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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^{-2} in SPF, 80% mortality was recorded, whereas at 60% RH insect mortality reached almost 60%. The survival time (LT_{50}) for *S. carpocapsae* applied in water was 36 h at 80% RH and only 3 h at 60% RH. With SPF the LT_{50} 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 morality 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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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_{50}) 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_{50} 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, $6 \times 5 \times 8$ 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^{-1} at 20^5 Pa and 15 µl cm^{-2}. 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^{-2} 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-

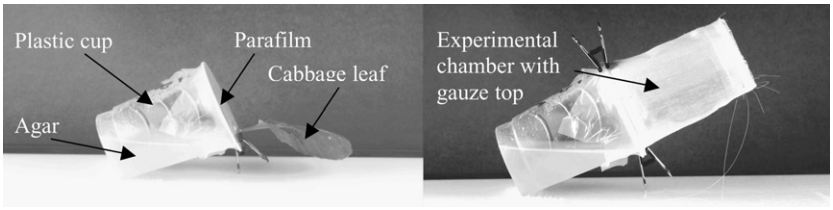

Fig. 1. Experimental cup to observe the performance of *Steinernema carpocapsae* on single cabbage leaves. Water supply for the leaf is provided by water agar in the bottom cup, which is divided from the experimental area with parafilm. Leaves are treated with one of the different nematode formulations 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.

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