

Aus dem Institut für Phytopathologie  
der Christian-Albrechts-Universität zu Kiel

**FOLIAR APPLICATION OF ENTOMOPATHOGENIC NEMATODES FOR INTEGRATION IN A  
BIOLOGICAL CONTROL MANAGEMENT AGAINST *PLUTELLA XYLOSTELLA*  
(LEPIDOPTERA; PLUTELLIDAE)**

**Dissertation**

zur Erlangung des Doktorgrades

der Agrar- und Ernährungswissenschaftlichen Fakultät

der Christian-Albrechts-Universität zu Kiel

vorgelegt von

**Dipl.-Ing. agr. (hort.) Sibylle Schroer**

aus Ulm

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**BLATTAPPLIKATION ENTOMOPATHOGENER NEMATODEN ZUR INTEGRATION IN EINE  
BIOLOGISCHE BEKÄMPFUNGSTRATEGIE GEGEN *PLUTELLA XYLOSTELLA*  
(LEPIDOPTERA; PLUTELLIDAE)**

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**Abbreviations:**

|     |                               |
|-----|-------------------------------|
| ha  | hectare                       |
| m   | meter                         |
| l   | liter                         |
| g   | gramme                        |
| DJ  | dauer juvenile                |
| EPN | entomopathogenic nematode     |
| DBM | diamondback moth              |
| RH  | relative humidity             |
| LT  | lethal time                   |
| LC  | lethal concentration          |
| C   | Celsius                       |
| Bt  | <i>Bacillus thuringiensis</i> |
| UV  | ultra violet                  |

**Weitere oder abweichende Abkürzungen im Deutschen:**

|      |                      |
|------|----------------------|
| RLF  | relative Luftfeuchte |
| DL   | Dauerlarve           |
| L3   | 3. Larvenstadium     |
| Std. | Stunde               |

## 1.1 Zusammenfassung

Die Kohlmotte *Plutella xylostella* (L.), ein weltweit verbreiteter Schädling an Kreuzblütlern, wird wegen ihrer Fähigkeit, Resistenzen gegen Insektizide zu bilden, sehr gefürchtet. Heute sind Resistenzen der Kohlmotte gegen jedes gegen sie eingesetzte Mittel bekannt, inklusive *Bacillus thuringiensis* Berliner (Bt), der einzige kommerziell weit verbreitete, biologische Wirkstoff. Aufgrund verminderter Wirksamkeit steigen die Aufwandmengen der Insektizide unverhältnismäßig, wodurch vor allem wirbellose Antagonisten geschädigt werden. Entomopathogene Nematoden (EPN) sind ungefährlich für den Anwender, das Ökosystem und vor allem für wirbellose Antagonisten. Im Labor wurde eine hohe Wirksamkeit des Nematoden *Steinernema carpocapsae* (Weiser) gegen das 3. und 4. Larvenstadium der Kohlmotte nachgewiesen. Doch werden die bodenbürtigen Nematoden durch fehlenden Schutz vor UV-Strahlen, Hitze und Trockenheit schon nach kurzer Zeit auf dem Blatt geschädigt. Ziel dieser Studie war durch Hilfsstoffe die entomopathogenen Dauerlarven (DL) zu schützen und damit ihre Wirksamkeit gegen die Kohlmotte zu optimieren. Als Blattapplikation könnten die Nematoden in ein nachhaltiges Bekämpfungssystem gegen die Kohlmotte integriert werden und für das Ökosystem schädliche Breitbandinsektizide ersetzen.

Zunächst wurde die Kompatibilität einer großen Auswahl verschiedener Zusatzstoffe mit Nematoden, Insekten und Pflanzen untersucht. Nur wenige Adjuvantien verursachten schädliche Effekte an Pflanzen oder Insekten und keines beeinflusste die Nematoden negativ. Das Verhalten von Kohlmottenlarven und Nematoden auf der Blattoberfläche wurde eingehend untersucht. In Wasser appliziert auf die Kohlpflanze waren Nematoden durch die Oberflächenspannung der Tropfen gefangen, die sie nicht selbständig durchbrechen konnten. Nur zufällig, wenn Kohlmottenlarven die Tropfen kreuzten, kamen die Nematoden in Kontakt mit dem Insekt. *S. carpocapsae* versuchten in diesem Fall an allen Körperöffnungen in das Insekt einzudringen. Eine erfolgreiche Invasion konnte aber nur durch den Anus beobachtet werden. Der Durchmesser der Tracheen der Kohlmotte ist zu klein, um Nematoden in die Larve passieren zu lassen. Versuche einer orale Invasion wurden durch Abwehrverhalten der Larven verhindert.

Die folgende Forschung richtete sich deshalb auf die Unterstützung des aktiven Eindringens der Nematoden in das Insekt unter Berücksichtigung der wächsernen

Kutikula des Kohls. In Versuchen auf 2 cm<sup>2</sup> Blattscheiben mit einzelnen Larven des 3. Stadiums (L3) wurde untersucht, die Wirksamkeit der Nematoden durch verschiedene Adjuvantien zu steigern. Fünf Tenside und vier Polymere wurden dazu in 0,3%iger Konzentration untersucht. Das Herabsetzen der Oberflächenspannung durch Tenside hatte keinen Einfluss auf die Wirksamkeit der Nematoden, doch durch Polymere konnte diese signifikant gesteigert werden. Die Wirksamkeit von 10 *S. carpocapsae* / Kohlmottenlarve wurde von 37% in Wasser durch Zugabe der Polymere auf 71% durch Xanthan und auf 82% durch Alginat gesteigert.

Weitere Zugabe von 0,3% Tensid zur Polymersuspension erhöhte den Wirkungsgrad der Nematoden auf > 90%. Die letale Konzentration (LC<sub>50</sub>) von *S. carpocapsae* auf das L3 bei 80% relativer Luftfeuchte (RLF) und 25°C wurde durch diese Tensid-Polymer-Formulierung von 13 auf 1 DL / Insektenlarve reduziert. Die LC<sub>50</sub> in Wasser bedingt bei 80% RLF und 25°C eine letale Zeit (LT<sub>50</sub>) von 42 Stunden (Std). Durch die Formulierung konnte die LT<sub>50</sub> auf nur 22 Std. reduziert werden.

Weiterhin wurde die Möglichkeit überprüft, die Sedimentation der Nematoden in Spritzbrühen und ihre Adhäsion am Blatt durch die Adjuvantien zu verbessern. In 0,5 l Wasser wurden Nematoden (1 DL / µl) gleichmäßig verteilt. In 5-minütigen Intervallen wurde aus 2 cm Tiefe 100 µl Proben entnommen und die Anzahl der enthaltenen Nematoden gezählt. In reinem Wasser wurden schon nach 5 Minuten 60% weniger *S. carpocapsae* als in der Ausgangsmenge wiedergefunden. Die Sedimentation der Nematoden konnte durch 0,3% Guarkernmehl, 0,1% Alginat oder 0,05% Xanthan für die Dauer einer Stunde verhindert werden. Auf der wachsigem Blattoberfläche des Kohls finden Wassertropfen mitsamt Nematoden keinen Halt und perlen ab. In Wasser appliziert auf Blättern, die sich in 60° Winkel zur Spritzdüse befanden, wurde 70% Verlust der Nematoden festgestellt. Durch 0,3%ige Zugabe von Polymeren wurde dieser Verlust verringert, auf 30% durch Gummi Arabicum und auf 26% durch Guarkernmehl. Noch stärkeren Einfluss auf die Adhäsion erzielten die Polymere Alginat und Xanthan, deren 0,3%ige Zugabe den Verlust auf ca. 20% verringerte.

Eine Untersuchung der Viskosität der Zusatzstoffe ergab eine Korrelation mit ihrem Potential, die Nematodenwirksamkeit zu verbessern, die Sedimentation zu verlangsamen und ihre Adhäsion am Blatt zu erhöhen. Die höchste Viskosität erreichten Xanthan und Alginat. Aufgrund des besseren Lösungsverhaltens von Xanthan wurde dieses Polymer für die folgenden Versuche gewählt. Die Tenside

verringerten weder die Sedimentation noch verbesserten sie die Adhäsion der Nematoden. Nur in Verbindung mit einem Polymer erhöhten sie die Wirksamkeit der Nematoden. Zwischen den Tensiden wurden keine signifikanten Unterschiede nachgewiesen. Für die folgenden Versuche wurde deshalb Rimulgan<sup>®</sup>, ein biologisch leicht abbaubares Tensid gewählt. Die bisherigen Wirksamkeitsuntersuchungen wurden in 2 cm<sup>2</sup> großen Versuchszellen durchgeführt, die das natürliche Verhalten der Kohlmottenlarven unberücksichtigt ließen. Deshalb wurde eine Versuchsarena entwickelt, in der sich die Larven frei bewegen konnten. Auf ganzen Kohlblättern inkubiert bei 80% RLF und 25°C erreichten Nematoden in Wasser appliziert einen Wirkungsgrad von 50% bei einer Konzentration von > 50 DL / cm<sup>2</sup>. Da der Effekt stark feuchtigkeitsabhängig war, wurde bei 60% RLF ein deutlich geringerer Wirkungsgrad erzielt. Erst ab einer Dichte von >100 DL / cm<sup>2</sup> konnten 50% Wirkung erzielt werden. Die Polymer-Tensid-Formulierung erhöhte die Wirkung der Nematoden auf dem Blatt. Bei 80% RLF wurden bis zu 80% Wirkung erzielt. Bei 60% RLF blieb der Wirkungsgrad zwar unter 60%, wurde aber schon durch 50 DL / cm<sup>2</sup> erreicht. Außerdem verbesserte die Formulierung das Überleben der Nematoden auf dem Blatt. Die letale Zeit (LT<sub>50</sub>) der Nematoden exponiert bei 80% RLF betrug 36 Std. und nur 3 Std. bei 60% RLF. Durch die Formulierung wurde die LT<sub>50</sub> auf 57 Std. bei 80% RLF und auf 23 Std. bei 60% RLF verlängert. Fast alle Nematoden überlebten bei 80% RLF 9 Std. auf dem Blatt. Ihre Aktivität nahm in dieser Zeit aber rapide ab. Kohlmottenlarven, die direkt nach der Behandlung der Blätter mit Nematoden in Wasser aufgesetzt wurden, wurden zu 40% kontrolliert. Wurden die Larven aber erst 9 Std. später aufgesetzt, wurden nur noch 13% Mortalität beobachtet. Appliziert in der Formulierung verringerte sich die Wirkung der Nematoden in 9 Std. von 70 auf 40%. Weitere Polymere, die sich durch ihre Wasserhaltungsmöglichkeiten als Austrocknungshemmer auszeichnen, wurden der Formulierung zu 0,25% zugefügt. Als Evaporationsschutz wurde ein Alginatgel, Polyacrylat und Silicat eingesetzt. Aber durch keinen dieser Austrocknungshemmer konnte der Aktivitätsverlust der Nematoden verhindert werden. Ein Versuch zur Ermittlung der Infektionszeit wurde an Einzelblattversuchen durchgeführt. Überraschend hoch war die Nematodeninfektion schon nach einer Std. Die Expositionszeit der Kohlmottenlarven auf nematodenbehandelten Blättern für 1 oder 20 Stunden zeigte keinen signifikanten Unterschied auf den Wirkungsgrad. Dieses Ergebnis bedeutet, dass auf dem Blatt schon nach einer Stunde die hauptsächlichsten



Aktivität der Nematoden stattgefunden hat. Selbst durch verbesserte Überlebensraten konnte der Aktivitätsverlust der Nematoden nicht beeinträchtigt werden. Als Konsequenz sollten Nematoden auf dem Blatt in ihrer Mobilität gefördert werden, da ihre Aktivität direkt nach der Applikation auf das Blatt größeren Einfluss auf ihre Wirksamkeit zeigt, als die Verlängerung ihrer Persistenz.

Die Resultate aus dem Labor wurden in Feldversuchen in Indonesien überprüft. Die Versuche wurden auf Java im Hochland > 2000 m über dem Meeresspiegel zur Regenzeit im Februar – April 2004 durchgeführt. Dabei wurde die Wirkung der Nematoden in Wasser und in der Polymer-Tensid-Formulierung verglichen und die Möglichkeit der Kombination von Nematoden mit Bt getestet.

Drei Tage nach der Applikation von 0,5 Millionen *S. carpocapsae* / m<sup>2</sup> wurde ein Wirkungsgrad von > 50% erzielt. Noch 10 Tage nach der Applikation wurde ein ähnlich hoher Wirkungsgrad gemessen. Die Formulierung erzielte nur wenig verbesserte Wirkung, wahrscheinlich wegen der hohen Luftfeuchte während der Regenzeit (> 90%). Vereinzelt wurde nach der Applikation der Nematoden in der Formulierung bis zu 70% Kohlmottenmortalität beobachtet. Während nach der Applikation von Nematoden in Wasser weiterhin die Anzahl der Kohlmotten pro Pflanze anstieg, blieb die Anzahl der Kohlmotten nach der Applikation von *S. carpocapsae* in der Formulierung konstant.

Wöchentliche Applikationen von Turex<sup>®</sup> (0,6g / m<sup>2</sup>) erzielten einen Wirkungsgrad von ca. 80%. Wöchentlich rotierende Applikationen von Bt und Nematoden (0,5 Millionen DL / m<sup>2</sup>) erreichten eine höhere Wirkung (ca. 90%), ohne sich signifikant von der reinen Bt-Applikation zu unterscheiden. Die gemeinsame Applikation beider biologischer Agenzien in doppeltem Zeitabstand erzielte einen leicht niedrigeren Wirkungsgrad (70%). Für eine nachhaltige Kohlmottenbekämpfung wird deshalb der Einsatz beider Agenzien appliziert als Rotation oder Mischung in geringen Zeitabständen empfohlen (1 Woche).

Die Ergebnisse dieser Studie zeigen, dass Nematoden, integriert in biologische Pflanzenschutzmaßnahmen hohe Wirksamkeiten gegen die Kohlmotte erzielen, und damit zukünftig für das Ökosystem gefährliche Breitbandinsektizide substituieren können. Die entwickelte Formulierung ermöglicht eine einfache Handhabung der Nematodenapplikation mit kommerzieller Spritztechnik. Durch die Formulierung wird ein Film auf der Pflanze gebildet, der die Mobilität von Nematoden fördert, wodurch

die Einwanderung in Insektenlarven begünstigt wird. Die Einsatzmöglichkeiten von Nematoden als Blattapplikation erweitern die Alternativen des biologischen Pflanzenschutzes und bieten damit eine neue nützlingsschonende Maßnahme, um Resistenzbildungen in Schadinsekten vorzubeugen.

## 1.2. Summary

The diamondback moth (DBM) *Plutella xylostella* (L.), a major pest of crucifers, is distributed world-wide and alarms by its distinct ability to develop resistance. Today, resistance against every insecticide applied for DBM control is documented, including resistance against the biological agent *Bacillus thuringiensis* Berliner (Bt). Due to the loss in susceptibility, insecticides are applied in excessive amounts and significantly affect the potential of invertebrate antagonists. The use of soil-born entomopathogenic nematodes (EPN) as a foliar application is a new attempt to substitute broad-spectrum insecticides for the sustainable control of the DBM. The nematode *Steinernema carpocapsae* (Weiser) provides high efficacy against 3<sup>rd</sup> and 4<sup>th</sup> instar of DBM. However, so far no sufficient protection from abiotic stress could be provided at the leaf surface as the soil-born organisms lack protection against UV-radiation, heat and drought. The present study aims to investigate the feasibility to support entomopathogenic dauer juveniles (DJ) with adjuvants for improved efficacy against the DBM on plant foliage.

A broad range of adjuvants were tested to support EPNs with their detrimental effects evaluated on DJs, plants and DBM. Only a few tested adjuvants were detrimental to insect larvae or phytotoxic, but none damaged DJs, indicating a broad range of agrochemicals suitable as compounds of a formulation for foliar application of nematodes. The performance of *S. carpocapsae* and DBM larvae on cabbage foliage was observed in detail, to identify the optimal chemical function of the adjuvants for the support of EPN on cabbage leaves. Applied in water DJs are captured in droplets, which they are not able to leave. In contact with DBM larvae, which randomly cross a droplet, they actively attempt to penetrate the insect body at every orifice. However, successful infection was observed only via the anus. The diameter of trachea is too small to allow DJ invasion into the larva and an oral uptake is repulsed by defending reactions of the DBM. Hence, further research focused on adjuvants which support EPN active penetration.

In 2 cm<sup>2</sup> leaf disc assays five surfactants and four polymers were evaluated to increase EPN mobility by either lowering the surface tension of water or thickening the drops. Minor improvements were recorded by the addition of surfactants but the use of polymers could increase DJ efficacy significantly. Considerably higher DBM mortality was recorded adding 0.3% xanthan gum (71%) or alginate (82%) to EPN in

water (37%). Additional supply with 0.3% surfactant to the polymer suspension further improved DJ efficacy from 50% to 90%. At 80% relative humidity (RH) and 25°C the lethal concentration (LC<sub>50</sub>) for *S. carpocapsae* against the 3<sup>rd</sup> instar was lowered by the surfactant- polymer-formulation (SPF) from 13 to 1 DJ / larva. The lethal time (LT<sub>50</sub>) evaluated with the LC<sub>50</sub> was 42 h when applied in water but was reduced to a LT<sub>50</sub> of only 22 h with the addition of the SPF.

The adjuvants were further evaluated on their potential to improve EPN application. The specific weight of EPN causes sedimentation in tank mixes and therefore inhibits homogenous distribution in the field. The addition of 0.3% guar gum, 0.1% alginate or 0.05% xanthan prevented sedimentation for 1 h. Applied to cabbage foliage water droplets bounce or run off the leaves due to the waxy cuticle. Adding 0.3% arabic gum, guar gum, xanthan or alginate reduces EPN run-off from 70% when applied in water to 30, 26, 22 and 18%, respectively.

The viscosity of the polymers was positively correlated with the potential of the adjuvants to improve EPN efficacy, retard sedimentation and enhance adhesion. Xanthan and alginate have the highest viscosity of the tested adjuvants. Xanthan was chosen for further research because it provides better solubility in water than alginate. The surfactants neither retarded sedimentation, nor enhanced adhesion, but improved EPN efficacy in combination with a polymer. As no significant difference was recorded between the different surfactants, Rimulgan<sup>®</sup> was recommended for further experiments due to its biological degradability.

Considering rather artificial conditions of leaf disc assays, experimental arenas were constructed for single cabbage leaf bioassays, which leave room for larval escape from EPN treated plant parts. Treating leaves with EPN in water resulted in 50% DBM mortality using > 50 DJs / cm<sup>2</sup> at 80% RH. As the effect strongly depends on humidity, EPN efficacy was lower at 60% RH. Only a density of > 100 DJs / cm<sup>2</sup> achieved 50% control. The addition of the SPF improved EPN efficacy up to 80%, applying 75 DJs / cm<sup>2</sup> at 80% RH. At 60% RH EPN efficacy did not surpass 60%, but the efficacy was achieved with only 50 DJs / cm<sup>2</sup>. Furthermore, DJ survival at foliage was improved by the SPF. Whereas the LT<sub>50</sub> of DJ in water was 36 h at 80% RH and only 3 h at 60% RH, it was prolonged to 57 h and 23 h, respectively, by the addition of SPF. At 80% RH almost all DJ survived 9 h, however, EPN activity decreased rapidly within this period. Of an initial 40% EPN efficacy in water, only 13% was

achieved after 9 h. Using the SPF nematode efficacy was reduced within 9 h from 70 to 40%.

The anti-desiccants alginate gel, polyacrylate and fumed silica were added at 0.25% to the SPF to retard evaporation. None of the anti-desiccants could sufficiently prevent the loss of EPN activity. In another experiment the time of DJ invasion into DBM larvae was observed. Surprisingly, DBM exposure time to EPN treated leaves had no impact on the mortality, indicating that the EPN activity takes place immediately after application. Hence, to improve EPN efficacy on plant foliage, their mobility must be supported to ensure invasion into insect larvae directly after application. The survival of the DJs is of minor importance as the activity declines rapidly after the application to plant foliage.

The promising laboratory results were evaluated during field trials in Indonesia. Three days after application of 0.5 million *S. carpocapsae*, > 50% efficacy was recorded. After 14 days an equal control effect was still observed. The impact of the SPF on EPN efficacy was low, probably due to optimal humidity during the rainy season (>90% RH). In some trials > 70% efficacy was recorded after the EPN treatments with SPF. Whereas the number of DBM larvae still increased after application of EPN in water, no further DBM increase was recorded until 10 days after SPF application.

In further field trials the combination of EPN with Bt was evaluated. Weekly applications of the Bt product Turex<sup>®</sup> achieved approximately 80% DBM control. The weekly rotation of Bt and EPN caused an even higher efficacy of up to 90%. The mixture of both agents applied every fortnight achieved less control (70%). Hence, the rotation of the agents with short time intervals is recommended for the sustainable control of DBM. The formulation enables simple application of EPN and allows the use of customary application technology, available in developing countries. The SPF provides a film layer which enables EPN mobility on plant foliage, thus active invasion into insect larvae is supported. The use of SPF enlarges the potential spectrum for EPN to pest insects on foliage and therefore offers a new strategy against the development of resistance.

## 2. Introduction

*Plutella xylostella* (L.), the diamondback moth (DBM) is commonly assumed to be native to the Palaearctic or Mediterranean area, but due to its ability to migrate over distances of > 3000 km (Talekar & Shelton, 1993), today the moth is distributed world wide. Oviposition and feeding is regulated by glycosinolates, secondary metabolites of crucifers (Ollsson & Jonasson, 1994). Hosts are the whole spectrum of Brassica crops and weeds, *Raphanus sativus* (L.) and *Nasurtium aquaticum* (L.). Eggs are mainly laid on the lower surface of protected plant parts. After less than one week the 1<sup>st</sup> instar hatches, which mines into soft leaf tissue. From the 2<sup>nd</sup> to 4<sup>th</sup> instar, larvae feed on the outside of the leaf. After the 4<sup>th</sup> instar larvae stop feeding and search for a protected area of the plant foliage to pupate. All instars take  $5 \pm 1$  days. Due to the short life cycle, up to 28 generations per year can appear in tropical regions (Talekar and Shelton, 1993). Temperatures ranging from 15° to 30°C are optimal for their life cycle. Larvae can not survive frost, and at  $\geq 35^\circ\text{C}$  they are killed (Shirai, 2000).

DBM larvae are affected by various cultural strategies. Inter-, trap- and rotation-cropping restricts the food source of the DBM (Talekar & Shelton, 1993). Numerous predators and parasitoids control the DBM. Predators are mainly polyphagous like birds, spiders or invertebrates (Chrysopidae). A more severe impact on DBM population is recorded by parasitoids. The ichneumonid *Diadegma* spp. and the braconid *Cotesia* spp. appear in various ecological niches. *Diadegma* spp. are most active at a temperature range from 15 to 25°C and *Cotesia* spp. develop the highest potential at 20° to 35°C. Therefore, in tropical regions *Diadegma* spp. occur mainly in the highlands and *Cotesia* spp. in the lowlands (Talekar & Yang, 1991). Verkerk and Wright (1997) observed a preference of the ichneumonid parasitoids to *Brassica oleracea* and of the braconids to *B. pekinensis*. The beneficial invertebrate antagonists were introduced into areas which lack natural antagonists. In Australia reiterated introduction of different parasitoids achieved 72-94% DBM control (Talekar & Shelton, 1993). Similar control rates were observed in Indonesia (Sastrosiswojo, 1996) and up to 60% in China (Liu *et al.*, 2000).

Extended cultivation of crucifer crops required enhanced control of the DBM, as crop rotation is rarely conducted due to the high price of crucifers (Wright, 2004). All classes of insecticides were applied against the DBM, including organochlorides, organophosphates, carbamates, pyrethroids, avermectins and acylureas (Sun,

1992). Broad-spectrum insecticides not only lost efficacy but also lowered the potential of invertebrate antagonists. Haseeb *et al.* (2000) investigated toxic effects of four different insecticides on a *Diadegma* sp. Hill and Foster (2000) compared the residual effect of different insecticides against *Diadegma* and found only *Bacillus thuringiensis* and teflubenzuron to be compatible with the beneficial invertebrate. Xu *et al.*, (2004) recommend Bt and neem-products emphasising the severe negative impact of broad-spectrum insecticides on the potential of DBM invertebrate antagonists.

While the potential of beneficial antagonists decreased, DBM developed resistance against every insecticide applied. In 1953, DBM was the first insect with decreased susceptibility to DDT (Tabashnik, 1994). Decades later the call for sustainable biological control strategies arose due to DBM loss of susceptibility to all kinds of active ingredients in chemical insecticides (Sun, 1992; Shelton *et al.*, 1998; Zhao *et al.*, 2002; Ninsin, 2004). *Bacillus thuringiensis* Berliner (Bt) is the major commercially available biocontrol agent against the DBM world-wide. *Bt kurstaki*, *Bt aizawai* and *Bt galleriae* provide different Cry toxins pathogenic to Lepidoptera (Navon, 1993). The use of Bt against the DBM started in the early 1970s and seemed to be the adequate solution for > 20 years, until the susceptibility to *Bt kurstaki* also started to decrease in the 1980s (Kirsch and Schmutterer, 1988). The DBM is the first insect that developed resistance against Bt in the field (Ferré & van Rie, 2002). Resistance to Bt was recorded from Hawaii, Florida, New York, the Philippines, Thailand and Malaysia. The susceptibility in the field decreased up to 330-fold. In the laboratory a > 1000-fold decrease of susceptibility to Bt was observed (Tabashnik, 1994). Fortunately, the development of resistance induces fitness costs to the DBM. Mating deficiency of resistant DBM males were observed (Groeters *et al.*, 1993), as well as loss in insect survival, fecundity and egg hatch. These fitness cost can be exploited for resistance management. DBM generations reared without exposure to Bt recovered the susceptibility to 100-fold (Tabashnik *et al.*, 1994). The introduction of novel control strategies is urgently required for resistance management and the conservation of beneficial invertebrates.

Entomopathogenic nematodes (EPN) of the genera Steinernematidae and Heterorhabditidae are soil-born organisms equipped with a bacterium, which kills the insect host (Han & Ehlers, 2000). EPN are safe to users and the environment (Ehlers & Hokkanen, 1996). In Petri dish bioassays, when EPN were applied in excessive

amounts, they had remote effects on non-target arthropods, but under more natural conditions these side-effects were negligible and no significant detrimental effects on the native insect fauna after EPN application have so far been reported (Bathon, 1996).

High susceptibility of *P. xylostella* larvae to *Steinernema* spp. and to a lesser amount to *Heterorhabditis* spp. was observed (Ratnasinghe & Hague, 1994; Baur *et al.*, 1995). Mason and Wright (1997) recorded infectivity of different nematode species against the DBM ranging from 3 to 100%. Cherry *et al.* (2004) summarised the efficacy of EPN indicating that *S. carpocapsae* (Weiser) is the most appropriate nematode species to control DBM. Dauer juveniles (DJ) search for hosts, which they invade via orifices (Poinar, 1990). The DJs can survive several months with no food but they are restricted by abiotic factors like UV-radiation, heat and drought (Glazer, 2002). Nickle and Shapiro (1992) observed drastic loss in EPN activity after only half an hour of exposure to sunlight. With an UV-protectant the time period to withstand sunlight was improved to 4h. UV-radiation can be avoided by spraying EPN at dusk. Additionally, EPN have to bear less heat during night time and the dew improves humidity on the plant canopy. But as heterorhabditids and steinernematids provide low tolerance to desiccation, they only survive for a few hours on the plant surface. After only 5 h on the foliage the survival of *S. carpocapsae* declined to < 20% (Begley, 1990). Glazer (1992) recorded 60%, 30% and 0% DJ survival after 6 h exposure to 80%, 60% and 45% relative humidity (RH), respectively. EPN species differ in their tolerance to desiccation. As *S. carpocapsae* is more tolerant to desiccation stress it is better adapted to host-finding on the soil surface, and therefore is the most suitable species for foliar application (Patel *et al.*, 1997; Patel & Wright, 1998). Several studies tried to improve DJ efficacy on foliage using different adjuvants. Glazer & Navon (1990) improved the efficacy of *S. feltiae* (Pye) against *Heliothis armigera* Hübner from 15% to 75% using glycerol (1% w/w) and to 95%, using "Folicote" (6% w/w). These anti-desiccants improved DJ survival up to 2-fold (Glazer, 1992). With the addition of "Biosys 627" (20% w/w) and a natural wax (18% w/w) a 4-fold increase in insect mortality was achieved. In the laboratory, the addition of 10% polypropylene achieved satisfying results increasing efficacy from 32% to 92% (Baur *et al.*, 1997). However, in the field EPN efficacy against the DBM was low due to limited survival or loss of EPN (Baur *et al.*, 1998). Navon *et al.* (1998) developed a calcium alginate formulation edible to insects. EPN were trapped in the



gel (500 *S. feltiae* / g), which contained yeast for insect attraction. The gel controlled 90% of *Spodoptera littoralis* Boisduval larvae (Navon *et al.*, 2002).

Due to increasing constraints in DBM control and the need to have available alternative strategies, the idea was borne to develop improved formulation technology to support the control potential of *S. carpocapsae* on foliage. The aim of the present study was the evaluation of variable adjuvants to support EPN on foliage and to improve dose transfer. Host finding and host invasion mechanisms were subjected to a detailed analysis to better understand, how a formulation can support nematode performance on the leaf. Adjuvants were improved under laboratory conditions and then tested in assays more closely simulating field conditions. Finally, a biological control strategy integrating EPN and Bt against DBM in cabbage fields in Indonesia was tested and the feasibility of this approach was evaluated to produce a cost-benefit analysis.

### 3. Results and Discussion

#### 3.1. Improving EPN efficacy on plant foliage

In order to identify adjuvants with the potential to increase EPN efficacy, 2 cm<sup>2</sup> leaf disc bioassays were conducted, testing the effects against single 3<sup>rd</sup> instar DBM larvae at 25°C and 60 or 80% RH. The high efficacy of *S. carpocapsae* against the DBM (Baur *et al.*, 1995; Wright & Mason, 1997; Ratnasinghe & Haque, 1997) was confirmed in the present study using an Egyptian isolate. The lethal concentration (LC<sub>50</sub>) was 13 (±6) DJs / 3<sup>rd</sup> instar DBM at 80% RH (**Publication 1**).

Adjuvants are used in pesticide formulation to assist or modify the action of the active ingredient. They may optimise the active agent enhancing the uptake by the pests, improving application and distribution or retaining physical properties to ensure successful application. The formulation of biological pesticides additionally must support viability and survival of the agent in the environment (Burgess, 1998). EPN are particularly compatible with several insecticides, but tank mixes with agrochemicals conceal the risk of detrimental effects to the nematodes (Koppenhöfer & Grewal, 2004). Thus toxicity tests were a prerequisite for the addition of adjuvants to nematode spray mixtures. A list of adjuvants was produced and divided according to their function as surfactants, binders and thickeners or oils and waxes (**Publication 1**). Detrimental effects were tested with up to 10% adjuvant concentration. All tested adjuvants were compatible with EPN. However, the surfactant Biodac<sup>®</sup> caused 55% mortality to DBM larvae. Phytotoxic effects were recorded using the surfactant Triton X-100 or a high concentration of a wetting agent used to support percolation of water in turf. Mason *et al.* (1998) improved EPN distribution on foliage adding the adjuvants Triton X-100. Considering the phytotoxic effects observed in the present study, this surfactant should not be used. Precaution for the use of adjuvants at field application is important as some surfactants are detrimental to soil-born organisms (Krogh *et al.*, 2003). Therefore this study focused on adjuvants registered for field use or food technology.

Five surfactants, Emulan ELP<sup>®</sup>, Rimulgan<sup>®</sup>, Tween 80<sup>®</sup>, Agrosom Net 5<sup>®</sup> and Adhäsit<sup>®</sup> as well as the four polymers xanthan, potassium alginate, guar and arabic gum were tested at 0.3% concentration. None of the surfactants improved DJ

efficacy compared to water. However, the polymers increased EPN efficacy. Significant effects were achieved with alginate resulting in a 5-fold increase of DBM mortality at 60% and 2.2-fold increase at 80% RH or with xanthan resulting in a 5-fold increase at 60% RH. The addition of any of the surfactants to the polymer suspension further improved EPN efficacy (**Publication 1**).

In water, 54% DJ efficacy was recorded and it was improved by the addition of the surfactant-polymer-formulation (SPF) to > 90%. The formulation reduced the  $LT_{50}$  for DBM control with *S. carpocapsae* at a concentration of 13 ( $\pm 6$ ) DJs / larva from 42 h when applied in water to 22 h with the addition of the SPF (**Publication 1**).

In a further step the combination of the surfactant and the polymer were improved. At 80% RH the addition of 0.1% polymer with 0.2% surfactant already doubled nematode efficacy compared to water. Efficacy increased with the proportion of a polymer up to a concentration of 0.3%, resulting in a 3.4-fold increase of the efficacy in comparison to water. At 60% RH nematode efficacy without adjuvants was less than 10%. Efficacy could be increased to 40% by adding 0.3% polymer plus 0.1% of the surfactant. The use of a lower polymer content required higher concentration of the surfactant. In general, a concentration for both adjuvants of 0.3% seemed to be optimal and was therefore used for the following investigation. Xanthan was chosen as a polymer due to its better solving attitudes in water compared with alginate. Although no significant effects were recorded between the surfactants tested, Rimulgan<sup>®</sup> was chosen for its composition of natural oils and resulting biodegradability (**Publication 1 + 2**).

The development had been made in leaf disc bioassay. This experimental design was considered to be rather artificial, as larvae cannot escape from the treated leaf and EPN find improved microclimatic conditions in the wells compared to the leaf surface. Therefore, an improved system was developed to test the nematode formulation against DBM larvae on whole cabbage leaves. In this assay a density of 50 DJ / cm<sup>2</sup> applied in water controlled 50% of DBM larvae at 80% RH, whereas no control was recorded at a humidity of 60%. Only a density > 100 DJ / cm<sup>2</sup> achieved 50% DBM control. The SPF significantly improved DBM control. At 80% RH the use of 75 DJ / cm<sup>2</sup> applied in SPF controlled up to 82% and at 60% RH 50% DBM control was achieved using only 50 DJ / cm<sup>2</sup> (**Publication 2**).

Humidity has a dramatic impact on EPN survival. Glazer (1992) recorded 100% survival 2 h after application of EPN at 80% RH, but only 20% when exposed to 45% RH. In the present study the SPF was evaluated for its potential to improve EPN survival on cabbage foliage. Applied in water, the  $LT_{50}$  for DJs was 36 h at 80% RH, but only 3 h at 60% RH. The addition of the SPF extended the  $LT_{50}$  to > 20 h at 60% RH and to 57 h at 80% RH (**Publication 3**).

Glazer (1992) also recorded a rapid loss of DJ activity on plant foliage at 65% RH. The mortality of *Spodoptera littoralis* larvae applied to EPN treated leaves 4 h after DJ application decreased from 80% to < 30%, when insect larvae were applied 2 h later. To investigate the effect of the SPF on the activity of *S. carpocapsae*, DBM was added to sprayed cabbage foliage after 9 h.

At 80% RH EPN applied in water caused 32.6% DBM mortality, when insect larvae were added immediately after DJ application, but only 13.3% mortality was recorded when larvae were added 9 h later. At 60% RH EPN efficacy in water was reduced during the time period of 9 h from 15.8% to 9.3%. At 80% RH with the addition of the SPF, the initial EPN efficacy of 67% decreased to 40%, and at 60% RH the efficacy decreased from 50.6% to 16.3% when the insects were added 9 h after EPN application (**Publication 3**). The results confirm that SPF can enhance EPN survival and improve efficacy, but the loss of activity could not be reduced sufficiently. Baur *et al.* (1997) recorded decreasing efficacy only after 12 h exposure to the leaf surface. The loss in activity decreased with DJ survival. In order to increase survival, the addition of different anti-desiccants at 0.25% was tested: an alginate gel according to Navon *et al.* (2002) was used to retard evaporation; cross-linked polyacrylate and fumed silica were added to improve swelling attitudes and thus water retention. However, none of these anti-desiccants significantly improved EPN survival compared to the SPF alone and the loss of activity within 9 h was only slightly reduced not justifying the further addition of costly anti-desiccants (**Publication 3**).

Due to the promising laboratory results obtained with the SPF, this formulation was evaluated under field conditions. The experiments were conducted in cabbage fields in the Indonesian highlands > 2000 m above sea level during the rainy season. EPN were applied at dusk. Three days after a single application of 0.5 million *S. carpocapsae* / m<sup>2</sup> DBM larvae were significantly controlled (**Publication 4**). EPN efficacy was > 50%. Even 14 days after application 45% control was recorded. The impact of the SPF on DBM control was low, probably due to the high humidity during

the rainy season (90-100%). But although the improvement of efficacy by the polymer was not significant, in some trials DBM control of > 70% was recorded with the use of SPF. The number of DBM per plant increased after EPN treatments in water, but no increase of the DBM population was recorded for the time period of 10 days after the application of EPN in the SPF. Baur *et al.* (1998) recorded only 41% EPN efficacy against the DBM on watercress. The addition of an anti-desiccant could improve the efficacy from 44 to 54% efficacy (Baur *et al.*, 1997). The higher efficacy recorded during this study was probably due to the humid microclimate encountered in bigger cabbage heads, which seem to support EPN performance in this crop.

### 3.2. Performance of EPN on plant foliage

Different species of EPN have distinct host searching behaviour (Lewis *et al.*, 1995). Whereas DJs of *S. feltiae* search for sedentary distributed hosts (cruiser-behaviour), *S. carpocapsae* has a sit-and-wait strategy to react to mobile hosts (ambush-behaviour). Latter performance seems to be more appropriate for foliar application. However, Baur *et al.* (1998) suggested lower control on foliage due to *S. carpocapsae* leaping off the leaves. Navon *et al.* (2002) successfully trapped DJs in an alginate gel at the same time protecting them from desiccation. Use of EPN in this formulation requires that insects are attracted by the formulation and take up EPN during feeding. In order to evaluate the impact of feeding on EPN infection, *S. carpocapsae* was applied to filter paper or cabbage leaf discs. On filter paper up to 100% DBM mortality was recorded after 48 h with a nematode concentration equal to the LC<sub>50</sub> of *S. carpocapsae* recorded on cabbage leaves (**Publication 2**). Thus feeding of the insects on the cabbage could not increase efficacy. To the contrary, the efficacy was much higher when no food was available. Consequently, the hypothesis was that DJs actively invade the insect on the foliage rather than being taken up by the insect during feeding. To investigate host finding behaviour, EPN were sprayed on leaves and the invasion was observed under the microscope. When applied on cabbage leaves, DJs were trapped in water droplets. The surface tension prevented EPN from reaching the host. They were only released when larvae crossed the water drops. These observations explained the higher efficacy on filter paper, on which water is absorbed providing better conditions for host finding. Once in contact with the insect body, *S. carpocapsae* attempted to penetrate via all

possible orifices. Host invasion was observed in detail using an invert microscope with cold light. Any attempt to invade third instars of the DBM through the trachea was in vain. The diameter of the trachea was too small to allow *S. carpocapsae* invasion. DJs trying to enter the mouth were repelled by larvae. Successful invasion was only observed via the anus (**Publication 2**). The results of the observation support the assumption that EPN need to actively invade the host and that uptake by DBM is rather improbable. The use of an edible formulation is therefore restricted to larvae that might not identify EPNs on the leaf surface.

*Heliothis armigera* control was increased by up to 95%, spraying EPN with an anti-desiccant (Glazer & Navon, 1990). The effect correlated with increased survival of the EPN on foliage. Baur *et al.* (1997) considered that the addition of anti-desiccants did not sufficiently increase EPN survival thus resulting in low DBM control in the field. However, as DBM avoid DJ uptake, maintenance of survival might be not enough to achieve higher DBM control. Conditions must be appropriate for DJs to develop their full activity. The results of this study indicate that although almost all DJs survive 9 h exposure to foliage at 80% RH, the activity declined by approximately 50%. The addition of anti-desiccants could not improve DJ activity (**Publication 3**). One possible explanation might be that anhydrobiosis is induced resulting in a quiescent state of the nematodes. Glazer (1992) recorded that EPN movement ceased when exposed to foliage at 65% RH only 45 minutes after application. Therefore an investigation was carried out to see how much time EPN require to invade DBM on cabbage leaves. When DBM was exposed to treated leaves for 1, 4 or 20 h the control effect did not differ significantly. Applied with water 40% and with SPF 75% efficacy was recorded. Full control activity thus was achieved already within 1 h exposure and no further increase in efficacy was observed at longer exposure time (**Publication 3**). These results confirm the hypothesis of Glazer (1992) that EPN efficacy against *S. litoralis* when exposed to EPN after 60 minutes is due to the uptake of the nematodes by the insect. As DBM refuse DJ uptake, the consequence for improvements of formulations to be used to control DBM is to provide optimal conditions for nematode host invasion rather than prolonging their survival. Increased efficacy with the SPF is a result of improved invasion conditions due to a film layer produced by the adjuvants on the leaf surface. *Steinernema* spp. were observed to better invade the host on filter paper (**Publication 2**) or plant surface with pubescent hair rather than on glabrous leaves. Conditions on the paper

or hairy surface seem to support the ambush-behaviour of the DJs (Baur *et al.*, 1995; Glazer, 1992) and support DJ invasion. Hence, the improvements observed with the use of the polymers xanthan and alginate are due to improved EPN attachment to the waxy cuticle of cabbage. Piggott (2000) added polyacrylamide, a polymer with swelling attributes used as an anti-desiccant. This polymer improved DJ distribution and efficacy on foliage. According to results of this study the effect was probably not obtained by the extension of DJ survival, but by the result of improved environmental conditions supporting DJ penetration. Anti-desiccants were added to the SPF in order to retard evaporation. But instead of extending the time of EPN activity, the compounds increased the efficacy immediately after application. Whether this effect is related to an increasing viscosity was investigated in this study. Results clearly indicated a positive correlation between EPN efficacy and the viscosity of the formulation (**Publication 2**).

The viscous character of the SPF provides additional advantages. In the spraying mixture, EPN sink to the bottom of the tank within a few minutes (Peters & Backes, 2003). The addition of methyl cellulose significantly reduced the sedimentation velocity. In this study 0.5 l polymer suspensions were prepared containing 1 *S. carpocapsae* DJ /  $\mu$ l. The nematodes were equally distributed in the suspension and then left for sedimentation. In intervals of 5 minutes 100  $\mu$ l were taken from 2 cm depth of the suspension and the number of DJs was counted. Without polymers, 60% *S. carpocapsae* sank during the first five minutes below 2 cm. The addition of 0.3% guar gum, 0.1% alginate and 0.05% xanthan prevented DJ sedimentation for 1 h (**Publication 2**).

Furthermore, the addition of a polymer improves adhesion to the leaf. Applied in water 70% DJs are lost by run-off, when leaves are positioned at an angle of 60° to the spraying nozzle. This loss was reduced by all tested polymers. Arabic gum, guar gum, xanthan and alginate reduced the run-off to 30%, 26%, 22% and 18%, respectively (**Publication 2**).

Lello *et al.* (1996) investigated reduced EPN application rates and achieved better efficacy when applying lower volumes using a spinning disc. Mason *et al.* (1999) considered spinning disc application for EPN to be unsuitable as the DJ were not applied into the cabbage plant, where most DBM are located. Piggott *et al.* (2003) developed a new application apparatus based on a spinning disc system, which

improved the droplet size for DJ deposition and prevented EPN clumping. But as modern technology rarely is available in developing countries, the aid of polymers to reduce EPN run-off and increased feasibility of DJ mobility on plant surface provides similar advantage.

### 3.3. EPN integration into a biological control strategy

Today, more than 500 different insect species have developed resistance against insecticides. Therefore, the use of novel biological pesticides is no longer restricted to industrialized countries that can afford costly biological control, but seems to provide the only chance to maintain food supply in developing countries. Today in Indonesia chemical insecticides are applied every second day. Due to the resistance of the DBM, applications are heavily overdosed and users are affected by the toxins (personal communication). Novel biological integrated control strategies urgently need to be introduced to the cabbage producing areas especially in developing countries to retard resistance development and conserve beneficial arthropods. Inheritance of DBM resistance is mainly recessive (Sayyed *et al.*, 2000). Lack of cross-resistance was recorded between abamectin, acylurea insect growth regulators and Bt- products (Iqbal *et al.*, 1996).

Reduced binding of Cry toxins at the Bt specific receptors of the brush border membrane vesicles is the major mechanism of field-evolved Bt resistance. Some toxins of the Bt serovars *aizawai* and *kurstaki* differ in their receptor-binding region thus the probability for a development of cross-resistance is lower (Ferré and van Rie 2002). These studies support the prospect to manage resistance development in DBM control by rotation or mixture of different Bt toxins or different biological control agents.

Baur *et al.* (1998) achieved 58% DBM control in the field combining EPN with Bt. They considered that the low effect achieved with Bt was due to resistance development of the DBM population in Hawaii. In the present study the feasibility to integrate EPN with Bt was investigated in Indonesia. As Bt was rarely known at the experimental area in Indonesia, Bt resistance was precluded. Weekly application of the Bt product Turex<sup>®</sup> (0.625 g / m<sup>2</sup>) achieved approximately 80% efficacy, confirming the suggestion. Applying EPN (50 DJs / m<sup>2</sup>) and Bt in weekly rotations caused even slightly higher control effects, occasionally reaching up to 90% control. However, insignificant lower effects (approximately 70%) were observed after



treatments with both biological agents applied at the same time every two weeks (**Publication 4**). Baur *et al.*, (1998) applied the mixture with only half the concentration of each biological agent. In the present study a single EPN treatment achieved sustained control effects for 14 days. Hence, low concentration of both agents applied in short time frequencies is recommended for DBM control. However, further research is urgently needed to detect the optimal concentration for this combination.

The time for EPN application was rather important for the control effect. During the first month after planting less than 5 DBM larvae, mainly 1<sup>st</sup> and 2<sup>nd</sup> instars, were recorded per plant. The low number was probably caused by washing off eggs and young larvae from the leaves by rain as observed by Wakisaka *et al.* (1992). After four weeks the DBM population increased, but 36 days after planting the EPN application caused higher efficacy than one week earlier, when DBM increase was still low. As no further DBM increase was recorded 82 days after planting, no further control effect was observed (**Publication 4**). Hence, observations of the number of DBM per plant by farmers is a prerequisite for an economical use of biological agents. Reddy and Guerro (2001) therefore recommend the use of pheromone traps to determine the date for insecticide application against the DBM.

Due to the high potential of *S. carpocapsae* against the DBM further research is urgently required to evaluate suitable application sequences for EPN to be integrated in biological control strategies.

The SPF proved to increase EPN efficacy and aids application of EPN, retarding sedimentation in the tank mix and increasing adhesion to the leaf. Today the commercial use of EPN as insecticides against variable soil-born pests surpasses ten million € annual turnover, but alternative use is considered to increase the economic potential (Ehlers, 1996).

Taking into consideration the results of this study, the use of the SPF to improve EPN performance on the foliage can be recommended as a novel, sustainable insecticide with particular potential against insects which have developed insecticide resistance, e.g., other small Lepidoptera, thrips or leafminers. The discovery that better EPN efficacy is achieved through increasing their mobility, is important for the control of those insects, which have developed defence mechanisms against EPN refusing an oral uptake. Initial extra expenses related with the use of costly biological control

agents compared to cheaper insecticide treatments will return, due to the benefits related with the repression of resistance development and conservation of invertebrate antagonists.

The developed formulation is easy to produce as the main compounds are a polymer providing high viscosity and an ordinary surfactant. The solubility of the polymer in water can be improved by mixing with equal percentages of sodium hydrogen carbonate and oxalic acid (98%) with the polymer (**Publication 4**). The SPF can be applied with standard nozzles, using orifice diameter > 0.3 mm. During the field trials, the formulation was applied using a customary knapsack sprayer as the addition of the SPF prevents EPN sedimentation (**Publication 2**). Adjuvants added at 0.3% achieve improvement in application handling and efficacy. However, the additional costs are high. Xanthan at 40 mesh costs 10.5 €/ kg (personal communication). The surfactant and additional chemicals to improve solubility of the polymer is estimated at 15 €/ kg. If applying EPN with high volume of water estimated at 500 l / ha, the costs for the SPF are about 50 €/ ha. The expense in applying 0.5 million *S. carpocapsae* per m<sup>2</sup> is 950 €/ ha (personal communication). However, prices of the adjuvants might be negotiated when purchasing large quantities and the amount of water applied per ha might possibly be reduced. Further research should determine the minimum water needed for successful DBM control to reach economically reasonable costs for the use of the SPF.

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**PUBLICATIONS**

**PUBLICATION 1**

Schroer, S., Yi, X. & Ehlers, R.-U.:

**Evaluation of adjuvants for foliar application of *Steinernema carpocapsae* against larvae of the diamondback moth (*Plutella xylostella*)**

Nematology, in press

**PUBLICATION 2**

Schroer S., Ziermann, D. and Ehlers, R.-U.:

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

Biocontrol Science and Technology, in press

**PUBLICATION 3**

Schroer, S. and Ehlers, R.-U.:

**Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*)**

Biological Control, in press

**PUBLICATION 4**

Schroer, S., Sulistyanto, D., Ehlers, R.-U.:

**Biological Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and *Bacillus thuringiensis* in cabbage fields**

Journal of Applied Entomology, in press

**Evaluation of adjuvants for foliar application of *Steinernema carpocapsae*  
against larvae of the diamondback moth (*Plutella xylostella*)**

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**Evaluation of adjuvants for foliar application of  
*Steinernema carpocapsae* against larvae  
of the diamondback moth (*Plutella xylostella*)**

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**Evaluation of adjuvants for foliar application of *Steinernema carpocapsae*  
against larvae of the diamondback moth (*Plutella xylostella*)**

1 **Summary** – Increasing resistance to chemical insecticides in field populations of the  
2 diamondback moth (*Plutella xylostella*) has stimulated research on alternative control  
3 measures. The entomopathogenic nematode *Steinernema carpocapsae* may be one such  
4 alternative, particularly against the third larval instar of *P. xylostella*. The LC<sub>50</sub> for the second  
5 instar is 38, the third 13 and the fourth 22 nematodes/larva. *P. xylostella* pupae were not  
6 affected by the nematodes. although mortality in leaf disc bioassays after application of  
7 nematodes in water seldom surpassed 50%. Therefore, additives were tested to improve  
8 nematode performance. Only Triton X-100 (0.3%). caused phytotoxic effects. The addition of  
9 xanthan gum or potassium alginate resulted in a two-fold increase of insect mortality at 80%  
10 RH and a five-fold increase at 60% RH. Mixtures of 0.3% xanthan or alginate with 0.3%  
11 surfactants further improved efficacy. In water the LT<sub>50</sub> for *S. carpocapsae* against *P.*  
12 *xylostella* larvae was > 40 h. Using a mixture of 0.3% xanthan or 0.3% alginate with 0.3%  
13 surfactant the LT<sub>50</sub> was reduced to < 25 h.

14

15 **Keywords** – surfactants, polymers, formulation, , *Brassica oleracea*, synergism,  
16 entomopathogenic nematodes, *Plutella*

17

18

19 **Introduction:**

20

21 The diamondback moth (DBM) *Plutella xylostella* (L.) is a major pest in crucifer crops  
22 worldwide. It has developed resistance against almost all chemical insecticides. Increasing  
23 application frequency severely reduced populations of naturally occurring invertebrate

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1 antagonists (Talekar & Shelton, 1993). Resistance has also become widespread to the  
2 *Bacillus thuringiensis* –based pesticides in field populations of DBM, particularly in Asia and  
3 the Americas (Ferre and van Rie, 2002). The integrated use of a broader spectrum of control  
4 measures would help prevent the development of resistance and provide more sustainable  
5 control Entomopathogenic nematodes (EPN) have a promising control potential against *P.*  
6 *xylostella* (Cherry *et al.*, 2004), however, the efficacy of the soil-born nematodes on foliage is  
7 limited by UV-radiation, heat and drought (Glazer, 2002). The different EPN species vary in  
8 their potential to resist environmental stress (Georgis & Kaya, 1998). *Steinernema*  
9 *carpocapsae* (Weiser) provides best desiccation tolerance for use above ground (Patel *et al.*,  
10 1997). There have been various attempts to improve nematode application, survival and  
11 activity on foliage to control DBM (Baur *et al.* 1995, 1997, 1998; Lello *et al.*, 1996; Mason *et*  
12 *al.*, 1998a,b, 1999). Beside the selection of desiccation tolerant strains and optimising spray-  
13 application equipment, the use of adjuvants can improve survival and activity of nematodes  
14 on foliage (Glazer, 1992; Baur *et al.*, 1997; Mason, *et al.*, 1998, 1999; Piggott *et al.*, 2000,  
15 2003). In this respect three functional groups of adjuvants offer potential: 1. Surfactants,  
16 which function as wetting agents and detergents, and include short-chain fatty acids and  
17 alcohols (Krogh *et al.*, 2003). 2. Binders, which enhance contact between the active ingredient  
18 with the target surface or thicken the film layer due to increased rheology; mainly  
19 polysaccharides are used to increase the viscosity of formulations, cross-linking of these  
20 polymers resulting in gel formation (Mollet & Grubenmann, 2001). 3. Oils and waxes, which  
21 coat the target surface and thus protect the active ingredient from desiccation, rain and UV-  
22 radiation (Jones & Burges, 1998). The aim of the present study was to improve nematode  
23 efficacy against DBM larvae on the foliage by the use of surfactants and polymers. Prior to  
24 testing the compounds in laboratory assays with DMB larvae, the compatibility of several

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1 potential adjuvants with EPN and the host plant was evaluated as well as possible direct  
2 effects on DBM larvae.

3

4

5 **Materials and methods**

6

7 INSECT, PLANT AND NEMATODE REARING

8

9 Cabbage plants (*Brassica oleracea* var. *capitata*), cultivar Amager, were grown on  
10 commercial peat substrate (Floragard<sup>®</sup>, Oldenburg, Germany) in the greenhouse. *Plutella*  
11 *xylostella* (population provided by Bayer Crop Science, Monheim, Germany) were reared on  
12 savoy cabbage leaves (*Brassica oleracea* convar. *capitata* var. *sabauda*) at 20°C and 80%RH.  
13 An Egyptian isolate (S2) of *S. carpocapsae* was reared *in vivo* according to Glazer and Lewis  
14 (2000).

15

16 EFFECT OF ADJUVANTS ON NEMATODES, CABBAGE LEAVES AND DBM

17 LARVAE

18

19 Adjuvants and suppliers are listed in Table 1. The compatibility of these chemicals with *S.*  
20 *carpocapsae*, DBM (third instar) and cabbage leaves was tested. Effects of additives on *S.*  
21 *carpocapsae* were examined following IOBC guidelines compiled by the working group  
22 “pesticides and beneficial organisms” (Peters, 2003). The additives were mixed at different  
23 concentrations (Table 1) in 25 ml tap water containing 5,000 dauer juveniles (DJs) of *S.*  
24 *carpocapsae* in a flask. DJ controls were kept in tap water. The flasks were sealed and

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1 incubated shaking at 200 rpm and 25°C. After 24 and 48 h nematode mortality was evaluated  
2 by taking samples of 100 nematodes at random. DJs not moving after mechanical stimulation  
3 were considered dead. The impact of the adjuvants on nematode infectivity was tested after 48  
4 h incubation of 10 DJs larva with a third instar larva of DBM. Each treatment was replicated  
5 10 times. Five hundred µl of each formulation-nematode suspension was applied per Petri  
6 dish (6 cm diam.) lined with filter paper. Pieces of cabbage leaves were added to supply DBM  
7 with food. Controls were without nematodes. Additionally, one dish was applied with the  
8 highest concentration of the adjuvant solution (Table 1) and without EPN to investigate  
9 possible effects on DBM larvae. After 48 h at 25°C DBM mortality was recorded. Four  
10 replicates were taken for each treatment and control.

11 The compatibility of the different adjuvants with cabbage plants was tested according to  
12 Baur *et al.* (1997). Plants were sprayed with a ten-fold rate of the adjuvant concentration  
13 recommended for field use, to simulate a worst-case scenario caused by desiccation of the  
14 adjuvant on foliage. The concentration was 10% (v/v) for the surfactants, waxes or oils and 3  
15 % (v/v) for thickeners. The plants were examined 1 and 2 days after application and any  
16 detrimental effects on the plant cuticle was recorded. Only if negative effects on plants,  
17 nematodes or DBM were observed, was the test repeated.

18

19 NEMATODE EFFICACY WITHOUT ADJUVANTS

20

21 Dauer juvenile efficacy was tested on 2 cm<sup>2</sup> cabbage leaf discs with different DBM  
22 instars. Discs were treated using a Teejet® (TP8003E) flat-fan nozzle with a volume-flow of  
23 0.96 l/min at 20<sup>5</sup> Pa (1430 l/ha) from a distance of 55 cm. Single leaf discs were then  
24 transferred into 24-well plates (2 cm<sup>2</sup> per well) and one DBM larva/well was added. The

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1 dishes were sealed with gauze to allow for gas exchange and incubated at 80% RH and 25°C.  
2 After 48 h the mortality of the DBM larvae was evaluated. Larvae not responding after a  
3 mechanical stimulus were considered dead. The LC<sub>50</sub> was evaluated for second, third and  
4 fourth instars and pupae using 0, 5, 10, 50 and 100 DJ per larva.

5

## 6 IMPACT OF ADJUVANTS ON NEMATODE EFFICACY

7

8 The surfactants Emulan ELP, Rimulgan, Tween 80, Agrosom Net 5 and Adhäsit and the  
9 polymers xanthan, potassium alginate, guar and arabic gum (Table 1) were chosen for testing  
10 their potential to improve nematode efficacy. The impact of the adjuvants on *S. carpocapsae*  
11 efficacy was observed using a concentration of 0.3% of the adjuvants. Effects of the adjuvants  
12 on the LC<sub>50</sub> value for DBM larvae were tested on cabbage leaf discs with third instar. The  
13 impact of the adjuvants was evaluated at 80% and 60% RH. After testing the single adjuvants  
14 each combination of the surfactants with one polymer was evaluated, following the same  
15 method (at 80% RH only). Additionally, the impact of the combinations on DBM lethal time  
16 (LT) was evaluated. Larval mortality was checked every 8h up to 48h at a concentration  
17 according to the LC<sub>50</sub> in water. Each treatment was tested with 24 larvae and the experiments  
18 were separately repeated twice.

19

## 20 DATA ANALYSIS

21

22 Nematode efficacy data were used to calculate the LC<sub>50</sub>- and LT<sub>50</sub> by Probit-analysis  
23 (Finney, 1971). Means of LT-data were compared for significant differences using ANOVA.  
24 (CSS StatSoft Inc., Tulsa, USA) with Duncan's multiple range test. Data on DBM was

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1 corrected for control mortality according to Abbott (1925) and the effect of different  
2 adjuvants and combinations separated using Duncan's multiple range test.

3

4 **Results**

5

6 EFFECT OF ADJUVANTS ON EPN, PLANTS AND DBM

7

8 With three exceptions, tested surfactants had no measurable effect on DBM, EPN or  
9 cabbage plants. The surfactant "Biodac" caused 55% mortality among DBM larvae at a  
10 concentration of 0.3%. "Kick", a wetting agent used to improve percolation of water in turf,  
11 and Triton X-100 had a detrimental effect on cabbage leaves. Using more than 0.3% of these  
12 surfactants caused injuries on the leaf cuticle surrounded by yellow areas. The detrimental  
13 effect extended to complete petal wilt when applying concentrations of 10% (Fig. 1).

14

15 NEMATODE EFFICACY

16

17 The efficacy of *S. carpocapsae* is restricted to larval stages of *P. xylostella*. No mortality  
18 was observed among *P. xylostella* pupae. The most susceptible stage was the third instar with  
19 a LC<sub>50</sub> of 13 DJs/larva (95% fiducial limits 5.5 – 19.9) followed by the fourth instar at 22 (7.6  
20 – 35.6) and the second instar at 38 (20.7 -63.6) DJs/larva (Fig. 2).

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**Evaluation of adjuvants for foliar application of *Steinernema carpocapsae*  
against larvae of the diamondback moth (*Plutella xylostella*)**

1 EFFECT OF ADJUVANTS ON EPN PERFORMANCE AGAINST DBM LARVAE

2

3 None of the surfactants increased EPN efficacy at 60 or 80% RH when compared to  
4 controls treated with water and EPN (data not shown). At 80% RH a mean control of 47.2%  
5 and at 60% RH almost no control (3.9%) of the DBM larvae was achieved. With the addition  
6 of polymers DBM mortality was increased compared to the control but significant effects  
7 were achieved only with alginate at 60% (5-fold) and 80% RH (2.2-fold) and with xanthan at  
8 60% RH (5-fold) (Table 2).

9 Mixing the five surfactants with each of the polymers, DBM mortality was also increased  
10 when compared with the application of EPN in water but this was only significant ( $P < 0.02$ )  
11 when the surfactants were combined with either xanthan gum or potassium alginate, where  
12 insect mortality was  $>90\%$  compared with 54% in the control. The combination of any  
13 surfactant with arabic- or guar gum did not improve efficacy significantly ( $P > 0.1$ ). (Fig. 3).

14 The combinations of polymers and surfactants also had an effect on the time necessary to  
15 kill the DBM larvae. The  $LT_{50}$  in water was 42 h. Using guar gum with any of the surfactants  
16 the  $LT_{50}$  decreased to 37 h and in combination with arabic gum to 36 h. ( $P > 0.3$ ) Again the  
17 combinations with xanthan gum or alginate were most effective with a  $LT_{50}$  of 21 in each case  
18 ( $P < 0.04$ ) (Fig. 4).

19

20 **Discussion**

21

22 The resistance of DBM to chemical insecticides has resulted in more widely spread use of  
23 *Bacillus thuringiensis* (Bt) to control this important cabbage pest (Talekar & Shelton, 1993).  
24 Most alarming, however, are reports on the development of resistance also against Bt toxins

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1 (Tabashnik *et al.*, 1990; Tabashnik 1994; Iqbal *et al.*, 1996; Sayed & Ferre (2000); Zhao *et*  
2 *al.* 2002). To prevent further increases in resistance against Bt alternative control measures  
3 are urgently needed. This study has shown that EPN are one possible alternative control  
4 strategy. With Bt, they share the lack of severe effects on beneficial arthropods, which can  
5 control up to 82% of DBM populations (Sastrosiswojo, 1996). As the results on the LC<sub>50</sub>  
6 indicate, the third instar seems to be the most susceptible stage (Fig. 2). As Bt and also  
7 baculoviruses with control potential against DMB larvae are most effective against the first  
8 instars (McGaughey, 1978; Tanada & Kaya, 1993), entomopathogenic nematodes have  
9 another major advantage as part of an integrated control strategy. *Steinernema carpocapsae*,  
10 due its desiccation tolerance (Patel *et al.*, 1997), seems to be well adapted for use on cabbage  
11 foliage. However, applied in water only, the potential of *S. carpocapsae* is by no means  
12 sufficient and did not surpass much beyond 50% control in our assays. Therefore, this study  
13 investigated the possibility to increase EPN efficacy on cabbage foliage by addition of  
14 adjuvants in leaf disk assays in the laboratory.

15 Prior to this investigation several adjuvants used in formulation technology (Bernhardt *et*  
16 *al.*, 1998) were tested for their feasibility to be used with EPN and the results indicate that  
17 with few exceptions all compounds can be used for the development of new formulations to  
18 enhance EPN performance on the leaf. Mason *et al.* (1998b) also considered the use of  
19 adjuvants including Triton X-100. However, our results indicate that this compound is  
20 phytotoxic and must therefore be excluded for use on cabbage foliage (Fig.1). Mason *et al.*  
21 (1998b) also recommended the use of surfactants to improve nematode deposition on the leaf  
22 using surfactants. The improvement obtained with combinations of surfactants with polymers  
23 might be a result of this effect. However, surfactants alone did not enhance nematode efficacy  
24 in our studies.

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1       The addition of polymers seem to be the key factor for improvement of EPN efficacy,  
2 although guar gum and arabic gum did not result in significant effects, but the addition of  
3 xanthan or alginate particularly in combination with surfactants increased efficacy to >90%.  
4 Unfortunately, the higher the polymers concentration, the lower is their solubility in water.  
5 For field application this can result in blocking of spray nozzles. Alginate in this aspect is a  
6 problematic compound, which can only be dissolved after intensive stirring of the  
7 formulation. With xanthan formulations this problem was solved by the addition of sodium  
8 hydrogen carbonate and oxalic acid (both at 0.3%) to the formulation. However, clumping of  
9 alginate could not be prevented with these additives.

10

11       One of the major advantages using the polymer formulations is the apparent reduction of  
12 the infection time (Fig. 4), since when nematodes cause mortality within 24 h, they must have  
13 entered the insect more rapidly. Hence, the formulation reduced the loss of nematodes due to  
14 detrimental effects on nematode survival caused by abiotic stress. The shorter the infection  
15 time the higher will be the efficacy at low relative humidity on the foliage. In this aspect,  
16 nematodes application should be scheduled in the evening short before sunset to provide high  
17 relative humidity during the night. The use of innovative formulation technology will thus  
18 enable nematodes to penetrate immediately after application before they are killed when  
19 exposed to more detrimental environmental conditions during the day. Considering the  
20 decrease of infection time caused by the formulation, the strategy of prolonging nematode  
21 survival on the canopy by the use of antidesiccants (Glazer, 1992; Piggott *et al.*, 2000) should  
22 be reconsidered. Instead, the provision of excellent conditions for host invasion might be the  
23 superior strategy.

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1 The presented results have been made under controlled conditions in leaf disk assays.

2 Whether similar effects will be achieved under field conditions remains to be tested.

3

4 **Acknowledgements**

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6 control of diamondback moth (*Plutella xylostella*) by conservation of natural enemies and  
7 application of biocontrol agents“. We acknowledge the financial support by the EU INCO  
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9

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**Evaluation of adjuvants for foliar application of *Steinernema carpocapsae*  
against larvae of the diamondback moth (*Plutella xylostella*)**

1 **Table. 1.** *Adjuvants tested for effects on nematode survival and infectivity, on Brassica*  
2 *oleracea leaves and diamondback moth third instar. Suppliers and tested concentrations in*  
3 *percent*

| Name                        | Supplier                          | Chemical  | Concentration (%) |
|-----------------------------|-----------------------------------|---|-------------------|
| <u>Surfactants:</u>         |                                   |   |                   |
| Adhäsit                     | Spiess Urania, Germany            | 10% marlopon and 15% methanol   | 0.3 & 3.0         |
| Agristick                   | Clariant, Indonesia               | Alkyl polyglycol ester  | 0.3 & 3.0         |
| Agrosom Net5                | Agrosom GmbH, Germany             | Rape-seed oil and emulsifier  | 0.3 & 3.0         |
| Biodac                      | Sasol S.p.A., Italy               | Polyethylene-polypropylene glycol-monodecyl ether                                   | 0.3 & 3.0         |
| Emulan ELP                  | BASF AG, Germany                  | Castor oil ethoxylat  | 0.3 & 3.0         |
| Eurogreen                   | Wolf-Garten GmbH & Co KG, Germany | Glycerol, ethylen-oxid, propylenoxid  | 0.3 & 3.0         |
| Glycerol                    | Walter DMB GmbH, Germany          | 86.5% Glycerol  | 0.1, 1.0 & 10.0   |
| Kick                        | Compo GmbH & Co KG, Germany       | Anionic and nonionic surfactants based on fatty alcohol and sulfonic carbon acid    | 0.3 & 3.0         |
| Magic Wet                   | Oilfield Chemicals, Germany       | Alkyl polyglucoside derivative lipid  | 0.3 & 3.0         |
| Rimulgan                    | Temmen GmbH, Germany              | 68% castor oil, 25% ionic oleic acid, 5% calcium and alcohol                        | 0.3 & 3.0         |
| Triton X-100                | Serva & Sigma Chemicals, Germany  | t-Octylphenoxy-ethanol  | 0.3 & 3.0         |
| Tween 80                    | Merck, Germany                    | Polyoxyethylene-sorbitan Monooleate   | 0.3 & 3.0         |
| Tween 85/ 60 /40            | Sigma-Aldrich, St. Louis, USA     | Polyoxyethylene-sorbitan -trioleate/ -monostearate / -monopalmitate                 | 0.3 & 3.0         |
| <u>Binders / Thickeners</u> |                                   |   |                   |
| Arabic Gum                  | Sigma, St. Louis, USA             | Acacia gum polymer  | 0.3 & 1.0         |
| Bevaloid 211                | C.H. Erbslöh KG, Germany          | Polyacrylate sodium salt  | 0.3               |
| Bond                        | Spiess Urania, Germany            | Synthetic Latex & alkoxyated alcohol  | 0.3 & 3.0         |
| Gum Xanthan                 | UD Chemie GmbH, Germany           | Fermentation-derived biopolymer from the bacterium Xanthomonas campestris (40 mesh) | 0.3 & 1.0         |
| Methocel J75MS              | Nordmann Rassmann, Germany        | Methyl cellulose  | 0.3 & 3.0         |
| Polyvinylalkohol            | Merck-Schuchard, Germany          | Ethenol homopolymer   | 0.3 & 3.0         |
| A: Protaweld EVR 200        | FMC Biopolymer, Norway            | A: Potassium salt of alginic acid   | 0.3 & 1.0         |
| B: Protamon S               |                                   | B: Ammonium salt of alginic acid  |                   |
| C: Protanal LF 20/40        |                                   | C: Sodium alginate (E-401)  |                   |
| Silica Fumed                | Sigma, St. Louis, USA             | Silicon dioxide   | 0.3 & 3.0         |
| Sorbitol                    | Merck-Schuchard, Germany          | D-sorbitol  | 0.3 & 3.0         |
| Stocksorb                   | Degussa, Germany                  | A: Potassium / ammonium polyacrylamide, cross-linked                                | 0.01 & 0.1        |
| A: C 10177                  |                                   |   |                   |
| B:C 10178                   |                                   | B: Sodium polyacrylate, cross-linked  |                   |
| <u>Oil and waxes:</u>       |                                   |   |                   |
| CereNat                     | Schumann Sasol, Germany           | Wax- / plant oil emulsion   | 0.1, 1.0 & 10     |
| TP Agrocer 010              | Clariant, Germany                 | Aqueous solution of montan wax  | 0.3 & 3.0         |
| Mustard oil                 | --                                | Oil expressed from mustard seeds  | 0.1, 1.0 & 10     |
| Rapeseed oil                | --                                | Oil expressed from seeds of Brassica campestris                                     | 0.1, 1.0 & 10     |

**Evaluation of adjuvants for foliar application of *Steinernema carpocapsae*  
against larvae of the diamondback moth (*Plutella xylostella*)**

1  
2 **Table 2.** Corrected mortality of third instar larvae of *Plutella xylostella* after application of  
3 *Steinernema carpocapsae* ( $13 \pm 6$  nematodes/larva) in water or 0.3% polymer at 25°C. Means  
4 ( $n = 3$ ) followed by the same letter do not differ significantly according to Duncan's multiple  
5 range test at  $P < 0.05$

6

| EPN suspension     | % efficacy $\pm$ 95% fiducial limits |                     |
|--------------------|--------------------------------------|---------------------|
|                    | 60% RH                               | 80% RH              |
| Xanthan gum        | 62.7 $\pm$ 23.1 bcd                  | 70.8 $\pm$ 6.9 cd   |
| Potassium alginate | 56.9 $\pm$ 28.0 bcd                  | 81.9 $\pm$ 11.7 d   |
| Guar gum           | 35.3 $\pm$ 24.9 abc                  | 42.9 $\pm$ 24.4 abc |
| Arabic gum         | 28.9 $\pm$ 22.4 ab                   | 53.9 $\pm$ 31.2 bcd |
| water              | 12.1 $\pm$ 23.7 a                    | 37.3 $\pm$ 22.8 abc |

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**Evaluation of adjuvants for foliar application of *Steinernema carpocapsae*  
against larvae of the diamondback moth (*Plutella xylostella*)**

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15 **Fig. 1.** *Detrimental effect caused by Triton X-100 70 h after application. (A) 0.3% applied to*  
16 *leaf of Brassica oleracea. (B) Effect on B. oleracea plants at 0, 0.3, 3 and 10% (from left to*  
17 *right)*

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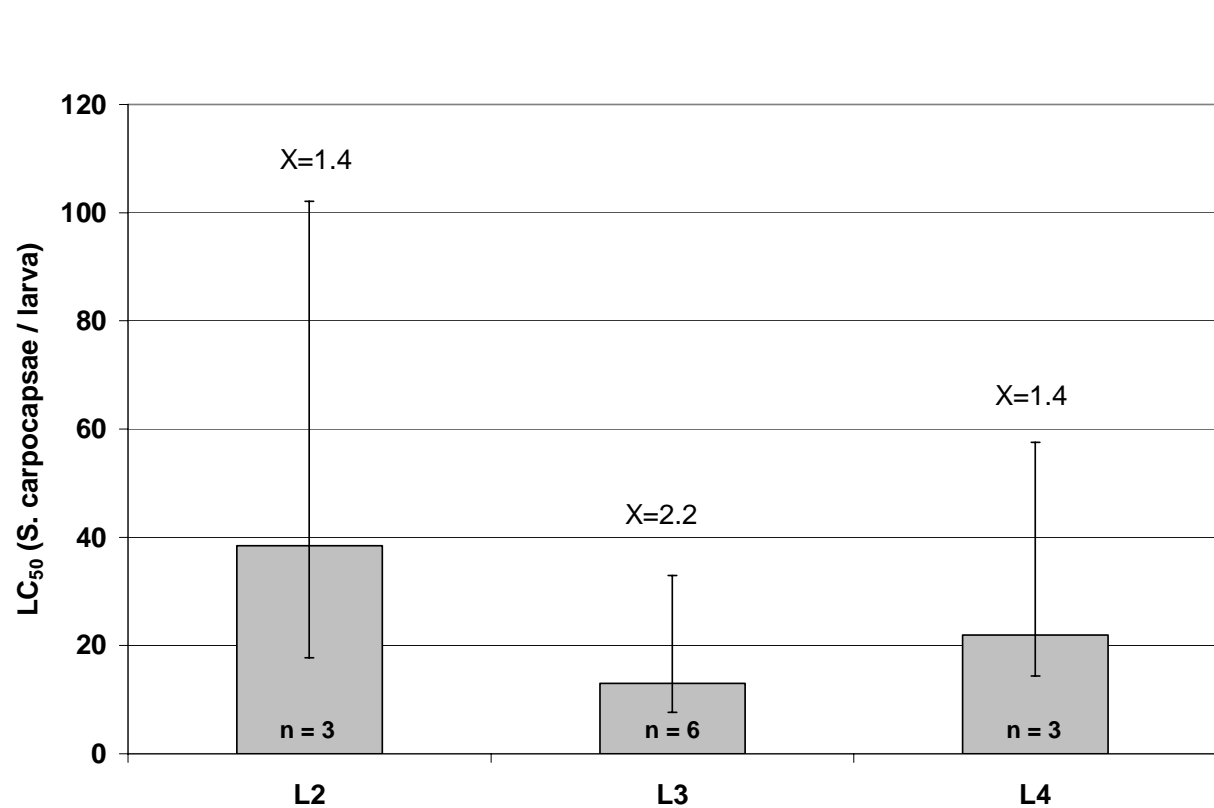
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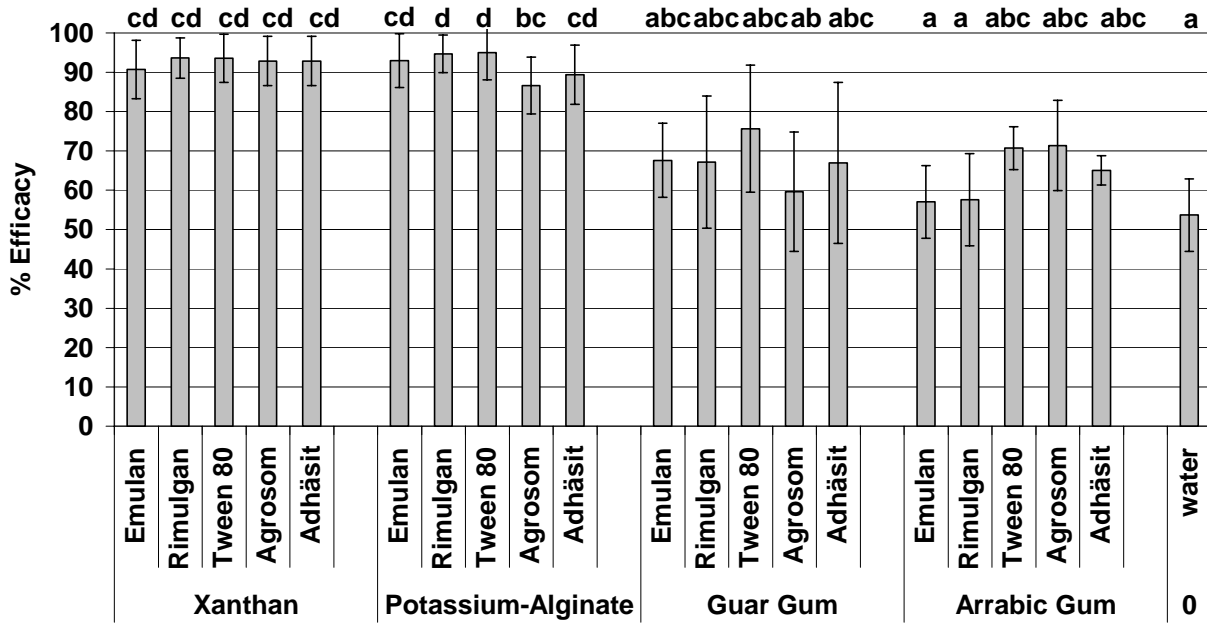
**Evaluation of adjuvants for foliar application of *Steinernema carpocapsae*  
against larvae of the diamondback moth (*Plutella xylostella*)**



**Fig. 2.**  $LC_{50}$  of *Steinernema carpocapsae* against *Plutella xylostella* second to fourth larval instars at 80% RH and 25°C. Error bars, indicate 95% fiducial limits and  $X$  = slope calculated according to probit analysis. Single larvae were treated with nematodes in water in 24 well dishes

**Evaluation of adjuvants for foliar application of *Steinernema carpocapsae* against larvae of the diamondback moth (*Plutella xylostella*)**

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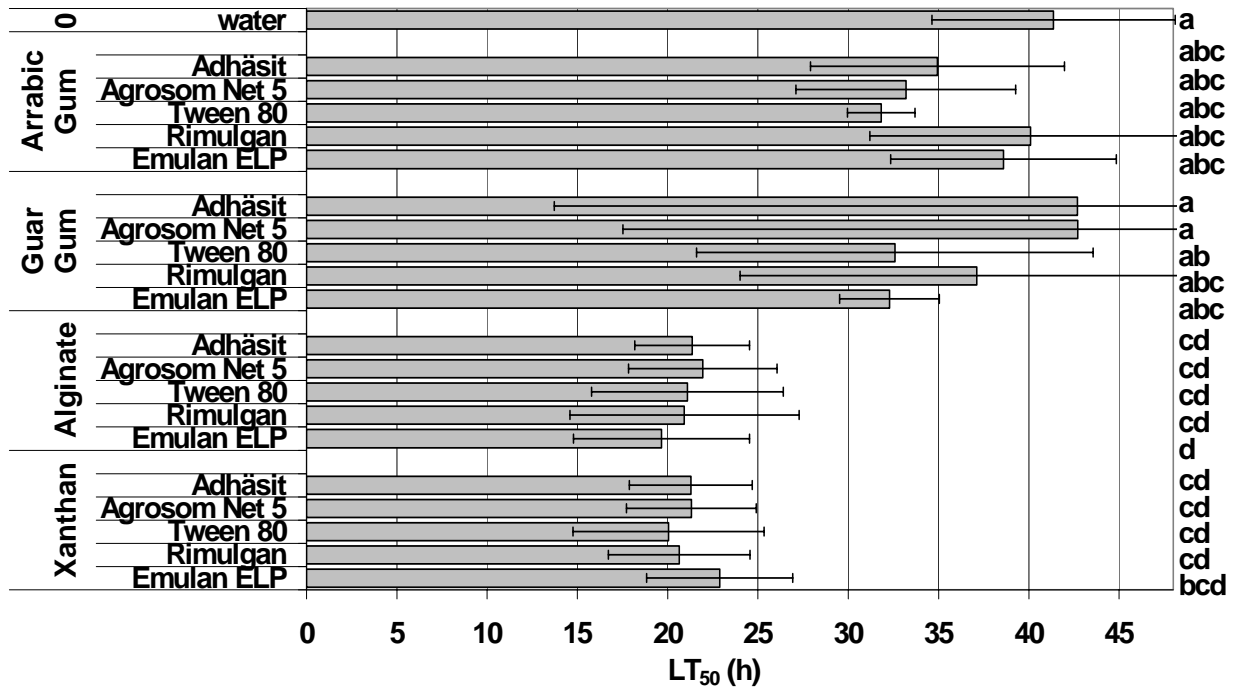


3  
4 **Fig. 3.** Corrected mortality of *Plutella xylostella* third instar larvae after application of  
5 *Steinernema carpocapsae* ( $13 \pm 6$  nematodes/larva) in water or in formulations of 0.3%  
6 surfactants and 0.3% polymers at 80% RH and 25°C ( $n = 3$ ). Bars indicated with the same  
7 letter do not differ significantly according to Duncan's multiple range test at  $P < 0.05$

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Evaluation of adjuvants for foliar application of *Steinernema carpocapsae*  
 against larvae of the diamondback moth (*Plutella xylostella*)

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**Fig. 4.** *LT<sub>50</sub>* of 3<sup>rd</sup> instar larvae of *Plutella xylostella* treated with *Steinernema carpocapsae* ( $13 \pm 6$  nematodes/larva) in 0.3% surfactant and 0.3% polymer at 80% RH and 25°C ( $n = 3$ ). Bars indicated with the same letter do not differ significantly according to Duncan's multiple range test at  $P < 0.05$

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

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**Mode of Action of a Surfactant-Polymer-Formulation to support**

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**Performance of the Entomopathogenic Nematode *Steinernema carpocapsae***

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**for Control of Diamondback Moth Larvae (*Plutella xylostella*)**

7

8

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**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

1 *The use of entomopathogenic nematodes on cabbage leaves against larvae of the*  
2 *diamondback moth (DBM) *Plutella xylostella* requires the addition of formulation adjuvants*  
3 *to achieve satisfying control. Without adjuvants nematodes settle in the tank mix of backpack*  
4 *sprayers causing uneven distribution. The polymers arabic and guar gum, alginate and*  
5 *xanthan were used in concentrations between 0.05 and 0.3% to retard sedimentation of*  
6 **Steinernema carpocapsae*. Arabic gum had no effect, guar gum prevented sedimentation at*  
7 *0.3% but the effect dropped significantly at lower concentration. At 0.05% xanthan prevented*  
8 *nematode sedimentation better than alginate. Deposition of nematodes on the leaves was*  
9 *significantly increased by the addition of any of the polymers. Spraying nematodes on leaves*  
10 *with an inclination of 45° without the addition of any formulation resulted in 70% run-off.*  
11 *Adding 0.2% alginate or xanthan reduced the losses to < 20%. The use of a surfactant-*  
12 *polymer formulation significantly reduced defoliation by DBM larvae. Visual examinations*  
13 *gave evidence that nematodes are not ingested by DBM larvae. Invasion of *S. carpocapsae* is*  
14 *an active process via the anus. The function of the formulation is not to prolong nematode*  
15 *survival, but to provide environmental conditions which enable rapid invasion of the*  
16 *nematodes. Nematode performance was improved by selection of the best surfactant in*  
17 *combination with xanthan and by optimisation of the concentrations of the surfactant*  
18 *Rimulgan<sup>®</sup> and the polymer xanthan. The best control results were achieved with Rimulgan<sup>®</sup>*  
19 *at 0.3% together with 0.3% xanthan, causing DBM mortality of >90% at 80% relative*  
20 *humidity and >70% at 60%. The formulation lowered the LC<sub>50</sub> from 12 to 1 nematode/larva.*  
21 *The viscosity of the surfactant-polymer-formulations correlated well with nematode efficacy,*  
22 *prevention of sedimentation and adherence to the leave. This physical parameter can*



**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

1 *therefore be recommended for improvement of nematode formulations to be used for foliar*  
2 *application against DBM.*

3 **Key words:** *Steinernema carpocapsae, foliar application, Plutella xylostella, surfactant-*  
4 *polymer-formulation, viscosity, nematode host invasion, Brassica oleracea, sedimentation,*  
5 *run-off*

6

7 INTRODUCTION

8 The diamondback moth (DBM) *Plutella xylostella* L. is a major pest of cabbage, particularly  
9 in tropical regions. The excessive use of chemical insecticides has promoted the development  
10 of insecticide resistance in DBM. As a consequence, frequency of insecticide applications  
11 increased with all negative impacts on naturally occurring invertebrate enemies of the DBM.  
12 Substitution of chemical compounds with *Bacillus thuringiensis* Berliner only has a limited  
13 potential as resistance against this biocontrol agent has already been recorded (Talekar &  
14 Shelton, 1993; Heckel, 1994). Therefore, other control measures are urgently needed to  
15 provide sustainable control of DBM, avoid resistance development against *B. thuringiensis*  
16 and promote the activity of naturally occurring invertebrate antagonists. Entomopathogenic  
17 nematodes (EPN) can be one alternative to substitute ineffective chemical insecticides  
18 (Cherry *et al.*, 2004). Baur *et al.* (1995) reported that *Steinernema carpocapsae* (Weiser), a  
19 desiccation tolerant nematode, is well adapted for foliage application against DBM larvae.  
20 However, the efficacy on foliage of soil-born nematodes is limited by abiotic factors like UV-  
21 radiation (Gaugler *et al.*, 1992; Nickle & Shapiro, 1992), extreme temperature or drought  
22 (Glazer, 2002). To prolong nematode survival on the leaf several adjuvants have been  
23 evaluated (Glazer, 1992; Baur *et al.*, 1997; Mason *et al.*, 1998), but the improvements were

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

1 not sufficient to increase the feasibility of nematode foliar application against DBM (Baur *et*  
2 *al.*, 1997). Other surfactant-polymer-formulations were therefore tested to improved  
3 nematode efficacy. The combination of 0.3% xanthan gum and 0.3% of different surfactants  
4 significantly enhanced nematode efficacy and reduced the time of infection in laboratory  
5 assays (Schroer *et al.*, in press). The objective of this study was to further improve the  
6 formulation and to understand how EPN performance is influenced by the use of different  
7 polymer formulations. Furthermore, the effect of the formulation on sedimentation of EPN in  
8 the spraying tank and deposition of EPN on the leaf surface was investigated.

9

## 10 MATERIALS AND METHODS

### 11 **Organisms and Adjuvants**

12 Cabbage plants (*Brassica oleracea* var. *capitata*) were grown on commercial peat substrate  
13 (Floragard<sup>®</sup>, Oldenburg, Germany) in the greenhouse. *P. xylostella* were reared to the third  
14 instar on savoy cabbage leaves (*B. oleracea* convar. *capitata* var. *sabauda*) at 20°C and 80%  
15 RH. The Egyptian isolate S2 of *S. carpocapsae* was reared *in vivo* according to Glazer and  
16 Lewis (2000). Adjuvants tested were polymers (galacto-polysaccharides) and surfactants  
17 (Table 1).

18

### 19 **Effect of Adjuvants on EPN Sedimentation and Deposition on the Leaf Surface**

20 Four different polymers were evaluated for the feasibility to retard sedimentation of infective  
21 dauer juveniles (DJs) in 500 ml spraying mixture. Sedimentation was recorded using the  
22 method described by Peters and Backes (2003). Polymers at a concentration of 0, 0.05, 0.1,  
23 0.2 or 0.3% were added to tap water. Nematodes (1/μl) were applied into the suspension and

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

1 the suspension was stirred in a flask until nematodes were equally distributed in the spraying  
2 mixture and then poured into a 13\*13.5\*6.5 cm (length, width, height) plastic cup.  
3 Sedimentation time was estimated by sampling 100 µl of the suspension from 2 cm depth at 0,  
4 5, 10, 15, 30 and 60 min after stirring of the suspension had been terminated.

5 Due to the glabrous and waxy cuticle of cabbage leaves, EPN are not retained on the  
6 surface of the leaves. The feasibility to stick DJs to foliage using an appropriate formulation  
7 was evaluated. Leaves were attached to a trestle exposing them in a 45° or 90° angle to the  
8 spraying nozzle. After spraying the leaves with nematodes in a formulation (0.3% Rimulgan  
9 in combination with 0.1, 0.2 and 0.3% arabic gum, guar gum, xanthan or alginate) or in water  
10 the cabbage leaves were removed and two discs of 2 cm<sup>2</sup> were cut from each leaf. The number  
11 of DJs on the discs was determined by washing them off the leaf surface into counting  
12 chambers. The amount of DJs counted from leaves attached in 45° angle were compared to  
13 the leave discs exposed at right angle to the spraying nozzle. The tests were conducted three  
14 times.

15

16 **Visual Examination of EPN Invasion**

17 Larval and DJ behaviour on the foliage was observed at 32-fold magnification in 6 cm dishes  
18 lined with cabbage leaf discs, which had been treated with EPN in water or a  
19 formulation. Nematodes were applied using a hand-hold sprayer. Adjuvants were added at  
20 0.3% concentration, using the polymer xanthan in order to thicken water drops and the  
21 surfactant Rimulgan<sup>®</sup> to lower the water tension.. Xanthan was chosen due to the feasibility to  
22 increase DJ efficacy (Schroer *et al.*, in press) and Rimulgan because of its biological

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

1 degradability. Three 3<sup>rd</sup> instar DBM were added to the dishes and their movements on the  
2 plant foliage were observed.

3 To record the way of DJ invasion into DBM larvae, single 3<sup>rd</sup> instars were dipped with their  
4 whole body into the formulation with >50 DJs/ $\mu$ l and placed into one 2 cm<sup>2</sup> well. DJ infection  
5 was observed visually examining the surface of the larva and recording EPN movements with  
6 an inverted microscope using cold light at 32-fold magnification.

7

8 **Single DBM Larva Bioassay to test EPN Performance**

9 Nematode efficacy was tested on 2 cm<sup>2</sup> filter paper discs or cabbage leaf discs treated with  
10 DJs water or formulation. In all applications nematodes were applied in 40 ml. The DJ dose  
11 was according to the LC<sub>50</sub> in water at 13  $\pm$  6 DJ/larva (Schroer *et al.*, in press). Nematodes  
12 were applied onto the discs using a spraying apparatus in 55 cm distance with a Teejet<sup>®</sup>  
13 (TP8003E) flat-fan nozzle at a volume-flow of 0.96 l/min and a pressure of 20<sup>5</sup> Pa. After  
14 treatment the leaf discs were transferred into 24-well plates (2 cm<sup>2</sup> per well) and one 3<sup>rd</sup> instar  
15 DBM was added per well. For each variable one cell well dish (24 wells) was prepared. The  
16 dishes were sealed with gaze for gas exchange and incubated at 80% or 60% RH and 25°C.  
17 After 48 h DBM mortality was evaluated. Larvae not moving when touched with pincers were  
18 recorded dead.

19 In 24-well plates (2 cm<sup>2</sup> per well) larvae were either exposed to filter paper or cabbage  
20 leaf discs treated with 13  $\pm$  6 DJ/disc, comparing DJ efficacy in presence or absence of food.  
21 At time intervals of 24 and 48 h dead larvae were dissected and infection was recorded.  
22 Defoliation of cabbage leaves was recorded: After exposure of DBM larvae to the discs for 20  
23 h, the leaf discs were photographed and the remaining foliage area was measured using image

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

1 analysis (Soft Imaging System GmbH, Münster, Germany). Defoliation tests were repeated  
2 twice.

3

#### 4 **Improvement of the Formulation**

5 To improve concentrations of the adjuvants, Rimulgan<sup>®</sup> was used at 0.1, 0.2 and 0.3 % in  
6 combination with 0.1, 0.2, 0.3 and 0.5% xanthan at 60 and 80% RH. Further, the impact of  
7 different surfactants in combination with xanthan was evaluated with or without the addition  
8 of nematodes. Three different surfactants (Table 1) were mixed at 0.3% with 0.3% xanthan.  
9 The surfactant-polymer-formulations were prepared in 80 ml volume and then divided for two  
10 application. To one 40 ml part nematodes were added, at the same concentration as in the  
11 previous experiments and treatments were handled as described above. All experiments were  
12 conducted three times.

13

#### 14 **Impact of the Formulation on the LC<sub>50</sub>**

15 A dose mortality response for *S. carpocapsae* against third instar *P. xylostella* was evaluated  
16 using 0.3% Rimulgan<sup>®</sup> and 0.3% xanthan with the addition of 0, 1, 3, 5, 10 and 20 DJs/larva.  
17 Controls were applied in water. Mortality was evaluated after 48 h. The experiments were  
18 conducted three times.

19

#### 20 **Viscosity**

21 The polymers performance in water was examined measuring the viscosity with a rotation  
22 viscometer (Thermo Elektron GmbH, Karlsruhe, Germany) at 0.1, 0.2 and 0.3% and  
23 additionally at the highest concentration with 0.3% Rimulgan<sup>®</sup>. The rotation spindles were set

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1 into 500 ml of each mixture. Spindles and rotation speed were adapted to the pressure in the  
2 solution. The viscosity was calculated from the rotation expended pressure in Pa (Mollet &  
3 Grubenmann, 2001). The viscosity measurements were conducted twice.

4

5 **Data analysis**

6 In all trials data were analysed using ANOVA (CSS StatSoft Inc., Tulsa, USA), with  
7 significance levels at 95%. Means were compared using Tukey's HSD test. Data of DJs loss  
8 by run-off from the leaves were expressed in percent since total number of applied nematodes  
9 differed between the treatments. Further was the efficacy of DJs in different adjuvant  
10 formulation expressed in percent. Percentage data were subjected to arcsine square root  
11 transformation test. . The LC<sub>50</sub>-value was determined using PROBIT analysis. The interaction  
12 between the formulation and nematodes were compared using the procedure described by  
13 Finney (1971).

14

15 **RESULTS**

16 **Effect of Formulation on Nematode Sedimentation**

17 Sedimentation of *S. carpocapsae* in water is fast. Five minutes after the stirring had ceased,  
18 50% of the nematodes had passed beyond a depth of 2 cm. After 1 h only 10% of the initial  
19 nematode number was recorded. Arabic gum increased the sedimentation velocity but without  
20 significant differences compared to water (Fig. 1). Guar gum retarded the sedimentation.  
21 Using 0.3% guar gum prevented *S. carpocapsae* from sedimentation for 1 h. xanthan and at  
22 0.05 or alginate at 0.1%, had similar effects (Fig. 1).

23

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

**1 Effect of the Formulation on Nematode Deposition on the Leaf Surface**

2 Compared to leaf discs applied in 90° angle to the spraying nozzle, at an inclination of 45° the  
3 majority of the nematodes were lost (70%) when applied in water. The addition of 0.3% of  
4 each of the tested polymers could significantly reduce the loss. Only 0.2% addition of xanthan  
5 and alginate reduced the nematode loss to < 20% (Table 2).

**7 Visual Examination of Dauer Juvenile Invasion**

8 Nematodes in water sprayed on cabbage leaf discs are captured in drops. DBM larvae were  
9 touching the drops at random. Due to the surface tension, nematodes got in contact with the  
10 host only when DBM larvae were crossing a drop. DBM larvae treated with the formulation  
11 showed decreased mobility, whereas *S. carpocapsae* DJs were moving without any obstacles.  
12 Attempts of DJs to enter the mouth, spiracles or the anus were observed. Spiracles appeared  
13 to be too small in diameter and the surrounding tissue too rigid to allow *S. carpocapsae*  
14 penetration. Single DJs were observed trying to enter the spiracles up to 1 h, then DJ  
15 movement ceased, but no successful invasion via the spiracles was observed.. Nematodes  
16 trying to enter the mouth were repulsed by the insect. Only larvae restricted in mobility by the  
17 formulation were invaded by EPN through the mouth. DJs accumulated around faecal pellets  
18 (Fig. 2) and successful penetration was recorded through the anus (Fig. 3). Excretion of DJs  
19 with faeces during defecation was also observed.

**21 Impact of Feeding on DBM Mortality**

22 Nematode infection into DBM larvae was observed on either cabbage leaf discs or filter  
23 paper. On both, 50% of 24 DBM larvae treated with nematodes were infected after an

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1 exposure of 24 h. Extending the exposure time to 48 h resulted in up to 100% mortality on  
2 filter paper, but on cabbage leaf discs mortality did not surpass 50%. Thus the availability of  
3 food could not increase EPN efficacy against DBM larvae.

4

5 **Influence of Formulation on Feeding**

6 Defoliation by 3<sup>rd</sup> instar DBM was measured after 20 h exposure to cabbage leaf discs treated  
7 with water or surfactant-polymer formulation with or without EPN (Fig. 4). Leaf consumption  
8 on water treated cabbage was 0.8 ( $\pm$  0.05) cm<sup>2</sup>/larva (n=72). Nematodes caused no significant  
9 difference in defoliation, with 0.84 ( $\pm$  0.06) cm<sup>2</sup>/larva (n = 72) loss of foliage on EPN treated  
10 leaves. Significant reduction in defoliation was recorded using the formulation. Only 0.43 ( $\pm$   
11 0.05) cm<sup>2</sup>/larva were consumed with or without the addition of EPN.

12

13 **Improving Concentrations of Adjuvants**

14 The impact of three surfactants in combination with xanthan on nematode efficacy was tested  
15 with or without the addition of nematodes at 60 and 80% RH. Nematodes applied in water  
16 control 50% of DBM larvae at 80% RH and only 20% at 60% RH (Fig. 5). Mortality caused  
17 by the addition of the surfactants Rimulgan<sup>®</sup>, Emulan ELP<sup>®</sup> or Tween to xanthan without  
18 nematodes was 29, 45 or 22%, respectively, at 80% RH and 46, 35 and 24%, respectively, at  
19 60% RH. The effect of all surfactant-polymer combinations was synergistic at 80% RH and  
20 additive at 60% RH. At 80% RH humidity only Rimulgan<sup>®</sup> and Emulan ELP<sup>®</sup> significantly  
21 improved nematode efficacy compared to the treatment with DJs in water, but no significant  
22 differences were recorded for EPN efficacy between the three surfactants (Fig. 5). At 60% RH  
23 no significant differences between any of the formulations were recorded.



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1           The impact of varying concentrations of Rimulgan<sup>®</sup> and xanthan on nematode efficacy  
2 was studied at 60 and 80% RH. At 80% RH the addition of 0.1% xanthan with 0.2%  
3 Rimulgan<sup>®</sup> already doubled nematode efficacy compared to water (Fig. 6). Efficacy increased  
4 with the proportion of xanthan up to a concentration of 0.3%, resulting in a 3.4-fold increase  
5 of the efficacy compared to water. At > 0.3% xanthan, a further increase of the Rimulgan<sup>®</sup>  
6 concentration did not significantly improve nematode efficacy. At 60% RH nematode efficacy  
7 without adjuvants was less than 10%. Efficacy could be increased to 40% by adding either  
8 0.3% or 0.5% xanthan plus any concentration of Rimulgan<sup>®</sup>, or at lower xanthan  
9 concentration with 0.3% Rimulgan<sup>®</sup>. But no significant differences between any of the  
10 formulations were recorded(Fig. 6).

11

### 12 **Impact of the Formulation on the LC<sub>50</sub>**

13 The LC<sub>50</sub> and 95% confidence interval of EPN formulated with 0.3% Rimulgan<sup>®</sup> and 0.3%  
14 xanthan was 0.1 # 1.54 # 3.1 DJs/larva at 80% RH and 25°C. There was a strong dose  
15 response on *P. xylostella* mortality (slope of the regression line: 1.6; Chi<sup>2</sup> = 0.8; df = 3) and  
16 the LC<sub>90</sub> = 5.5 # 10.0 # 58.9 DJs/larva. The LC<sub>50</sub> in the control applied with water was 7.3 #  
17 11.3 # 15.7 and the LC<sub>90</sub> = 31.0 # 51.2 # 174.1.

18

### 19 **Viscosity**

20 Obvious differences were recorded in the rheology of the different polymers. The lowest  
21 viscosity was recorded with arabic gum. At all tested concentration of arabic gum 6 mPa was  
22 measured and even the addition of the surfactant did not change the viscosity. The viscosity of  
23 the guar gum correlated with the concentration of the compound. Higher viscosity was

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1 measured with xanthan and alginate. The viscosity of xanthan was further increased by the  
2 addition of Rimulgan<sup>®</sup> (Table 3).

3

4 DISCUSSION

5 The investigation has shown that the use of a polymer formulation together with the  
6 entomopathogenic nematode *S. carpocapsae* has several advantages. The addition of the  
7 surfactant-polymer formulation prevents nematodes from settling in the spray tank thus  
8 securing an even distribution, it deposits nematodes on the leaf reducing loss into the soil by  
9 run-off, it produces optimal conditions for host invasion by reducing the mobility of the insect  
10 and produces ideal conditions for DJ host seeking and invasion.

11 When applying nematodes, the first constraint is sedimentation of DJs in the tank.  
12 Peters & Backes (2003) prevented DJ sedimentation using carboxy-methyl cellulose at 0.3%.  
13 The choice of the polymer is rather important, because some polymers even act as  
14 precipitants, *e.g.* arabic gum. Already a small amount of xanthan prevented DJ sedimentation  
15 sufficiently. The same polymer also provided ideal properties to bind DJs on the foliage. The  
16 use of 0.2 or 0.3% xanthan can significantly reduce the loss of DJs by run-off and deposit DJs  
17 next to the target host.

18 Another advantage of the formulation is the provision of humidity, which enables nematodes  
19 to survive, move, seek for and invade the host insect. Low humidity leads to rapid desiccation  
20 of DJs and they are usually not able to survive for longer than a few hours (Glazer, 1992).  
21 Improving formulation technology therefore aims at prolonging DJ survival on the leaf (*e.g.*,  
22 Baur *et al.*, 1995; Navon *et al.*, 1998). The use of the surfactant-polymer formulation (0.3%  
23 xanthan and 0.3% Rimulgan<sup>®</sup>) has the same function, but it also provides ideal conditions for

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1 host invasion. Navon *et al.* (1998) developed an edible polymer formulation to control  
2 *Spodoptera littoralis*. Alchantis *et al.* (2000) fed *S. littoralis* larvae with the gel and recorded  
3 DJs inside the insect after gel consumption. However, this formulation would probably not be  
4 useful for control of DBM larvae as host invasion into this small insect is not a passive  
5 process requiring ingestion of the DJs, indicated by the strong defending behaviour of DBM  
6 larvae. Further the results of defoliation present less food consumption at leaves treated with  
7 the formulation (Fig. 4) but increased DJ efficacy. Accordingly, we conclude that host  
8 infestation in DBM requires nematode activity like host seeking and invasion through the  
9 anus. Thus a major function of the formulation for DBM control is to enable DJs to invade  
10 more quickly and thus escape from the exposure to detrimental environmental conditions on  
11 the foliage. The formulation reduces the time necessary to kill DBM larvae significantly  
12 (Schroer *et al.*, 2004, in press), thus lowering the risk for the DJs to die from desiccation.

13 The observations indicate that host invasion is an active process and not a result of  
14 ingestion of the DJs. This is supported by results on DBM mortality obtained with EPN on  
15 filter paper or cabbage leaf discs. Considering DBM would ingest DJs, nematode caused  
16 mortality would be higher on cabbage leaf discs than on filter paper, however, the opposite  
17 was recorded. The porous structure of the filter paper might have supported host invasion  
18 even better than the cabbage leaf. Water drops are sucked up by the paper enabling DJ  
19 movement and providing a humid environment. In contrast, the waxy surface of cabbage  
20 might be less favourable when nematodes are applied in water and they are trapped in the  
21 drops. According to Glazer (1992) and Baur *et al.* (1995) nematodes survive longer on hairy,  
22 pubescent plant canopies than on glabrous foliage. *S. carpocapsae* have ambushing attitudes

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1 in host finding. Thus the structure of filter paper seems to support host finding and invasion  
2 (Grewal *et al.*, 1994).

3 According to these results there are two options to enhance infectivity on foliage: To  
4 immobilise DBM larvae or to support nematode survival and movement. Reduced leaf  
5 damage when using the formulation (Fig. 4) indicates that DBM are immobilised so damage  
6 by feeding is avoided. Reducing DBM mobility will also result in a limited ability to escape  
7 from nematode attack.

8 Host finding and invasion thus seem to be a major factor in DBM control with EPN.  
9 Baur *et al.* (1995) explain the lower infection of *S. carpocapsae* into DBM than into *Heliothis*  
10 *armigera* larvae (Glazer & Navon, 1990) with blocked spiracles due to the use of excessive  
11 amounts of water during nematode application. However, our visual observations indicate that  
12 the spiracles are simply too small for nematode penetration. Infection into DBM was most  
13 successful through the anus. *S. carpocapsae* show dismissive reaction to the faeces of *Blatella*  
14 *germanica*, which then excluded invasion through the anus as well (Grewal *et al.*, 1993) Our  
15 results indicate that *S. carpocapsae* is attracted by DBM faeces as DJs accumulate around  
16 particles of faeces (Fig. 2). Another consequence of the observation is, that improved  
17 application technology with better DJ distribution is not a matter of droplet size, but rather a  
18 matter of producing a viscous film layer to support the active penetration of *S. carpocapsae*  
19 into DBM larvae and at the same time reduce insect mobility. Compared with the application  
20 in water, the use of the formulation considerably lowered the LC<sub>50</sub> from 12 to 1 DJ/larva. The  
21 support of nematode mobility and the provision of water by the polymers makes infection  
22 feasible even at 60% RH.

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1           The results on the rheology of the compound suggests that all favourable characters of  
2 the formulation, prevention of sedimentation and run-off, provision of ideal conditions for  
3 host seeking and invasion and efficacy, seem to be correlated with the viscosity of the  
4 surfactant-polymer formulation. Thus this physical parameter provides an excellent measure  
5 the further improvement of formulations.

6           A mixture of 0.3% Rimulgan combined with 0.2 to 0.3% xanthan provided satisfying  
7 control results against DBM in the laboratory. Experiments will follow to test the formulation  
8 under field conditions.

9

10   ACKNOWLEDGMENTS

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12 control of Diamondback Moth (*Plutella xylostella*) by conservation of natural enemies and  
13 application of biocontrol agents“. We acknowledge the financial support by the EU INCO  
14 programme (ICA4-2001-10003) and by the Technology Foundation Schleswig-Holstein.  
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16 University Kiel, who measured the viscosity of the polymers.

17

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- 11  
12



**Mode of action of a surfactant-polymer-formulation to support performance of the  
entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae  
(*Plutella xylostella*)**

1 TABLE 1. List of tested adjuvants  
2  
3

| Name                    | Use        | Chemical composition   | Supplier                                 |
|-------------------------|------------|--|--|
| Rimulgan <sup>®</sup>   | surfactant | 68% castor oil, 25% ionic oleic acid, 5% calcium and alcohol                     | Temmen GmbH,<br>Hattersheim, Germany     |
| Emulan ELP <sup>®</sup> | surfactant | castor oil ethoxylat   | BASF AG,<br>Ludwigshafen,<br>Germany     |
| Tween 80                | surfactant | polyoxyethylene-sorbitan monooleate  | Merck-Schuchardt,<br>Darmstadt, Germany  |
| Xanthan                 | thickener  | Fermentation-derived biopolymer from the bacterium <i>Xanthomonas campestris</i> | UD Chemie GmbH,<br>Wörrstadt, Germany    |
| Arabic gum              | thickener  | acacia gum polymer   | Sigma-Aldrich CO.,<br>St. Louis, MO, USA |
| Guar gum                | thickener  | endosperm flour of <i>Cyamopsis tetragonolobis</i>                               | UD Chemie GmbH,<br>Wörrstadt, Germany    |
| Potassium-alginate      | thickener  | potassium salt of alginic acid   | FMC Biopolymer,<br>Brussels, Belgium     |

4

5

**Mode of action of a surfactant-polymer-formulation to support performance of the  
entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae  
(*Plutella xylostella*)**

1 TABLE 2. Impact of different polymers each mixed with the surfactant Rimulgan<sup>®</sup> at 0.3% on the  
2 loss of nematodes by run-off from cabbage leave discs attached at an angle of 45° to  
3 the application nozzle. Means (n = 3) followed by the same letter are not significantly  
4 different according to Tukey's HSD test at P < 0.05 (arc sine transformed data)

5  
6  
7

| Polymer    | %<br>concentration | % EPN loss<br>(± 95% fiducial limit) |
|------------|--------------------|--------------------------------------|
| Water      |                    | 70.0 (12.4) a                        |
| Arabic gum | 0.1                | 51.9 (12.2) ab                       |
|            | 0.2                | 52.9 (15.2)ab                        |
|            | 0.3                | 39.1 (12.0) bc                       |
| Guar gum   | 0.1                | 55.7 (11.9) ab                       |
|            | 0.2                | 56.6 (5.3) ab                        |
|            | 0.3                | 26.8 (22.8) c                        |
| Xanthan    | 0.1                | 69.2 (2.6) a                         |
|            | 0.2                | 17.1 (16.8) c                        |
|            | 0.3                | 22.2 (15.1) c                        |
| Alginate   | 0.1                | 32.9 (23.8) bc                       |
|            | 0.2                | 12.6 (5.9) c                         |
|            | 0.3                | 17.8 (23.1)c                         |

8  
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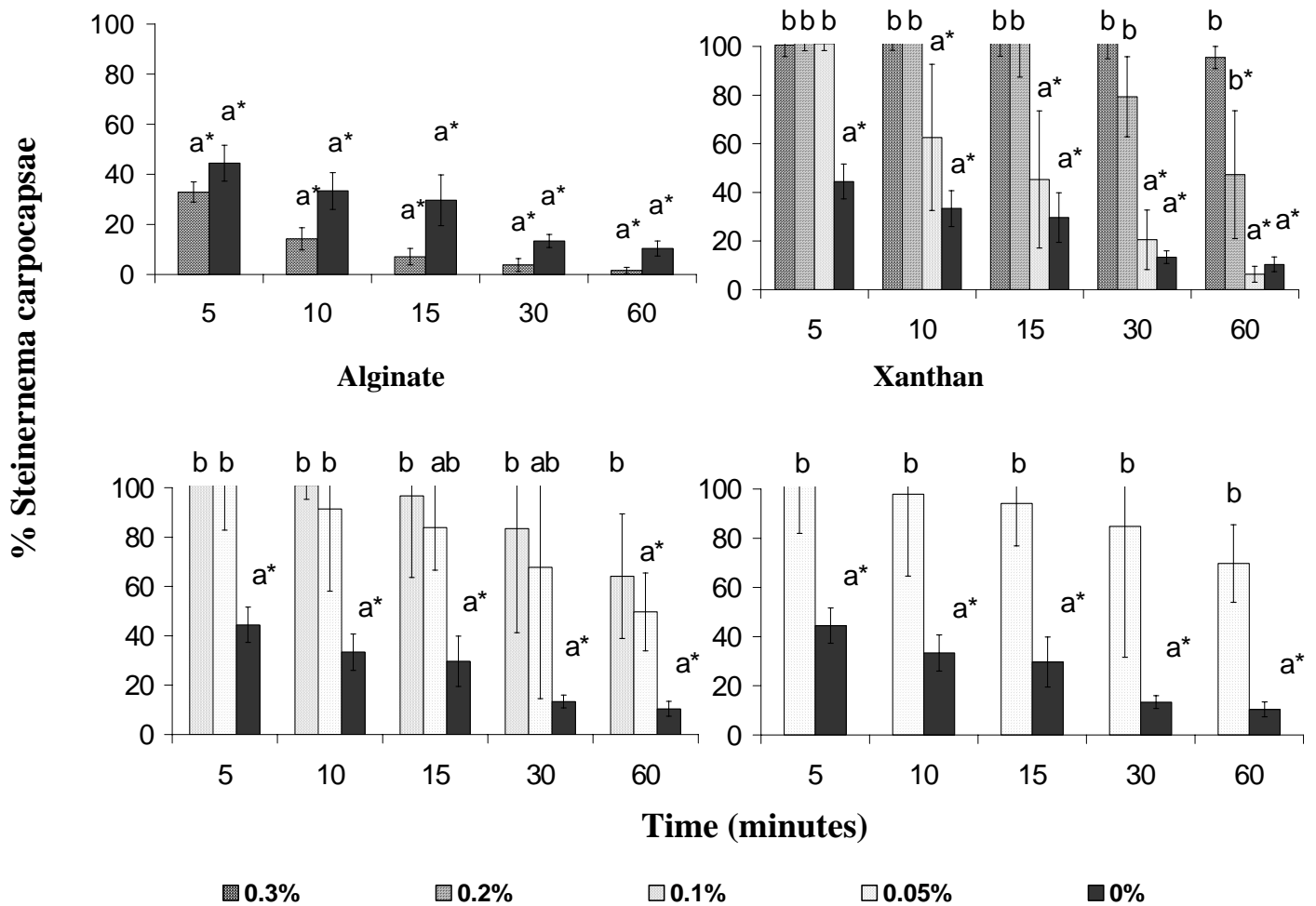
**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

1 TABLE 3. Viscosity of polymer solutions at different concentration and with the addition of the  
 2 surfactant Rimulgan<sup>®</sup> (0.3%), measured with a rotation viscometer at 20°C. Spindles  
 3 (L) and rotation (U) were adapted according to the pressure in the different solutions  
 4

|             | Viscosity (mPa) (spindle, rotation) |               |               |                      |
|-------------|-------------------------------------|---------------|---------------|----------------------|
|             | 0.1%                                | 0.2%          | 0.3%          | 0.3%<br>+ surfactant |
| Arabic gum  | 6 (L1, U200)                        | 6 (L1, U200)  | 6 (L1, U200)  | 6 (L1, U200)         |
| Guar gum    | 9 (L1, U200)                        | 22 (L1, U100) | 80 (L1, U30)  | 80 (L1, U30)         |
| Xanthan gum | 26 (L1, U100)                       | 380 (L1, U10) | 410 (L2, U20) | 450 (L2, U20)        |
| Alginate    | 6 (L1, U200)                        | 16 (L1, U200) | 450 (L2, U30) | 430 (L2, U30)        |

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**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**



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FIGURE 1. Sedimentation of *S. carpocapsae* in water and polymer solutions of different concentrations measured as mean percentage (n = 3) of DJs recovered in 2 cm depth of suspensions at different periods after ending of mixing the nematode suspension. Reference set to 100% was the number of nematodes recorded immediately after ending of mixing. Bars indicated with the same letter are not significantly different and those indicated with \* differ significantly from the reference number according to Tukey's HSD test at P < 0.05

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

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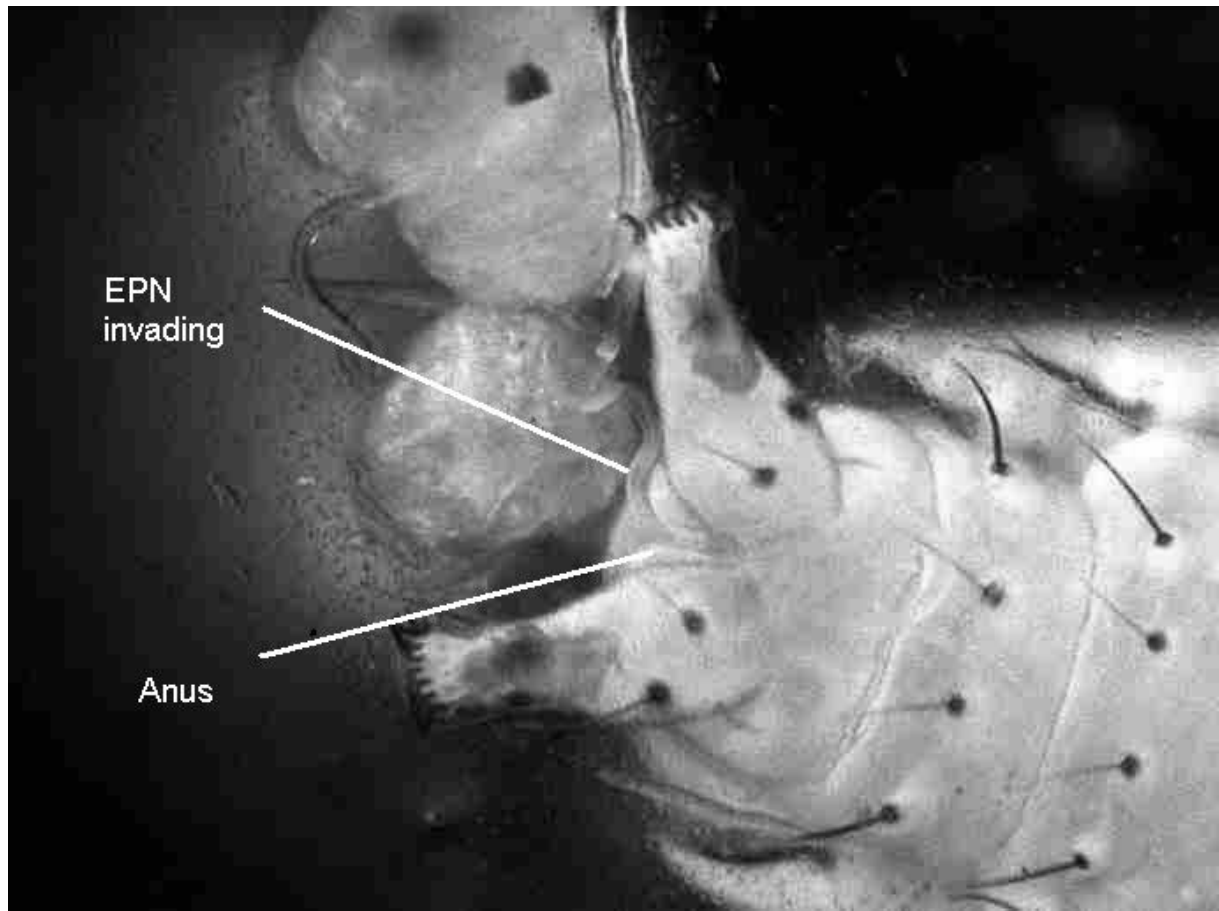


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FIGURE 2. *S. carpocapsae* dauer juvenile accumulation at diamondback moth faeces. Observations with an inverted microscope at 32-fold magnification

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

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5 **FIGURE 3.** *S. carpocapsae* invading 3<sup>rd</sup> instar *Plutella xylostella* via the anus treatment. Nematode  
6 application in surfactant-polymer-formulation. Observations with an inverted  
7 microscope at 32-fold magnification

8

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

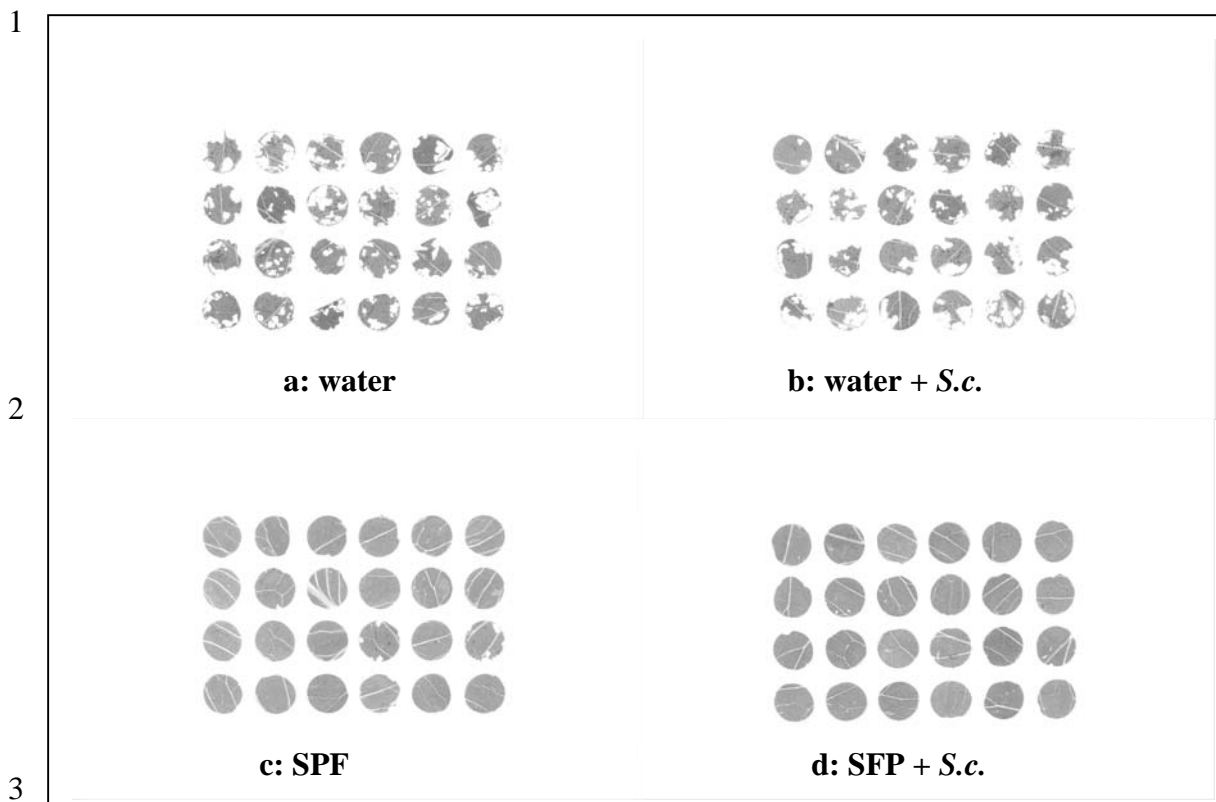
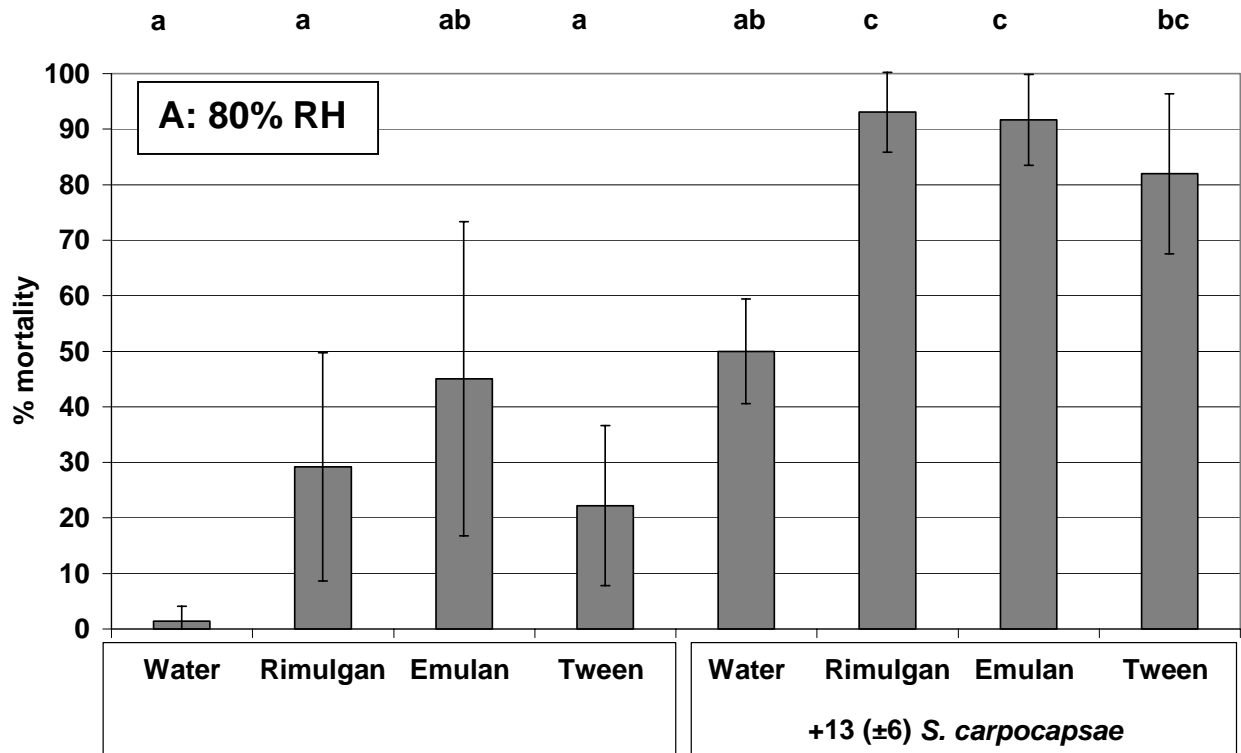


FIGURE 4. Defoliation of cabbage leaf discs by 3<sup>rd</sup> instar *Plutella xylostella* after 20 h at 80% RH and 25°C exposed to a: water; b: water with nematodes; c: surfactant-polymer-formulation (0.3% Rimulgan<sup>®</sup> and 0.3% xanthan or d: formulation with nematodes in 24-well dishes. *S. carpocapsae* was applied at 6 DJs/cm<sup>2</sup>

**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

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3 **FIGURE 5.** Selection of surfactant. Mean *Plutella xylostella* mortality after 48 h exposure to  
 4 cabbage leaf discs treated with water or Rimulgan<sup>®</sup>, Emulan ELP<sup>®</sup> or Tween 80 each  
 5 combined with 0.3% xanthan with or without *Steinernema carpocapsae* at 80% (A) or  
 6 60% (B) RH and 25°C. Bars indicated with the same letters are not significantly  
 7 different according to Tukey’s HSD test at P < 0.05 (arc sine transformed data)

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**Mode of action of a surfactant-polymer-formulation to support performance of the entomopathogenic nematode *Steinernema carpocapsae* for control of diamondback moth larvae (*Plutella xylostella*)**

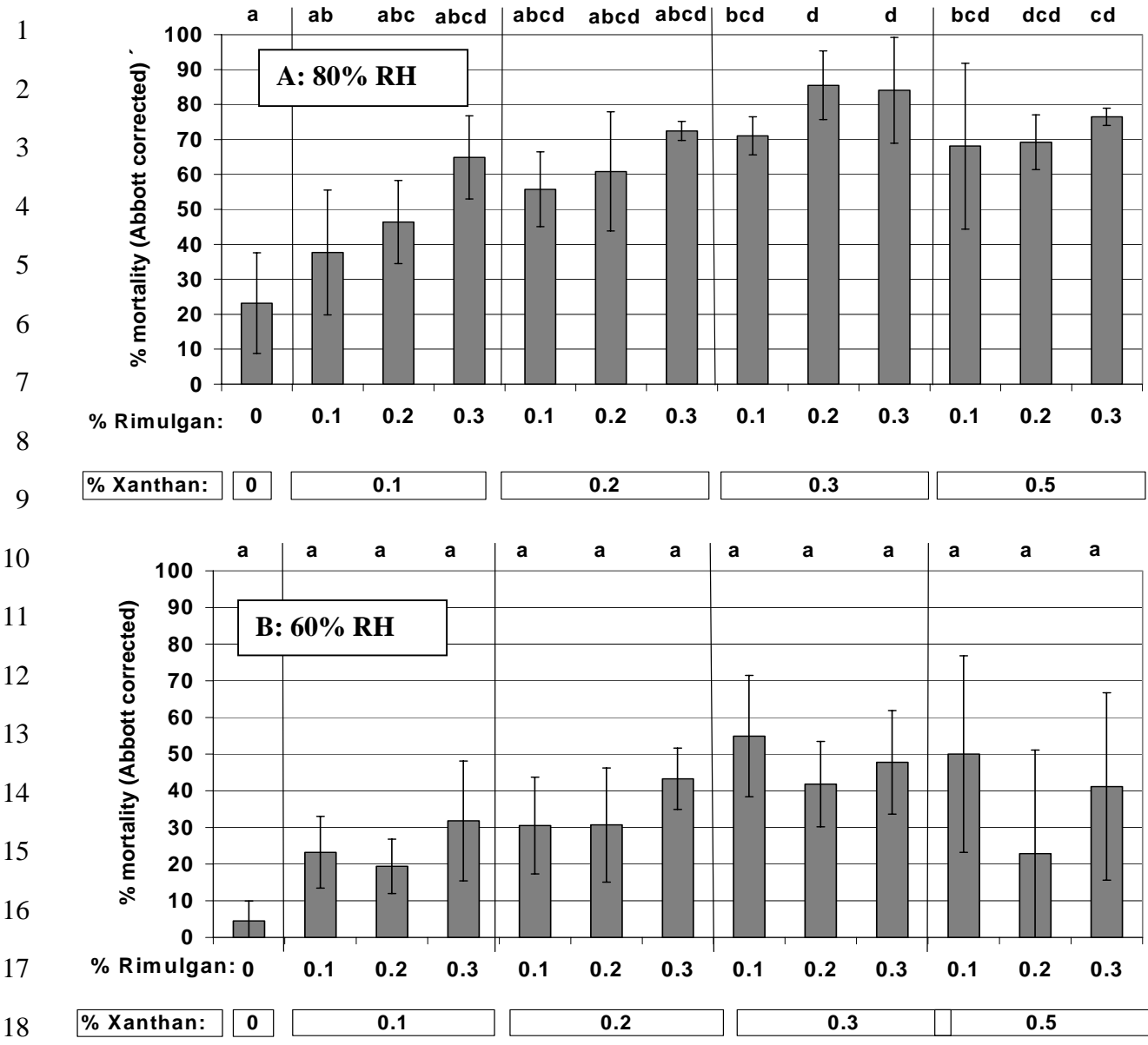


FIGURE 6. Improvement of surfactant (Rimulgan®) and polymer (xanthan) concentration. Abbot corrected mortality of *Plutella xylostella* after 48 h exposure to cabbage leaf discs treated with 13 (± 6) *Steinernema carpocapsae* in different adjuvants at 80% (A) and 60% (B) RH and 25°C. Bars indicated with the same letters are not significantly different according to Tukey’s HSD test at P < 0.05 (arc sine transformed data)



# Foliar application of the entomopathogenic nematode *Steinernema carpocapsae* for biological control of diamondback moth larvae (*Plutella xylostella*)

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## Abstract

For the use of the entomopathogenic nematode *Steinernema carpocapsae* on cabbage foliage to control larvae of the diamondback moth (DBM), *Plutella xylostella*, a formulation containing 0.3% of the surfactant Rimulgan and 0.3% of the polymer xanthan was tested in leaf bioassays and compared to nematodes applied in water. Compared to water, the surfactant–polymer formulation (SPF) significantly improved efficacy. Using 75 dauer juveniles (DJs) cm<sup>-2</sup> in SPF, 80% mortality was recorded, whereas at 60% RH insect mortality reached almost 60%. The survival time (LT<sub>50</sub>) for *S. carpocapsae* applied in water was 36 h at 80% RH and only 3 h at 60% RH. With SPF the LT<sub>50</sub> was prolonged to 58 h at 80% RH and >20 h at 60% RH. The addition of fumed silica, cross-linked sodium polyacrylate or alginate gel did not significantly improve DJ survival compared to SPF alone. Nematode caused mortality decreased when DBM larvae were added 9 h after DJ application. As 98% of the nematodes were still alive after 9 h, the nematodes must have lost efficacy. No significant increase in DBM mortality was recorded when insects were exposed to DJs for 1, 4 or 20 h. The results indicate that host penetration on the leaf occurs within the first hour after application. Thus, the major advantage using the formulation is not to enhance nematode survival but rather to provide optimal environmental conditions that support nematode invasion of the host on foliage.

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**Keywords:** Anti-desiccant; *Brassica oleracea*; Foliar application; *Plutella xylostella*; Polymer; Surfactant; Survival; Time of infection

## 1. Introduction

*Plutella xylostella* (L.), the diamondback moth (DBM), is the major pest of cabbage crops (Talekar and Shelton, 1993). The overuse of insecticides has caused development of resistance (Shelton et al., 1993; Tabashnik, 1994) particularly in sub-tropical and tropical countries, where farmers tend to grow cabbage continuously and apply mixtures of chemical insecticides sometimes more than twice a week (Wright, 2004). As a result, natural antagonists are sacrificed (Xu et al., 2004), and the

efficacy of the treatment is declining with considerable negative effect on the profit margin of growers. As an alternative biological control measure, *Bacillus thuringiensis* Berliner (Bt) is used (Cherry et al., 2004). Since Bt use was extended, resistant DBM populations were recorded first in 1990 from Malaysia and are now widespread in Asia and the Americas (Ferré and van Rie, 2002).

To avoid further resistance development against Bt and to exploit the control potential of natural enemies, other biocontrol agents are urgently needed to substitute broad-spectrum insecticides as well as Bt. Entomopathogenic nematodes can be one alternative to control DBM. Baur et al. (1995) used *Steinernema carpocapsae* Weiser

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in leaf disc assays and achieved >95% control. Mason and Wright (1997) screened indigenous nematode strains from Malaysia and identified some *Steinernema* spp. to be better adapted to extreme environmental conditions. Baur et al. (1997) tested different adjuvants to prolong survival of *S. carpocapsae* and increase DBM control in water cress *Nasurtium aquaticum* (L.). They concluded that although entomopathogenic nematode efficacy and persistence could be improved by the addition of adjuvants, the effects in the field were not significant and recommended further screening of adjuvants. Schroer et al. (2005a) screened several adjuvants for toxicity on nematodes, plants or insects and tested different combinations of surfactants and polymers for their potential to improve nematode efficacy. They recorded a twofold increase of DBM mortality at 80% relative humidity (RH) and fivefold increase at 60% RH in leaf disc assays when spraying nematodes in 0.3% xanthan. The addition of 0.3% surfactant (Rimulgan) further improved efficacy. The surfactant–polymer formulation (SPF) reduced the time to kill 50% (LT<sub>50</sub>) DBM from >40 to <25 h. Schroer et al. (2005b) recorded that the same formulation decreased mobility of DBM larvae and at the same time provided conditions, which enhanced nematode host seeking and invasion of the target insect. Visual observations indicated an active invasion of *S. carpocapsae* dauer juveniles (DJs) into DBM larvae mainly via the anus. A passive uptake by the insect during feeding was never observed. SPF caused >90% at 80% RH and >70% at 60% and reduced the LC<sub>50</sub> from 12 to 1 nematode/larva in leaf disc bioassays.

Previous studies (Schroer et al., 2005a,b) focused on the screening of adjuvants and investigations to understand the impact of adjuvants on sedimentation of DJs in the spraying suspension, the run-off from the leaf surface and on host invasion. All of these investigations were conducted in leaf disc assays, a rather artificial setup not reflecting conditions in the field. The purpose of this study was to investigate the influence of SPF on nematode survival and efficacy in leaf bioassays, which provide conditions more adapted to the natural environment of DBM. It was also investigated when infection occurred and whether the addition of anti-desiccants could further enhance efficacy.

## 2. Materials and methods

### 2.1. Insect, plant, and nematode rearing

Cabbage plants (*Brassica oleracea* (L.) convar. *capitata* Alef.) were grown on commercial peat substrate (Floragard, Oldenburg, Germany) in the greenhouse. *P. xylostella* were reared on savoy cabbage leaves (*B. oleracea* convar. *capitata* var. *sabauda* L.) at 20 °C and 80% RH. The Egyptian isolate (S2) of *S. carpocapsae* was reared in vivo according to Glazer and Lewis (2000).

### 2.2. Leaf bioassay

A single leaf bioassay was developed to investigate nematode efficacy and survival at 80 or 60% RH and 25 °C. Experimental chambers, 6 × 5 × 8 cm plastic cups, were filled with 1.1% (w/w) water agar solidified at an angle of 45° and sealed with parafilm (Fig. 1). Cabbage leaves were removed from the plants and sprayed with different formulations and DJ concentration on the top side from 55 cm distance with a Teejet (TP8003E) flat-fan nozzle at a flow rate of 0.96 L min<sup>-1</sup> at 20<sup>5</sup> Pa and 15 μl cm<sup>-2</sup>. Untreated controls were sprayed with water. Treated leaves were immediately transferred into the cups with their stalks passed through the parafilm into the agar to ensure water supply. An identical cup was used to close the chamber after ten third instars of the DBM had been added to each cup. For gas exchange most parts of the side walls of the cups were removed and substituted with gauze (Fig. 1).

### 2.3. Testing different DJ concentrations at variable humidity

*Steinernema carpocapsae* was sprayed at a concentration of 25, 50, 75 or 100 DJs cm<sup>-2</sup> in water or in SPF, containing 0.3% Rimulgan (Themmen GmbH, Hattersheim, Germany), a surfactant based on castor oil and 0.3% xanthan gum (UD Chemie, Wörrstadt, Germany). Each concentration was tested on three leaves. The experiments were conducted in growth chambers at 80% RH and at 60% RH. DBM larvae were added immedi-

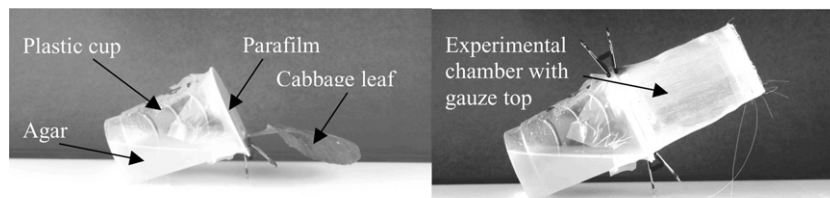


Fig. 1. Experimental cup to observe the performance of *Steinernema carpocapsae* on single cabbage leaves. Water supply for the leaf is provided by water agar in the bottom cup, which is divided from the experimental area with parafilm. Leaves are treated with one of the different nematode formulations and then the stalks are put through the parafilm into the water agar. After nematode application diamondback moth larvae were added and the top cup was covered with a 300 μm mesh to allow gas exchange.

ately after nematode application and mortality was recorded after 48 h. Experiments at 60% RH were repeated twice and at 80% RH were repeated three times.

#### 2.4. Testing anti-desiccants to prolong DJ survival

Cabbage leaves were treated with 50 DJscm<sup>-2</sup> in water, SPF or SPF with addition of one of the following anti-desiccants at 0.25%: cross-linked sodium polyacrylate (PA) (Stocksorb, Degussa, Krefeld, Germany), silica fumed (Sigma–Aldrich, St. Louis, USA), alginate gel (FMC Biopolymers, Brussels, Belgium). The alginate gel was prepared according to Navon et al. (2002) with 0.04% calcium carbonate (Merck, Darmstadt, Germany) and 0.125% D-gluconic acid lactone (Sigma–Aldrich). After incubation for 0, 3, 6, 9, 24 or 48 h, *S. carpocapsae* were washed off the leaves into counting chambers and nematode mortality in percent was recorded. DJs not moving when mechanically stimulated were recorded as dead. Tests were conducted at 80 and 60% RH and 25°C. The experiment was repeated twice.

#### 2.5. Persistence of DJ activity

To investigate the influence of nematode survival on the efficacy against DBM, 10 insect larvae were added to each cup immediately after treatment of the leaves or 9 h later. Spraying of the leaves and assay conditions were as described in 2.4. The mortality was recorded 48 h after addition of DBM. Insect larvae not moving when touched with tweezers were recorded dead. The experiment was repeated twice.

#### 2.6. Influence of exposure time on DBM mortality

To investigate how long the nematodes need to achieve DBM control, cabbage leaves were treated with 50 DJscm<sup>-2</sup> in water or in SPF and DBM larvae were removed from treated leaves 1, 4, and 20 h after addition of DBM larvae. Assay conditions were as described in Section 2.4. Removed DBM larvae were washed in tap water, dried on cellulose tissue, and transferred into the cover cup together with an untreated cabbage leaf. Mortality was recorded after 48 h exposure of DBM to the nematode treated leaves. The experiment was repeated twice.

#### 2.7. Data analysis

Percentage data were subjected to arcsine square root transformation and then analyzed using Tukey honest significant difference test (HSD) of ANOVA (CSS StatSoft, Tulsa, OK, USA). The significant levels were at 5%. The LT<sub>50</sub> of nematodes applied in different formulation was calculated using Probit analysis (Finney, 1971).

### 3. Results

#### 3.1. Nematode performance on the leaf

When applied in SPF and exposed to 80% RH, the mortality of DBM larvae increased with increasing number of *S. carpocapsae* up to a concentration of 75 DJscm<sup>-2</sup> (Fig. 2). DBM mortality was significantly lower when applied in water compared to SPF using  $\geq 75$  DJscm<sup>-2</sup> ( $F=26.06$ ,  $df=9$ , 108;  $p \leq 0.0004$ ). Efficacy at 60% RH in water was below 30% mortality with no concentration effect. Only a concentration of 100 DJscm<sup>-2</sup> controlled nearly 50% of DBM. The addition of SPF significantly increased efficacy at a concentration of 50 DJscm<sup>-2</sup> ( $F=11.65$ ,  $df=9$ , 67;  $p=0.01$ ). DBM mortality did not surpass 60% at any concentration.

#### 3.2. Effect of anti-desiccants

Formulated nematodes survived longer on plant foliage than nematodes applied in water at 80 and 60% RH (Fig. 3). However, the addition of anti-desiccants to SPF did not significantly increase nematode survival. Applied in water and exposed to 80% RH the LT<sub>50</sub> of *S. carpocapsae* was 36 h (95% fiducial limit: 31.9–41.5), whereas in SPF it was prolonged to 57 h (49.4–68.3).

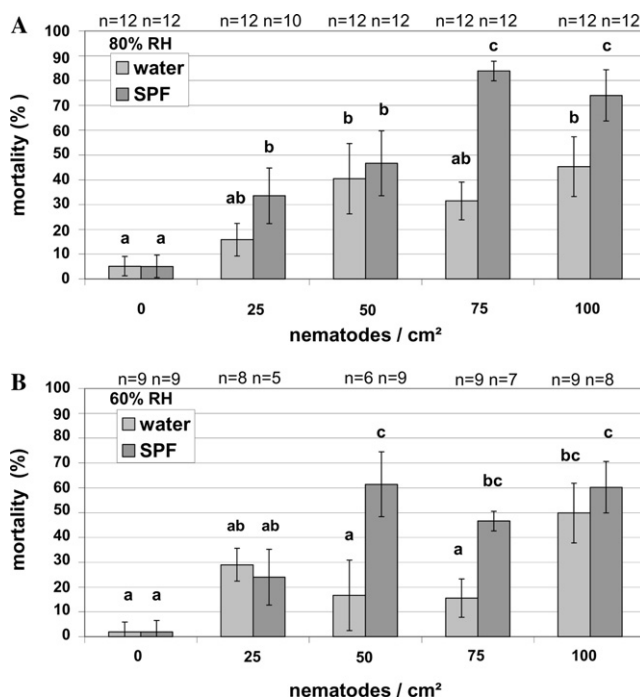


Fig. 2. Mortality of 3rd instar *Plutella xylostella* 48 h after application of different densities of *Steinernema carpocapsae* applied in water or SPF (0.3% Rimulgan and 0.3% xanthan) at (A) 80% RH or (B) 60% RH and 25°C. Means ( $n=9$ ) indicated with the same letter do not differ significantly according to Tukey HSD test at  $p < 0.05$  (arcsine transformed data).

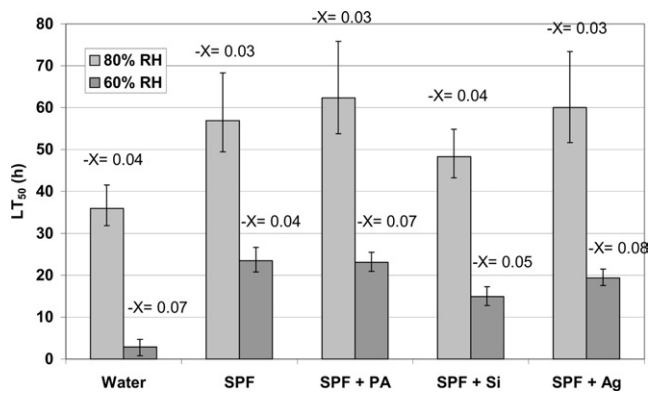


Fig. 3. Survival of *Steinernema carpocapsae* applied in water on cabbage leaf foliage, SPF, SPF plus 0.25% of the anti-desiccants cross-linked sodium acrylate (PA), fumed silica (SI) or potassium-alginate gel (AG). Bars indicate the  $LT_{50}$  ( $n=9$ ) of nematodes on single cabbage leaves at 80 or 60% RH and 25 °C. Values on top of the bars indicate the slope of the probit curves.

When applied in water and exposed to 60% RH nematode survival declined to 50% after 3 h. The  $LT_{50}$  in water was 2.9 h (0.9–4.7), but when applied with SPF, it increased to 23.5 h (20.8–26.7).

### 3.3. Persistence of DJ activity

When adding DBM immediately after DJ application, insect mortality was significantly increased using SPF compared to the untreated control ( $F=11.73$ ,  $df=5, 12$ ;  $p=0.02$ ), whereas no significant effect was recorded when DJs were applied in water ( $p=0.7$ ) (Table 1). The addition of anti-desiccants further increased DBM mortality significantly compared to water at 80% RH ( $p \leq 0.04$ ). DJs applied in water achieved 32% control at 80% RH, whereas the addition of adjuvants caused 67–92% mortality. At 60% RH DBM mortality was 15% when applied in water and 51–74% with the addition of adjuvants.

Table 1

Mortality (%) of 3rd instar *Plutella xylostella* after 48 h caused by *Steinernema carpocapsae* ( $50 \text{ cm}^{-2}$ ) applied in water, surfactant–polymer formulation (SPF) or SPF with the addition of 0.25% of the anti-desiccants fumed silica (SI), cross-linked sodium acrylate (PA) or potassium-alginate gel (AG)

| Treatment | Mortality and reduction of efficacy in 9 h (%) |               |      |                |                |      |
|-----------|--|---------------|------|----------------|----------------|------|
|           | 80% RH   |               |      | 60% RH         |                |      |
|           | 0  | 9             | Red  | 0              | 9              | Red  |
| Control   | 5.7 ± 9.5 a                                    | 7.0 ± 9.4 a   |      | 2.2 ± 3.8 a    | 1.1 ± 1.9 a    |      |
| Water     | 32.6 ± 2.3 ab                                  | 13.3 ± 3.3 a  | 59.1 | 15.8 ± 10.9 ab | 9.3 ± 3.5 a    | 41.2 |
| SPF       | 67.0 ± 20.2 bc                                 | 40.0 ± 25.2 a | 40.2 | 50.6 ± 9.6 bc  | 16.3 ± 10.3 ab | 67.8 |
| SPF + SI  | 81.1 ± 10.7 bc                                 | 41.4 ± 19.2 a | 48.9 | 53.9 ± 19.6 c  | 13.3 ± 8.8 ab  | 75.3 |
| SPF + PA  | 92.1 ± 8.4 c                                   | 50.1 ± 37.4 a | 45.6 | 67.0 ± 22.8 c  | 37.6 ± 15.7 b  | 43.8 |
| SPF + AG  | 82.0 ± 12.8 c                                  | 50.1 ± 10.4 a | 38.8 | 74.0 ± 10.3 c  | 39.0 ± 9.6 b   | 47.3 |

Insects were added 0 or 9 h after the nematode treatment. Red, reduction of efficacy (%) after 9 h exposure of leaves to 80 or 60% RH and 25 °C.

Means ( $n=9$ ) within a column followed by the same letter do not differ significantly according to Tukey HSD test at  $p < 0.05$  (arc sine transformed data).

Efficacy of DJs rapidly declined 9 h after DJ application on the foliage. This decline can be retarded by the addition of polymers. At 80% RH with DJs applied in water, nematode efficacy was reduced almost 60%. The addition of the SPF lowered the reduction to 40%. But no significant DBM control was recorded after 9 h with the addition of any of the anti-desiccants. At 60% RH applied in water the reduction of efficacy was only 41%. This effect can be expected, as DBM control was already low immediately after application. The efficacy of DJs in SPF was reduced by 68% in the period of 9 h. However, the reduction was only 44 or 47% of DJs applied with the addition of PA or alginate, respectively.

### 3.4. Influence of exposure time on DBM mortality

*Steinernema carpocapsae* applied in water achieved 40% DBM mortality. The use of SPF significantly increased mortality to 75% ( $F=20.16$ ,  $df=8, 72$ ;  $p \leq 0.03$ ) (Fig. 4). No significant differences were recorded when the larvae were exposed for 1, 4 or 20 h to the treated leaves (SPF:  $p > 0.08$ ; water:  $p > 0.75$ ). Accordingly, nematode infectivity had occurred within the first hour after application.

## 4. Discussion

Low humidity is a major limiting factor for nematode survival on foliage (Glazer, 2002). Using entomopathogenic nematodes against pest insects on the leaf requires the addition of adjuvants. Our results indicate that the function of the adjuvants is to support DJ performance on the leaf surface rather than enhance DJ survival. SPF can significantly prolong DJ survival (Fig. 3), but DBM mortality was not increased over time. After 9 h, the period used to investigate persistence (Table 1), 98% of the DJs were alive in SPF (calculated from probits for

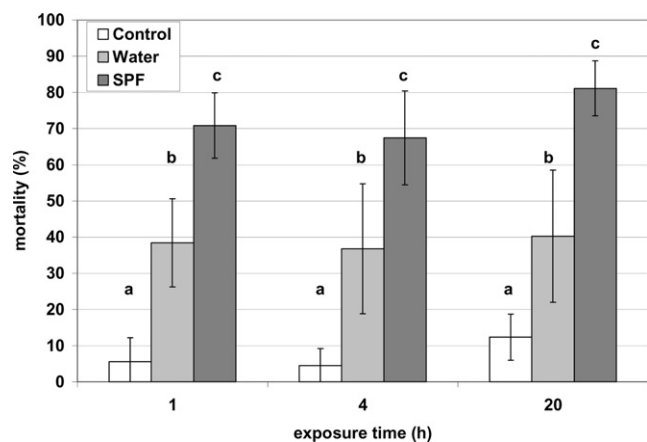


Fig. 4. Mortality of 3rd instar *Plutella xylostella* exposed to *Steinernema carpocapsae* ( $50 \text{ DJ cm}^{-2}$ ) for different time periods at 80% RH and 25°C. Nematodes were formulated in water or SPF (0.3% Rimulgan and 0.3% xanthan gum). Means ( $n=9$ ) indicated with the same letter do not differ significantly according to Tukey HSD test at  $p < 0.05$  (arcsine transformed data).

LT<sub>50</sub>). Should these nematodes all be active, DBM mortality should not be reduced when insects were added to the leaf 9 h after nematodes had been applied. However, we recorded a 40% reduction in mortality even at high RH of 80%. Similar results were obtained with the other adjuvants. From these findings we conclude that DJs either lost activity over time or that changing environmental conditions made it harder for nematodes to seek out and penetrate into host insects. Our conclusions are supported by the fact that DBM mortality did not increase over time. Nematodes invade the host within 1 h after application on the leaf as no increase in mortality was recorded after that time (Fig. 4). Our results indicate that on the leaf, nematodes are active shortly after application and any enhancement of survival is of minor importance for efficacy on the foliage. On the other hand, when applying the host after 9 h, many insects get killed, indicating that at least some nematodes must have regained their activity in the presence of a host. The use of SPF enabled nematodes to penetrate more quickly and thus escape from detrimental environmental conditions on the foliage. This effect correlated with the viscosity of the adjuvants (Schroer et al., 2005b). Consequently, the key to enhance nematode efficacy on the foliage to control DBM is to improve conditions to enable a rapid host invasion. Prolonging nematode survival is of less importance. Although the formulation provides better conditions for survival, this is not needed as the activity seems to be restricted to a few hours after application.

The results of our investigation confirm the potential of the SPF for foliar application in whole leaf assays and provide more realistic conditions to the natural environment than leaf discs (Fig. 2). Other than using leaf disc assays, results from whole leaf assays were too variable to enable the calculation of the LC<sub>50</sub> for DBM. Mortality recorded on leaves was also lower compared to leaf

disc assays (Schroer et al., 2005a). In disc assays insects have no chance to escape from nematode attack, whereas whole leaves sprayed only from one side provide more room for avoidance. Another reason might be that nematodes are not lost in leaf disc assays but have the chance to migrate from the leaves in the assay used for this study. Baur et al. (1998) assumed that low nematode efficacy on water cress was caused by DJs leaping off the leaves. This assumption is supported by the data obtained with nematodes sprayed in water to determine the LT<sub>50</sub>. Over time the total number of DJs on the leaves decreased (data not shown). This effect might as well have caused the lower efficacy at 60% RH. At 80% RH, DJs formulated in SPF have optimal conditions for survival and therefore they do not have to move to more favorable environments. At 60% RH, they possibly moved into the agar below the leaf.

DJ efficacy in SPF was consistently high at 80% RH in all tests, whereas at low humidity, efficacy never surpassed 60%. Another reason might be that at 60% RH, water is immediately evaporating from the film layer on the leaf, producing conditions that reduce nematode movement and host invasion. Glazer (1992) recorded a drastic reduction in nematode efficacy 6 h after application to foliage. He observed cessation in DJ movement 1 h after application to plant foliage, suggesting that further entomopathogenic nematode uptake happened by insects consuming the DJs. In our study, sufficient nematodes to kill the hosts invaded within 1 h after application on the leaf. No further increase in mortality was recorded after that time (Fig. 4).

The formulation reduces larval mobility and at the same time improves conditions for nematode invasion (Schroer et al., 2005b). Consequently, a high DBM control should be observed when nematodes in SPF are sprayed directly onto the insects as encountered under field conditions. In our investigation, insects were added after spraying thus enabling movement on the surface of the polymer layer.

Piggott et al. (2000) prolonged nematode survival using cross-linked polyacrylamide. In our study, we tested the benefit of anti-desiccants combined with SPF. Anti-desiccants are polymers with water swelling capacity and thus provide humidity for a longer period. Although all adjuvants significantly increased the efficacy against DBM on the leaf, the differences were not significant among the adjuvants. Considering that the addition of anti-desiccants will further increase costs of a nematode formulation and taking into account the relatively low additional effect, we cannot recommend the use of anti-desiccants.

Our experiments were conducted with DBM, a small lepidopteran. Conditions might differ when larger insects are controlled with nematodes. Navon et al. (2002) developed an edible alginate-nematode-gel formulation, which is deposited as droplets on the leaves for the

control of *Spodoptera littoralis* Boisduval and *Helicoverpa armigera* Hübner. These insects seem to feed on the formulation and get infected. In this case nematode survival is important as host invasion is occurring in the gut after DJs have been consumed. Schroer et al. (2005b) observed that nematode invasion is an active process with DBM and that this insect can detect nematodes in the diet and raise its head to prevent penetration. The invasion process of different nematode species into variable pest insects can differ (Grewal et al., 1993; Lewis et al., 1992). The nematode behavior towards the target pest must be the focus to overcome constraints of foliar application. Nematodes actively invading the host insect need support to enable their mobility; improvement of persistence can then be neglected. When a passive uptake is the main entrance into the host, prolonging survival is of priority. DJs can be entrapped in a formulation that prevents them from emigration and at the same time will preserve their energy resources (McGuire and Shasha, 1992; Navon et al., 2002).

Improved formulation technology enables the integration of entomopathogenic nematodes into a biological control strategy for the control of foliage-feeding insects. The feasibility of the SPF has been tested in leaf disc and complete leaf bioassays. Experiments are now needed to check whether the technology is feasible in the field to substitute chemical insecticides and retard the development of resistance also against Bt products.

## Acknowledgments

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**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
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4 **Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and**

5 ***Bacillus thuringiensis* in cabbage fields**

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**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
*Bacillus thuringiensis* in cabbage fields**

1 **Abstract:** Field trials evaluating the potential of the entomopathogenic nematode  
2 *Steinernema carpocapsae* and the feasibility to combine nematodes with *Bacillus*  
3 *thuringiensis* for sustainable control of the diamondback moth *Plutella xylostella* (DBM)  
4 were conducted in cabbage cultivated in the province Probolinggo, East Java, Indonesia. A  
5 single use of 0.5 million *Steinernema carpocapsae* m<sup>-2</sup> applied with a surfactant-polymer-  
6 formulation containing 0.3% xanthan and 0.3% Rimulgan<sup>®</sup> achieved a significant reduction of  
7 the insects per plant with > 50% control after 7 days. Even 14 days after the application about  
8 45% control was recorded and dead larvae containing nematodes were found. No significant  
9 effects were recorded when the formulation was compared to nematodes applied in water or  
10 with a surfactant alone. This was attributed to high humidity in the experimental area at the  
11 end of the rainy season and a microclimate in the cabbage heads favouring nematode survival.  
12 Weekly applications of *B. thuringiensis* (Turex<sup>®</sup>) or alternating applications of Turex<sup>®</sup> and  
13 the nematodes achieved > 80% control. The application of both biological agents together  
14 every second week reached insignificant lower efficacy (70%). Nematodes can be used to  
15 substitute ineffective chemical insecticides and alterations with *B. thuringiensis* can prevent  
16 the further development of resistance against the bacterial control agent.

17

18 Keywords: diamondback moth, entomopathogenic nematodes, foliar application, surfactant,  
19 integrated pest management, Indonesia

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**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
*Bacillus thuringiensis* in cabbage fields**

**1 Introduction**

2 *Plutella xylostella* (L.), the diamondback moth (DBM), is a major pest of crucifer crops  
3 recorded from almost all over the world. Promoted by the immense misuse of ineffective  
4 pesticides, DBM has a history of developing resistance against every chemical insecticide  
5 (TALEKAR and SHELTON, 1993). In the past, biological control strategies mainly focused on  
6 the introduction of endolarval parasitoids (VERKERK and WRIGHT, 1997; ZHAO et al, 2002;  
7 SAYYED et al., 2004). However, the potential of introduced invertebrate antagonists from  
8 Europe into Indonesia was limited due to the intensive use of broad-spectrum insecticides  
9 (WRIGHT, 2004; VERKERK and WRIGHT, 1997; SASTROSISWOJO, 1996). XU et al. (2004)  
10 investigated the detrimental effect of chemical insecticides on the DBM parasitoid *Diadegma*  
11 *insulare* (Cresson) and recommended the use of other biological agents *Bacillus thuringiensis*  
12 Berliner (*Bt*) to avoid reduction of antagonist invertebrates in cabbage fields. In some  
13 countries, the bacterial biological control agent Bt is applied against Lepidoptera. As Bt was  
14 the only available biological alternative for >20 years, many DBM field populations have now  
15 developed resistance against the insecticidal crystal toxin (TABASHNIK, 1994; FERRÉ and VAN  
16 RIE, 2002). To prevent a further development of resistance against Bt novel biological control  
17 strategy are urgently needed also to conserve the potential of invertebrate antagonists. Besides  
18 Bt, viruses and fungi, entomopathogenic nematodes (EPN) can be used to control DBM  
19 (BAUR et al., 1995; CHERRY et al., 2004). As EPN are soil inhabiting organisms, their  
20 potential on the foliage is limited by heat, desiccation and UV-radiation (GLAZER, 1992).  
21 RATNASINGHE and HAGUE (1994) compared different nematode species and recommended  
22 *Steinernema carpocapsae* Weiser for control of DBM larvae. This species is more tolerant to  
23 desiccation than others (PATEL et al., 1997) and provided effective control of DBM larvae in  
24 laboratory tests (MASON and WRIGHT, 1997). BAUR et al. (1998) improved EPN activity in

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1 the field by adding a wetting agent to prolong nematode survival on the leaf and a stilbene  
2 brightener to protect nematodes against UV-radiation. However, field efficacy still was  
3 insufficient to achieve adequate control. SCHROER et al. (2005a) screened several polymers  
4 and surfactants to improve EPN performance on the foliage and developed a formulation  
5 containing 0.3% xanthan and 0.3% of the surfactant Rimulgan<sup>®</sup>. Although the formulation  
6 was able to increase EPN survival on the leaf, SCHROER and EHLERS (2005) observed a host  
7 invasion within the first hour after application and concluded that the function of a foliar  
8 formulation for EPN is not to prolong survival of the nematodes but to provide adequate  
9 conditions for nematode host invasion on the leaf surface. SCHROER et al. (2005) b observed  
10 that host invasion is an active process via the anus. In the presence of EPN they observed that  
11 DBM larvae tried to escape invasion through the mouth. The formulation reduced insect  
12 mobility and at the same time significantly lowered the lethal EPN concentration and time  
13 necessary to kill DBM third instars. The promising laboratory results justified testing of the  
14 formulation in field experiments. Tests were conducted in cabbage fields in the province of  
15 Probolinggo, East Java, Indonesia, where farmers used chemical insecticides against DBM  
16 almost every two days without any economic benefit. Consequently, the other objective of the  
17 study was to test a biological control strategy combining EPN with Bt. BAUR et al. (1998)  
18 achieved 48% DBM control in a field trial on water cress combining EPN with Bt and  
19 recommended the search for novel formulations to increase control with EPN. Therefore, *S.*  
20 *carpocapsae* applied with an improved formulation (SCHROER et al. 2005 a, b; SCHROER and  
21 EHLERS, 2005) was tested in the field.

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**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
*Bacillus thuringiensis* in cabbage fields**

1   **2 Materials and Methods**

2   **2.1 Experimental conditions**

3   The province Probolinggo, East Java, Indonesia is a rural commune at the volcano Bromo and  
4   is a typical cabbage growing region with sandy silt soils and a high percentage of volcanic  
5   ashes. Cabbage is cultivated mainly on 0.5 to 1 ha fields at the slopes of the hills up to a  
6   height of 2,400 m above sea level. The trials were conducted in March 2004 at the end of the  
7   rainy season, the main cabbage production time in this area. Relative humidity and  
8   temperature measured every half hour using a hygrometer with thermo-sensor (Escort Data  
9   Login System LTD, IUL Instruments GmbH, Königswinter, Germany) was usually 90 - 100%  
10   RH and dropped to 75 or 50 % RH for no longer than 2 hours during the day. The temperature  
11   varied between 24°C during day time and 15°C during night time. Four weeks old cabbage  
12   seedlings (F1-hybrid *Brassica oleracea* var. *capitata* "Ecuador") were planted in 40 x 40 cm  
13   distance and fields were fertilized with chicken manure prior to planting. Cabbage heads are  
14   usually harvested 90 days after planting. In three different fields the development of the DBM  
15   population was frequently observed by sampling 5 untreated plants at random. These were  
16   transferred to the laboratory, defoliated and the total number of living larval stages per plant  
17   was counted.

18

19   **2.2 Biocontrol agents and adjuvants**

20   *S. carpocapsae* Weiser (All strain) produced in liquid culture was provided by the company e-  
21   nema GmbH (Raisdorf, Germany). Quality of EPN was tested prior to application in  
22   Indonesia according to GLAZER and LEWIS (2000) in 24-well plates (2 cm<sup>2</sup> per well). Filter  
23   paper was added to each well and one last instar *Tenebrio molitor* larva, reared at the  
24   University of Jember, was added. Five nematode dauer juveniles (DJs) were applied into each

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1 well and controls received water only. After 48 h incubation at 20°C the mortality of the  
2 larvae was recorded. In two independent tests the mortality reached >90 % indicating  
3 sufficient activity of the DJs.

4 Prior to application in the field tests, nematodes were mixed with the surfactant-  
5 polymer formulation (SPF). All components were used at 0.3 % (w/v). Galacto-mannane  
6 polysaccharid xanthan gum (40 mesh), a secondary metabolite of the bacterium *Xanthomonas*  
7 *campestris*, was purchased from UD Chemie GmbH (Wörrstadt, Germany) and mixed with  
8 sodium hydrogen carbonate and oxalic acid (98%) (Sigma-Aldrich, St. Louis, USA). The  
9 powder was then mixed with water. Afterwards the surfactant Rimulgan® (Themmen,  
10 Hattersheim, Germany), based on castor oil, was mixed into the water at 0.3 % (v/v). The  
11 different field plots were treated before sunset (4.30 p.m.) using a Swan® knapsack sprayer  
12 with 6 mm flat fan nozzle. The Bt product Turex® (Certis, Columbia, SC, USA) was  
13 purchased in Jember, Indonesia and was applied at 0.1g per plant water dispersible powder,  
14 38g delta-endotoxin kg<sup>-1</sup> Bt *aizawai* (GC91), 25,000 IU mg<sup>-1</sup> (*Trichoplusia ni*). Water without  
15 nematodes or Bt was used in controls. In all treatments 100 ml water per cabbage plant was  
16 used.

17

### 18 **2.3 Testing different DJ concentration**

19 Cabbage was planted on January 4, 2004 in field B. One pesticide application with fenvalerate  
20 (Sumicidin®, Syngenta, Basel, Switzerland) at the recommended dose had been applied at the  
21 beginning of February, farmers communication. Plots of fifty plants were marked in the field.  
22 On March plots were randomly selected and treated with *S. carpocapsae* in SPF at 0.25, 0.5  
23 or 1 million DJ m<sup>-2</sup> as described in 2.2. One control plot was sprayed with water. Before  
24 application and at 2, 3, 7 and 14 days after application 5 plants per plot were randomly

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1 selected and transferred to the laboratory where the cabbage plants were defoliated and the  
2 number of living DBM larvae per plant was recorded. Larvae not moving when mechanically  
3 stimulated were recorded as dead.

4

5 **2.4 Testing the formulation under field conditions**

6 Nematodes were applied at 0.5 million m<sup>-2</sup> in water, with the surfactant Rimulgan<sup>®</sup> or with  
7 SPF as described in 2.2. The SPF prevents nematodes from settling in the spraying tank  
8 (SCHROER et al. 2005b). To prevent sedimentation when EPN were sprayed in water, the  
9 knapsack sprayer was constantly shaken during application. The trials were conducted once at  
10 a field A with 31 day-old cabbage planted February 9, 2004. This field had not received any  
11 treatment before nematodes were applied on March 10, 2004. The other application was done  
12 on March 19, 2004 in field C with 79 day-old cabbage planted January 2, 2004. The latter  
13 field had been intensively treated with a variety of different chemical insecticides but had  
14 been abandoned as treatments were no longer successful, farmers communication. The  
15 experimental design was as described in 2.3. Evaluations were prior to application and on  
16 days 3, 7 and 10 after treatment.

17

18 **2.5 Testing the combined application of EPN and Bt**

19 Plots each containing 100 plants, were randomly selected in fields A (planted February 9,  
20 2004) and C (planted January 2, 2004). Treatments were weekly alternating beginning with  
21 80,000 *S. carpocapsae* per plant (corresponding to 0.5 million DJs m<sup>-2</sup>) as described in 2.2,  
22 followed by 0.1 g Turex<sup>®</sup> per plant (recommended dose). The other treatments received Bt in  
23 weekly intervals at the same concentration. Another treatment received the same amount of  
24 EPN and Bt together in one treatment every fortnight. Control trials were treated with water

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1 also at fortnight. The trial in field A was repeated once, the first sprayed on March 9, 2004  
2 with 30 days-old plants and the second on March 15, 2004 with 36 days-old plants.  
3 Treatments in field C were conducted with 66 days-old cabbage treated on March 6, 2004.  
4 Evaluations were done as described in 2.2 before the treatment and 3, 7, 10, 14 and 21 days  
5 after the first treatment.

6

## 7 **2.6 Statistical analysis**

8 To evaluate the effect of different EPN concentrations on the number of DBM larvae per  
9 plant, means of the treatments were compared with the control using the Mann-Whitney's  
10 test. Data recorded in the other trials were analysed using ANOVA with significant level at P  
11 < 5 % and analysed using Duncan's multiple range test. The impact of different nematode  
12 formulations on the number of DBM larvae per plant was evaluated with Kendall's  
13 correlation coefficient test at significant levels P < 5%. As the number of DBM per plant did  
14 not significantly differ between the plots before the treatments the control mortality was  
15 calculated using Abbott's formula (1925).

16

## 17 **3 Results**

### 18 **3.1 Testing different DJ concentration**

19 Before the treatment in field B, 8.2 ( $\pm$  2.2) DBM larvae per plant were counted in all plots  
20 (Figure 1). In the control plot the number of DBM larvae increased to 32.6 per plant, whereas  
21 the increase was lower in all nematode treated plots (Tab. 1). Two days after application  
22 hardly any effect was noticeable. Three days after the application of 0.5 or 1 million DJ m<sup>-2</sup>, a  
23 significant reduction of DBM larvae per plant was recorded (P = 0.03). Although no  
24 significant difference of the treatments to the control was detected 7 days after the application

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1 (P ≥ 0.06), a significant reduction to 18.4 per plant (P = 0.03) was again recorded on plants  
2 treated with 0.5 million 14 days after application (Tab. 1). The efficacy for 0.5 million *S.*  
3 *carpocapsae* increased from 10% two days after application to 50% seven days after the  
4 treatment and even after 14 days 45% control was recorded. Dissection of dead larvae  
5 indicated nematode infection even 14 days after the treatment. As significant effects were  
6 recorded with 0.5 million DJ m<sup>-2</sup> the field experiments were continued with this DJ  
7 concentration.

8

9 **3.2 Testing the formulation under field conditions**

10 Results from field trials evaluating nematodes sprayed in water, surfactant or SPF are  
11 presented in Tab. 2 for plants treated at an age of 30 days (field A) and in Table 3 for plants  
12 treated at an age of 79 days (field C). Before application 3 (± 1.4) DBM per plant were  
13 counted in all plots. In control plots the mean number of DBM increased significantly to 23.6  
14 at 10 days after application (P = 0.00 ). In the control and treatments with EPN in water or  
15 surfactant, the increase of DBM larvae correlated significantly with the time (P = 0.03, 0.02,  
16 respectively), whereas in treatments with nematodes in SPF no significant correlation was  
17 recorded (P = 0.06). A significant reduction of the number of DBM in all nematode  
18 treatments compared with the control was recorded 7 days after the application (P < 0.21).  
19 The effect lasted until 14 days after application only in treatments with SPF (Tab. 2), but did  
20 not significantly differ from treatments with water or surfactant (P > 0.7). The mortality  
21 reached a maximum of 72.4% in treatments with SPF 10 days after the treatment.

22 On 79 days-old plants (field C) 40.6 (± 8.4) DBM were recorded per plant before the  
23 treatment (Fig. 1C). The number increased to 97 (± 45.5) three days later and decreased again  
24 afterwards. Significant correlations between the number of DBM larvae and the time after



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1 application were not recorded, neither in control plots nor at any nematode treated plots ( $P \geq$   
2 0.3). Treatments with nematodes applied in surfactants and SPF achieved significant DBM  
3 mortality compared to the control, reaching effects of 46.2 ( $P = 0.04$ ) and 63.9% ( $P = 0.008$ ),  
4 respectively, only the treatment using nematodes in water was not significant compared to the  
5 control ( $P > 0.12$ ).

6

7 **3.3 Testing the combined application of EPN and Bt**

8 The results obtained in plots in field A with 30-days old cabbage plants are presented in Tab.  
9 4. On control plants the mean number of DBM per plant increased from 4.2 ( $\pm 1.2$ ) 30 days  
10 after planting to 38.4 ( $\pm 10.2$ ) at 47 days-old plants (Figure 1A). On all treated plots <12  
11 DBM per plant were recorded with one exception at 7 days after the second Bt treatment. In  
12 plots which had received Bt every week the results were quite variable with no significant  
13 control on days 3, 10 and 14 after the first application. On the other hand, compared to all  
14 other treatments, the highest control of 84.2% was recorded in this treatment 21 days after the  
15 first application. At this time all treatments achieved significant control ( $P < 0.0001$ ). In the  
16 plot receiving alternating applications with EPN and Bt the control was 28.9% three days  
17 after the first nematode application and it reached 68.4% on day 21 after this application. On  
18 plants treated with EPN and Bt together a significant effect was recorded 3 days after  
19 application reaching 47.4% control ( $P = 0.48$ ). It dropped in the following weeks when the  
20 application was suspended and increased after the second treatment, reaching 75.3% on day  
21 21 after the first application.

22 Treatments in the same field (A), which started 6 days later, reached an even higher  
23 mortality of DBM with a maximum of 91.7% in the plot with alternating treatments of EPN  
24 and Bt, followed by 80.7 in the plot treated with Bt only. The number of DBM per plant

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1 recorded in the control differed significantly to all treated plots at any time of the evaluation  
2 ( $P < 0.002$ ), except in plots treated with EPN and Bt on day 7 after the first application ( $P >$   
3  $0.3$ ). The effects between the different treatments did not significantly differ from each other.

4 Table 6 presents the results obtained in field C with 66 day-old cabbage plants. The  
5 number of DBM increased within 3 weeks from  $19.6 (\pm 14.0)$  on 66 days-old plants to  $130.6$   
6  $(\pm 87.8)$  on 87 days-old plants (Figure 1C). In general, the results confirm what was recorded  
7 in the second trial in field A. The treatments with Bt alone and with alternating treatments of  
8 EPN and Bt reached the highest mortality with 84.6 and 82.2 % control 21 days after the first  
9 application, respectively. Again, no significant differences were recorded between the  
10 different treatments.

11

#### 12 **4. Discussion**

13 The diamondback moth is the major pest in cabbage plants in Indonesia. The results of this  
14 study indicate that EPN are a valuable alternative control measure to manage DBM larval  
15 populations. With a single treatment of 0.5 million per  $m^2$  up to 72.4 % mortality was reached  
16 (Tab.2). The variability of DBM mortality recorded at the different days after treatment is  
17 considerably high, which was also caused by the constant oviposition of DBM. Although  
18 nematode-containing cadavers of DBM were found even 14 days after application this  
19 observation was exceptional. In general, persistence of EPN on the canopy is short and thus  
20 the effect of constant oviposition is even more pronounced.

21 EPNs have several advantages over non-effective chemical insecticides. They have  
22 almost no effect on other invertebrate biological control agents (EHLERS and HOKKANEN,  
23 1996) which can thus contribute significantly to the management of DBM populations. Using  
24 biological control agents the number of sprays can be reduced from 3-4 per week to a single

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1 treatment once a week. The integration of EPN into a biological control strategy in  
2 combination with Bt reached an even higher mortality of 91.7%. Thus this control strategy is  
3 a feasible approach to produce cabbage at reasonable costs, reduce residues of chemical  
4 insecticides and prevent the negative impact of ineffective chemicals on the environment.  
5 Highly promising results were obtained from trials using nematodes against other cabbage  
6 pests like *Crociodolomia binotalis* Zeller (NURDIANA and SULISTYANTO, 1999).

7       Previous studies have evaluated the feasibility of using improved formulations to  
8 enhance the effect of EPN against DBM in laboratory assays (SCHROER et al. a, in press;  
9 SCHROER et al. 2005b; SCHROER and EHLERS, 2005). In this study we were able to show that  
10 EPN can significantly reduce DBM larvae on cabbage (Tab. 2 and 3), but no significant  
11 differences were recorded in the number of DBM between the treatments water, surfactant or  
12 SPF. However, in the plots treated with SPF no significant increase in DBM density was  
13 recorded over time, whereas in the other treatments significant increases were recorded. In the  
14 laboratory the SPF was always superior to EPNs applied in water or surfactants (SCHROER et  
15 al. 2005a) at relative humidity of 60 and 80%. The reason why these results could not be  
16 repeated under field conditions can be attributed to the climatic conditions (> 90% RH)  
17 recorded during the end of the rainy season, which provides good conditions for nematode  
18 survival on the canopy. Nematode application at sunset further prevent nematodes from  
19 desiccation as humidity rises during the night and nematodes are also protected from UV-  
20 radiation for at least 9 h in tropical regions. In general, conditions in cabbage heads seem to  
21 be favourable for EPN as we observed nematode-infected cadavers even 10 days after EPN  
22 application. When the RH reached lower levels these were recorded only for very few hours.  
23 Considering the microclimate within the cabbage heads, one can assume that sufficient  
24 humidity is provided other than for instance on the leaves of young cabbage plants or of

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1 cruciferous leaf vegetable, like Pak Choi (*Brassica chinensis* L.), which have an open leaf  
2 architecture. Consequently, in our study the formulation was not tested under extreme  
3 environmental conditions, which would probably result in more pronounced differences.  
4 Future investigations should therefore test the SPF under more stressful conditions for the  
5 nematodes than encountered at the end of the rainy season at the Bromo mountain. SCHROER  
6 et al. 2005b report of other major advantages of the SPF. It significantly retarded  
7 sedimentation in knapsack spray tanks, thus providing improved distribution of EPN in the  
8 field and it increased significantly the deposit of EPN on the leaf. Applied in water, > 70% of  
9 the DJs were lost due to increasing run-off from the canopy. These advantages alone already  
10 let us recommend the use of the SPF also on cabbage.

11         Highly promising results were obtained from trials combining Bt with EPN. Further  
12 studies should test the possibility that alternating applications of Bt and EPN can fully  
13 substitute ineffective chemicals, which pollute the environment and can be dangerous for  
14 farmers and consumers. In the region insecticides are often applied 3-4 times a week and still  
15 cabbage heads very often only reach the size of an apple. Consequently the chemical  
16 compounds cannot provide any commercial benefit to the farmers anymore due to severe  
17 resistance development.

18         Today DBM populations have developed resistance against every insecticide,  
19 including Bt, abamectin, fipronil and Spinosad (IQBAL et al., 1996; ZHAO et al., 2002; SAYYED  
20 and WRIGHT, 2004). Resistance development under field conditions against a new insecticide  
21 is estimated by WRIGHT (2004) with 2 years if cross-resistance is not present. In our study the  
22 effect of weekly rotation of EPN and Bt achieved the same effect as Bt applications alone  
23 indicating that EPN are an appropriate complementation in an integrated biological system

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1 and can thus be recommended for resistance management as proposed by FERRÉ and VAN RIE  
2 (2002).

3         Although the biological agents might be more expensive than chemical insecticides  
4 they only need to be applied every week. Consequently, the cost will be almost comparable.  
5 In this study we could not investigate the effect of the biological control strategy on the  
6 parasitoid antagonists of DBM. However, it can be assumed that their impact will increase as  
7 chemicals are substituted. Investigations by TALEKAR and SHELTON (1993) reported of up to  
8 94 % parasitizing of DBM. If the control potential of invertebrate antagonists will add to the  
9 effect of the specific control agents Bt and nematodes, the spraying intervals may possibly be  
10 enlarged further and the biological strategy will be even more cost effective.

11

12 **Acknowledgments**

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14 control of diamondback moth (*Plutella xylostella*) by conservation of natural enemies and  
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**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
*Bacillus thuringiensis* in cabbage fields**

2

3 *Table 1.* Number of *Plutella xylostella* larvae per plant ( $\Sigma$ ) and corrected mortality (%) at different time after a treatment with *Steinernema*  
4 *carpocapsae*. Nematodes were applied in different concentration on cabbage 60 days after planting. Plants had been treated with one  
5 Sumicidin<sup>®</sup> application 28 days after planting (farmers communication).  
6

| Nematode dose in<br>million m <sup>-2</sup> | <i>Plutella xylostella</i> larvae per plant ( $\Sigma$ ) and control (%) |                  |      |                  |      |                  |      |                  |      |     |
|---|--|------------------|------|------------------|------|------------------|------|------------------|------|-----|
|   | Days after application   |                  |      |                  |      |                  |      |                  |      |     |
|   | 0  |                  | 2    |                  | 3    |                  | 7    |                  | 14   |     |
|   | $\Sigma(\pm$ SD)   | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  | (%) |
| Control                                     | 12.8 (3.9)   | 12.6 (6.0)       |      | 28.4 (10.2)      |      | 25.8 (11.6)      |      | 32.6 (10.8)      |      |     |
| 0.25  | 8.8 (2.0)  | 11.4 (3.7)       | 9.5  | 22.6 (13.8)      | 20.4 | 17.4 (12.6)      | 32.5 | 21.4 (4.4)       | 34.3 |     |
| 0.5   | 13.2 (9.6)   | 11.2 (5.4)       | 11.1 | 15.6 (4.8)*      | 45.1 | 12.8 (14.0)      | 50.4 | 18.4 (5.8)*      | 43.5 |     |
| 1   | 10 (1.2)   | 11.4 (4.3)       | 9.5  | 13 (6.2)*        | 54.2 | 15.2 (3.7)       | 41.1 | 18 (2.8)         | 44.8 |     |

7 Means (n=5) indicated with \* differ from the control according to Mann-Whitney's range test at P < 0.05.  
8

**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
*Bacillus thuringiensis* in cabbage fields**

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5 *Table 2.* Number of *Plutella xylostella* larvae per plant ( $\Sigma$ ) and corrected mortality (%) at different time after a treatment with *Steinernema*  
6 *carpocapsae*. Nematodes (0.5 million m<sup>-2</sup>) were applied in water, with the surfactant Rimulgan<sup>®</sup> or with the surfactant-polymer-  
7 formulation (SPF) on cabbage plants 30 days after planting. Plants had not been treated with any insecticides before the experiment.

| Nematode<br>formulation | <i>Plutella xylostella</i> larvae per plant ( $\Sigma$ ) and control (%) |                  |      |                  |      |                  |      |
|-------------------------|--|------------------|------|------------------|------|------------------|------|
|                         | Days after application   |                  |      |                  |      |                  |      |
|                         | 0  | 3                | 7    | 10               |      |                  |      |
|                         | $\Sigma(\pm$ SD)   | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  |
| Control                 | 5.7 (2.7)a   | 5.2 (2.2)a       |      | 17.8 (6.3)a      |      | 23.6 (11.8)a     |      |
| Water                   | 5.5 (3.7)a   | 6.4 (3.4)a       | 0    | 9.2 (5.6)b       | 48.3 | 15.2 (4.5)ab     | 34.5 |
| Surfactant              | 5.5 (2.1)a   | 6.4 (4.8)a       | 0    | 8.4 (5.4)b       | 51.7 | 7.8 (6.1)ab      | 40.5 |
| SPF                     | 3.2 (2.1)a   | 3.2 (1.3)a       | 38.5 | 7.8 (3.4)b       | 56.2 | 6.4 (1.1)b       | 72.4 |

8 Means (n=5) indicated with the same letter within the same column do not differ according to Duncan's multiple range test at P < 0.05.

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**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
*Bacillus thuringiensis* in cabbage fields**

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3 *Table 3.* Number of *Plutella xylostella* larvae per plant ( $\Sigma$ ) and corrected mortality (%) at different time after a treatment of 0.5 million  
4 *Steinernema carpocapsae* m<sup>-2</sup> applied in water, with the surfactant Rimulgan® or with the surfactant-polymer-formulation (SPF) on  
5 cabbage plants 79 days after planting. Until 33 days before the experiment, plants had been treated with different insecticides, farmers  
6 communication).

| Nematode<br>formulation | <i>Plutella xylostella</i> larvae per plant ( $\Sigma$ ) and control (%) |                  |      |                  |      |                  |      |
|-------------------------|--|------------------|------|------------------|------|------------------|------|
|                         | Days after application   |                  |      |                  |      |                  |      |
|                         | 3  |                  | 7    |                  | 10   |                  |      |
|                         | $\Sigma(\pm$ SD)   | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  |
| Control                 | 41.2 (29.8)a   | 97 (45.5)a       |      | 66.8 (20.2)a     |      | 63.4 (28.5)a     |      |
| Water                   | 43.2 (27.8)a   | 69 (14.9)a       | 28.9 | 79.6 (47.4)a     | 0    | 53.8 (9.5)a      | 15.1 |
| Surfactant              | 56 (48.0)a   | 52.2 (32.9)b     | 46.2 | 36.2 (21.7)a     | 45.8 | 41.2 (11.7)a     | 35   |
| SPF                     | 38.2 (15.2)a   | 35 (9.7)b        | 63.9 | 53.2 (22.6)a     | 20.4 | 40.8 (12.8)a     | 35.6 |

7 Means (n=5) indicated with the same letter within the same column do not differ according to Duncan's multiple range test at P < 0.05.

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**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
*Bacillus thuringiensis* in cabbage fields**

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3 *Table 4.* Number of *Plutella xylostella* larvae per plant ( $\Sigma$ ) and Abbott corrected mortality (%) at different time after treatment with *Bacillus thuringiensis*  
4 alone or in combination with *Steinernema carpocapsae* (in surfactant-polymer-formulation). Thirty days-old plants received a weekly treatment  
5 of 0.625 g m<sup>-2</sup> of Turex<sup>®</sup> (BT), alternating treatments with 0.5 million *Steinernema carpocapsae* m<sup>-2</sup> followed by 0.625 g m<sup>-2</sup> Turex<sup>®</sup> (EPN / BT)  
6 or one treatment with the same amount of Turex<sup>®</sup> and nematodes (EPN + BT) every fortnight. Number of larvae was recorded selecting 5 plants  
7 at random per plot. Plants had been treated with any insecticides before the experiment.

| Treatment | <i>Plutella xylostella</i> larvae per plant ( $\Sigma$ ) and control (%) |                  |      |                  |      |                  |      |                  |      |                  |      |
|-----------|--|------------------|------|------------------|------|------------------|------|------------------|------|------------------|------|
|           | Days after the first treatment   |                  |      |                  |      |                  |      |                  |      |                  |      |
|           | 0  |                  | 3    |                  | 7    |                  | 10   |                  | 14   |                  | 17   |
|           | $\Sigma(\pm$ SD)   | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  | $\Sigma(\pm$ SD) | (%)  |
| Control   | 5 (2.2)a   | 7.8 (4.1)a       |      | 11.0 (2.5)a      |      | 13.4 (9.6)a      |      | 17.4 (4.0)a      |      | 38.4 (10.2)a     |      |
| BT        | 6.5 (3.4)a   | 4.2 (2.0)ab      | 44.7 | 4.8 (1.9)b       | 66.7 | 5.4 (2.7)a       | 59.7 | 19.0 (10.2)a     | 0    | 6.0 (3.6)b       | 84.2 |
| EPN / Bt  | 9.2 (6.3)a   | 5.4 (1.8)ab      | 28.9 | 5.2 (2.4)b       | 48.1 | 6 (3.2)a         | 55.2 | 8.4 (2.7)b       | 51.7 | 12.2 (6.9)b      | 68.4 |
| BT+EPN    | 4.5 (2.1)a   | 4 (1.5)b         | 47.4 | 8.4 (2.5)a       | 14.8 | 7 (4.3)a         | 47.8 | 11.2 (5.4)ab     | 35.6 | 9.4 (3.4)b       | 75.3 |

8 Means (n=5) indicated with the same letter within the same column do not differ according to Duncan's multiple range test at P < 0.05.

**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
*Bacillus thuringiensis* in cabbage fields**

2

3 *Table 5.* Number of *Plutella xylostella* larvae per plant ( $\Sigma$ ) and Abbott corrected mortality (%) at different time after treatment with *Bacillus thuringiensis*  
4 alone or in combination with *Steinernema carpocapsae* (in surfactant-polymer-formulation). Thirty-six days-old plants received a weekly  
5 treatment of 0.625 g m<sup>-2</sup> of Turex<sup>®</sup> (BT), alternating treatments with 0.5 million *Steinernema carpocapsae* m<sup>-2</sup> followed by 0.625 g m<sup>-2</sup> Turex<sup>®</sup>  
6 (EPN / BT) or one treatment with the same amount of Turex<sup>®</sup> and nematodes (EPN + BT) every fortnight. Number of larvae was recorded  
7 selecting 5 plants at random per plot. Plants had been treated with any insecticides before the experiment.

| Treatment | <i>Plutella xylostella</i> larvae per plant ( $\Sigma$ ) and control (%) |                  |      |                  |      |                  |      |                  |      |  |
|-----------|--|------------------|------|------------------|------|------------------|------|------------------|------|--|
|           | Days after the first treatment   |                  |      |                  |      |                  |      |                  |      |  |
|           | 0  |                  | 3    |                  | 7    |                  | 10   |                  | 14   |  |
|           | $\Sigma(\pm SD)$   | $\Sigma(\pm SD)$ | (%)  | $\Sigma(\pm SD)$ | (%)  | $\Sigma(\pm SD)$ | (%)  | $\Sigma(\pm SD)$ | (%)  |  |
| Control   | 11.2 (4.8)a  | 24.6 (6.8)a      |      | 19.6 (8.0)a      |      | 23.2 (9.0)a      |      | 29.6 (11.2)a     |      |  |
| BT        | 10 (3.2)a  | 7.6 (2.2)b       | 68.8 | 10.0 (4.8)b      | 47.4 | 14.4 (6.6)b      | 37.4 | 5.6 (3.2)b       | 80.7 |  |
| EPN / Bt  | 11.7 (5.4)a  | 9.4 (5.0)b       | 61.5 | 14.6 (6.7)ab     | 23.2 | 13.2 (3.4)b      | 45.2 | 2.6 (1.5)b       | 91.7 |  |
| BT+EPN    | 12.7 (6.4)a  | 6.6 (2.1)b       | 72.9 | 8.4 (2.3)b       | 55.8 | 12.6 (4.1)b      | 45.2 | 7.4 (7.7)b       | 74.5 |  |

8 Means (n=5) indicated with the same letter within the same column do not differ according to Duncan's multiple range test at P < 0.05.

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**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and  
*Bacillus thuringiensis* in cabbage fields**

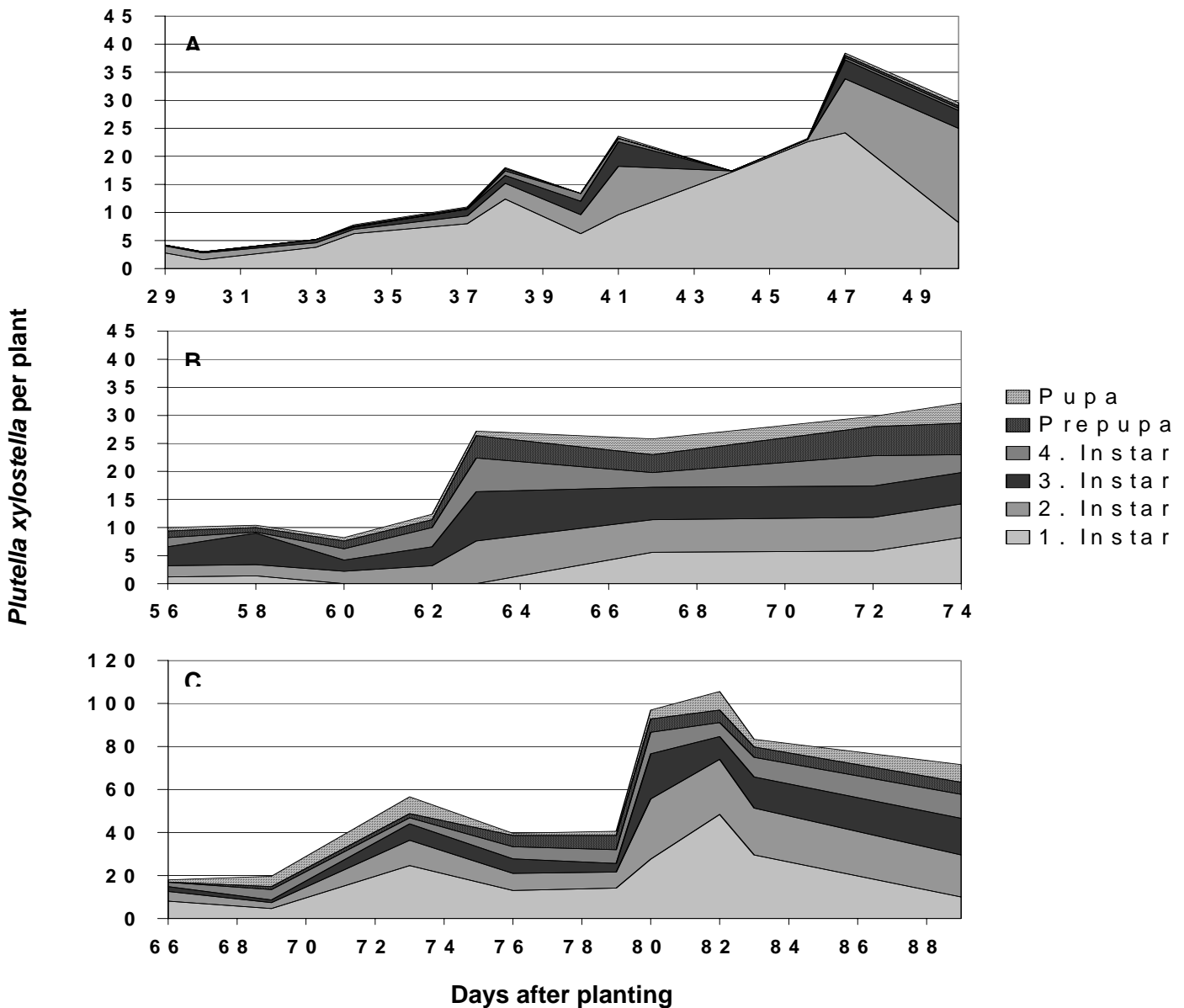
2

3 *Table 6.* Number of *Plutella xylostella* larvae per plant ( $\Sigma$ ) and Abbott corrected mortality (%) at different time after treatment with *Bacillus thuringiensis*  
4 alone or in combination with *Steinernema carpocapsae* (in surfactant-polymer-formulation). Sixty-six days-old plants received a weekly  
5 treatment of 0.625 g m<sup>-2</sup> of Turex<sup>®</sup> (BT), alternating treatments with 0.5 million *Steinernema carpocapsae* m<sup>-2</sup> followed by 0.625 g m<sup>-2</sup> Turex<sup>®</sup>  
6 (EPN / BT) or one treatment with the same amount of Turex<sup>®</sup> and nematodes (EPN + BT) every fortnight. Number of larvae was recorded  
7 selecting 5 plants at random per plot. Plants had been treated with any insecticides before the experiment.

| <i>Plutella xylostella</i> larvae per plant ( $\Sigma$ ) and control (%) |                                |              |     |                  |             |     |                  |             |     |                  |             |     |                  |             |               |      |
|--|--------------------------------|--------------|-----|------------------|-------------|-----|------------------|-------------|-----|------------------|-------------|-----|------------------|-------------|---------------|------|
| Treatment  | Days after the first treatment |              |     |                  |             |     |                  |             |     |                  |             |     |                  |             |               |      |
|  | 0                              |              | 3   |                  | 7           |     | 10               |             | 14  |                  | 17          |     | 21               |             |               |      |
|  | $\Sigma(\pm$ SD)               | ( $\pm$ SD)  | (%) | $\Sigma(\pm$ SD) | ( $\pm$ SD) | (%) | $\Sigma(\pm$ SD) | ( $\pm$ SD) | (%) | $\Sigma(\pm$ SD) | ( $\pm$ SD) | (%) | $\Sigma(\pm$ SD) | ( $\pm$ SD) | (%)           |      |
| Control  | 24 (10.4)a                     | 19.6 (14.0)a |     | 56.6 (29.0)a     |             |     | 39.6 (18.4)a     |             |     | 97.0 (24.3)a     |             |     | 83.4 (35.1)a     |             | 130.6 (87.8)a |      |
| BT   | 23.5 (4.0)a                    | 22.6 (15.6)a | 0   | 32.0 (16.7)b     | 45.1        |     | 17.8 (9.6)b      | 58.5        |     | 17.4 (6.6)b      | 68.4        |     | 39.8 (18.7)b     | 55.4        | 19.0 (9.7)b   | 84.6 |
| EPN / Bt   | 17 (10.8)a                     | 19.8 (19.9)a | 0   | 12.8 (7.0)b      | 80.7        |     | 10 (4.6)b        | 74.6        |     | 24.2 (8.7)b      | 79.3        |     | 37.0 (24.0)b     | 57.4        | 22.4 (4.2)b   | 82.2 |
| BT+EPN   | 20.5 (15.2)a                   | 19.6 (17.3)a | 0   | 12.4 (5.0)b      | 77          |     | 28 (15.6)ab      | 29.5        |     | 36.4 (27.5)b     | 61.4        |     | 29.4 (12.3)b     | 64.6        | 51 (20.2)b    | 58.5 |

8 Means (n=5) indicated with the same letter within the same column do not differ according to Duncan's multiple range test at P < 0.05.

**Control of *Plutella xylostella* using polymer-formulated *Steinernema carpocapsae* and *Bacillus thuringiensis* in cabbage fields**



*Figure 1.* Mean number ( $n=5$ ) of *Plutella xylostella* per untreated plant of three different fields during the experimental time in March 2004. The number of larvae is presented considering the instars. Fields were planted at the beginning of January (C + B) or at the beginning of February (A). Field A was not treated with any insecticides, field B was supplied with one Sumicidin<sup>®</sup> application four weeks after planting and field C was treated with different insecticides but was abandoned at mid-February.



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