EFFICACY OF FOLIAR APPLICATIONS OF ENTOMOPATHOGENIC NEMATODES AGAINST THE CRUCIFER DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* - A REVIEW

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Summary. Diamondback moth (*Plutella xylostella* L.) is a serious pest of cruciferous crops. It has developed resistance against several chemical pesticides and Bt toxins. Management of diamondback moth through suitable bio-agents is an effective alternative to chemical pesticides. The present review highlights the potential of foliar applications of entomopathogenic nematodes for the management of diamondback moth on cruciferous crops and describes factors affecting nematode efficacy, developments in application technology, and field application of entomopathogenic nematodes against this pest. Since the first specific work on diamondback moth management through entomopathogenic nematodes in 1995, considerable progress has been made in understanding and improving the performance of these nematodes against this pest. Progress has been systematic, from standardization of dosages to research on application technology, including uses of different sprayers and adjuvants, and actual application of entomopathogenic nematodes in the field. However, integration of entomopathogenic nematodes with Integrated Pest Management (IPM) programmes still needs to be worked upon, and will determine the direction of future research.

Key words: Application technology, biocontrol, cabbage, environmental factors, heterorhabditids, steinernematids.

DIAMONDBACK MOTH, A NOXIOUS PEST OF CRUCIFERS

Diamondback moth (DBM) (*Plutella xylostella* L., Lepidoptera: Plutellidae) is one of the most serious pest of cruciferous crops and causes huge economic losses of more than 1 billion US dollars in terms of annual management costs (Talekar, 1992; Talekar and Shelton, 1993). It occurs wherever crucifers are grown, and is believed to be the most universally distributed of all Lepidoptera (Meyrick, 1928), especially in tropical and sub-tropical regions.

The DBM completes an average of 7-8 generations in one year, and is a very prolific insect. In tropical climates, it can have more than 20 generations a year (Roux *et al.,* 2006). It takes an average of 32 days to develop from egg to adult, but may take from 3 to 6 weeks to complete a generation depending upon the environmental conditions. The eggs are minute (<1mm), yellow and attached mostly on the lower side of the leaves (AVRDC, 1987; Harcourt, 1954). The eggs hatch in about 5-6 days, and the emerging larvae begin feeding on the leaves immediately. The larvae of DBM are dark green (8-10 mm) when fully grown. The larvae moult three times in 10-21 days (Hsu and Wang, 1971; Lu and Lee, 1984; Bhalla and Dubey, 1986; Salinas, 1986; Sarnthoy *et al.*, 1989) and then pupate in delicate white fibrous cocoons, measuring about 10-12 mm. The pupal stage lasts for one to two weeks depending upon the environmental conditions (Harcourt, 1957; Chelliah and Sriniwasan, 1986). The adult moth emerging from the pupa is about 8-10 mm long, grayish or brownish in colour. It is distinguished by having three pale, triangular markings along the inner margins of the wings, because of which it is named "diamondback moth". Each moth lays about 150 eggs (Harcourt, 1954) during the life span of about 15 days. A scheme of life cycle stages and durations is presented in Fig. 1.

DBM has a wide host range (Talekar and Shelton, 1993). It feeds not only on the commonly cultivated cruciferous crops such as cabbage (*Brassica oleracea* L. var. *capitata*), cauliflower (*B. oleracea* L. var. *botrytis*), broccoli (*B. oleracea* L. var. *italica*), radish (*Raphanus sativus* L.), turnip (*B. rapa* L. *pekinesis*), brussels sprouts (*B. oleracea* L. var. *gemmifera*), Chinese cabbage (*B. rapa* L. cv. gr. *pekinensis*), kohlrabi (*B. oleracea* L. var. *gongylodes*), mustard (*B. juncea* L.), rapeseed (*B. napus* L.), collard (*B. oleracea* L. *acephala*), pak choi (*B. rapa* L. cv. gr. *pakchoi*), saishin (*B. rapa* L. cv. gr. *saishin*), watercress (*Nasturtium officinale* R. Br.) and kale (*B. oleracea* L. var. *alboglabra*), but also on a large number of cruciferous weeds, which serve as alternate hosts for DBM before the main host crops are planted (Talekar and Shelton, 1993). The damage threshold level of DBM is one caterpillar/plant (Shelton *et al.,* 1983; Maltais *et al.,* 1998). DBM larvae feed on leaves, buds, flowers, seed pods, the green outer layer of the stems and, occasionally, the developing seeds within the older seed pods of canola and mustard. The amount of damage varies

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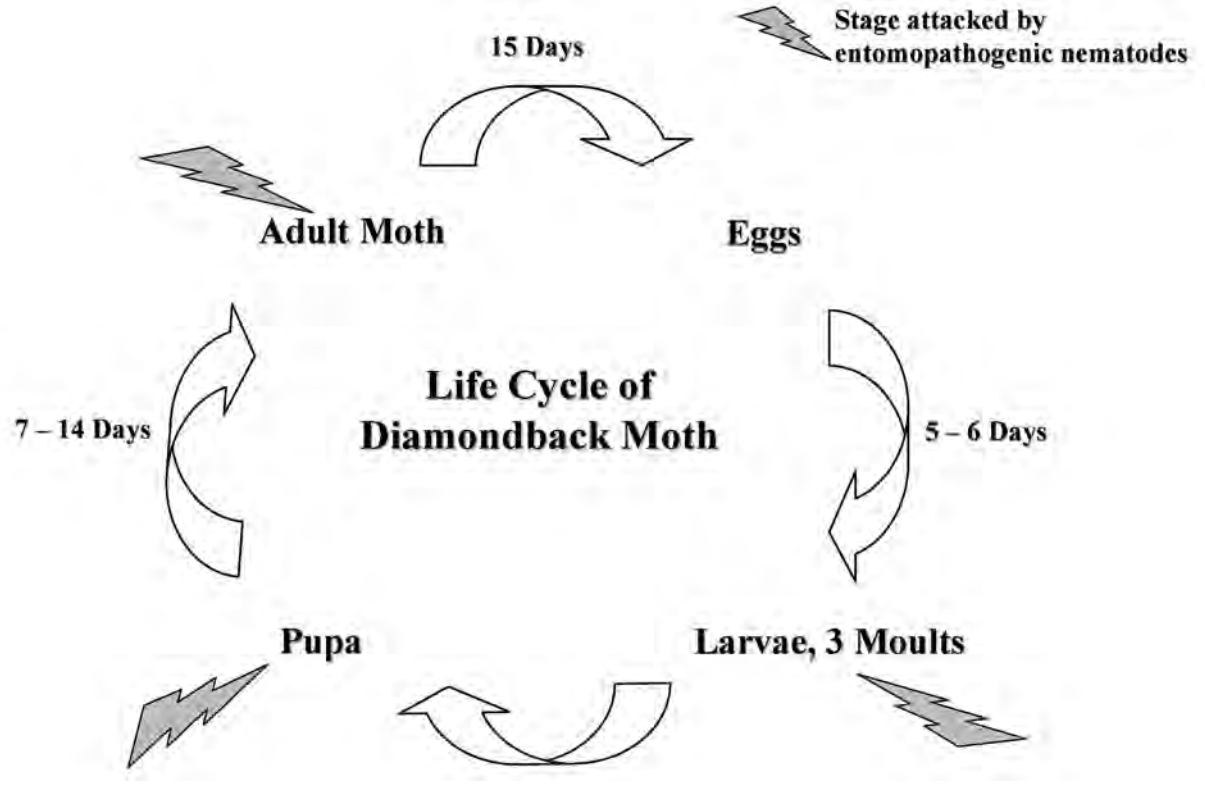

Fig. 1. Diagrammatic representation of life cycle of diamondback moth. Stages susceptible to entomopathogenic nematode infection are indicated with bolt arrows.

greatly, depending on plant growth stage, larval densities and size. Larval feeding causes typical symptoms in the form of shot holes of irregular shape in the leaves. If larvae are numerous they may eat the entire leaf, leaving only the veins and causing more than 90% crop loss (Verkerk and Wright, 1996). It is a serious pest as it feeds on the marketable portion of the plant, the leaves of cabbage and other leafy brassicas; only a few fourth stage larvae on a cabbage can make it un-saleable (Shelton *et al.*, 1983; Maltais *et al.*, 1998).

DBM became a major pest of crops only after the introduction of synthetic insecticides in the late 1940s. It is supposed that widespread use of synthetic insecticides on crucifers in the mid-1950s eliminated important natural enemies of DBM, turning DBM into a noxious pest in most parts of the world (Lim, 1986; Talekar and Griggs, 1986). In 1953, DBM became the first crop pest in the world to develop resistance to DDT (Ankersmit, 1953; Johnson, 1953), and has now become resistant to every synthetic insecticide used against it in the field in many countries (Talekar *et al.,* 1985, 1990). In addition, DBM has the distinction of being the first insect to develop resistance in the field to the bacterial insecticide, *Bacillus thuringiensis* Berliner (Kirsch and Schmutterer, 1988; Tabashnik *et al.*, 1990; Shelton and Wyman, 1992; Tabashnik, 1994). Information on the biology, ecology and management methods for DBM can be obtained from the excellent review by Talekar and Shelton (1993).

DBM has always been a difficult pest to manage, and even now there is no single management strategy that works effectively against it. Therefore, the development of novel and effective management programmes has always been a priority amongst researchers working on this insect. In recent years, another group of insect parasites, the entomopathogenic nematodes, have emerged as potent insect controlling bio-agents (Gaugler, 2002). The present review summarizes recent developments in the efforts to control DBM through use of entomopathogenic nematodes (Table I).

ENTOMOPATHOGENIC NEMATODES AS BIO-CONTROL AGENTS

Entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae have good bio-control potential against several insect pests of crops, including the diamondback moth (Gaugler, 2002; Kaya *et al.*, 2006). These nematodes have also been proved to be efficacious against some household and veterinary insect pests (Gaugler, 2002; Georgis *et al.*, 2006). Presently, there are more than a hundred companies in the USA alone that are mass-producing, formulating and marketing these nematodes for use against various insect pests of fruit tree crops, mushrooms, turf grass, etc. (http://www.oardc.ohio-state.edu/nematodes/nematode_suppliers.htm).

Table I. Timeline of addition in knowledge about Diamondback moth (DBM) management through entomopathogenic nematodes.

Type of study	Entomopathogenic nematode species	Salient results and recommendations	Reference
Leaf disc assays	*Steinernema carpocapsae* and *S. riobrave*	95% control of DBM by 2100 to 2500 IJs/ml spray	Baur *et al.*, 1995
Laboratory tests on Chinese cabbage	*S. carpocapsae*	High output nozzles such as standard cone and full cone deposited more IJs and caused 98% mortality	Lello *et al.*, 1996
Laboratory assays	Comparative efficacy of *Steinernema* and *Heterorhabditis* spp.	*Steinernema* spp. more effective than *Heterorhabditis* spp. against DBM	Ratansinghe and Hague, 1995, 1998
	Effect of temperature using two isolates of *Steinernema* (SSL85 and M87), and two species of *Heterorhabditis*	*(i)* Max infectivity between 20-25 ºC, for both the isolates	Mason and Wright, 1997
		(ii) Infection of DBM started within 3 hours post exposure	
		(iii) Only 1-18% of the IJs applied infected DBM larvae	
Field efficacy against DBM infecting *Nasturtium aquaticum*	*S. carpocapsae*	Adjuvants increased the field efficacy, but the effects were not significant	Baur *et al.*, 1997
Application technology under laboratory conditions	*S. carpocapsae*	*(i)* Spinning disc nozzles not good for nematode spray	Mason *et al.*, 1998a, 1998b, 1999
		(ii) Adjuvants and increased flow rate resulted in greater deposition of the nematodes per unit area	
		(iii) Increased initial concentration of nematodes deposited more nematodes, resulting in greater mortality of DBM	
		(iv) More infection in 150 min following spray application	
Laboratory assays	Entomopathogenic nematode species and strains	*H. bacteriophora* is most pathogenic against DBM	Shinde and Singh, 2000
	S. carpocapsae and *H. indica*	*(i)* 96 to 98% mortality on DBM 72 h post-infection	Hussaini *et al.*, 2003
		(ii) Recycling of *H. indica* better because of higher progeny production	
	S. thermophilum	100% mortality of DBM larvae within 48 h after infection, and found to be a good host for this species.	Ganguly and Gavas, 2004
	S. carpocapsae	*(i)* Tests on screening of adjuvants showed 2- to 5-fold increase in DBM moratlity at 80% to 60% RH by addition of Xanthan gum	Schröer *et al.*, 2005b
		(ii) Additives reduced the LT50 dose of nematode	
	S. carpocapsae	*(i)* Surfactant polymer combination (0.3% surfactant Rimulgan and 0.3% polymer Xanthan) increased nematode efficacy	Schröer *et al.*, 2005a
		(ii) DBM penetration by EPNs on the leaves occurred within the first hour after their application	
		(iii) Major thrust of the formulation should be on providing optimal environmental conditions to support nematode invasion	
		Same formulation reduced DBM mobility and so improved infectivity.	Schröer *et al.*, 2005a
Field efficacy against DBM on cabbage	*S. carpocapsae*	>50% control of DBM with IJs @ 0.5 million per m^2 in the surfactant polymer formulation containing 0.3% Xanthan and 0.3% Rimulgan as foliar spray in Cabbage fields	Schröer *et al.*, 2005a
	S. thermophilum	*(i)* 35-46% mortality in DBM with foliar spray @ 1,000 to 3,000 IJs/ml + adjuvant 0.033% APSA80, on cabbage	Somvanshi *et al.*, 2006
		(ii) Crop damage reduced by 9.7 to 49.5%	

In their gut, these *Steinernema* and *Heterorhabditis* nematodes harbour symbiotic bacteria, belonging to the family Enterobacteriaceae, i.e. *Xenorhabdus* and *Photorhabdus*, respectively (Boemare, 2002; Adams *et al.,* 2006). The nematodes carry the bacteria into the insect and release them in the insect hemocoel, where the bacteria multiply and cause septicemia thus resulting in the death of the insect host within 24-48 h (Boemare, 2002). Thereafter, the nematodes develop further, multiply, complete 2-3 generations and then emerge from the insect body *en masse* as infective juveniles (IJs), ready to infect another host. The life cycle of an entomopathogenic nematode is represented in Fig. 2. To date, more than 40 species of *Steinernema* and ten of *Heterorhabditis* have been described from various parts of the world (Adams *et al.*, 2006). All these nematodes species have specific host ranges and environmental requirements, and differ in their insect-parasitism strategies and foraging behaviour (Lewis *et al.*, 2006).

In the soil, the nematodes are continuously under different kinds of biotic and abiotic stresses (Glazer, 2002). Biotic stresses include the parasites and predators of nematodes. Desiccation, temperature extremes, relative humidity, pesticides, agro-chemicals, and ultraviolet light are some examples of abiotic stresses. Any stress is potentially disastrous for the nematode, and can severely impair its ability to find and infect the insect pest. The success of any insect biological control programme by EPNs depends heavily on the nematode species, the target insect pest and environmental conditions (Lewis *et al.*, 2006; Shapiro-Ilan *et al.*, 2006).

Much work has been done on biological control of different insect pests using entomopathogenic nematodes and has been described in detail elsewhere (Georgis, 2002; Georgis *et al.*, 2006; Grewal, 2002; Shapiro-Ilan *et al.*, 2006). Surprisingly, in spite of being an important pest, DBM is conspicuously absent from recently published papers on insect pest control using entomopathogenic nematodes (Grewal, 2002; Arthurs *et al.*, 2004; Georgis *et al.*, 2006; Shapiro-Ilan *et al.*, 2006). In this review, we have tried to summarize the recent works pertaining to application of EPNs for the management of DBM on crucifers (Table I). The literature on utilizing different species of EPNs against DBM proves their utility in DBM management. In the current situation, when DBM continues to defeat all the more traditional approaches deployed for its management, application of EPNs may be a very useful alternative, and might turn out to be an important component of IPM programmes in crucifers.

INTERACTIONS BETWEEN EPN AND DBM

Nematodes are hydrophilic organisms and need a film of water around their body for their survival (Glaz-

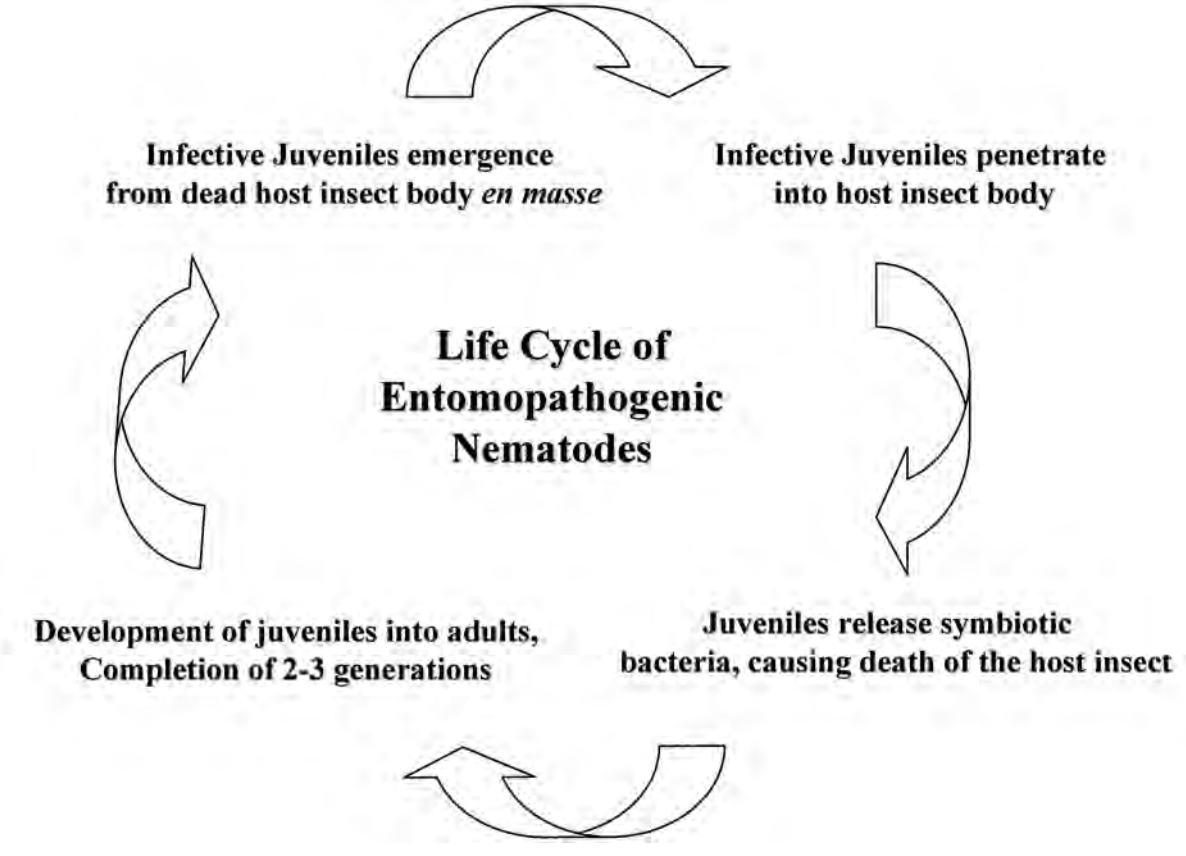

Fig. 2. Diagrammatic representation of life cycle of entomopathogenic nematodes belonging to families Steinernematidae and Heterorhabditidae.

er, 2002). This makes EPNs very susceptible to environmental factors such as temperature and humidity, and to the time of application etc. when they are spray applied on crop foliage, so influencing their efficacy. DBM larvae feed and pupate on the leaf surface; hence foliar application of the nematodes is the natural choice for DBM management. Most invasion of the DBM larvae takes place on the leaves within 1-3 hours of application of the nematodes (Mason and Wright, 1997; Schröer and Ehlers, 2005) so, to be successful, the spray application strategy should aim to help the nematodes survive for this period of time on the leaf surface so that the nematodes have ample time and optimal conditions to parasitize the insect larvae.

Baur *et al.* (1995) studied the effect of relative humidity and leaf type on steinernematid nematode infectivity on DBM in leaf disc assays and achieved more than 95% control by foliar application of *Steinernema carpocapsae* (Weiser) Wouts, Mracek, Gerdin *et* Bedding and *S. riobrave* Cabanillas, Poinar *et* Raulston at the rate of 2100-2500 IJs/ml concentration in the spray mixture. Mason and Wright (1997) recorded the effects of other abiotic factors such as temperature range on the infective juveniles of two isolates of *Steinernema* spp. (SSL85 and M87) and two species of *Heterorhabditis*, and recorded greatest infectivity at 20-25 ºC, which was found to be the optimal temperature range. Prior exposure of the IJs suspended in water droplets to simulated solar radiation also resulted in relatively constant DBM mortality in subsequent bioassays (Mason and Wright, 1997). This defined the limits within which EPNs could tolerate the major abiotic factors. Furthermore, they found that infection of DBM larvae commenced within 3 hours of exposure, though maximal infection did not occur until 24 hours and only 1 to 18% of the IJs applied infected the DBM larvae (Mason and Wright, 1997).

Schröer and Ehlers (2005) performed a leaf bioassay to test the efficacy of foliar application of the EPN *S. carpocapsae* against DBM and observed that penetration of the DBM larvae by EPNs on the leaves first occurred within the first hour after application of the nematodes, and so the major thrust of the formulation should be on providing optimal conditions for nematode invasion of the host on the foliage. The chosen formulation (0.3% surfactant Rimulgan + 0.3% polymer Xanthan) also reduced the mobility of the DBM larvae, and thus improved conditions for the invasion of the nematode into the insect (Schröer *et al.*, 2005a).

To obtain better control of DBM through the use of EPNs, the nematodes should be sprayed onto the foliage at a suitable time of the day when UV light levels are low, relative humidity is high, and temperature is optimal for their survival. Generally, early morning or late evenings after sunset are the best times to spray. As DBM feeds nocturnally on leaf surfaces, late evening foliar applications are better for DBM infection on the leaf. Native and indigenous strains of the nematodes are better adapted to local environmental conditions, so should be used in preference to exotic strains whenever possible. Suitable application formulations can enhance the efficacy of the nematodes.

PATHOGENICITY OF EPN SPECIES/STRAINS ON DBM

Like many other lepidopteran larvae, DBM larvae are parasitized by most of the entomopathogenic species and strains tested. However, comparative studies on the efficacy of different EPNs have produced contradictory results. Some studies have shown *Steinernema* species to be more effective than *Heterorhabditis* species against DBM (Ratansinghe and Hague, 1995, 1998). Contrary to this, in their experiment on desiccation survival cited above, Mason and Wright (1997) reported no significant differences between the isolates. However, Shinde and Singh (2000) tested eight nematode species/strains against DBM and found that all of them were pathogenic but that *Heterorhabditis bacteriophora* Poinar exhibited the greatest pathogenic potential due to its lowest LD_{50} (9.16 IJs/larvae), LT_{50} (43.26 hr) and Lex T50 (3.24 hr), and its greatest propagation potential (average of 271.4 IJs/mg host body weight). A laboratory study to test bio-efficiency and progeny production on various insects revealed 96 to 98% larval mortality in DBM 72 hours after infection by both *S. carpocapsae* and *Heterorhabditis indica* Poinar, Karunaka *et* David, but the recycling ability of *H. indica* was better than *S. carpocapsae* due to its greater progeny production (Hussaini, 2003). Twenty-one insect species belonging to six orders were tested by Ganguly and Gavas (2004) and seventeen species, including DBM, were found to be good hosts for *S. thermophilum* Ganguly *et* Singh.

APPLICATION TECHNOLOGY AND FIELD TRIALS

An excellent review of the various application technologies currently in use for EPNs and a description of all the factors needed for successful application of nematodes can be found in Sapiro-Ilan *et al.* (2006). Here, we focus on efforts made to standardize the application technology for the use of EPNs as foliar sprays against DBM.

Bio-efficacy of EPNs depends on the various features of the application methodology, viz., *i)* type of sprayer, *ii)* type and size of spray nozzles, *iii)* operating pressures, *iv*) screens of sprayers, *v)* the choice of nematode species, adjuvants and surfactants to be used in spray mixtures, and *vi)* the concentration of nematodes, adjuvant and surfactant in the spray mixture.

Adjuvants. Generally, adjuvants are found to enhance the efficacy of EPNs. Researchers have investigated various adjuvant-nematode systems for control of DBM, and the results have sometimes been contradictory. The chemical compositions of all the adjuvants used with EPNs for DBM management are given in Table II.

Table II. Adjuvants used with entomopathogenic nematodes in experiments to control diamondback moth and their chemical composition.

Name	Supplier	Chemical
Agral	Zeneca Plant Protection, Surrey, UK	Nonyl phenol ethylene oxide condensate
Triton X100	BDH, Merck Ltd, Dorset, UK	Polyethylene Glycol p-tert-Octylphenyl Ether
Triton X155	BDH, Merck Ltd, Dorset, UK	Polyethylene Glycol p-tert-Octylphenyl Ether
Tween 60	Sigma-Aldrich, St Louis, USA	Polyoxyethylene-sorbitan-monostearate
Croduvant	Croda Chemicals Ltd, Cowick Hall, Snaith, Goole, North Humberside, UK	Glycerol Based
Crovol 127	Croda Chemicals Ltd, Cowick Hall, Snaith, Goole, North Humberside, UK	Linseed oil based
Crovol L40	Croda Chemicals Ltd, Cowick Hall, Snaith, Goole, North Humberside, UK	Linseed oil based
Rimulgan	Temmen GmbH, Germany	68% castor oil, 25% ionic oleic acid, 5% calcium and alcohol
Xanthan	Gum Xanthan, UD Chemie GmbH, Germany	Fermentation-derived biopolymer 0.3 & 1.0 from the bacterium *Xanthomonas campestris* (40 mesh)
APSA80	Amway Ltd., India	Poly(oxy-1,2-ethanediyl), alpha.-(nonylphenyl)-omega hydroxyl-(60 – 100%) 1-Butanol (10-30 %) Fatty acids, tall-oil (1-5%)
Glycerol	Walter DMB GmbH, Germany	86.5% Glycerol

An experiment to test efficacy of different adjuvants to prolong survival of the nematode *S. carpocapsae* and increase DBM control in water cress, *Nasturtium aquaticum* L., revealed that adjuvants (Agral, Triton X100, Triton X155, Tween 60) could improve the field efficacy, but that the effects were not significant and perhaps should be examined further (Baur *et al.*, 1997). However, Mason *et al.* (1998a) found that addition of adjuvants Triton X100, Glycerol, Croduvant, Crovol 127 or Crovol L40 increased flow rate and resulted in greater number of nematodes being deposited per unit area.

Schröer *et al.* (2005b) screened several adjuvants for toxicity to nematodes, plants and insects, and also different combinations of the surfactants and polymers to improve nematode efficacy, and recorded a two- to five-fold increase in DBM mortality at 80 to 60% relative humidity by addition of Xanthan gum. The additives reduced the LT50 from >40 h in water to <25 h in the 0.3% Xanthan or 0.3% surfactant mixture. Compared to water, the surfactant-polymer formulation (0.3% surfactant Rimulgan + 0.3% polymer Xanthan) significantly improved the efficacy of the nematodes (Schröer *et al.*, 2005a, b).

Somvanshi *et al.* (2006) tested the effects of four different concentrations of the adjuvant APSA80 on survival and infectivity of *S. thermophilum* and *H. indica* at different time intervals (24-72 h), under laboratory conditions. They found that higher concentrations (0.33 to 2%) of the adjuvant significantly affected the survival and infectivity of both species. *Heterorhabditis indica* suffered significantly higher mortality than *S. thermophilum*, a trend that was observed at all the concentrations and time intervals. The lowest concentration of adjuvant (0.033%) was found to be the most appropri-

ate for *S. thermophilum* and was used in the spray mixture in the subsequent field trial experiments.

Adjuvants help to achieve uniform spreading and distribution of the spray droplets containing nematodes on the crop canopy. Other additives, such as gums and chemicals, used in formulations help to increase the droplet retention on the leaf surfaces, and also protect the nematodes from environmental factors. Nematode strains vary in susceptibility to different adjuvants, so all adjuvants may not be equally good for each nematode strain. Therefore, it is essential to test the toxicity of the adjuvants and to assess their optimal dosages for the nematode to be used in bio-control programmes.

Nozzles and spray equipment. Lello *et al.* (1996) assessed adjuvant toxicity and nozzle type, and analyzed droplet spectra with *S. carpocapsae* infective juveniles against DBM. They reported that higher output hydraulic nozzles, e.g. standard cone and full cone, deposited more nematodes and gave up to 98% mortality of DBM on Chinese cabbage. Mason *et al.* (1998a, b) studied spray nozzles, screening and selection of suitable entomopathgenic nematodes against DBM larvae and suggested spinning disc nozzles to be inappropriate because, in 90% of cases, the nematodes were not released in spray mist. Further studies by Mason *et al.* (1999) to standardize the application methodology for EPNs using a spinning disc spray system showed greater deposition of nematodes with increased initial concentration, and consequently better infection and mortality of the DBM. Some of the adjuvants significantly improved infection of DBM by the nematodes. In desiccation survival studies, they recorded 50% survival of the IJs for over 3 h with or without adjuvants on Chinese cabbage leaf discs, and high levels of DBM infection within 150 min of spray application (Mason *et al.,* 1999).

Studies on EPN application technology for DBM control represent only a small percentage of published reports. However, as application technology depends more on the nematode strain used and the crop to be protected, results from other studies could be adopted for DBM management, with appropriate modifications based on DBM behaviour.

Field trials. In field trials to evaluate the potential of entomopathogenic nematodes and *Bacillus thuringiensis* (Bt) for DBM control in East Java and Indonesia, Schröer *et al.* (2005c) recorded >50% control of DBM by use of 0.5 million *S. carpocapsae* per m^2 in the surfactant polymer formulation containing 0.3% Xanthan and 0.3% Rimulgan. The control achieved by weekly application of *B. thuringiensis* or alternating applications of Bt with nematodes was >80%. These researchers have worked systematically towards control of DBM on cabbage, by first generating a suitable surfactant polymer combination in laboratory trials and then taking it into the field.

In India, Somvanshi *et al.* (2006) conducted tests on the field efficacy of *S. thermophilum* against DBM infesting cabbage between 2002 and 2004. Cabbage plants were inoculated with 10 DBM larvae/plant and then sprayed with *S. thermophilum* infective juveniles (IJs) at three concentrations (1000, 2000 or 3000 IJs/ml) containing 0.033% APSA80. An insecticide (Lambda Cyahalothrin at 0.0025%) and a no nematode treatment served as controls. Averaged over two years, the tests showed that *S. thermophilum* at 3000 IJs/ml caused the highest mortality of 46%, whereas 2000 IJs/ml and the insecticide treatment caused 40.5 and 40% mortality in DBM larvae, respectively. The differences between the treatments were not significant. DBM mortality in the treatments showed a slight increase with increasing temperatures between years. The IJs treatments reduced crop damage by 9.7 to 43.1%, whereas the insecticide treatment reduced damage by 49.5%. Interestingly, despite being heat tolerant, *S. thermophilum* performed well during the cropping season, when minimum temperatures of 5-10 °C were recorded.

In spite of the noxious pest status of DBM, there has been only one major EPN field trial project against DBM. This project, called DIABOLO, was funded by the European Union (http://cordis.europa.eu/data/PROJ_FP5/ACTIONeqDndSESSIONeq112122005919ndDOCeq57ndTBLeqEN_PROJ.htm) for DBM control in Indonesia and China, in a collaboration with Ireland and Germany. The results from the Indonesian trials are encouraging (Schröer *et al.*, 2005b), and should promote further use of the EPNs in controlling DBM.

INTEGRATION OF EPN IN INTEGRATED PEST MANAGEMENT PROGRAMMES

We could not find any published record for integrated pest management (IPM) of DBM that included EPNs as one of its components. To exploit the bio-control potential of EPNs in IPM, it is imperative to know their compatibility with other management strategies. The compatibility of EPNs with agrochemicals, including herbicides, fungicides, acaricides, insecticides, surfactants, fertilizers and soil amendments, have been explored by some workers, but the results have been variable. It has been found that infective juveniles are tolerant of short exposures (2-6 hours) to most agrochemicals, and so can be tank-mixed (Rovesti and Deseo, 1990; Ishibashi, 1993). However, other workers (Patel and Wright, 1996; Grewal *et al.*, 1998) reported that some pesticides could be toxic to the nematodes. In contrast, some other studies demonstrated synergism between EPNs and imidacloprid (Koppenhöfer and Kaya, 1998), tefluthrin (Nishimatsu and Jackson, 1998) and pathogens such as *Bacillus thuringiensis* (Koppenhöfer and Kaya, 1997; Schröer *et al.*, 2005b).

Baur *et al.* (1998) tested the field efficacy of the entomopathogenic nematode *S. carpocapsae* and *B.*

thuringiensis against DBM and achieved 41% control using the nematodes alone, as against 58% in the combined (Nematode + Bt) treatment. They suggested that the nematodes could be included as a component in IPM schedules for the control of DBM resistant to Bt. Similar conclusions were drawn by Schröer *et al.* (2005b), so the use of EPN and Bt together could be a highly effective strategy for DBM management.

Insect mortality following the use of EPNs is mainly due to septicemia caused by their symbiotic bacteria. Direct toxicity of symbiotic bacteria against some insect pests, without involving the nematodes, has also been documented (Dudney, 1997; Elawad *et al.*, 1999a, 1999b). Mahar *et al.* (2004) reported lethality of the cell and cell-free filtrates of the bacterium *Xenorhabdus nematophila* isolated from *S. carpocapsae* against DBM on Chinese cabbage leaves, thus raising the possibility of insect control without the nematode vector of the bacteria.

NEED FOR FUTURE RESEARCH

All the studies reported indicate that EPNs could be very effective bio-control agents against DBM, especially when used in combination with other management strategies such as insecticides and other biocontrol agents such as Bt. A local area-based research and development approach is needed for each target insect. Indigenous EPNs should be used whenever possible. For better DBM management, the nematode application technology should be carefully tested before using it in the field. A major thrust of EPN-based management strategies should be to keep the nematodes alive and active after spraying by avoiding desiccation and minimizing UV exposure. The compatibility of EPNs with other management strategies also needs more research. Selection and breeding approaches will also aid in these objectives. The whole IPM strategy should be carefully planned and carried out to optimise the management of DBM. Attention should be given to avoiding the development of resistance in DBM against the management methods.

Lack of commercial availability of EPNs, poor communication and transfer of research results to the farmers, and lack of public awareness about the benefits of using EPNs are still the major hurdles to exploitation of EPNs for control of insect pests, especially in Asian countries like India and China. Huge 'potential' demand for EPNs exists in these countries because of their large areas of land under cultivation and the associated insect pest problems. A coordinated effort between scientists, extension workers and the commercial producers of EPNs is needed to promote the use of EPNs as an environmentally friendly and effective solution for the management of DBM and other pests in world agriculture. Bearing in mind the increasing markets for organic agricultural products, the economic prospects for these nematodes are bright.

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