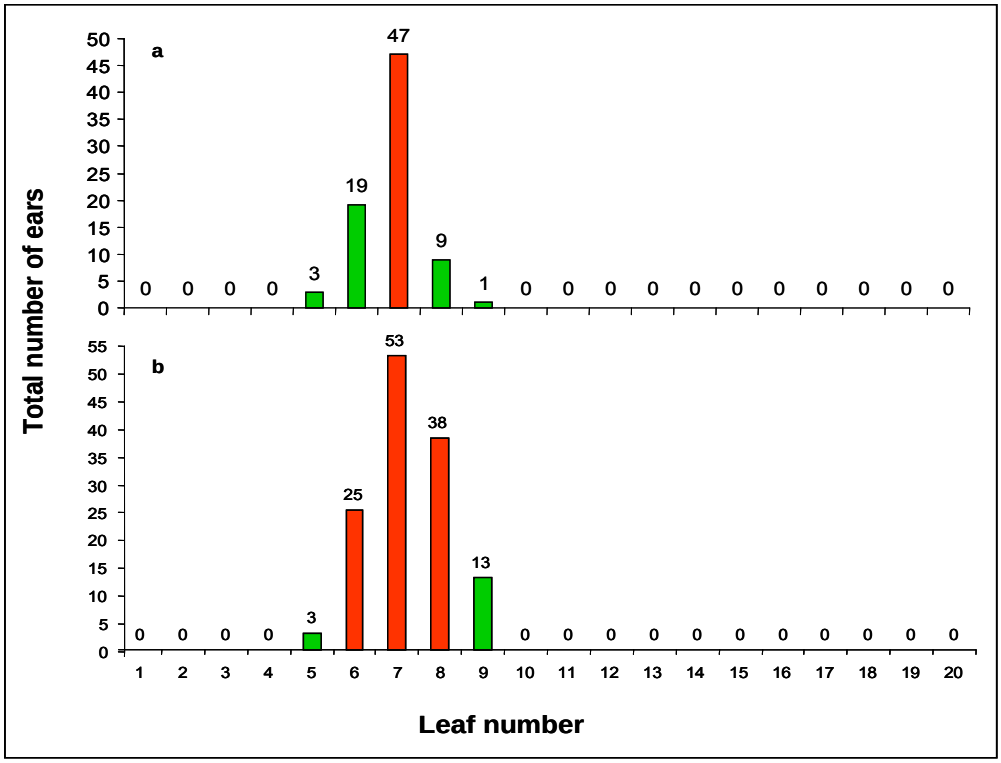

Fig. 2.3 Maize ear positioning on plants. a. Vaalharts irrigation scheme, b. Tshiombo irrigation scheme. Orange bars indicate the leaf position (axil) at which most maize ears occurred.

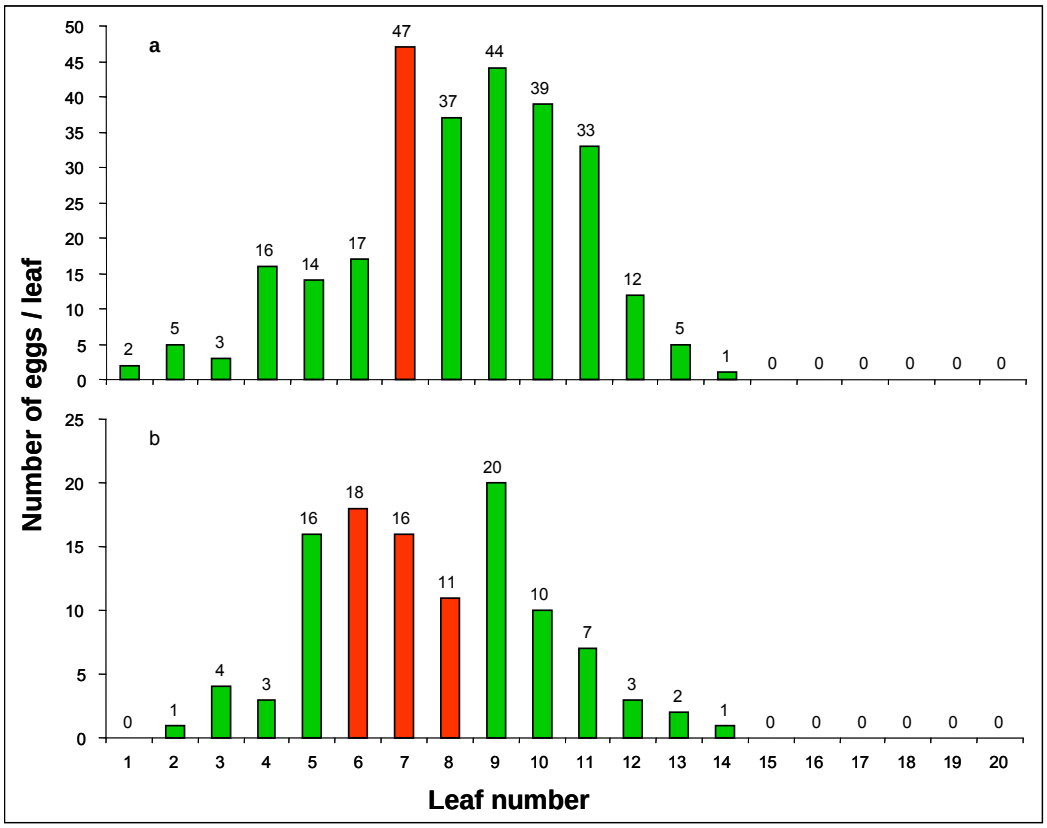

Fig. 2.4 Mean number of *Chrysoperla* spp. eggs found per leaf. a. Vaalharts irrigation scheme, b. Tshiombo irrigation scheme. Orange bars indicate the leaf position (axil) at which maize ears occurred.

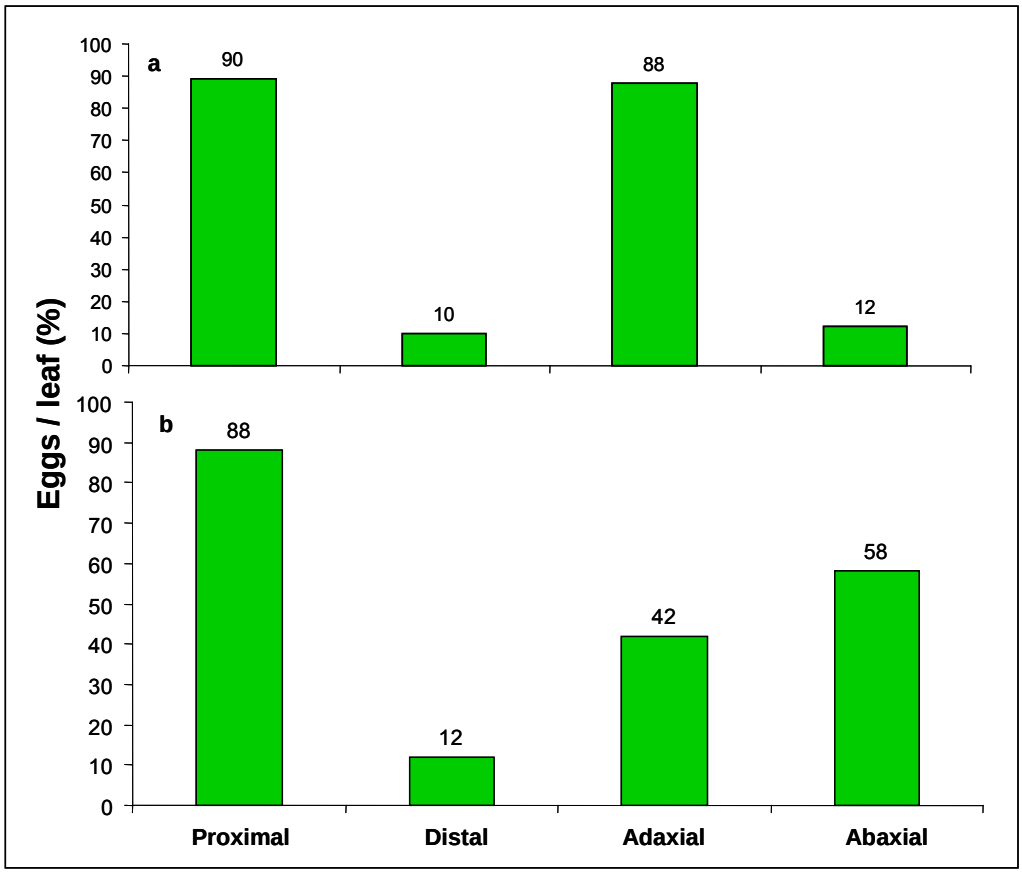

Fig. 2.5 Horizontal distribution of *Chrysoperla* spp. eggs on maize leaves. a. Vaalharts irrigation scheme, b. Tshiombo irrigation scheme.

Chapter 3

Effect of *Cry* 1Ab protein from Bt maize on the biology of *Chrysoperla pudica* (Neuroptera: Chrysopidae)

3.1 Abstract

Resistance development and possible non-target effects have been of concern since the first deployment of genetically modified crops with insecticidal properties. It is especially at the third trophic level and with important predators such as lacewings (*Chrysoperla* spp.) where negative effects of *Cry* 1Ab protein could have adverse effects in agro-ecosystems. Previous studies with *C. carnea* showed contradicting results. Some studies ascribed poor performance of *C. carnea* to poor quality prey, caused by the effect of *Cry* 1Ab protein on the prey, while others indicated no effect. Evolution of Bt-resistant pests facilitates a new pathway of exposure of entomophagous arthropods to *Cry* 1Ab proteins. Since Bt-resistant maize stem borer (*Busseola fusca*) (Lepidoptera: Noctuidae) larvae were available in a rearing colony, it provided a unique opportunity to study the effect of *Cry* 1Ab protein, consumed by healthy prey, on the biology of *Chrysoperla pudica.* An experiment was conducted in which chrysopid larvae were separated into five diet groups (50 individuals per group) receiving different diets. Diets consisted of *B. fusca* larvae that fed on Bt- or non-Bt maize (treatment group 1) and two groups that also received the latter food alternated with *Ephestia cautella* (Lepidoptera: Pyralidae) eggs at 2-day intervals (treatment group 2). One group was fed only on eggs of *E. cautella.* Larval mass, larval and pupal development time, larval mortality and overall mortality were determined. The optimum diet was *E. cautella* eggs on which larval survival was highest (94%) and development time shortest (9.8 days). Results showed no significant differences in larval development period within treatment groups 1 and 2. The pupal period and percentage adult emergence of treatment group 1 was, however, significantly shorter and lower respectively for the group that was fed with stem borer larvae that were

exposed to Bt maize. The overall result of this study, in which the possible effect of food quality was excluded, showed that *Cry* 1Ab protein had an adverse affected on only certain fitness components during the life cycle of *C. pudica*. However, since this study represented a worst-case scenario where diverse prey was not available to *C. pudica*, negligible effects is expected under field conditions where prey is more diverse.

Key words: Lacewings, natural enemies, predator, risk assessment, third trophic level

3.2 Introduction

Since the first deployment of genetically modified (GM) crops with insecticidal properties, there has been concern with regard to resistance development of target pests and possible non-target effects (Meeusen & Warren, 1989; Tabashnik, 1994; Gould, 1998; Dutton *et al.,* 2002). Although Bt proteins are considered safe due to their selective mode of action, there are concerns since the insecticidal protein in most tissues of the plant is continuously expressed throughout the growing season (Dutton *et al.,* 2002). The potential effect of Bt proteins, such as *Cry* 1Ab expressed in Bt maize, at the third trophic level is of specific concern. Evolution of Bt-resistant pests for example, facilitates a new pathway of exposure of entomophagous arthropods to *Cry* 1Ab proteins. Natural enemies play an important role as biological control agents and are considered important natural resources. If the Bt protein produced by GM crops affects natural enemies such as green lacewings, *Chrysoperla* spp. (Neuroptera: Chrysopidae), it may disrupt beneficial interactions in agro-ecosystems where they are important predators (Dutton *et al.,* 2003). *Chrysoperla pudica* (Navás) is a common chrysopid species in maize ecosystems in South Africa (Keulder & Van den Berg, in review) and because of its polyphagous feeding habits, will be exposed to Bt protein consumed by larvae of resistant target pests, as well as other non-target arthropods that feed on Bt maize (Van Wyk *et al.,* 2007).

The green lacewing *C. carnea* (Stephens) is frequently used as test species in insecticide non-target effect studies as well as studies with Bt proteins (Hilbeck *et al.,* 1998a; Hilbeck *et al.,* 1998b; Romeis *et al.,* 2003; Lawo & Romeis, 2007; Li *et al.,* 2008; Li *et al.,* 2010). *Chrysoperla carnea* does, however, not occur in the southern hemisphere where *C. zastrowi* (Esben-Petersen) is the only representative of the Carnea-group (Duelli, 2007). The chrysopid species complex occurring in maize, in the southern hemisphere, which is also a receiving environment of GM Bt maize, is dominated by the Pudica-group (Duelli, 2007), of which *C. pudica* is common. When non-target evaluation of products such as Bt maize or cotton is done, it is important to

test species that occur in the receiving environment (Andow & Hilbeck, 2004; Van Wyk *et al.,* 2007).

Lacewing larvae are voracious predators with a high effective searching capacity and very high prey consumption rate. *Chrysoperla* spp. feed on large and small soft-bodied arthropods. Among the prey consumed, are pests of economic importance such as aphids, whitefly, mites and mealy bugs (Senior & McEwen, 2007). *Chrysoperla zastrowi* for example consume an average of 488 aphids or 906 potato tuber moth eggs during its larval stages. The capacity for food consumption increases as larvae grow and by the third larval stage 84 aphids or 200 potato tuber moth eggs can be consumed per day (Barnes, 1975). These characteristics enable larvae of *Chrysoperla* species to be effective biological control agents (Senior & McEwen, 2007).

Studies showed that duration of the larval period of *C. carnea* increased and the predation rate decreased when fed Bt-sprayed *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) caterpillars and *Aphis durantae* (Theobald) (Hemiptera: Aphididae) (Salama, 1982, cited by Szentkiralyi, 2007).

Dutton *et al.* (2002) and Hilbeck *et al.* (1998a) also reported an indirect negative effect of *Cry*1Ab protein which negatively affected development and survival of *C. carnea* larvae when rearing the larvae on herbivores that fed on Bt plants. They did, however, indicate that it could have been as a result of the low prey quality (sick prey) and not because of the Bt protein.

Evolution of resistance of the maize stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) to Bt maize in South-Africa (Van Rensburg, 2007; Kruger *et al.,* 2009) facilitated the rearing of *B. fusca* larvae on Bt maize without observable negative effects to stem borer larvae. These larvae provided a unique opportunity to exclude food quality as a mortality factor and to study the effect of *Cry* 1Ab protein, consumed by healthy prey, on the biology and fitness of *C. pudica.*

The aim of this study was to evaluate the effect of indirect exposure of *C. pudica* to *Cry* 1Ab protein, through healthy Bt-maize feeding prey, on its biology.

3.3 Material and methods

Chrysoperla, as well as their food sources used in this study, i.e. *B. fusca* larvae and *Ephestia cautella* eggs (Walker) (Lepidoptera: Pyralidae), were reared in the entomology laboratory at North-West University, Potchefstroom, South Africa.

3.3.1 Insect rearing

3.3.1.1 *Chrysoperla pudica* rearing

Adults were collected from a cotton field at the Vaalharts irrigation scheme (27°50'S; 24°50'E), (Northern Cape Province) in South Africa. This experiment was done with the F1-generation obtained from these adults. Adults were kept in glass containers (30 x 20 x 20 cm) covered with a fine mesh. A mixture of honey, sugar and water was provided as a food source on cotton wool. Eggs were laid on the mesh covering the glass container and occasionally on the glass surface. Glass containers were kept at room temperature and natural day/night photoperiod. Detailed information on rearing is provided in Appendix I.

3.3.1.2 *Ephestia cautella* rearing

Ephestia cautella eggs (0.1 g) were placed in plastic jars, each containing 100g of grain based diet. Larval and pupal development was completed inside jars. When moths emerged, they were transferred to oviposition chambers from which large numbers of eggs could be collected on a daily basis. These eggs were used as food for lacewing larvae in this study. The jars were kept at room temperature and natural day/night photoperiod. Detailed information on rearing is provided in Appendix II.

3.3.1.3 *Busseola fusca* rearing

Pairs of male and female moths were kept in plastic containers (25 x 15 cm), in which a maize stem was provided as oviposition substrate. Eggs were later removed and placed in petri dishes until they hatched. First-instar larvae were placed on whorl leaf tissue of either Bt maize (MON 810) (hybrid DKC 78-15B) or its iso-hybrid (CRN 3505), a non-Bt maize variety that served as food. When larvae reached the second to third instar on the maize plant material, after feeding for a period of 4 – 10 days, they were used as prey for chrysopid larvae. Rearing was done in an incubator at 26 ± 1 °C, with relative humidity of 60-70%. Detailed information on rearing is provided in Appendix III.

3.3.2 Chrysoperla pudica feeding experiment

Chrysoperla eggs were removed from glass containers by carefully cutting the stalks. Eggs were placed individually into test tubes (75 x 10 mm) where hatching took place. Test tubes were kept in an incubator at 26 ± 1 °C, with relative humidity between 60-70% and under natural day/night photo period. Test tubes were plugged with water-damped cotton wool to prevent larvae from escaping and to provide humidity. In the rare occasion that larvae did escape, the individual was removed from the experiment.

The day that eggs hatched was recorded as day zero. During the first three days each larvae received 0.003g newly laid *E. cautella* eggs per day. On the third day, each individual was weighed, put into a clean test tube and individuals randomly divided into five diet groups (Table 1). Each group consisted of 50 3-day old individuals.

Diet Group 1 received the highest exposure to Bt protein since they were fed only Bt-resistant *B. fusca* larvae that were reared on Bt maize. Group 2 served as a control for Group 1 and were not exposed to any Bt protein since their prey consisted of *B. fusca* larvae reared only on non-Bt maize. Group 3 was less exposed to Bt protein and, for two days, received larvae of Bt-resistant *B. fusca* that were reared on Bt maize, after which the lacewing larvae were again provided with 0.003 g *E. cautella* eggs per day for two

more days. This feeding cycle was repeated until a lacewing pupa formed. Group 4 served as control treatment for Group 3 and also received food in two-day cycles. The only difference was that they received larvae reared on non-Bt maize. Group 5 received 0.003 g of newly laid *E. cautella* eggs daily.

Second- to third-instar *B. fusca* larvae were fed to *Chrysoperla* larvae in ample quantities of four to ten larvae per day, depending on larval size. After chrysopid larvae were ten days old, they received only third instar *B. fusca* larvae as food. The mouth parts of these larvae were damaged with a pinset before introducing them as food into test tubes to prevent them from injuring the *Chrysoperla* larvae. The *Chrysoperla* larvae were transferred to clean test tubes every third day. Debris was removed from each test tube daily before fresh food was provided.

Individual chrysopid larvae were weighed at three-day intervals until pupation and larval mortality was recorded daily. The number of days until the onset of the pupal stage, as well as adult emergence was recorded. Larval mortality, as well as overall mortality, which included mortality factors during the pupal en adult emergence stage, was calculated for each diet group. Pupal mortality was taken as individuals that died during the pupal phase inside the cocoon while unsuccessful emergence was recorded when individuals that did emerge from cocoons, did not expand their wings. Mortality during the pupal as well as adult emergence phase was expressed as percentages of the actual number of pupae.

An attempt was made to determine the fecundity of females that emerged successfully from their cocoons. This was, however, not successful since the numbers that emerged were low and males were not always available when females were.

3.4 Data analysis

Data on larval mass at three-day intervals, days to pupation and days in pupation were analyzed by means of t-tests. Data were compared between Groups 1 and 2 and between Groups 3 and 4. Two-sided Fisher tests were used to compare other life stage parameters. An analysis of variance was used to determine whether the mass of larvae assigned to the different diet groups on day three was similar.

3.5 Results

3.5.1 Larval mass

Since all larvae fed on *E. cautella* eggs during the first three days prior to assigning them to different diet groups, there were no differences in mean larval mass on day three (p=0.13). Three days after the onset of the experiment, (day six of larval development) Group 5, which were fed *E. cautella* eggs only, showed the highest larval mass. There were significant differences between larval mass of diet Groups 3 and 4, at days six and nine (Table 2). However, on days 12 and 15 there were no differences between any of the treatments. The larval stage of Groups 1 and 2 lasted for 15 days without any difference in mean larval mass between these groups.

3.5.2 Larval period and pupation period

No significant differences were observed in the duration of the larval stage between the diet groups (Table 3). The most rapid mean larval development period of 9.8 days was observed for Group 5 which fed on *E. cautella* eggs only (Table 3). The larval development of Groups 1 and 2 took 13.11 and 13.02 days respectively. This was 14.6 and 12.2% longer than larvae from the groups which received *E. cautella* eggs together with the Bt- or non-Bt consuming borer larvae as part of their diet.

A significant difference was observed between the duration of the pupal periods of individuals in Groups 1 and 2 (p=0.0017) (Table 3). Group 1, which only fed on Bt-fed *B. fusca*, had a significantly shorter pupal period (8.85 days) compared to its control group (Group 2) which had the longest pupation period (10.02 days). This results could not be explained and is contrary to what was expected. There was no significant difference between the mean duration of the pupal period of Groups 3 and 4.

3.5.3 Larval mortality, overall mortality and pupal formation

Larval survival in the experiment was high and ranged between 68 % and 94 % for Groups 1 and 5 respectively (Table 4). There was no significant difference in larval survival of the group that fed on Bt-maize reared larvae only (Group 1) and that of the group that were fed non-Bt reared larvae (Group 2) (Table 4). There was also no significant difference between larval survival of the two groups that fed on both stem borer larvae and *E. cautella* eggs (Groups 3 and 4).

Percentage unsuccessful emergence from cocoons and the percentage mortality in the pupal stage did not differ significantly between the different diet groups (Table 4). The percentage adult emergence differed between groups 1 and 2 with only nine of the 50 individuals surviving to adulthood in Group 1 compared to 17 individuals in Group 2 (Table 4).

3.6 Discussion

The differences in larval mass and development times between different diet groups were expected since the *E. cautella* egg-diet is an optimum diet for rearing of *Chrysoperla*. Eggs of *Ephestia* moths were used as the comparative diet or to maintain rearing colonies in various studies (Li *et al.,* 2010; Hilbeck *et al.,* 1998b; Li *et al.,* 2008; Corrales & Campos, 2004; Meier & Hilbeck, 2001;).

Since the aim of this study was to evaluate the effect of indirect exposure of *C*. *pudica* to *Cry* 1Ab protein, comparisons should be made between Groups 1 and 2 and between 3 and 4 and not with larvae that only fed on *E. cautella* eggs.

The differences between larval mass of different diet Groups 3 and 4, at days six and nine cannot be explained since the chrysopid larvae that were exposed to Bt protein were heavier than its control on day six, while the opposite was observed on day nine (Table 2). An interesting result was that the larval stage of Groups 1 and 2, was longer than that of other groups, indicating that a diet of stem borer larvae only, resulted in delayed onset of the pupal stage (Table 3). The shorter duration of the larval stages of diet groups 3 and 4 is therefore ascribed to improved food quality and the presence of *E. cautella* eggs in their diet.

Results also showed that the delayed onset of the pupal period resulted in heavier chrysopid larvae with the onset of pupation (day 15) (Table 2), most likely because they had more time to feed than those pupating earlier. Zheng *et al.* (1993) showed that limited food resources resulted in development of smaller adults with lower fecundity. Unlimited sub-optimal food sources (borer larvae compared to insect eggs) therefore resulted in delayed pupation but also in development of larger individuals.

The duration of the pupal stage was the shortest for chrysopid larvae that were fed with only Bt-feeding stem borer larvae indicating that exposure to Bt protein did not have an adverse effect on pupal development, unless a shorter duration of the pupal period affects another fitness component. The percentage of adults that successfully emerged from cocoons was observed to be significantly less in Group 1, which was exposed only to Bt-feeding stem borer larvae. However, under more realistic conditions, with reduced exposure to Bt protein (Groups 3 and 4) no differences were observed between duration of the pupal period, mortality during pupal stage or adult emergence.

This study represents a worst-case scenario where diverse prey was not available to *C. pudica*. Even under these conditions C*. pudica* was only affected by *Cry* 1Ab expressed in maize during the pupal development phase and adult emergence. At field level, where prey is more diverse, this effect is suspected to be negligible. A further important aspect that may affect chrysopid food selection was highlighted in choice-tests done by Meier and Hilbeck (2001) which showed that *C. carnea* larvae preferred aphids to Lepidoptera larvae. The cryptic behaviour of stem borer larvae also makes them less likely to be exploited as food by chrysopid larvae. A 2-year field study by Bourguet *et al.* (2002) indicated that *C. carnea* numbers were not affected by Bt maize and numbers were similar to those inside non-Bt maize fields.

Movement of *Cry* proteins through the food chain was reported by Obrist *et al.* (2006) who indicated the presence of *Cry* 1Ab proteins in chrysopid larvae that fed on *S. liitoralis* larvae or mites that consumed Bt maize. Several factors need to be investigated in the analysis of tri-trophic effects that *Cry* 1Ab proteins may have on natural enemies such as *Chrysoperla* spp. Dutton *et al*. (2002) summarized and analysed these factors and showed that knowledge of suitability and importance of the available prey in the field is important. It is also important to know if both prey and predator ingested the Bt proteins, which was unfortunately not known in this study. The Bt protein can be ingested either by feeding directly on the genetically modified plant (prey) or indirectly at the third trophic level when a predator feeds on contaminated prey. Depending on the life-history of the predator it can also ingest Bt proteins directly by feeding on plant tissue, for example chrysopid adults feeding on Bt pollen. Aphids, which are phloem sucking arthropods that do not ingest the Bt protein (Head *et al.,* 2001) are the major prey of chrysopids (Coderre, 1988). It is furthermore important to establish whether the Bt protein affects the prey itself. In some cases it is believed that the Bt protein affects prey quality which in turn affects the predator because of the combination of poor prey quality (sick prey) and Bt protein (Dutton *et al.,* 2002; Hilbeck *et al*., 1998a).

Feeding by chrysopids on aphids (*Rhopalosiphum padi*) (L.) (Homoptera: Aphididae) and spider mites (*Tetranychus urticae*) (Koch) (Acari: Tetranychidae) which consumed high and low concentrations of Bt protein respectively, did not affect predator development and survival (Dutton *et al*., 2002). However, predator development and survival was also shown to be adversely affected when feeding on Lepidoptera larvae (*S. littoralis*) that consumed a low concentration of Bt protein, leading to the conclusions that a combined interaction of poor prey quality and *Cry* 1Ab toxin may be the reason for the negative effects observed by Dutton *et al*. (2002).

Several high-dose toxicity tests in which the effect of Bt protein on *Chrysoperla* spp. was determined, were done by Lawo & Romeis (2007), Rodrigo-Simón *et al.* (2006) and Romeis *et al.* (2003). Romeis *et al.* (2003) tested the hazard potential of a transgenic protein using high-dose toxicity tests by mixing purified protein into an artificial diet and concluded "that transgenic maize expressing *Cry* 1Ab poses a negligible risk for this predator". Lawo and Romeis (2007) found that *Cry* proteins did not affect development of *C. carnea.* Rodrigo-Simón *et al.* (2006) found similar results and concluded that the *Cry* toxins, even at concentrations higher than those expected in real-life situations, did not have detrimental effects on *C. carnea*. Obrist *et al.* (2006) indicated that chrysopid survival and development time was, however, only adversely affected when they fed on *S. littoralis* larvae as compared to mites containing higher concentrations of Bt protein, also leading to conclusions that adverse effects were not due to the presence of *Cry* 1Ab protein but to poor prey quality.

Hilbeck *et al.* (1998a) assessed the impact of Bt proteins (*Cry* 1Ab) on *C. carnea* larvae by using *Ostrinia nubilalis* (Hübner) (Lepidoptera: Noctuidae) and *S. littoralis* larvae as prey. Their observations indicated that chrysopid larvae that fed on Bt-reared prey had a higher mortality and prolonged development time compared to prey that was reared on non-Bt maize. They concluded that the poor quality of the prey used in combination with the Bt proteins could have been the reason for the negative effects observed. In a follow-up study Hilbeck *et al.* (1998b) used a bioassay technique

incorporating the *Cry* 1Ab protein into a liquid diet that was then encapsulated within small paraffin spheres, that was with or without the protein. The study showed that chrysopid larvae that were fed *Cry* 1Ab protein spheres had higher larval mortalities than the control groups and required more time to complete the larval stage which lead to the conclusion that the Bt protein was toxic to chrysopid larvae.

Recent studies by Li *et al.* (2008) on pollen feeding adult *C. carnea* concluded that *Cry* 1Ab protein in pollen does not affect fitness of adult chrysopids even at concentrations exceeding the levels in pollen. A follow-up study showed that even though the pollen grains were not fully digested, the insects were exposed to transgenic insecticidal proteins contained in the pollen (Li *et al.,* 2010). Mason *et al.* (2008) found that maize cultivars containing Bt protein (Event 176 and TC1507 (*Cry*1F)) may prolong the adult stages of *C. plorabunda* (Fitch), compared to their non-Bt isolines. More importantly they found that the mean number of eggs produced per female was significantly lower for the group that was fed MON810 pollen compared to their non-Bt isoline (Mason *et al.,* 2008).

The overall result of this study, in which the possible effect of food quality was excluded, showed that *Cry* 1Ab protein had an adverse affected only on certain fitness components during the life cycle of *C. pudica*. However, since this study represented a worst-case scenario where diverse prey was not available to *C. pudica*, negligible effects are expected under field conditions where prey is more diverse.

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Table 1. Diet groups used in the *Chrysoperla pudica* feeding experiment.

Diet group	Bt exposure	Diet	Number per day
1	Bt +	2nd-3rd instar Bt-resistant *B. fusca* reared on Bt-maize	4-10 larvae
2	Bt -	2nd-3rd instar *B. fusca* reared on conventional maize	4-10 larvae
3	Bt +	2nd-3rd instar Bt-resistant *B. fusca* reared on Bt-maize for 2 days followed by *E. cautella* eggs for 2 days	4-10 larvae/ 0.003g eggs
4	Bt -	2nd-3rd instar *B. fusca* reared on conventional maize for 2 days followed by *E. cautella* eggs for 2 days	4-10 larvae/ 0.003g eggs
5	Bt -	*Ephestia cautella* eggs	0.003g eggs

Table 2. Mean mass (mg) of *Chrysoperla pudica* larvae over time after feeding on different diets consisting of Bt-fed and Bt-free prey (±S.E).

Groups*	Day 6	Day 9	Day 12	Day 15
1	1.560 (±0.079) t-value **=1.112	3.834 (±0.406) t-value=-1.221	7.391 (±0.553) t-value=1.657	8.400 (±0.743) t-value=1.479
2	1.437 (±0.076) p=0.2688	4.500 (±0.366) p=0.2257	6.019 (±0.619) p= 0.1049	6.275 (±1.323) p=0.1825
3	2.188 (±0.080) t-value=2.105	5.050 (±0.285) t-value=-2.686	6.307 (±0.312) t-value=-0.639	
4	1.945 (±0.082) p=0.0381	6.328 (±0.378) p=0.0087	6.867 (±1.328) p=0.5321	
5	3.306 (±0.385)	6.297 (±0.284)		

*****Gr 1 only received Bt-fed *B. fusca* larvae. Gr 2 only received non-Bt fed *B. fusca* larvae. Gr 3 received Bt-fed *B. fusca* larvae and *E. cautella* eggs on alternate days. Gr 4 received non-Bt fed *B. fusca* larvae and *E. cautella* eggs on alternate days. Gr 5 only received *E. cautella* eggs.
****** t-tests indicate comparison between pairs of diet groups only.

Table 3. Duration of the larval period (days to pupation) and pupal period (days in pupation) of *Chrysoperla pudica* fed on different diets.

Diet group *	Larval period days (±S.E)	Pupal period days (±S.E)
1	13.11 (±0.3) t-value=0.1957**	8.85 (±0.2) t-value=-3.280
2	13.02 (±0.3) p=0.8453	10.02 (±0.2) p=0.0017
3	11.53 (±0.2) t-value=1.3294	9.71 (±0.1) t-value=1.36
4	11.14 (±0.1) p=0.1874	9.47 (±0.1) p=0.1782
5	9.82 (±0.1)	9.86 (±0.2)

*****Gr 1 only received Bt-fed *B. fusca* larvae. Gr 2 only received non-Bt fed *B. fusca* larvae. Gr 3 received Bt-fed *B. fusca* larvae and *E. cautella* eggs on alternate days. Gr 4 received non-Bt fed *B. fusca* larvae and *E. cautella* eggs on alternate days. Gr 5 only received *E. cautella* eggs.
****** t-test indicates comparison between pairs of diet groups only.

Table 4. Life stage parameters of *Chrysoperla pudica* fed on different diets.

Diet group*	% Larval survival (n)	% Unsuccessful emergence (n)	% Mortality in pupal stage (n)	% Adult emergence (n)
1	68.0 (34) p=0.0756**	55.9 (18) p=0.0894	20.6 (7) p=0.3576	18.0 (9) p=0.0151
2	80.0 (40)	42.5 (17)	15.0 (6)	34.0 (17)
3	82.0 (41) p=1.1458	26.8 (11) p=0.3569	14.6 (6) p=0.6796	48.0 (24) p=0.6705
4	82.0 (41)	34.1 (14)	12.2 (5)	44.0 (22)
5	94.0 (47)	17.0 (8)	4.3 (2)	74.0 (37)

*****Gr 1 only received Bt-fed *B. fusca* larvae. Gr 2 only received non-Bt fed *B. fusca* larvae. Gr 3 received Bt-fed *B. fusca* larvae and *E. cautella* eggs on alternate days. Gr 4 received non-Bt fed *B. fusca* larvae and *E. cautella* eggs on alternate days. Gr 5 only received *E. cautella* eggs.
** Fisher-test used to compare between pairs of diet groups only.

Chapter 4

Flight activity patterns, flight height and migration of *Chrysoperla pudica* (Neuroptera*:* Chrysopidae)

4.1 Abstract

In order to survive lacewings must be able to migrate between vegetation types to follow their prey as crops and seasons change. Lacewings are predominantly arboreal, but a few species are able to live in patchy and temporary environments, making them highly successful predators in field crops. The aim of this study was to determine the daily flight activity pattern of chrysopids, to determine how high above the crop canopy they fly and to determine the movement of adult *Chrysoperla* spp. between maize fields and surrounding headlands. Flight activity and flight height data were collected by erecting five 6 m-high sticky traps that were monitored for a total of 10 days at hourly intervals between 16:00 – 08:00. Migration patterns were determined by erecting 40 sticky traps in five transects from inside a maize field through a lucerne field and into a grassy headland. These traps were monitored for seven days at daily intervals. Sweep net samples were collected to determine chrysopid population densities in each vegetation type. It was determined that chrysopids was most active between 16:00 - 23:00 and that they fly largely between 0.5 m - 2.5 m above ground level. An attempt was also made to quantify migration between different vegetations types. This aspect of the study was terminated because of bad weather conditions at all occasions when traps were erected. Chrysopids were never present in grassland vegetation, but the lucerne field maintained a large population. As the maize crop developed and reached the reproductive stage, chrysopid population numbers increased inside the field, presumably originating from the adjacent lucerne field.

Key words: sticky traps, lacewings, migration, natural enemies

4.2 Introduction

Insects have developed a number of ecophysiological and behavioural mechanisms to avoid or escape from temporary adverse environmental conditions. The two most important strategies are migration and diapause (Duelli, 1980).

Field crops are temporary habitats. Some times of the year crops are unsuitable for predatory insects such as Chrysopidae (Neuroptera), since crop habitats change from season to season. Larvae of chrysopid species are active predators with a high effective searching capacity and high prey consumption rate (Senior & McEwen, 2007) and they must therefore be able to migrate between vegetation types to follow their prey as the crop and season changes.

Migration of lacewings for example can be described as a movement process away from the crop field, because of a decrease in food or unfavourable environmental conditions, such as the harvest process, and then back into the crop the next season. For practical reasons, migration patterns are difficult to quantify (Dingle, 1996). Migrating insects use airstreams to travel hundreds of kilometres in a single flight. Duelli (1980) described flight patterns of *Chrysoperla carnea* (Stephens) (Neuroptera; Chrysopidae) and found that shortly after emergence the adults perform adaptive dispersal flights which are straight downwind flights. Adults therefore use the wind to move. This use of wind to migrate, combined with the fact that the adults are small, and that flight activity is nocturnal means that it is very difficult to observe and study the migration and flight activity patterns of lacewings.

This study was motivated by the renewed interest in lacewings as biological control agents of agricultural pests and the possible effect that genetically modified Bt maize could have on their populations. Neuroptera are excellent indicators of environmental- and habitat transformation, and also include key species for signifying areas and faunas that require priority protection (Mansell, 2002). *Chrysoperla* species are also commonly used for testing

pesticide effects that genetically modified crops could have on non-target species (Hilbeck *et al.,* 1998a; Hilbeck *et al*., 1998b; Dutton *et al.,* 2002; Dutton *et al.,* 2003).

The aim of this study was to determine the flight activity patterns of chrysopids in a lucerne field and to determine how high above the crop canopy they fly. An attempt was also made to study the movement of adult *Chrysoperla* spp. between maize fields and surrounding habitats.

4.3 Material and methods

Field experiments were conducted at the Vaalharts irrigation scheme (27°50'S; 24°50'E) located in the Northern Cape Province. The Vaalharts irrigation scheme is a high input irrigation scheme where no intercropping is done.

4.3.1 Flight activity and flight height

Five 6 m-high sticky traps were placed inside a lucerne field (± 2 ha) (Fig. 4.1). Traps were constructed of wooden frames (6 m x 30 cm) covered with mesh and attached by means of a pulley system between two PVC pipes (Fig. 4.2). The mesh was covered with sticky glue (stickem) and marked at 50 cm intervals from bottom to top. The traps were erected at five occasions (May 2009, August 2009, September 2009, November 2009 and March 2010) and left in the field to be monitored for two night-cycles at each occasion. The nocturnal flight activity of lacewings was monitored every hour from 16:00 through the night until 08:00. The number of lacewings caught at different heights was determined by lowering the mesh-covered panels and counting the number of adults trapped on both sides of the mesh. Individuals on the mesh were not removed, but marked at each hourly interval. Traps were maintained during the day, but hourly monitoring was not done. At commencement of daily monitoring (16:00) traps were checked for presence of lacewing adults to determine if any individuals were trapped during the day.

Fig. 4.1 Five sticky traps erected in lucerne field (Vaalharts irrigation scheme).

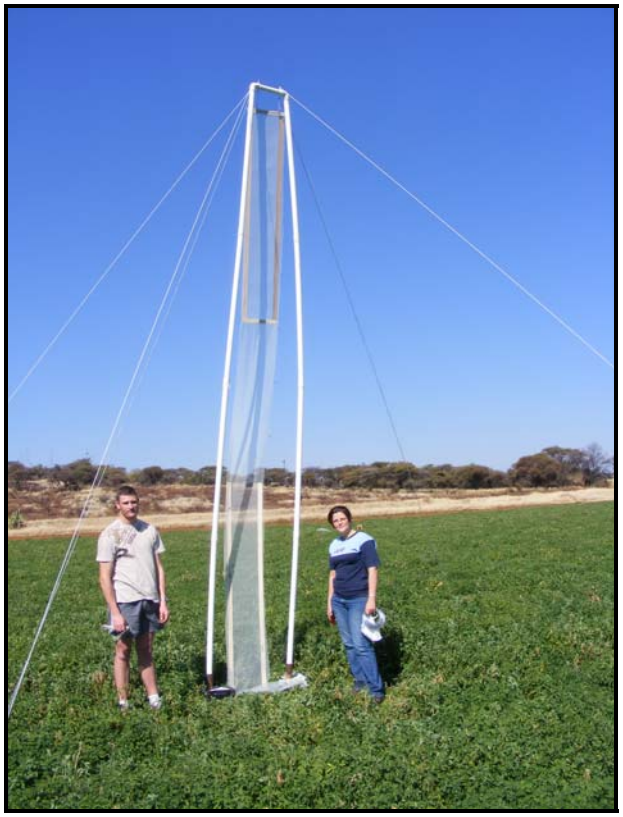

Fig. 4.2 Six meter-high sticky trap.

4.3.2 Flight direction

Five transects of traps each consisting of eight 3 m-high sticky traps (40 traps in total) were erected (Fig. 4.3). The transects stretched from inside a maize field through a lucerne field and into a grass headland (Fig. 4.4). This was done at two occasions (January 2010 and March 2010). Traps were placed inside the field for five days at a time and monitored daily for presence of lacewings on traps. The distance between traps within the rows was 20 m and between the rows 30 m.

Fig. 4.3 Three meter-high sticky traps erected in transects from inside a maize field through a lucerne field and into the grassy headland.

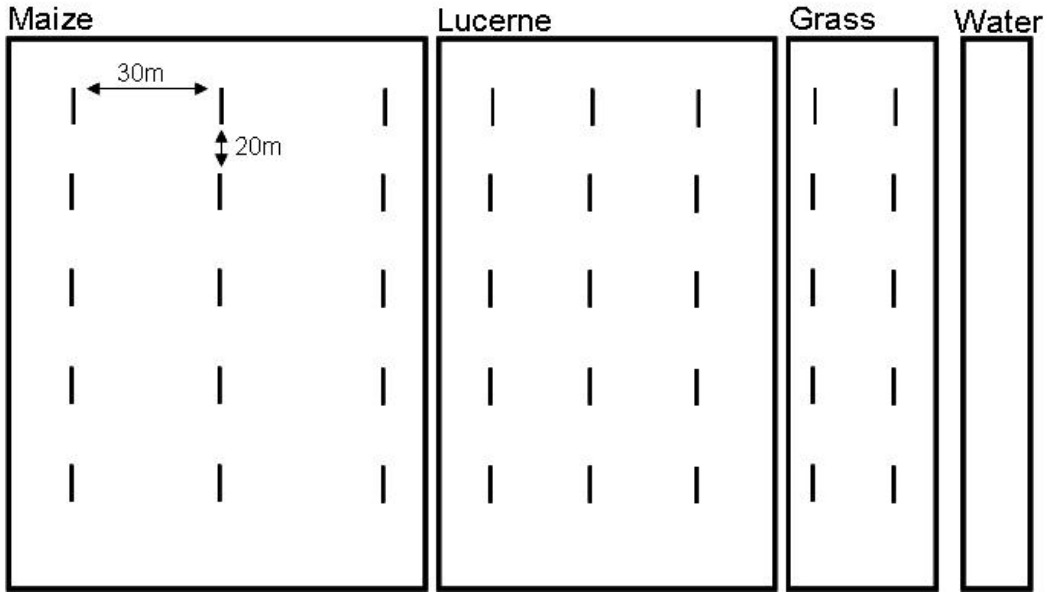

Fig 4.4 Schematic representation of transects of traps inside a maize field, lucerne field and a grassy headland.

4.3.3 Sweep net samples

During the 2009/2010 season sweep net samples were taken at regular intervals inside lucerne- and maize fields, as well as inside the grassy headland. Five blocks were randomly selected inside each field. Ten 180^{0-} sweeps were taken in each block by means of a sweep net and lacewings were removed from sweep nets with a pooter. The sampled surface area inside each block was calculated to be 36 m^2.

4.4 Data analysis

Data on flight patterns were calculated as a 2-hour moving average for lacewing catches and nightly flight activity was presented graphically. Flight height was calculated as proportions of catches at specific heights above soil level. The mean number of lacewings collected with sweep nets per field was calculated and also expressed as number per hectare.

4.5 Results and discussion

4.5.1 Flight activity and flight height

The dominant chrysopid species that occurred at Vaalharts irrigation scheme was *C. pudica* (Navàs) (Keulder & Van den Berg, in review). Since the traps were maintained during the day, between the two night-cycles, day-flight activity could also be recorded. However, no lacewing adults were found on the traps between 8:00 and 16:00 in this study, which was expected since Neuroptera are nocturnal (Duelli, 1986; New, 2007). During the daytime, lacewings rest on the under side of leaves and twigs (Duelli, 2007). Results showed that the lacewings started flying after 16:00 and reached peak activity by 23:00. From 23:00 the activity decreased rapidly and by 08:00 flight activity ceased (Fig. 4.5). Duelli (1986) studied flight activity patterns of chrysopids under laboratory conditions and observed that most lacewing species started flying after sunset, before it was completely dark, and that they have a short but consistent activity peak after the onset of flight. In this study it was observed that environmental conditions remained favourable, flight activity was observed until dawn, when flight activity decreases sharply (Duelli, 1986).

The highest position that lacewings were captured on traps was at 5.5 m. Fifty three of the 73 chrysopids sampled (72.6%) was between 0.5 m and 2.5 m above soil level (Fig. 4.6). Duelli (1980) estimated an average height of flight of females during the first 2-3 nights after eclosion was between 6 to 12 m, where the average for females in the oviposition stage was around 3 m. The mean height of flight, for males and female was calculated to be between 6 and 12 m (Duelli, 1980).

4.5.2 Flight direction

At both occasions when this experiment was attempted (14 days of field work) several difficulties were experienced. The trap structures were not able to withstand adverse weather conditions and some of the traps were stolen over

night. At each occasion when monitoring was done, traps were demolished due to bad weather conditions. No data could be recorded. After two weeks of field work, it was decided to terminate this part of the study.

4.5.3 Sweep net samples

No lacewings were collected in any of the sweep net samples taken in the grassland vegetation at this study site. This may be due to of a more suitable environment (lucerne) nearby, which provided food in the form of aphids for the largest part of the year. Lacewings were present in the lucerne field throughout the season. However, population size inside the lucerne field changed during the season and was highest in April to May and October to December (Fig. 4.7). From May until September the adult numbers declined which may be as a result of the decreasing temperatures from autumn to winter. In September no adults were caught in the sweep nets inside lucerne fields. As the temperatures increased with the onset of spring (September to November) so did the population numbers and by November a mean number of three adults were found per 36 m^2 which equates to 833 adults per hectare. As the growth season of crops progressed the population numbers in the lucerne field decreased. No adults were found in the maize field from the onset of winter (May) until December, when plants were still in the vegetative stage of developmeny. This was because of the change in season and since the maize was drying off and the general abundance of arthropods on maize decreased. In August the farmer harvested the maize and the field was ploughed in October. For this reason there were no adults found inside maize. In December the farmer planted the new season's maize and in January chrysopid adults could be found in the maize. By February when the maize flowered there was again a rapid increase in chrysopid numbers inside maize with a mean number of 2.8 adults per 36 m^2 which equates to 777 adults per ha. The lacewing population in the maize field most likely originates from the population that is sustained in the adjacent lucerne field and their movement to maize is most likely because of an increasing amount of food becoming available inside maize fields.

4.6 Conclusion

This study showed that *Chrysoperla* populations at the Vaalharts irrigation scheme, which consisted largely of *C. pudica*, is mainly active during 16:00 – 23:00 and that flight activity is largely in the 2.5 m above the crop canopy. Although limited data were collected on the incidence of chrysopids inside the different vegetation types and movement between vegetation types, results seemed to indicate that no chrysopids adults occurred in the wild habitat and that lucerne acted as source of lacewings that colonized maize. Since this grassland was situated in an agricultural area, surrounded by maize and lucerne crops, the distribution of lacewings in more natural areas needs to be investigated further.

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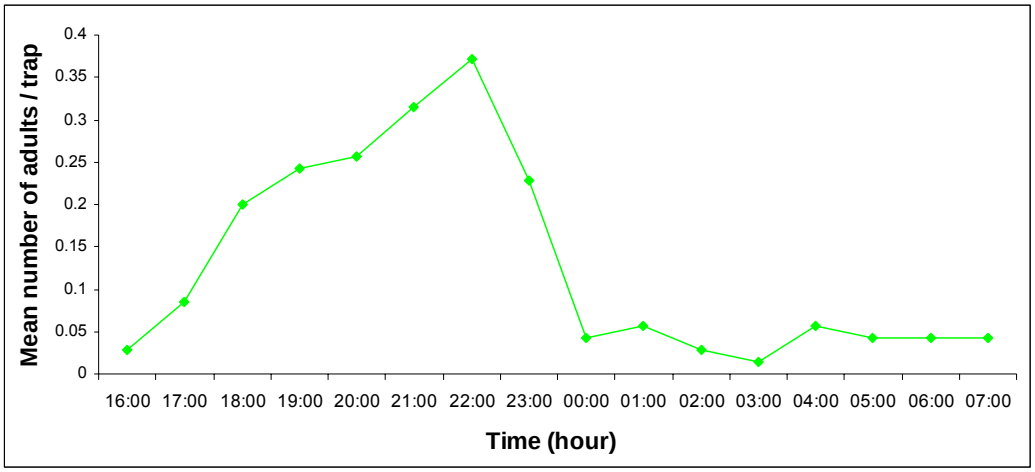

Fig. 4.5 Mean daily flight activity pattern of lacewing adults at the Vaalharts irrigation scheme (7 days during 2009-2010 growing season)

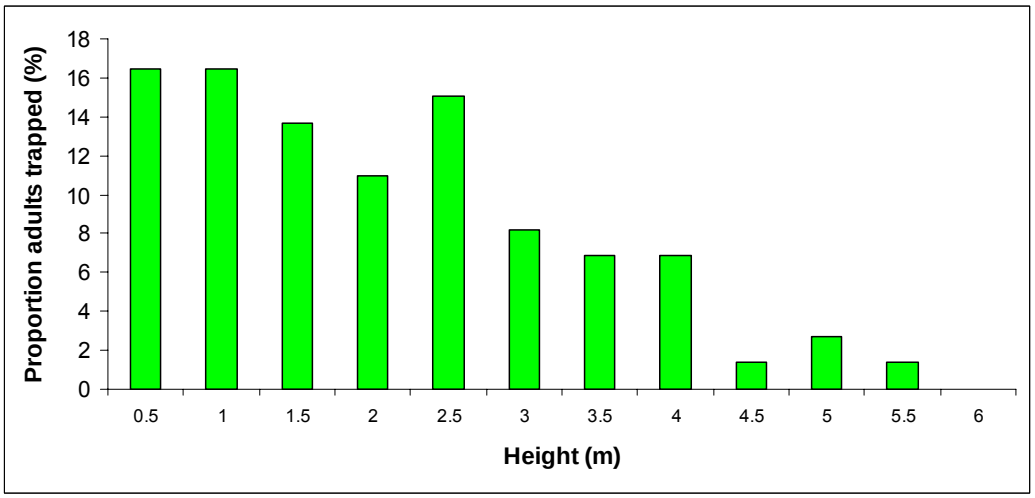

Fig. 4.6 Flight height of lacewing adults at the Vaalharts irrigation scheme (7 days during 2009-2010 growing season).

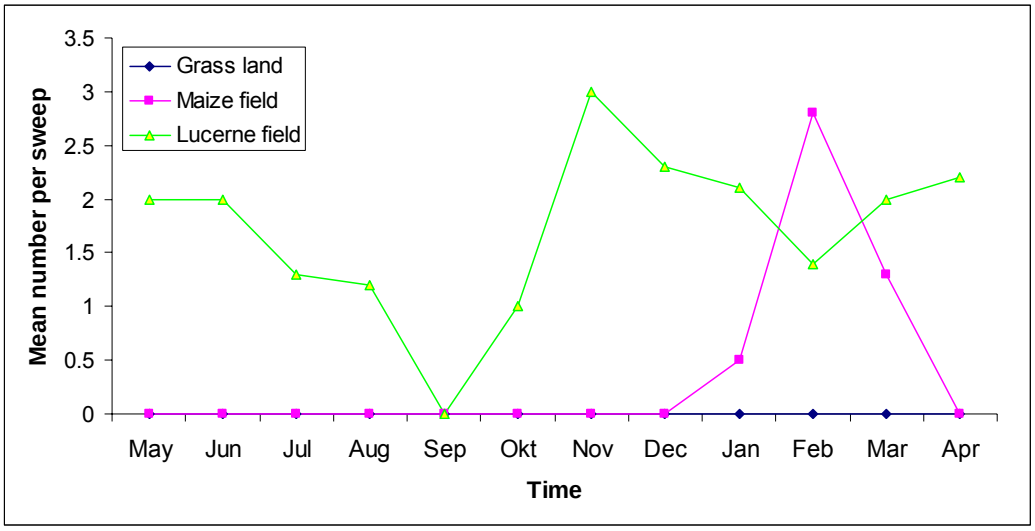

Fig. 4.7 Mean number of lacewing adults caught in sweep nets over time, in adjacent grass, maize and lucerne field habitats (At the Vaalharts irrigation scheme during 2009-2010 growing season).

Chapter 5

Conclusion

Genetically modified (GM) Bt maize which produces an insecticidal *Cry* protein is planted in South Africa for control of lepidopterous stem borers of which *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is the most important (Van Wyk *et al*., 2007). Since the first deployment of GM crops with insecticidal properties, there has been concern with regard to possible non-target effects (Meeusen & Warren, 1989; Gould, 1998). Post-release monitoring of non-target effects and possible environmental impact of genetically modified crops with insecticidal properties on non-target organisms in South-Africa is required by law (Biodiversity act, 2004, Act No. 10 of 2004).

Lacewings, *Chrysoperla* spp. (Neuroptera: Chrysopidae), is one of the potential non-target species that could be indirectly affected by feading on herbivorous prey that consumed Bt protein aimed at the target pest (Hilbeck *et al*., 2006). Natural enemies such as chrysopids play an important role in agro- ecosystems and are considered important natural resources. If the Bt protein produced by GM crops affects natural enemies such as green lacewings, it may disrupt beneficial interactions in agro-ecosystems where they are important predators (Dutton *et al.,* 2003).

Monitoring of insect numbers, especially in terms of insect eggs, is often labour intensive and time consuming. Prior to this study the need existed to develop time-effective searching/monitoring techniques, for important beneficial insect species such as lacewings. Results from this study provided information that can be used in accurate sampling of eggs of *Chrysoperla* species in maize. Results showed a clear pattern with regard to vertical distribution of eggs on the plants relative to the position of maize ears. This study indicated that *Chrysoperla* spp. that occurs in maize fields were not indiscriminate in their choice of oviposition sites in the field and that they seem to have preferences for specific areas on plants. Our results also

showed that female chrysopids preferred to lay there eggs near maize ears. Choice-tests done with a Y-tube olfactometer, to investigate if the presence of aphids on maize plants had any influence on *Chrysoperla* spp. behaviour, showed that females responded positively to host plants that were infested with aphids.

Field crops are temporary habitats. Some times of the year crops do not host pray for chrysopids and its environment is therfore unsuitable for chrysopids, and often crop types change from season to season. Because lacewings have a high prey consumption rate they must be able to migrate between vegetations types to follow their prey as the crop and season change, in order to survive. Lacewings are predominantly arboreal, but a few species are able to live in patchy and temporary environments. These species are the most successful in field crops (Duelli, 1984). Migrating insects use moving airstreams to travel hundreds of kilometres in a single flight (Duelli, 1984). This usage of wind to migrate and move, combined with the fact that the adults are small, and that flight activity is nocturnal, means that it is very difficult to observe and study these migration and flight activity patterns.

Chrysoperla carnea (Stephens), a lacewing species that occurs in North America and Europe is used as test species in risk assessment with insecticidal GM crops. However, previous studies with *C. carnea* showed contradicting results. Some studies ascribed poor performance of this species to poor quality prey, caused by the effect of *Cry* 1Ab protein on the prey, while others indicated no effect. In this study an experiment was conducted in which the possible effect that food quality could have on lacewing performance was excluded. *Chrysoperla pudica* (Navàs) larvae were separated into five diet groups (50 individuals per group) receiving different diets to determine the effect of *Cry* 1Ab protein on their biology. Larval mass, larval and pupal development time, larval mortality and overall mortality were determined. Results showed that indirect exposure to Bt toxin had no significant effect on larval development period of *C. pudica*. The pupal period and adult emergence of individuals exposed to unrealistically high levels of Bt toxin, through feeding on stem borer larvae, was significantly shorter and

lower respectively. The overall result of this study, in which the possible effect of food quality did not play a role, showed that *Cry* 1Ab protein had an adverse affect on only certain fitness components during the life cycle of *C. pudica*. However, since this study represented a worst-case scenario where diverse prey was not available to *C. pudica*, negligible effects is expected under field conditions.

In order to study lacewing movements between habitats/vegetation types information is needed on daily flight activity patterns, as well as height at which individuals fly inside crop fields. In this study flight activity was investigated by means of sticky traps. Sweep net samples were collected to determine chrysopid population densities in each vegetation type. It was determined that chrysopids were most active between 16:00 and 23:00 and that they fly largely between 0.5 m - 2.5 m above ground level. An attempt was also made to quantify migration between different vegetations types. This part of the study was terminated because of bad weather conditions throughout the experimental period. Chrysopids were never present in grassland vegetation, but the lucerne field maintained a large population. As the maize crop developed chrysopid population numbers increased inside the maize field, presumably originating from the lucerne field. Although limited data were collected on the incidence of chrysopids in the different vegetation types and movement between vegetation types, it seems no chrysopid adults occurred in the wild habitat and that lucerne acted as source of lacewings that colonized maize.

Lacewings have long been appreciated as natural enemies of pests. However, their biology, behaviour and migration are not well understood. As indicator species it is important to study their ecology, biology and the possible effects that long-term exposure to Bt maize may have on populations of these beneficial species.

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Appendix I:

Chrysoperla pudica rearing

A rearing colony was started by collecting *Chrysoperla* adults from a cotton field in the Vaalharts irrigation scheme in South Africa. Adults were kept in glass containers covered with a fine mesh (Fig. 1). A mixture of honey, sugar and water was provided as food source on cotton wool soaked in the mixture. Eggs were laid on the mesh covering the glass container and occasionally on the glass surface. Eggs were collected every third and placed individually into test tubes (Fig. 2) (75 x 10 mm) where hatching took place. Test tubes were kept in an incubator at 26 $\pm$ 1 °C and relative humidity between 60-70%. Test tubes were plugged with water-damped cotton wool, to prevent larvae from escaping and to provide humidity. These individuals were either used in experiments or put back in the rearing colony. *Ephestia cautella* eggs (0.003 g) were provided daily as food and test tubes were cleaned every second day. Pupation and adult emergence took place in test tubes. Newly emerged adults were moved to the glass containers to continue the rearing cycle.

Fig. 1 Glass containers used in rearing of *Chrysoperla pudica* adults.

Fig. 2 Test tubes containing *Chrysoperla pudica* larvae.

Appendix II

Ephestia cautella rearing

Ephestia cautella (0.1 g) eggs were placed in bottles with 100 g of artificial diet (Fig. 3). The recipe of the diet is provided in Table 1. Larval and pupal stages were spent inside the bottle where the larvae fed on the diet and pupated between the debris. When moths emerged, they were placed in a refrigerator to decrease their movement and then removed from the bottles by using a pooter and a suction pump. They were placed in a special container (Fig. 4) which consisted of two chambers that were divided with mosquito gauze. Adults were put into the top chamber, where mating took place. When the adults laid their eggs, the eggs fell through the net to the bottom chamber where it was collected to serve as food for the lacewings or used for the next rearing cycle.

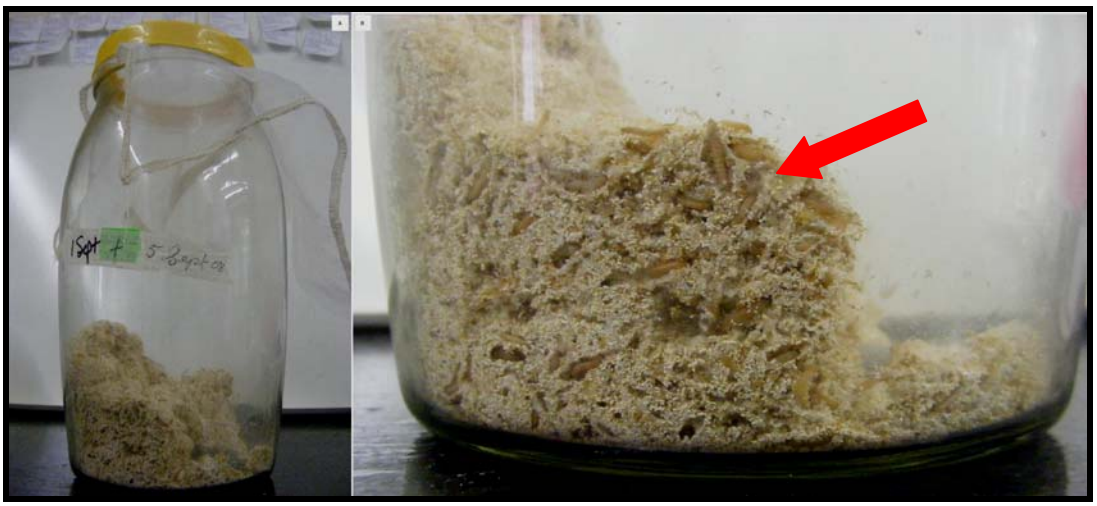

Fig. 3 Glass containers, containing diet material and *Ephestia cautella* larvae indicated by the red arrow.

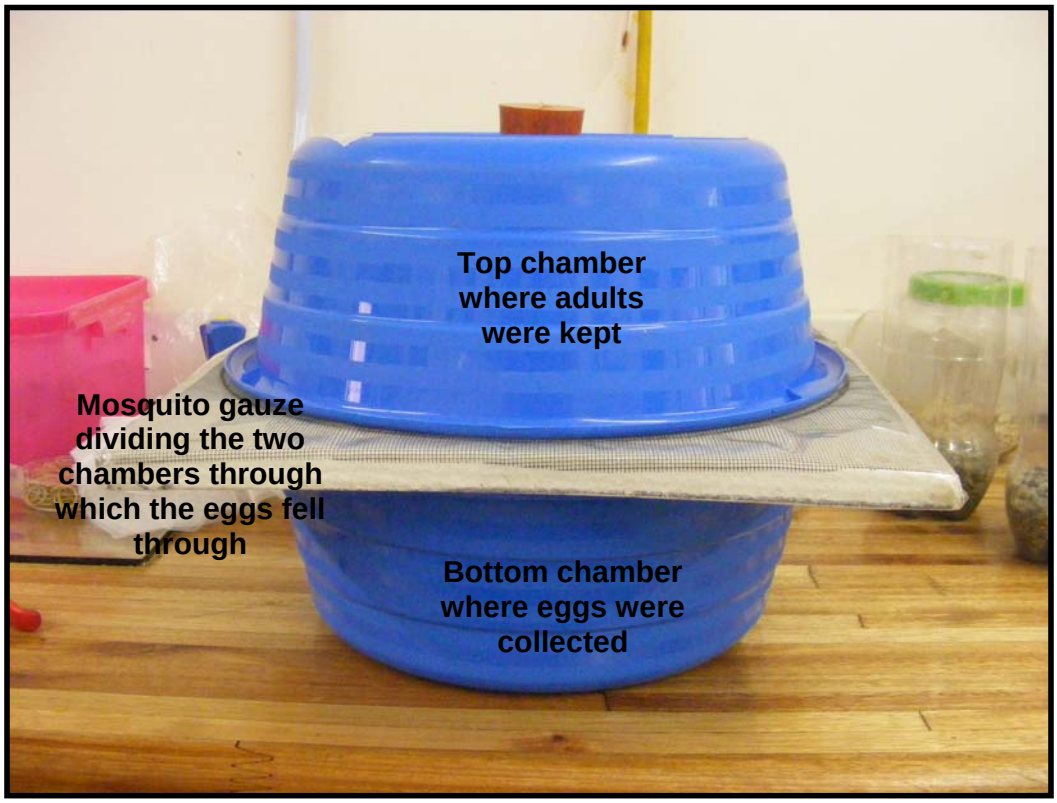

Fig. 4 Container in which *Ephestia cautella* adults were kept to mate and lay eggs.

Table 1. Ingredients of the artificial diet provided to *Ephestia cautella* larvae.

Ingredient	Mass / volume
Maize meal	2198 g
Wheat flower	2198 g
Oats	466 g
Brewers yeast	410 g
Glycerine	700 ml
Honey	465 ml
Distilled water	465 ml

Appendix III

Busseola fusca rearing

After emergence of moths, pairs of males and females were kept in oviposition chambers. Oviposition chambers were 30 cm high and 15 cm in diameter and covered with a fine gauze mesh to prevent escape of moths (Fig. 5). A 20 cm long piece of maize stem with bases of leaves intact was placed in an upright position in the oviposition chamber, serving as the oviposition substrate. Plastic containers that served as oviposition chambers were filled with approximately 5 cm of crusher stone (7 mm diameter) as substrate to keep the maize stems upright. Water was added up to a level three-quarters of the height of the substrate to provide humidity to moths and to keep stems fresh.

Egg batches were carefully removed from each stem at 2-day intervals by cutting off a small piece of the leaf with the egg batch attached to it. In order not to inhibit oviposition, stems were removed and replaced with a freshly-cut stem if there were two or more egg batches on it. After removal, each egg batch was placed into a test-tube and the opening covered with damp cotton wool to prevent desiccation. Eggs were kept in an incubator at 26 (± 1°C) until they hatched.

First-instar larvae were placed on Bt- or non-Bt maize whorls that served as food in plastic containers (35 cm x 20 cm) covered with a mesh lid (Fig. 6). The containers were cleaned and fresh food provided at weekly intervals. When larvae reached the 2nd to 3rd instar the young maize whorls were replaced with maize stems. Second to 3rd instar larvae were removed from this laboratory colony for use in the feeding experiments.

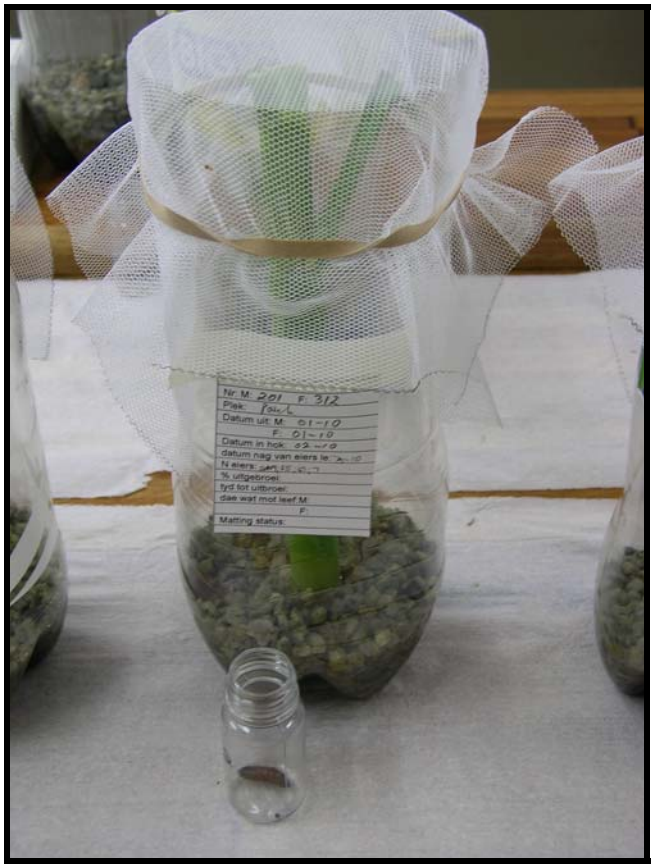

Fig. 5 Oviposition chamber containing a cut maize stem.

Fig. 6 Plastic container used for rearing *Busseola fusca* larvae on maize plant material.