Research Article

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Improving the biocontrol potential of entomopathogenic nematodes against *Mamestra brassicae*: effect of spray application technique, adjuvants and an attractant

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Abstract

BACKGROUND: *Steinernema carpocapsae* Weiser, an entomopathogenic nematode (EPN), is a potential biological control agent for the cabbage moth (*Mamestra brassicae* L.). This research aimed to identify a suitable spray application technique, and to determine whether yeast extract added to an EPN spray has an attracting and/or a feeding stimulant effect on *M. brassicae*. The biological control capabilities of EPN against this pest were examined in the field.

RESULTS: Good coverage of the underside of cauliflower leaves, the habitat of young instar larvae (L1–L4) of *M. brassicae* was obtained using different spray boom configurations with vertical extensions that carried underleaf spraying nozzles. One of the configurations was selected for field testing with an EPN spray. Brewer's yeast extract stimulated larval feeding on leaves, and increased the mortality of these larvae when exposed to EPN. The field trial showed that a spray application with *S. carpocapsae*, Addit and xanthan gum can effectively lower the numbers of cabbage heads damaged by *M. brassicae*. Brewer's yeast extract did not significantly increase this field performance of EPN.

CONCLUSION: *Steinernema carpocapsae*, applied with an appropriate spray technique, can be used within biological control schemes as part of a resistance management programme for Bt.

Keywords: biological control; brewer's yeast extract; cabbage moth; spray boom modifications; spray nozzle; *Steinernema carpocapsae*

1 INTRODUCTION

Crops of *Brassica oleracea* (e.g. cauliflower, savoy cabbage) are vulnerable to attacks by various lepidopteran insects. The economically most important species are the diamondback moth, *Plutella xylostella* L., the small white butterfly, *Pieris rapae* L., the cabbage looper, *Trichoplusia ni* Hübner, and the cabbage moth, *Mamestra brassicae* L.[1–5] These pests are commonly controlled with chemical pesticides. However, due to toxicological and environmental concerns, such as resistance development of target organisms and unwanted side effects on natural enemies, policies are developed worldwide to reduce the use of chemical pesticides.[6]

Entomopathogenic nematodes (EPN) might be a biological alternative. *Steinernema carpocapsae* Weiser has a high potential for use on foliage, because it shows a superior desiccation survival under the fast drying conditions occurring on foliage, when compared to other EPN species.[7] Much work has been done on assessing the usability of *S. carpocapsae* sprays against *P. xylostella* on leaves.[8–19] In contrast, almost no information is available about using this nematode against *M. brassicae*, although it is known that the larvae of cabbage moth are relatively susceptible to EPN.[20]

The cabbage moth is a polyphagous pest insect, widely distributed across Europe and Asia.[21] It feeds preferably on cruciferous crops, but attacks also many other crops like sugar beet, spinach and tomato. In organic farming, lepidopteran pests are commonly controlled with *Bacillus thuringiensis* (Bt) products; however, caterpillars of *M. brassicae* seem to be less susceptible to the toxins of Bt.[22] EPN could be a good alternative to reduce damage by *M. brassicae*.

There is, however, a major obstacle for a successful application of EPN against *M. brassicae*, i.e. young larvae live predominantly

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on the underside of the cabbage leaves,[23] whereas older and larger caterpillars enter inside the crown of the plant and hide between the leaves.[24] This makes them hard-to-reach targets for spray applications.[25] Brusselman *et al.* concluded that the use of modified spray application techniques, which direct the spray to the target site are necessary to increase the chances of contact of EPN with their target.[26] Even with adapted spraying equipment, it is difficult to get a good coverage with EPN on the lower side of cauliflower leaves. This is caused by the hydrophobicity of the leaf surface, which leads to bouncing and run-off of droplets.[27] Leaf deposition of spray liquids can be significantly improved by adding a spreading agent to the spray suspension.[28,29] Improved coverage with EPN can be obtained by adding a surfactant and a polymer to the spray suspension.[19] Preliminary research confirmed that a spreading agent (Addit, 2.5 mL L^{-1}; Koppert, Berkel en Rodenrijs, The Netherlands), and the polymer xanthan gum (0.3 g L^{-1}; Carl Roth, Karlsruhe, Germany) improve deposition of EPN on cauliflower leaves (Beck B *et al.*, unpublished).

To optimise the control of *M. brassicae*, a first experiment was performed to select a spray application technique that leads to a maximum spray coverage on specific parts of the cauliflower plant. The targeted spraying areas were (1) the central part of the plant, where the cabbage moth larvae can cause direct economic damage to the cauliflower head; and (2) the undersides of the leaves, where the young larvae hide during the day and feed during the night.[23]

The second laboratory experiment, verified whether brewer's yeast extract (powdered, produced through aqueous extraction of autolysed brewer's yeast; Carl Roth) could influence the behaviour of *M. brassicae* larvae, and whether this attractant improved the mortality caused by EPN to *M. brassicae*. Yeast extract is known to stimulate the feeding of larvae of *Spodoptera littoralis*.[30] If the feeding rate of *M. brassicae* would be stimulated likewise, the contacts between the larvae and the EPN would increase, possibly improving infectivity.

The last experiment determined whether *S. carpocapsae*, applied in the field with the selected technique, could effectively reduce the cabbage moth numbers in a cauliflower crop or the damage to this crop. It also tested if these parameters could be further reduced by adding yeast extract and/or the two above-mentioned spray retention adjuvants to the tank suspension.

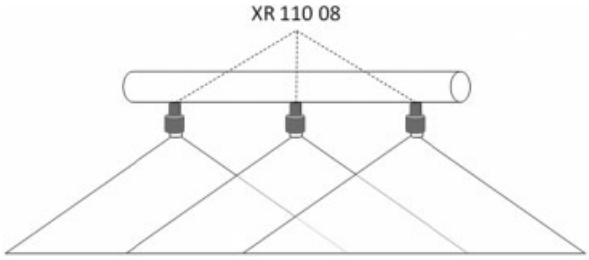

Figure 1. Scheme of spray boom configuration 1 (C1): TeeJet XR 110 08 nozzles mounted on a standard spray boom with 50 cm nozzle spacing.

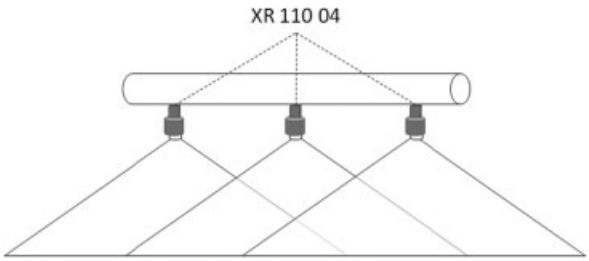

Figure 2. Scheme of spray boom configuration 2 (C2): TeeJet XR 110 04 nozzles mounted on a standard spray boom with 50 cm nozzle spacing.

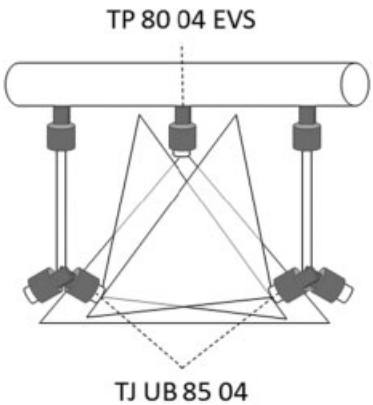

Figure 3. Scheme of spray boom configuration 3 (C3): TeeJet TP 80 04 EVS mounted on a spray boom and TeeJet UB 85 04 nozzles mounted 38 cm long vertical extensions. Nozzles and extensions were spaced 35 cm apart.

2 EXPERIMENTAL METHODS

2.1 Spray application tools test

Cauliflower plants were planted on 6 July 2011 (Rumbeke-Beitem, Belgium). The plots consisted of four crop rows, each consisting of 14 plants. Crop rows were spaced 70 cm apart.

2.1.1 Spray boom configurations

Two standard broadcast spray boom configurations (C1 and C2; Fig. 1 and Fig. 2) and three configurations with spray booms adapted for band spray applications (C3, C4 and C5; Fig. 3, Fig. 4 and Fig. 5) were selected for spray deposition tests with water in field conditions. C1 was a standard spray boom, equipped with TeeJet XR 110 08 extended range flat fan nozzles (TeeJet Technologies, Wheaton, Illinois, USA), whereas C2 was equipped with TeeJet XR 110 04 extended range flat fan nozzles. Nozzle spacing and spray boom height were 50 cm in both configurations.

In C3 and C4, nozzles fixed to the spray boom were alternated by vertical extensions of 38 cm (TeeJet Hose drop). These extensions ended in two sideward spraying nozzles, each one spraying to an opposite side. Nozzles and extensions were spaced 35 cm apart. C3 used ISO 04 band spraying nozzles (TeeJet TP 80 04 EVS) directly mounted on the boom, and two ISO 04 underleaf band spraying nozzles (TeeJet UB 85 04) on the extensions. C4 used the same nozzles as C3 on the extensions, but the nozzles mounted on the boom were replaced by ISO 04 twinjet band spraying nozzles (TeeJet TJ 60 40 04 EVS) that provided two even flat spray fans. One spray fan was angled 30° forward; the other one sprayed at 30° backwards. Both spray fans had a top angle of 40°, instead of the 80° top angle of the band spraying nozzles in C3.

In C5, the spray boom was equipped with TeeJet row application kits at distance intervals of 0.70 m with three TeeJet XR 110 04 flat fan nozzles (four) per kit: one central nozzle sprayed directly downwards, while the other two sprayed downwards at an angle of 45° towards the central nozzle.

All spray boom configurations functioned at a spray pressure of 4.0 bar, and at a speed of 4.0 km h^{-1}. These parameters resulted

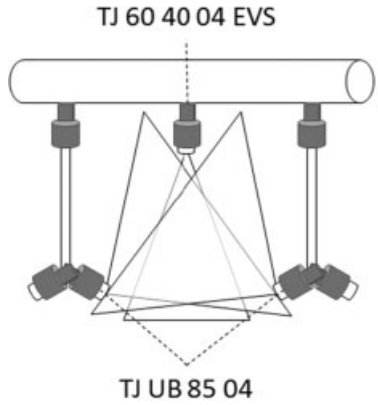

Figure 4. Scheme of spray boom configuration 4 (C4): TeeJet TJ 60 40 04 mounted on a spray boom and TeeJet UB 85 04 nozzles mounted on 38 cm long vertical extensions. Nozzles and extensions were spaced 35 cm apart.

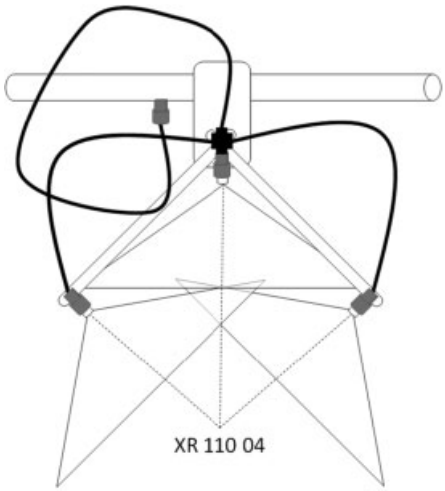

Figure 5. Scheme of spray boom configuration 5 (C5): TeeJet XR 110 04 nozzles mounted on a row application kit. The row application kits were spaced 70 cm apart.

in an application rate of 1095 L ha^{-1} for C1, 546 L ha^{-1} for C2, and 1170 L ha^{-1} for the three other configurations.

Each configuration was tested on six different plots of 7.0 m × 2.8 m (= replicates). For configurations C3–C5, the central nozzle was positioned directly above a crop row. Water sensitive papers were fitted on three randomly chosen cauliflower plants.

2.1.2 *Observations*

The water sensitive papers (7.6 × 2.6 cm^2; TeeJet) were folded around the leaf margins, so that one half of the paper covered a part of the upper side of a leaf, and the other half covered the lower side. On every sampled cauliflower plant, a paper was folded around a leaf near the cauliflower head, another one was folded around a leaf at the edge of the plant. Immediately after spraying, the water sensitive papers were collected and dried. They were scanned afterwards at a resolution of 600 dpi. The percentages of initially wetted, and thus blue surface on the water sensitive papers, were measured using image analysis software written in Halcon 8.0 (MVTec Software GmbH, Munich, Germany).[25] The wetted surface was measured separately for the parts covering the upperside and the underside of a leaf.

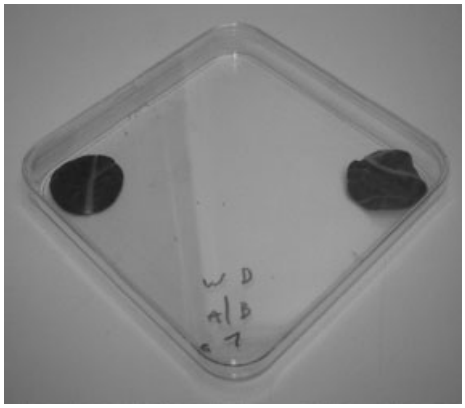

Figure 6. Petri dish with one *Mamestra brassicae* second instar larva (under corner) and two cauliflower leaf discs.

2.1.3 *Statistics*

Coverage results of the upperside and underside were analysed separately. These results were analysed statistically with the non-parametric Kruskal–Wallis test. This test was chosen since there was no equality of variances between groups of data and since there was a significant interaction between the factors spray boom configuration (C1–C2–C3–C4–C5) and location (centre–edge). Comparisons of specific treatments were made with the non-parametric Dunnett T3 test. All tests were performed with SPSS Statistics 20. Statistical significance was considered at $P < 0.05$.

2.2 Attractant test

The attractant test determined the influence of brewer's yeast extract on the survival, leaf preference and feeding rate (measured as the decrease in leaf surface over time) of second instar larvae (L2) of *M. brassicae*.

This test was carried out in 120 Petri dishes, divided over three replicates. One replicate consisted of four sets of 10 square (120 mm × 120 mm × 17 mm) Petri dishes (Fig. 6) each filled with two cauliflower leaf discs (A and B, 3 cm diameter). Within one set of 10 Petri dishes, 10 discs 'A' were sprayed with water and one disc was put into a corner of each of the 10 Petri dishes. A set of 10 discs 'B' received one of the spray treatments, summarised in Table 1. One 'B' disc was put in opposite corners of the Petri dishes containing an 'A' disc. One L2 of *M. brassicae* was put in an empty corner of each dish. Subsequently, the dishes were sealed with Parafilm to prevent moisture loss, and put in an incubator at 24 °C.

All spray applications were carried out with an automated spray boom with configuration C2 in controlled laboratory conditions.[31,32] Yeast extract was applied at 1 g L^{-1}. EPN were represented by infective juveniles (IJ) of *S. carpocapsae* (Biobest, Westerlo, Belgium – Becker Underwood, Littlehampton, UK). They were applied at an intended concentration of 5 × 10^6 IJ L^{-1}. This corresponds to a theoretical deposition of 27 EPN cm^{-2} on the sprayed surface. The actual concentration and survival percentage of EPN in the tank suspensions with and without yeast extract was recorded after every application. The actual number of EPN deposited on the leaf discs, and their survival (expressed as percentage of applied number) was recorded as described by Brusselman *et al.*[31] and compared between treatments.

2.2.1 *Observations*

Larval survival was recorded 24, 48, 72, 96 and 120 h after application. At the same time, feeding rate was estimated by

Table 1. Different spray treatments for discs (diameter 3 cm) A and B within all sets of 4 × 10 Petri dishes

Set	Disc A (control)	Disc B
1	Sprayed with water	Not sprayed
2	Sprayed with water	Sprayed with *S. carpocapsae* suspension
3	Sprayed with water	Sprayed with *S. carpocapsae* suspension + yeast extract
4	Sprayed with water	Sprayed with yeast extract

Leaf discs (diameter 3 cm) were sprayed with a spray boom fitted with Teejet XR 110 04 nozzles spaced 50 cm apart, 50 cm above the leaf discs, at a rate of 4 km h^{-1} and a pressure of 4 bar. Brewer's yeast extract was applied at 1 g L^{-1}. *S. carpocapsae* was applied at 27 EPN cm^{-2} onto the sprayed surface.

measuring the unconsumed surface of each leaf disc. This was quantified by image analysis of the photos taken before and after the feeding, using ImageJ digital software.[33] Leaf preference was recorded 24 h after the start of the experiment by observing which leaf discs showed feeding damage, and which were unscathed.

2.2.2 Statistics

Tank concentrations, deposition of EPN on leaf discs and survival rates of EPN were compared between spray treatments with a one-way ANOVA. Data on larval survival were analysed with a Kaplan–Meier log rank survival test with pair-wise comparisons. First, for each set of 40 Petri dishes that received the same treatment, leaf area measurements (independent variable) of the leaf discs A (water) were compared with the leaf discs B (treated) during five consecutive days (0, 24, 48, 72, 96 and 120 h) using the generalised linear mixed model (GLMM). Leaf disc was considered as a random factor to correct for repeated measurements over time within one leaf disc. Bonferroni correction for multiple comparisons was applied. Further, the effect of the treatment of leaf disc B on the total leaf disc area within a Petri dish (independent variable) was studied through generalised linear mixed model with leaf disc as random factor and treatment of leaf disc B (1, 2, 3 and 4), time (0, 24, 48, 72, 96 and 120 h) and the interaction between treatment and time as fixed factors. In addition, the influence of treatment on the leaf preference within dishes (presence of damaged versus undamaged leaf discs) was analysed with binary logistic regression. SAS 9.3 was used for the GLMM calculations. SPSS Statistics 20 was used for all other calculations. Statistical significance was considered at $P < 0.05$.

2.3 Field trial

This trial was conducted on the same site as the application test. Cauliflower plants, cv. Korlanu, were planted on 6 July 2011. The soil type was sandy loam. The experiment was arranged as a randomised complete block design of seven treatments (Table 2), each replicated four times. Plots were 7 m long by 2.8 m wide. Plants were spaced 51 cm apart on the row and 70 cm between rows. During the first 3 weeks after planting, the plants were covered with a net (7 × 7 mm mesh) to prevent damage caused by birds and small game. All plots were harvested between 26 September and 3 October.

The nets were removed on 28 July to allow natural deposition of eggs by the cabbage moth. To supplement natural infestation, 20 laboratory bred cabbage moth larvae (L1–L2) were set out on 10 plants (two larvae per plant) in every plot on 26 August.

Spray treatments with *S. carpocapsae* and *B. thuringiensis* (Bt) subsp. *aizawai* (Xentari®; Valent Biosciences, Libertyville, IL, USA) started on 30 August. EPN were applied twice a week, and six times in total; Bt was applied once a week, and three times in total. EPN were sprayed at a rate of 1170 L ha^{-1} corresponding with a theoretical EPN deposition of 58 EPN cm^{-2} of ground surface (treatments 2–5). Bt was sprayed at a rate of 1 kg ha^{-1}, independent of the amount of water with which it was sprayed. All EPN applications were performed with the band application technique C3; Bt was applied with the same technique (C3) as well as with the broadcast (C2) application. Treatments 3, 4 and 5 also included adjuvants (Addit at 2.5 mL L^{-1} and xanthan gum at 0.3 g L^{-1}) and/or an attractant (brewer's yeast extract at 1 g L^{-1}) in the EPN spray liquid. Cauliflowers were harvested between 26 September and 3 October.

2.3.1 Observations

The tank temperature was measured at the start and at the end of each spray round. Before the first spray, cauliflower leaf discs (Ø: 3 cm) were attached to both the upperside and underside of a fully grown leaf on three plants per plot. After spraying, the number of EPN deposited on the exposed side of these leaf discs were counted in all treatments with EPN (i.e. treatments 2–5), and the relative deposition (i.e. the percentage of the expected deposition of 58 EPN cm^{-2}) was calculated and compared between treatments.[26]

At the beginning and at the end of each treatment on the first treatment day, a sample was taken of all suspensions in the tank; allowing the concentration of EPN to be calculated and to check whether their survival was affected or not by passing several times through the pumping system.

The number of living *M. brassicae* L3–L6 was counted on all plants that were supplemented with larvae. These counts were performed the day before the first treatment (29 August), and again one and two weeks later (on 5 September and 12 September, respectively). To reduce possible observational error, stages L1 and L2 were not counted and other larval stages were grouped in two classes: L3–L4 and L5–L6. The numbers of living larvae of other damaging insect species were also counted on these three dates, to check if these might have influenced the observed parameters.

On 21 September, 5 days before harvesting, leaf damage was scored per plot, on a scale from 1 (no damage) to 9 (severe damage). The number of marketable cauliflower heads and the number of cauliflower heads damaged by *M. brassicae* were counted per plot.

2.3.2 Statistics

The effect of treatment (treated groups versus control group) on the presence of marketable cabbage heads (compared to damaged cabbage heads) was analysed with a binary logistic regression. The influence of treatment on damage scores was analysed using a one-way ANOVA. Finally, the effect of treatment on the numbers of L3 and L4 larvae (dependent variable) for the three different time points (29 August 2011, 5 September 2011 and 12 September 2011) were compared with a generalised linear mixed model (GLMM). 'Plant' was considered as random factor to correct for repeated measurements within plants. Bonferroni correction for multiple comparisons was applied. The same analyses (GLMM with 'plant' as random factor) were performed on the number of L5 and L6 larvae as dependent variable. SAS 9.3 was used for the GLMM

Table 2. Overview of all applied treatments and treatment dates

Nr	Treatment	Dose	Application technique	Treatment days
1	Water (control)	1170 L ha^{-1}	C3, Pressure 4.0 bar, speed: 4.0 km h^{-1}	1, 4, 8, 10, 15, 18
2	*S. carpocapsae*	58 EPN cm^{-2}, 1170 L ha^{-1}	C3, Pressure 4.0 bar, speed: 4.0 km h^{-1}	1, 4, 8, 10, 15, 18
3	*S. carpocapsae* + yeast extract[a]	58 EPN cm^{-2}, 1170 L ha^{-1}	C3, Pressure 4.0 bar, speed: 4.0 km h^{-1}	1, 4, 8, 10, 15, 18
4	*S. carpocapsae* + Addit[b] + xanthan gum[c] + yeast extract[a]	58 EPN cm^{-2}, 1170 L ha^{-1}	C3, Pressure 4.0 bar, speed: 4.0 km h^{-1}	1, 4, 8, 10, 15, 18
5	*S. carpocapsae* + Addit[b] + xanthan gum[c]	58 EPN cm^{-2}, 1170 L ha^{-1}	C3, Pressure 4.0 bar, speed: 4.0 km h^{-1}	1, 4, 8, 10, 15, 18
6	Bt	1 kg ha^{-1}, 1170 L ha^{-1}	C3, Pressure 4.0 bar, speed: 4.0 km h^{-1}	1, 8, 15
7	Bt	1 kg ha^{-1}, 1170 L ha^{-1}	C2, Pressure 4.0 bar, speed: 1.9 km h^{-1}	1, 8, 15

Treatments started on 30 August 2011 (= day 1)
[a] Brewer's yeast extract, 1 g L^{-1}.
[b] Addit, 2.5 mL L^{-1}.
[c] Xanthan gum, 0.3 g L^{-1}.
Bt, *Bacillus thuringiensis* toxin.

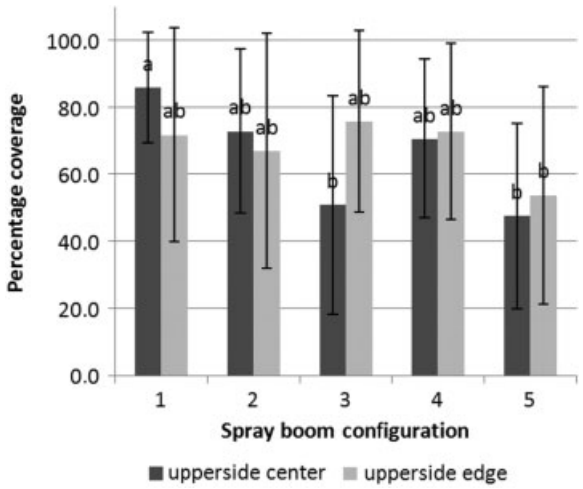

Figure 7. Percentage of water coverage (±SD) on the upper side of leaves in the centre and at the edge of the cauliflower plants as obtained with the five selected spray boom configurations (1: reference spray boom with XR110 08 nozzles, 2: reference spray boom with XR110 04 nozzles, 3: spray boom with TP 80 04 EVS nozzles above rows and UB 85 04 nozzles on extensions between rows, 4: spray boom with TJ 60 40 04 nozzles above rows and UB 85 04 nozzles on extensions between rows, 5: row application kits with XR 110 04 nozzles). Different letters indicate statistical differences between data columns ($P < 0.05$).

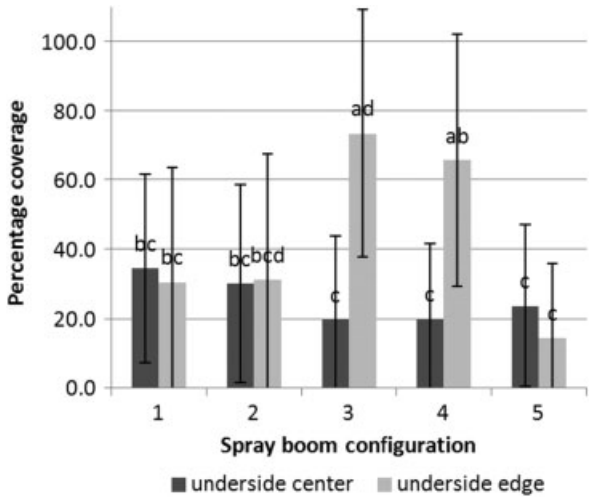

Figure 8. Percentage of coverage (±SD) on the underside of cauliflower leaves in the centre and on the edge of the plants, obtained with the five selected spray boom configurations (1: reference spray boom with XR110 08 nozzles, 2: reference spray boom with XR110 04 nozzles, 3: spray boom with TP 80 04 EVS nozzles above rows and UB 85 04 nozzles on extensions between rows, 4: spray boom with TJ 60 40 04 nozzles above rows and UB 85 04 nozzles on extensions between rows, 5: row application kits with XR 110 04 nozzles). Different letters indicate statistical differences between data columns ($P < 0.05$).

calculations. SPSS Statistics 20 was used for all other calculations. Statistical significance was considered at $P < 0.05$.

3 RESULTS

3.1 Spray application tools test

The coverage of the upper side of leaves was highest for the standard spray boom configuration with XR 110 08 nozzles (C1), and lowest for the row application system (C5) (Fig. 7). With the standard spray boom configuration, no significant difference was found between the 1095 L ha^{-1} (C1) and the 546 L ha^{-1} (C2) application. Except for the row application system C5, all configurations covered more than 50% on average of the water sensitive papers on the upper side of the leaves, both in the centre and at the edge of the plants (Fig. 7). No significant differences between centre and edge of the plant were observed within the different techniques.

The coverage at the underside of the outer leaves, the normal habitat of the young larvae of the cabbage moth, was markedly lower for the standard spray booms (C1 and C2) and for the row application system (C5) than for the spray boom configurations with extensions in the crop (C3 and C4) (Fig. 8). These spray boom configurations managed to cover the underside of the outer leaves for more than 65% on average (73.5% for C3 and 65.7% for C4). The other configurations obtained only less than half of this coverage (30.5%, 31.2% and 14.4% on average for C1, C2 and C5, respectively). Coverage on the underside of centre leaves was less variable over the different techniques than the coverage on the underside of the outer leaves. Coverage here ranged from 19.7% (C3) to 34.4% (C1).

3.2 Attractant test

No significant differences were found between nematode concentrations in the tank, the number of nematodes deposited

Table 3. Tank concentrations, deposition of *Steinernema carpocapsae* on cauliflower leaf discs and survival rates of EPN

Parameter	EPN suspension	EPN + yeast extract
Tank concentration (EPN mL^{-1})	5333 ± 1135	5740 ± 1162
Survival in tank (%)	99.2 ± 0.5	98.9 ± 0.2
Deposition (EPN cm^{-2})	10.4 ± 2.1	9.2 ± 3.8
Survival on leaf disc (%)	98.3 ± 1.7	99.0 ± 1.6

No statistically significant differences were noted ($P < 0.05$).

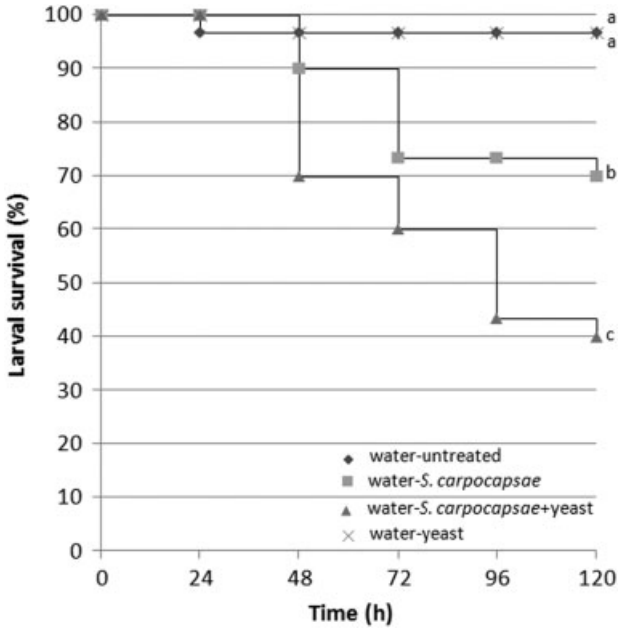

Figure 9. Kaplan–Meier plot of the survival dynamics of second instar larvae of *Mamestra brassicae* (%) in the four sets of Petri dishes. Each dish contained two leaf discs (A and B). Disc A was sprayed with water in every set. Disc B was untreated in set 1, treated with a suspension of *S. carpocapsae* (27 EPN cm^{-2}) in set 2, with a suspension of *S. carpocapsae* (27 EPN cm^{-2}) and brewer's yeast extract (1 g L^{-1}) in set 3 and with a suspension of brewer's yeast extract (1 g L^{-1}) in set 4. Different letters indicate statistical differences between the survival percentages ($P < 0.05$).

on the leaf discs, or between the survival percentages in the tank and those on the leaves (Table 3).

The dynamics of the larval survival of *M. brassicae* is depicted in Fig. 9. Larval survival remained near 100% on average in the Petri dishes without *S. carpocapsae* treatment (water/water and water/yeast) on disc B. In the dishes in which disc B was sprayed with a suspension of *S. carpocapsae* without yeast, the larval survival dropped after 3 days and reached 70% on average after 5 days. When disc B was sprayed with a suspension of *S. carpocapsae* with yeast extract, the larval survival already dropped after 2 days and was only 40%, on average, after 5 days.

No significant differences between the leaf area of discs A and B were observed for the sets without *S. carpocapsae* treated discs, i.e. set 1 (Fig. 10a) and 4 (Fig. 10d). Figure 10b reveals that the *M. brassicae* larvae consumed significantly more from the control discs than from those treated with the *S. carpocapsae* suspension in set 2. In set 3 (Fig. 10c), this behaviour is reversed: discs treated with the *S. carpocapsae* + yeast extract suspension were significantly more damaged than the control ones. From the

Table 4. Percentage of leaf discs (±SD) showing feeding damage, after 24 h in each set of 4 × 10 Petri dishes

Set	Discs	Damaged leaf discs (%)
1	Water	46.7 ± 5.8
	Untreated	50.0 ± 0.0
2	Water	50.0 ± 0.0
	S. carpocapsae	50.0 ± 0.0
3	Water	56.7 ± 25.2
	S.carpocapsae + yeast extract	53.3 ± 28.9
4	Water	46.7 ± 11.5
	Yeast extract	56.7 ± 5.8

Leaf discs (diameter 3 cm) were sprayed with a spray boom fitted with Teejet XR 110 04 nozzles spaced 50 cm apart, 50 cm above the leaf discs, at a speed of 4 km h^{-1} and at a pressure of 4 bar. Brewer's yeast extract was applied at 1 g L^{-1}. *S carpocapsae* were applied at 27 EPN cm^{-2} on the sprayed surface. No statistically significant differences were noted ($P < 0.05$).

measurements of the summed leaf areas of the discs A and B within the same Petri dishes (Fig. 11), it can be concluded that the leaf discs in set 3 (i.e. the set in which the B discs were treated with the *S. carpocapsae* + yeast extract suspension) were least damaged, followed by the leaf discs in set 2 (B discs treated with *S. carpocapsae* suspension). Almost all of the leaf area was consumed in set 4 (B discs treated with yeast extract suspension) and set 1 (B discs untreated).

Leaf preference, measured as the percentage of damaged leaf discs after 24 h, was not influenced by the treatment of disc B in the different sets (Table 4). The digital images taken of the leaf discs at later sample times showed that the *M. brassicae* larvae generally stayed at the leaf disc chosen after 24 h until the disc of their choice was completely consumed (not shown). Infected larvae often died before the disc of their choice was completely consumed.

3.3 Field trial

The temperature of the spray liquid containing *S. carpocapsae* ranged between 16 °C and 23 °C. Spray liquid temperatures did not differ more than 2 °C between measurements made before and after a single treatment.

Survival of *S. carpocapsae* in the tank ranged between 94.1% and 96.9% on average and did not significantly differ between treatments (data not shown). Survival of *S. carpocapsae* was not significantly affected by the passage through the pump during treatments (data not shown). Relative deposition on the upper side of the leaves ranged between 13.9% and 18.4% on average over the treatments. Relative deposition on the underside was much more variable within and between treatments and varied between 3.0% and 21.3% on average (results not shown). Due to the large variation between the repeated observations, no significant differences were recorded between depositions on the same side of the leaves between different treatments.

The average percentage of marketable cauliflower heads and the average leaf damage scores per treatment are shown in Table 5. Both treatments with *S. carpocapsae*, Addit and xanthan gum and both treatments with Bt resulted in a statistically higher proportion of marketable cabbage heads, in comparison to the control. The leaf damage was not significantly affected by any of the treatments. The treatments with *S. carpocapsae* without

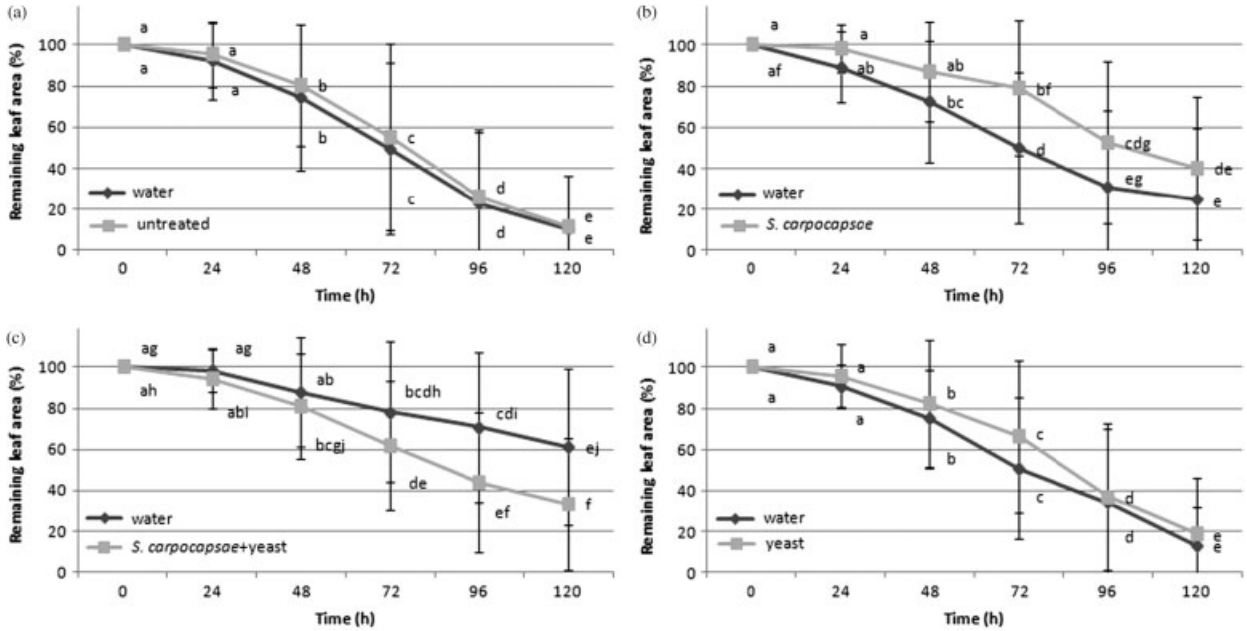

Figure 10. Decrease over time of the leaf area (±SD) of cauliflower discs (3 cm^2 diameter) exposed to two treatments. (a) Control discs (A) treated with water vs. untreated B discs. (b) Control discs (A) treated with water vs. B discs treated with an *Steinernema carpocapsae* (27 EPN cm^{-2}) suspension. (c) Control discs (A) treated with water vs. B discs treated with an *S. carpocapsae* (27 EPN cm^{-2}) + yeast extract (1 g L^{-1}) suspension. (d) Control discs (A) treated with water vs. B discs treated with a yeast extract (1 g L^{-1}) suspension. Different letters indicate statistical differences between data points within the same graph ($P < 0.05$).

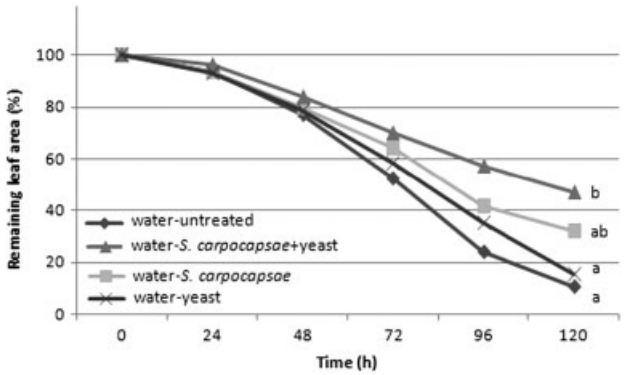

Figure 11. Comparison between sets of the evolution of the total remaining leaf area *summed per Petri dish*. Different letters indicate statistical differences between the evolution of the remaining leaf area of different treatments over the 0–120 h period ($P < 0.05$).

adjuvants and with *S. carpocapsae* combined with yeast extract also resulted in a higher proportion of marketable cabbage heads, although not significantly different from the control.

Due to the large variation of the measurements, the average numbers of L3–L4 and L5–L6 at specific sample dates were statistically similar for all treatments (Fig. 12a and b). The total number of L3–L4 peaked at the second sampling date. One week later, the total number of L5–L6 was at its highest level.

4 DISCUSSION

This research shows an alternative approach to the conventional control of *M. brassicae* in cauliflower. Such an alternative is needed, due to the potential for insecticide resistance and unwanted side effects encountered with synthetic pesticides, and due to the relatively low susceptibility of *M. brassicae* to Bt (*vide supra*).

4.1 Spray application tools test

The choice of a spray application technique suitable to control *M. brassicae* in cauliflower depends on the life stages of the insect targeted. It is known that most (>95%) of the feeding by *M. brassicae* larvae is done by L5 and L6.[34] These stages bore towards and into the cabbage head, causing economic damage. All earlier larval stages (L1–L4) cause less economic damage in cauliflower, since they mostly reside on the outer leaves, especially on the underside of these leaves.[24,35] Based on this information, we decided to select an application technique suitable for the control of the L1–L4 of *M. brassicae* in order to prevent economic damage caused by L5 and L6.

Our results show that the row application system (C5) and the standard spray booms (C1 and C2) have little potential for providing a good coverage of the lower side of cauliflower leaves. However, both spray boom configurations with extensions (C3 and C4) covered the lower side of the outer leaves very well (C3, 73.5%; C4, 65.7%). These configurations also secured statistically equal coverage of the underside of the centre leaves, when compared to all other configurations. They are therefore equivalent options for the control of *M. brassicae* at this target zone. For the above reasons, C3 was selected for the field trial with *S. carpocapsae*.

4.2 Attractant test

The addition of yeast extract to the suspension of *S. carpocapsae* clearly increased the mortality of L2 of *M. brassicae*. The consumption by the insect of leaf discs sprayed with a suspension of nematodes without additional yeast was visibly lower than the consumption of the untreated discs. This suggests that insect larvae are aware of the presence of *S. carpocapsae* by the cues emitted by the nematodes and consume less of the leaves, thereby preventing contact with their antagonist. When yeast was added to the nematode suspension, leaf consumption by the insect larvae was noticeably higher on the EPN–yeast sprayed discs than on the

Table 5. Numbers and odds ratios (+ confidence intervals) of marketable and damaged cabbage heads and damage scores (±SD) per treatment.

Nr	Treatment	Spray application	Marketable/damaged cabbage heads		Odds ratio	95% Confidence interval	Leaf damage score
1	Water (control)	C3	43	29	1.00[a]		6.0[a] ± 1.2
2	*Steinernema carpocapsae*	C3	47	25	1.27[a]	0.65, 2.49	6.5[a] ± 0.6
3	*S. carpocapsae* + yeast extract	C3	53	19	1.88[a]	0.93, 3.81	6.3[a] ± 0.5
4	*S. carpocapsae* + Addit + xanthan gum + yeast extract	C3	55	16	2.32[b]	1.12, 4.81	6.3[a] ± 0.5
5	*S. carpocapsae* + Addit + xanthan gum	C3	54	17	2.14[b]	1.04, 4.40	6.3[a] ± 0.5
6	Bt	C3	60	10	4.05[b]	1.79, 9.17	7.3[a] ± 0.5
7	Bt	C2	57	14	2.75[b]	1.30, 5.82	6.0[a] ± 0.8

Damage was scored per plot, on a scale from 1 (severe damage) to 9 (no damage).
[a,b]Different superscriptletters indicate statistical differences between rows ($P < 0.05$).
Bt, *Bacillus thuringiensis* toxin.

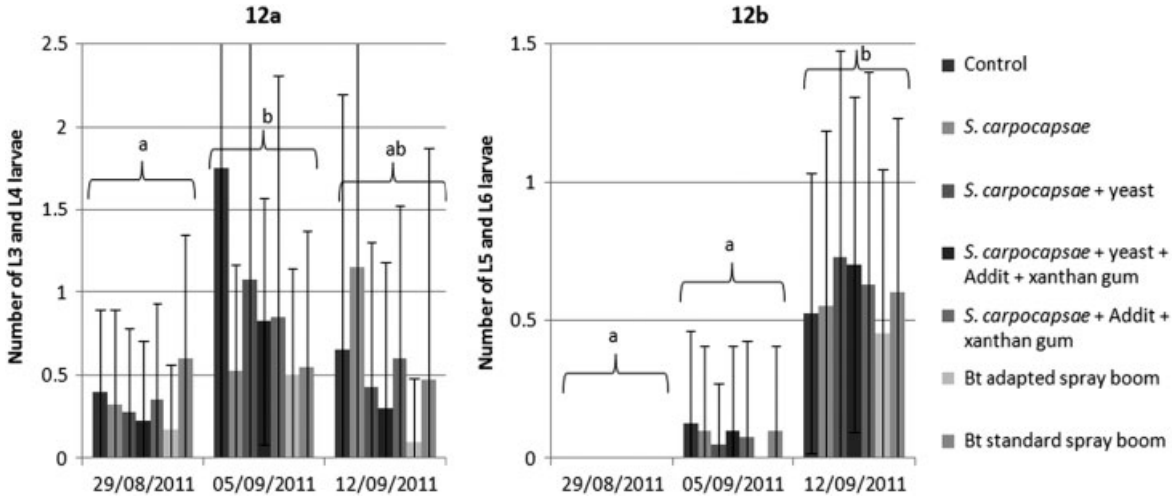

Figure 12. Number of L3 and L4 larvae (a) and L5 and L6 larvae (b) of *Mamestra brassicae* per cauliflower plant as influenced by different treatments and time. All treatments used 1170 l water ha^{-1} and were sprayed at 4 bar. All treatments except for both treatments including Bt were applied twice a week and six times in total. Both treatments with Bt were applied once a week and three times in total. All treatments, except for one treatment with Bt, were applied with a spray boom fitted with TeeJet TP 80 04 EVS nozzles above the crop rows, and with vertical extensions, ending in two sideward spraying TeeJet UB 85 04 nozzles between rows. One Bt treatment was applied with a standard spray boom fitted with TeeJet XR 110 04 nozzles. Active ingredients and additives were sprayed at the following concentrations: *Steinernema carpocapsae* at 58 EPN cm^{-2} of ground surface, brewer's yeast extract at 1 g L^{-1}, Addit at 2.5 mL L^{-1}, xanthan gum at 0.3 g L^{-1}, Bt at 1 kg ha^{-1}. Different letters indicate statistical differences between the total number of L3–L4 over all treatments at different sample times ($P < 0.05$).

control ones. However, when yeast extract was sprayed without *S. carpocapsae*, leaf consumption did not differ from the control. These are desirable traits from a crop protection point of view, but require more research to elucidate the mechanism behind these effects. Yeast extract is an important ingredient in the artificial feed of EPN[36,37] and of *M. brassicae*,[38] so the source of the effects of yeast extract on the mortality of *M. brassicae* could derive from an interaction with the EPN as well as with the larvae. It is clear, however, that yeast extract has positive effects on the control of the cabbage moth: adding yeast to an EPN spray suspension effectively increases insect mortality (Fig. 9), and thereby also decreases total leaf consumption (Fig. 11). Therefore, the practical use of yeast extract was tested in the field trial.

4.3 Field trial

The spray application equipment was well suited for applying *S. carpocapsae* on foliage for several reasons. First, the temperature in the tank remained well below 37 °C, the temperature above which detrimental effects on the survival of *S. carpocapsae* can occur.[39] Second, the high survival percentage of *S. carpocapsae* in the tank samples proved that the mixing system is suitable for *S. carpocapsae* applications. Third, the spray pressure (4 bar) was well below 20 bar, the advised upper pressure limit for spraying *S. carpocapsae*.[40] Fourth, the *S. carpocapsae* suspension did not clog the nozzles. Finally, the nozzles did not cause mortality to *S. carpocapsae*.

Due to the high variability between the numbers of caterpillars counted per plant, no significant differences could be noted between these numbers per treatment. However, the data on the numbers of damaged cabbage heads clearly demonstrate a protective effect of spraying with a suspension of *S. carpocapsae*, when the nematodes are combined with the retention adjuvants Addit and xanthan gum. Brewer's yeast extract seems to add a small additional protection, although this effect could not be proven statistically. It is noticeable that Bt applications outperform all other treatments. However, the difference in marketable/damaged cabbage heads between Bt treatments, the treatment with *S. carpocapsae* + retention adjuvants and the treatment with *S. carpocapsae* + retention adjuvants + yeast, could not be proven

statistically. Since Bt was only applied three times, unlike *S. carpocapsae*, which was applied six times, and because EPN are far more expensive than Bt, the use of the bacterium should still be considered as the preferred biological control option. However, if the efficacy of EPN treatments can be enhanced, EPN applications might be fitted into biological control schemes to prevent resistance development of *M. brassicae* against Bt. Resistance to Bt has not yet been reported for *M. brassicae*, but it is already documented for several other Lepidoptera: the Indian meal moth, the diamondback moth and the cabbage looper.[41] On the other hand, *M. brassicae* seems to possess some innate resistance against Bt products: the susceptibility of *M. brassicae* to one of the toxins of Bt (Cry1Ac) is low: 2000 times lower than the susceptibility of *Pieris brassicae*, cultured cell lines of *M. brassicae* are relatively insensitive to the Cry1C toxin and the dose of *B. thuringiensis* kurstaki required to kill 30–40% of *M. brassicae* larvae is approximately 20 times greater than that required to kill a similar percentage of *Lacanobia oleracea* L. larvae.[22,41,42]

5 CONCLUSIONS

Current research shows that there is room for improvement in the foliar application of *S. carpocapsae* against *M. brassicae*. EPN are some of the most expensive active ingredients used for insect control, so an efficient delivery of these EPN from the spray tank to the target pest is of key importance.[43] Research is still needed to make *S. carpocapsae* applications as effective as Bt applications against the cabbage moth. Furthermore, end-user education about the specific problems encountered with the application of these EPN (e.g. sedimentation in the tank suspension, damage by temperature increase due to extensive tank recirculation, drought sensitivity of EPN on foliage[39,44]) is badly needed. When these conditions are met, spray applications with *S. carpocapsae* can be fitted into biological control schemes to prevent resistance problems of *M. brassicae* to Bt.

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