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Foliar applied entomopathogenic nematodes *Steinernema feltiae* are not suitable for controlling *Thrips tabaci* in leek

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Abstract

Two field trials in leek were set up to determine and, if possible, improve the effect of spray applications of *Steinernema feltiae* (Filipjev) Wouts, Mracek, Gerdin et Bedding against *Thrips tabaci* Lindeman. The first trial focused on selecting a suitable application technique and on testing the effect of an attractant on thrips control in leek. The second trial focused on incorporating entomopathogenic nematodes (EPN) in a conventional insecticide scheme, and on the effect of mixing a surfactant in the spray suspension. *S. feltiae* proved to be ineffective against the foliar inhabiting life stages of *T. tabaci*, despite adding a surfactant and/or an attractant to the spray suspension and despite adapting the equipment. Compared with a traditional spray boom, a row application technique ensured a more even EPN deposition on the upper side and underside of both old and new leek leaves. This technique may improve the applications of fungicides and contact insecticides in leek.

Key words: entomopathogenic nematodes, biological control, application technique, adjuvants, *Steinernema feltiae*, *Thrips tabaci*.

Introduction

During the past two decades, the economic importance of onion thrips, *Thrips tabaci* Lindeman (Thysanoptera Thripidae), has risen, because the species has developed resistance against various insecticides (e.g., pyrethroids, organophosphates and carbamates) (Gao *et al.*, 2012), because it is spreading plant pathogens (e.g., *Alternaria porri*, Iris Yellow Spot Virus, Tomato Spotted Wilt Virus) (Rueda and Shelton, 1995; Drees and Jackman, 1999; Jenser *et al.*, 2003), and, bearing global warming in mind, since it produces more generations at higher temperatures (Bergant *et al.*, 2004; Diaz-Montano *et al.*, 2011).

These factors have stimulated research towards biological control alternatives like entomopathogenic nematodes (EPN), microscopically small roundworms that are a cross-over between insect parasitoids and pathogens (Beck *et al.*, 2013c). Past research on controlling thrips with EPN has mainly been directed against the western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera Thripidae) (Buitenhuis and Shipp, 2005; Trdan *et al.*, 2007). Since EPN are soil-dwelling organisms, which are not accustomed to drought and light, most research focused on controlling the soil-dwelling life stages of WFT (Beck, 2013). However, due the widening knowledge on application technology and formulations for EPN, nematode applications are becoming feasible against some foliar pests (Laznik *et* al., 2011; Lanzoni *et al.*, 2014).

Previous research has shown that the use of various species and strains of steinernematids and heterorhabditids against soil-dwelling prepupae and pupae produced low and inconsistent control results (Tomalak *et al.*, 2005; Georgis *et al.*, 2006). Foliar applications on the other hand, which are directed against adults and young nymphs of thrips, proved more promising (Tomalak *et al.*, 2005; Georgis *et al.*, 2006).

Thrips are an attractive goal for foliar EPN applications for two reasons: (1) since thrips mostly reside in cryptic habitats (in flowers, flower buds, growing points of plants, etc.), relatively large biocontrol organisms like predatory bugs may have difficulties reaching the enclosed feeding areas where thrips tend to accumulate (Arthurs and Heinz, 2006). EPN are much smaller, which makes them more suitable to reach these target areas. (2) The cryptic habitats may offer protection from harmful environmental conditions, namely UV radiation and drought (Shapiro-Ilan *et al.*, 2006; Jung, 2008). These habitats are, however, not easy to reach with conventional spraying equipment. The use of a wetting agent seems to be essential to spread the EPN into the growing points of plants (Georgis *et al.*, 2006).

Repeated applications seem to be a key factor for successful control of WFT with EPN (Trdan *et al.*, 2007). This statement is corroborated by other studies (Tomalak, 1994; Belay *et al.*, 2005), stating that EPN can kill WFT, but that they cannot reproduce inside this (and probably other) thrips species due to the small host size. Since EPN can only stay infective for a limited period on foliage (Brusselman *et al.*, 2012a), repeated applications are necessary to combat new generations.

Three studies examining the potential of EPN against *T. tabaci*, were found in literature. A laboratory study (Saffari *et al.*, 2013) showed that an isolate of *Steinernema feltiae* (Filipjev) Wouts, Mrácek, Gerdin et Bedding (Rhabditida Steinernematidae) has high potential to infect the soil-dwelling life stages of *T. tabaci*. A field study (Jung, 2008) showed good possibilities for foliar applications of *S. feltiae* against *T. tabaci* on three outdoor crops: *viz.* onion, leek and chives. In the leek trial, the number of thrips was reduced by over 40%

when compared to the control and the individual weight of plants receiving six applications of a commercial product of *S. feltiae* (Nemaplus®, E-Nema, Germany) was 20% higher than the individual weight of control plants. In a greenhouse test with *Heterorhabditis indica* against *T. tabaci* in sweet pepper, comparing both foliar and soil applications (Al-Siyabi *et al.*, 2006), both applications reduced the number of adult *T. tabaci* on sticky traps. Results of this study seemed to favour soil applications, showing less adults on the sticky traps in the soil treatment than in the foliar treatment. These results should, however, be handled with caution, since no direct damage assessment was carried out on the crop. A foliar application killing as much thrips is probably more effective at reducing crop damage than a soil application, since soil applications can only kill prepupae and pupae of thrips, while leaving the damaging L1 and L2 nymphs unscathed (Arthurs and Heinz, 2006). Furthermore, the EPN were sprayed without a wetting agent, which might have caused low nematode deposition and/or limited spreading of the EPN on the plants, thereby hampering control results.

Considering the above and keeping in mind that onion thrips have become the most important pest of leek crops in southern and western Europe (Brusselman, 2010), two field trials in leek were set up to determine and, if possible, to improve the effect of spray applications of *S. feltiae* against *T. tabaci* in leek. The first trial was carried out on an organic farm and focused on selecting a suitable spray application technique and on testing the effect of an attractant on thrips control. The second trial focused on incorporating spray applications of EPN in a conventional insecticide scheme, and on the effect of mixing the surfactant Addit in the spray suspension on the control of *T. tabaci*.

Materials and methods

Organic trial

Experimental setup

Leek seeds, cv. Antiope (Syngenta, Basel, Switzerland), were sown on March 15th, 2012 in a 5-cm layer compost on a field at the experimental farm of Inagro (Rumbeke-Beitem, Belgium: 50°54'16.0"N 3°07'39.3"E, 21 m a.s.l.). The plants were transplanted on June 6th on a field with a sandy loam soil. Plants were spaced 10 cm in the crop row and 70 cm between rows. The experiment was arranged in a randomized complete block design with four replicates per treatment. Plots were 11.0 m long and 4.2 m wide with 660 plants per plot. No fungicides were applied throughout the trial, to avoid an effect of these fungicides on the measured parameters in the experiment. All plots were harvested on October 23rd.

The nematode used in this experiment was a commercial strain of *S. feltiae* (Entonem®, Koppert B.V., Berkel en Rodenrijs, The Netherlands). Infective juveniles of *S. feltiae* were applied as foliar sprays at a rate of 6×10^6 IJ/l suspension and a volume application rate of 800 l/ha. This corresponds with a theoretical deposition of 48 IJ/cm² of ground surface. In all EPN treatments, the surfactant Addit (Koppert B.V.) was added at a rate of 2.5 ml per l of spray suspension (Beck *et al.*, 2013a; 2013b). EPN applications were performed with a mounted sprayer (Delvano NV, Hulste, Belgium), with a tank capacity of 200 l. This spraying machine was equipped with a diaphragm pump with a flow rate of 72 l/min (AR 70 BP/C, Annovi Reverberi, Modena, Italy). Different spray boom configurations were used in the field trial, as discussed below. One EPN treatment also included the attractant Biosweet (Biobest, Westerlo, Belgium), i.e. a sugar solution that is marketed for improvement of thrips control. Two control treatments were included: (1) an untreated control and (2) foliar sprays with water, the wetting agent Addit and the attractant Biosweet. As a reference treatment, four plots were sprayed with spinosad (Conserve Pro®, Dow Agrosciences). All treatments with spinosad occurred in the evening, to avoid breakdown of spinosad through photolysis. Spinosad is known to kill thrips within one to three days after contact or ingestion, and has up to two weeks of residual activity (Cloyd, 2009). The spinosad-treated plots were sprayed three times with a 2- or 3-week interval. The first application happened one week later than the EPN applications. All seven treatments are listed in table 1.

The nematode applications started on August 2nd. The next two months, EPN were applied weekly. EPN were applied in the evening, to avoid heat inactivation. In the first week of October, no spray applications were carried out due to stormy weather conditions. On October 10th (= day 70 after the first applications were made), EPN were applied for the last time. A total of 10 EPN applications were made on the appropriate plots.

Table 1. Overview of all applied treatments and treatment days in the organic thrips trial. All treatments were applied with 800 l of spray suspension per ha. Treatments started on August 2nd, 2012 (= day 1).

Nr	Treatment	Application rate	Application technique	Treatment days
1	untreated (control)	-	-	-
2	*S. feltiae* + Addit[a]	48 IJ/cm^2	C1, speed: 4.7 km/h	1, 7, 15, 22, 29, 35, 42, 50, 56, 70
3	*S. feltiae* + Addit[a]	48 IJ/cm^2	C2, speed: 3.4 km/h	1, 7, 15, 22, 29, 35, 42, 50, 56, 70
4	*S. feltiae* + Addit[a]	48 IJ/cm^2	C3, speed: 10.2 km/h	1, 7, 15, 22, 29, 35, 42, 50, 56, 70
5	*S. feltiae* + Addit[a] + Biosweet[b]	48 IJ/cm^2	C1, speed: 4.7 km/h	1, 7, 15, 22, 29, 35, 42, 50, 56, 70
6	Addit[a] + Biosweet[b]	-	C1, speed: 4.7 km/h	1, 7, 15, 22, 29, 35, 42, 50, 56, 70
7	Spinosad	96 g/ha	C1, speed: 4.7 km/h	7, 22, 42

[a] Addit: 2.5 ml/l of spray suspension; [b] Biosweet: 5.0 ml/l of spray suspension.

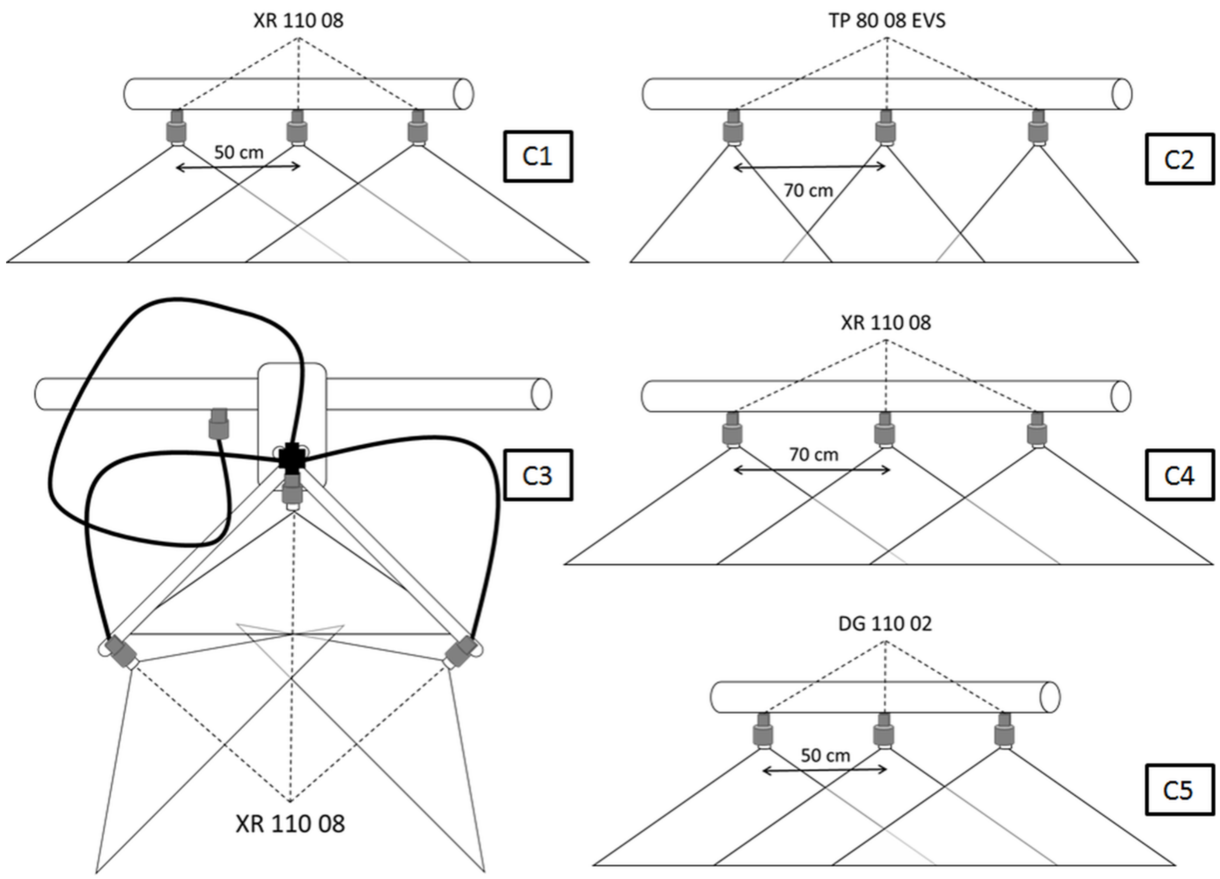

Figure 1. Scheme of all spray boom configurations: standard broadcast spray boom configuration C1: TeeJet XR 110 08 nozzles (seven in total) were mounted on a standard spray boom with 50 cm nozzle spacing; band spray boom configuration C2: TeeJet TP 80 08 EVS band spray nozzles (six in total) were mounted on a standard spray boom with 70 cm nozzle spacing; spray boom configuration C3: three TeeJet XR 110 08 nozzles were mounted on a TeeJet row application kit. The row application kits were spaced 70 cm apart. The complete spray boom consisted of three row application kits; spray boom configuration C4: TeeJet XR 110 08 nozzles (four in total) were mounted on a standard spray boom with 70 cm nozzle spacing. Spray screens (not shown) were mounted on both ends, to avoid spraying nearby fields; spray boom configuration C5: TeeJet DG 110 02 nozzles (five in total) were mounted on a standard spray boom with 50 cm nozzle spacing. Spray screens (not shown) were mounted on both ends, to avoid spraying nearby fields.

Spray boom configurations

Three foliar spray application techniques were tested in this field experiment: (1) the standard broadcast application (C1, figure 1), (2) a band spray application (C2, figure 1), and (3) a row spray application (C3, figure 1).

The standard spray boom was equipped with TeeJet XR 110 08 extended range flat fan nozzles (TeeJet Technologies, Wheaton, USA) with 50 cm nozzle spacing. The control and the reference treatment were also sprayed with this standard equipment. On the band spray boom, TeeJet TP 80 08 EVS band nozzles with 70 cm nozzle spacing were positioned directly above the crop row. The spray boom for row application was equipped with three TeeJet XR 110 08 flat fan nozzles mounted on TeeJet row application kits: one central nozzle spraying directly downwards and two nozzles spraying downwards at an angle of 45° towards the central nozzle. The row application kits were spaced 70 cm apart with the central nozzle positioned above the crop row. All spray boom configurations functioned at a rather low spray pressure of 3.0 bar, to promote formation of bigger droplets, which can more easily penetrate into the shaft of the leek plants. The speed was adjusted for each application in order to attain an application rate of 800 l/ha.

Evaluation methodology

Throughout the experimental period, temperature and rainfall were registered at a nearby KMI/RMI weather station (50°53'30.2"N 3°06'53.5"E, 29 m a.s.l.). On the first four treatment dates, two tank samples were taken of the EPN suspension: one just before spraying and one at the end of all spray applications with EPN. From these samples, three 50-µl subsamples were taken to count the number of live and dead nematodes, to calculate the concentration of EPN in the tank and to check if the agitation system caused damage to the EPN.

Starting on July 31st, one week after the first spray treatments, five clusters of four plants were harvested per plot from the four middle rows. On each of these 20 plants the percentage of leaf area damaged by onion thrips was assessed through visual (non-destructive) examination of the green parts of all leaves on each plant. Based on the damage found on these green parts, a damage severity score was given according to the following logarithmic 9-point rating scale: 1 = 0 − 1% green leaf surface damaged by thrips; 2 = 1 – 3%; 3 = 3 − 6%; 4 = 6 – 10%; 5 = 10 – 15%; 6 = 15 – 25%; 7 = 25 – 40%; 8 = 40 – 60%; 9 = more than 60%. This scale provides sufficient detail at the low end of the damage spectrum. It was developed in accordance with the Weber-Fechner law. This law states that an observer's ability to see differences decreases by the logarithm of the intensity of the stimulus (Bowen, 2008). In this case: the stimulus is the percentage of leaf area covered with thrips damage. Logarithmic rating scales are most often used in determining the severity of diseases (e.g. rust) on plants, but are also very useful for determining the damage done by foliage damaging insects such as thrips.

Subsequently, the number of live 2nd stage instar and adult thrips were counted on each of the harvested leek plants by destructive sampling (i.e. peeling the leaf sheaths one by one of the plants). These measurements were repeated on a weekly basis, until October 17th, a week before harvest. After harvest, damage rating measurements were also carried out on a random set of 20 harvested and cleaned plants per plot. Thrips counts were not performed on these cleaned plants.

On the last treatment day, October 10th, the absolute and relative deposition (i.e. the percentage of the expected deposition of 48 IJ/cm^2) on the leek leaves was measured by attaching leek leaf discs (⌀ 3 cm) (Brusselman *et al.*, 2012b) to both the upper side and underside of one young leaf and one older leaf of three plants per plot, in all plots receiving EPN treatments. All leaf discs were attached as close to the stalk as possible, without touching other nearby leaves. After spraying, the EPN deposited on these leaf discs were washed off into a Petri dish and were counted, and the relative deposition was calculated and compared between treatments.

At harvest, the marketable yield (in kg/ha) and the total weight of 60 plants per plot were assessed, to see if the treatments against thrips had any effect on the size (= diameter) of the leek stalks and on the weight of the harvest, since both factors have commercial implications for the farmer. These 60 harvested plants were divided into four different stalk size categories (< 2 cm, 2-3 cm, 3-4 cm, > 4 cm), categories which are also used for commercial purposes in Belgium. All plants of one plot that fell in one stalk size category were weighed together.

Conventional trial

Experimental setup

Leek plants cvs Harston and Aylton (Nunhems BV, Nunhem, the Netherlands) were planted July 27th, 2012 on a field with a sandy loam soil (50°54'16.9"N 3°7'21.7"E, 19 m a.s.l.). Plants were spaced 10 cm apart in the row and 65 cm between rows. The experiment was arranged in a randomized complete block design with four replicates per treatment. Replicates 1 and 2 were planted with cv Harston, while replicates 3 and 4 were planted with cv Aylton. Plots were 11.0 m long by 2.6 m wide with 440 plants per plot. All plots were harvested on February 18th.

The nematode used in this experiment was a commercial strain of *S. feltiae* (Entonem®, Koppert B.V., Berkel en Rodenrijs, The Netherlands), the same species and strain as used in the organic trial. Infective juveniles of *S. feltiae* were applied by foliar sprays at 6 × 10^6 IJ/l suspension and a volume of 800 l/ha. This corresponds with a theoretical EPN deposition of 48 IJ/cm² of ground surface.

Eight treatments were selected for this test (table 2). In the control treatment water was sprayed at 800 l/ha. The reference treatment alternately sprayed spinosad (Tracer®, Dow Agrosciences), abamectin (Vertimec®, Syngenta) and methiocarb (Mesurol® SC 500, Bayer CropScience),

Table 2. Overview of all applied treatments and treatment days in the conventional thrips trial. Treatments started on August 7th, 2012 (= day 1).

Nr	Treatment	Application rate	Application technique	Treatment days
1	water (control)	800 l/ha	C4, speed: 3.4 km/h	1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 74
2	spinosad (s), abamectin (a), methiocarb (m)	96 g/ha 9 g/ha 750 g/ha	C5, speed: 1.2 km/h C5, speed: 1.2 km/h C5, speed: 1.2 km/h	1 (s), 15 (a), 29 (m), 43 (s), 57 (a), 74 (m)
3	spinosad (s), abamectin (a), methiocarb (m), *S. feltiae* (EPN)	96 g/ha 9 g/ha 750 g/ha 48 IJ/cm^2	[a]C5, speed: 1.2 km/h [a]C5, speed: 1.2 km/h [a]C5, speed: 1.2 km/h [b]C4, speed: 3.4 km/h	1 (s), 15 (EPN), 29 (m), 43 (EPN), 57 (a), 74 (EPN)
4	spinosad (s), abamectin (a), methiocarb (m), water (w)	96 g/ha 9 g/ha 750 g/ha -	[a]C5, speed: 1.2 km/h [a]C5, speed: 1.2 km/h [a]C5, speed: 1.2 km/h [b]C4, speed: 3.4 km/h	1 (s), 15 (w), 29 (m), 43 (w), 57 (a), 74 (w)
5	*S. feltiae*	48 IJ/cm^2	C4, speed: 3.4 km/h	1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 74
6	*S. feltiae* + Addit[c]	48 IJ/cm^2	C4, speed: 3.4 km/h	1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 74
7	Addit[c]	-	C4, speed: 3.4 km/h	1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 74
8	*S. feltiae*	48 IJ/cm^2	C4, speed: 3.4 km/h	1, 15, 29, 43

[a] used for application of chemicals; [b] only used for application of *S. feltiae* or water; [c] Addit: 2.5 ml/l of spray suspension.

with two weeks between applications. Treatments 3 and 4 replaced three of the six chemical insecticide applications either by applications of *S. feltiae* or water. Treatments 5, 6 and 7 were weekly applications of *S. feltiae*, *S. feltiae* + Addit and Addit, respectively. The *S. feltiae* applications in treatment 8 were timed according to a warning system that uses temperature data and sticky traps population measurements.

Spray boom configurations

All applications containing EPN and/or Addit and all applications spraying solely water, were carried out with a spray boom fitted with TeeJet XR 110 08 nozzles with 70 cm nozzle spacing (C4, figure 1). This boom sprayed at 3.0 bar and at a speed of 3.4 km/h. All applications with spinosad, abamectin or methiocarb, were carried out with a spray boom fitted with TeeJet DG 110 02 nozzles, with 50 cm spacing between two nozzles, spraying at 3.0 bar and at a speed of 1.2 km/h (C5, figure 1). At these speeds both spray boom configurations sprayed 800 l/ha. The ends of both spray booms were fitted with spray screens mounted perpendicular to the spray boom, to avoid spraying adjacent fields.

Evaluation methodology

Throughout the experimental period, temperature and rainfall were registered by the same weather station as in the organic trial. The thrips population assessment on the plants and the leaf damage assessment were carried out in the same way as in the organic trial. These observations started on August 6th and ended on October 22nd. On the last treatment day, October 19th, the absolute and relative deposition (i.e. the percentage of the expected deposition of 48 IJ/cm^2) on the leek leaves was measured in treatments 5 (*S. feltiae*, weekly) and 6 (*S. feltiae* + Addit, weekly). This was done in the same way as in the organic trial.

Statistical analysis

All statistical analyses were completely analogous in both trials. The tank concentrations and the survival percentages of the EPN before and after applications were compared with a one-way ANOVA. Absolute and relative deposition of EPN were compared between treatments using a one-way ANOVA; comparisons between specific treatments were made with the non-parametric Dunnett T3 test. The effect of the treatment on both the number of thrips per plant and the measured damage scale per plant was analysed every week by a Kruskal-Wallis test followed by a Dunn's test for multiple comparisons. SPSS Statistics 21 was used for all calculations. Statistical significance was considered at $P < 0.05$. The marketable yield and the weights of leek stalks in the four size categories were compared between treatments with a one-way ANOVA. SPSS Statistics 21 was used for all calculations. Statistical significance was considered at $P < 0.05$.

Results

Organic trial

Average daily temperatures and precipitation quantities are detailed in figure 2. During the trial, the absolute maximum (shaded) temperature of 33.4 °C was recorded on August 18th. The absolute minimum (shaded) temperature of 2.2 °C was recorded on October 11th.

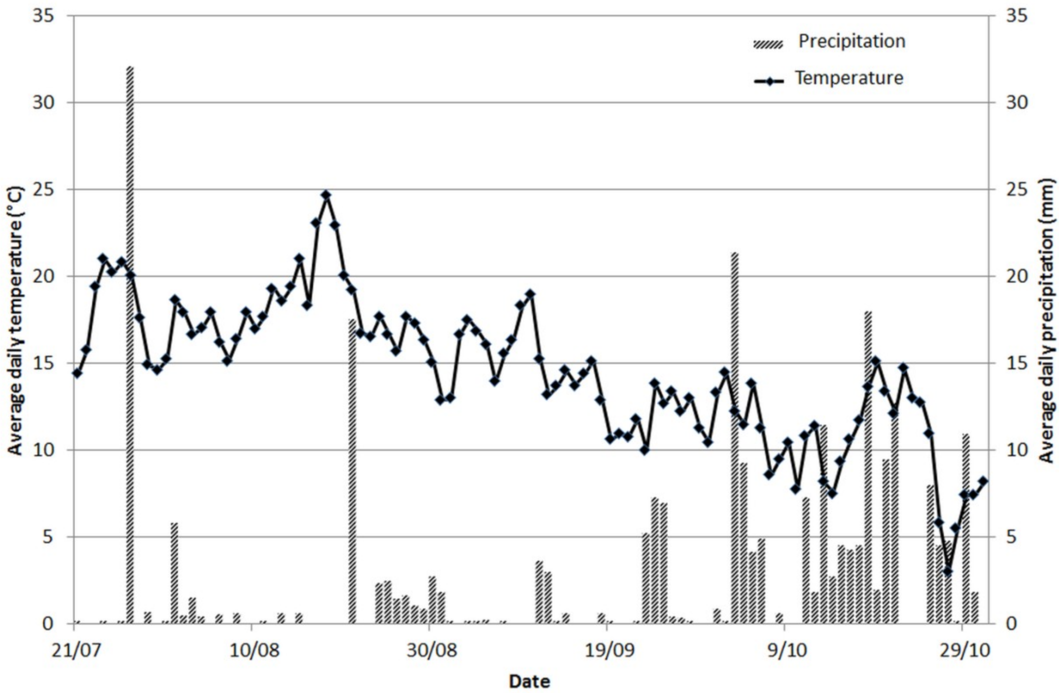

Figure 2. Average daily temperature and precipitation data for both trials. Data source: KMI/RMI weather station, Beitem.

Table 3. Deposition of infective juveniles (IJ) of *S. feltiae* in the organic (O) and conventional (C) trial. Deposition was measured on leaf disc collectors, expressed in absolute terms (IJ/cm²) and relative to the theoretical deposition per unit of ground surface (% ± SD). Different letters indicate significant differences within columns between treatments of the same experiment on all collector positions located on the same side of the plant (P < 0.05).

O/C	Nr	Treatment	Application technique	Collector position	Absolute deposition (IJ/cm² ± SD)				Relative deposition (% ± SD)			
O	2	*S. feltiae* + Addit	C1	upper side old leaf	7.2	±	4.7	ad	15.5	±	10.0	ac
				upper side new leaf	15.8	±	4.6	b	33.7	±	9.8	b
O	3	*S. feltiae* + Addit	C2	upper side old leaf	18.5	±	11.8	bcd	39.5	±	25.3	bcd
				upper side new leaf	10.8	±	9.8	ab	23.2	±	21.0	ab
O	4	*S. feltiae* + Addit	C3	upper side old leaf	11.5	±	6.6	ab	24.7	±	14.1	ab
				upper side new leaf	10.9	±	8.3	ab	23.3	±	17.7	ab
O	5	*S. feltiae* + Addit + Biosweet	C1	upper side old leaf	6.5	±	5.6	ac	17.3	±	14.8	ad
				upper side new leaf	15.6	±	5.8	b	41.5	±	15.3	bcd
O	2	*S. feltiae* + Addit	C1	underside old leaf	4.5	±	2.8	a	9.7	±	5.9	acd
				underside new leaf	1.0	±	1.0	abd	2.2	±	2.2	a
O	3	*S. feltiae* + Addit	C2	underside old leaf	5.4	±	8.2	ab	11.6	±	17.5	aef
				underside new leaf	1.0	±	1.8	abe	2.2	±	3.8	a
O	4	*S. feltiae* + Addit	C3	underside old leaf	13.3	±	9.0	abc	28.5	±	19.3	bdf
				underside new leaf	4.8	±	7.7	cde	10.2	±	16.4	ab
O	5	*S. feltiae* + Addit + Biosweet	C1	underside old leaf	4.0	±	2.4	a	10.7	±	6.4	bce
				underside new leaf	1.0	±	1.0	c	2.7	±	2.7	a
C	5	*S. feltiae*, weekly		upper side old leaf	16.3	±	4.0	a	33.2	±	8.3	a
				upper side new leaf	12.7	±	4.4	a	25.9	±	9.1	a
C	6	*S. feltiae* + Addit, weekly		upper side old leaf	15.5	±	5.1	a	31.1	±	10.3	a
				upper side new leaf	11.2	±	6.5	a	22.6	±	13.2	a
C	5	*S. feltiae*, weekly		underside old leaf	2.7	±	2.0	a	5.4	±	4.0	a
				underside new leaf	3.4	±	1.9	ab	7.0	±	3.9	ab
C	6	*S. feltiae* + Addit, weekly		underside old leaf	4.6	±	2.9	ab	9.2	±	5.8	ab
				underside new leaf	5.7	±	2.9	b	11.4	±	5.9	b

Concentration measurements of the tank samples taken on the first four treatment dates revealed that just before spraying, EPN concentrations ranged between 5753 and 6473 IJ/ml in the tank suspension. This is in the range of the intended concentration of 6000 IJ/ml. After spraying the last EPN plots (with the same tank suspension), the concentration of EPN in the tank residue was consistently lower than the starting concentration on the same date, and ranged between 2080 and 5740 IJ/ml. The difference with the starting concentration was only statistically significant on the second (df = 1, F = 319.627, p < 0.010 and the fourth (df = 1, F = 28.300, p = 0.006) treatment date. Survival ranged between 80.2 and 88.3% and did not differ statistically (df = 1, F ≤ 6.669, p ≥ 0.061) before and after applications.

The results of the absolute and relative deposition (table 3) reveal that spray boom configuration C1 and C2 performed well at covering the upper side of both old and new leaves. However, these configurations performed less good on the underside of leaves, especially on the new leaves in the centre of the plant. With the row application technique (C3) a relative deposition greater than 10% was obtained on the underside of new leaves; a relative deposition of 28% was observed on the underside of old leaves. This technique also performed well on both upper side collector positions. Despite high variability of the deposition data, significant differences between absolute and relative deposition results were noted, both on the upper side (absolute deposition: df = 7, F = 3.320, p = 0.004; relative deposition: df = 7, F = 3,648, p = 0.002) as on the underside (absolute deposition: df = 7, F = 6.429, p = 0.000; relative deposition: df = 7, F = 6.221, p < 0.001) of leaves.

The number of thrips (adult and juvenile) counted per plant (figure 3) was highly variable between individual plants, ranging from 0 on more than half of the plants up to 14 thrips on one individual plant. In the control treatment, the recorded number of thrips rose after the start of the observations to a low peak (0.73 ± 0.97 standard deviation) on August 22nd and a high peak (1.58 ± 1.83) on September 5th after which it declined towards the last counting on October 10th. On most sampling dates, no significant differences were observed between the numbers of thrips in different treatments. The only exceptions were noticed on July 31st (df = 6, χ^2 = 16.619, p = 0.011), which was caused by an exceptionally low number of EPN in the reference treatment with spinosad and on October 10th (df = 6, χ^2 = 23.598, p = 0.001), which was caused by an exceptionally high number of EPN in the reference treatment with *S. feltiae* + Addit, applied with spray boom C3.

The evolution of the damage showed much less variation than the numbers of thrips on individual plants (figure 4). On August 29th and in all following weeks,

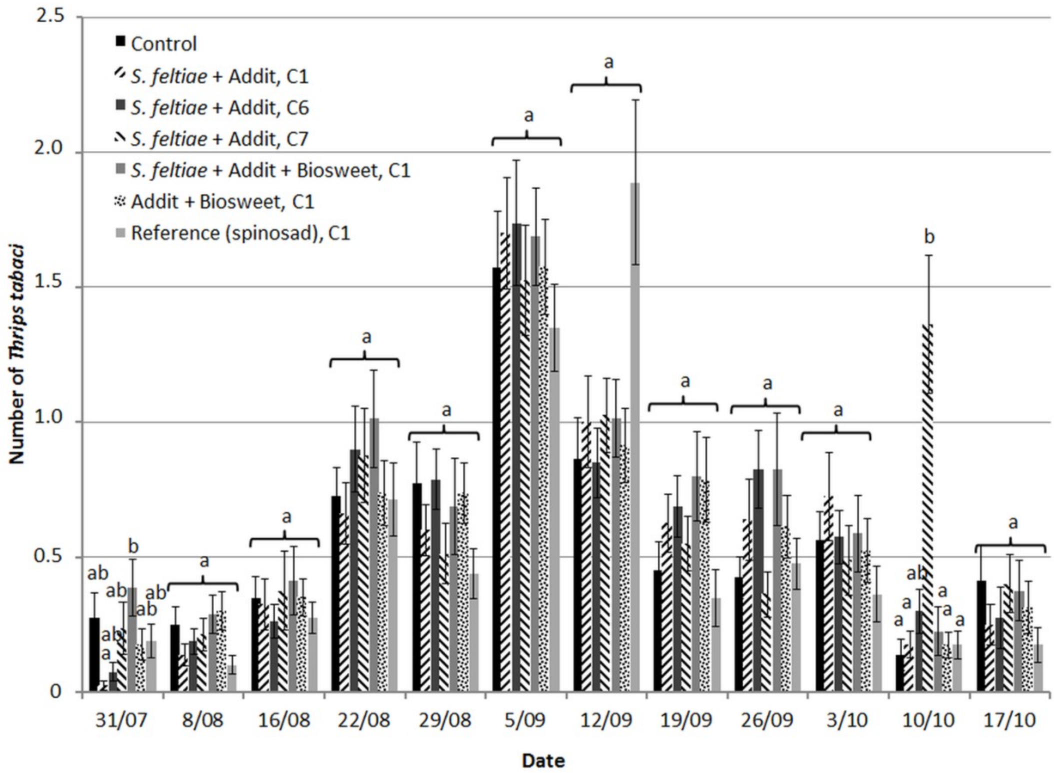

Figure 3. Evolution of the *T. tabaci* population per plant (± SE) in different treatments of the organic trial. Different letters indicate statistical differences between the number of thrips per plant in different treatments per date (P < 0.05).

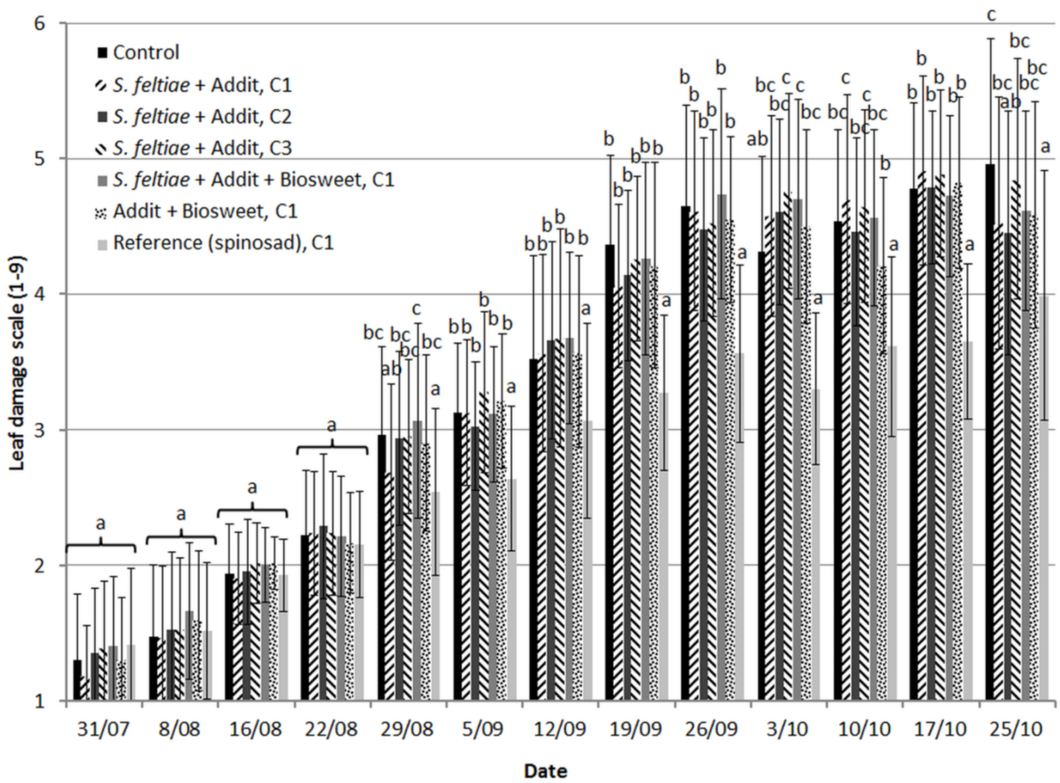

Figure 4. Evolution over time of the leaf damage caused by *T. tabaci* (average leaf damage scale ± SD) in different treatments of the organic trial. Different letters indicate statistical differences between the average leaf damage in different treatments per date (P < 0.05).

the average leaf damage differed between plots (df = 6, 35.624 ≤ F ≤ 164.780, p < 0.001) This was caused by the lower damage observed in field treated with spinosad. On October 25th, the damage observed in the treatment with *S. feltiae* applied with the band spray boom configuration (C2), was also significantly lower than in the control treatment. The total marketable yield ranged between 24978 and 27467 kg/ha for all treatments, and did not differ significantly (df = 6, F = 0.759, p = 0.610) between treatments. Also, the stalk size distribution of the harvested leek was not affected by the treatments for thrips control (df = 6, 0.332 ≤ F ≤ 1.443, 0.245 ≤ p ≤ 0.918 for all stalk size categories). In all treatments, 48 to 57% of the total harvested weight consisted of leek stalks with a diameter between 2 and 3 cm, while 30 to 40% of the harvested weight consisted of leek stalks with a diameter between 3 and 4 cm. 10% or less of the total harvested weight in each treatment consisted of leek stalks in the < 2 cm size category, while less than 1% of the weight in each treatment consisted of leek stalks in the > 4 cm size category.

Conventional trial

The average weekly temperature (day + night) and rainfall measurements of the organic trial also apply to the conventional thrips trial, since the cropping period was roughly the same, and since the two fields were located less than 500 m from each other.

The deposition results (table 3) on the upper side of both old and new leaves did not differ between treatments (absolute deposition: df = 3, F = 2.524, p = 0.070; relative deposition: df = 3, F = 2.625, p = 0.062). The significant differences observed between treatments on the underside of leaves (absolute deposition: df = 3, F = 3.484, p = 0.024; relative deposition: df = 3, F = 3.306, p = 0.029) point to positive effects of adding Addit to the spray suspension. More specific, the deposition on the underside of new leaves in the treatment with EPN + Addit was significantly higher than the deposition on the upper side of old leaves in the treatment with EPN.

Again, the thrips counts on individual plants were highly variable (figure 5). The thrips population peaked on 10th of September with 0.81 ± 1.04 thrips per leek plant observed in the control treatment. No significant differences between the numbers of thrips in different treatments were recorded in all but the last week (df = 7, χ^2 = 15.822, p = 0.027). In that week, the number of thrips in the reference treatment plots was significantly lower than the number in the plots that were treated weekly with Addit.

The leaf damage results (figure 6) clearly show an effect of the chemical insecticides in all weeks after 3 September (df = 7, 33.302 ≤ χ^2 ≤ 134.139, p < 0.001). Plants in the reference treatment plots showed the lowest damage ratings and were closely followed by the treatments in which one in three insecticide applications was replaced either by an application of *S. feltiae* or by water. The treatment with only Addit showed the highest leaf damage rating in some weeks, but at the end of the measurements, leaf damage ratings in this treatment, and in all treatments applying only *S. feltiae*, did not differ significantly from the leaf damage rating in the control treatment.

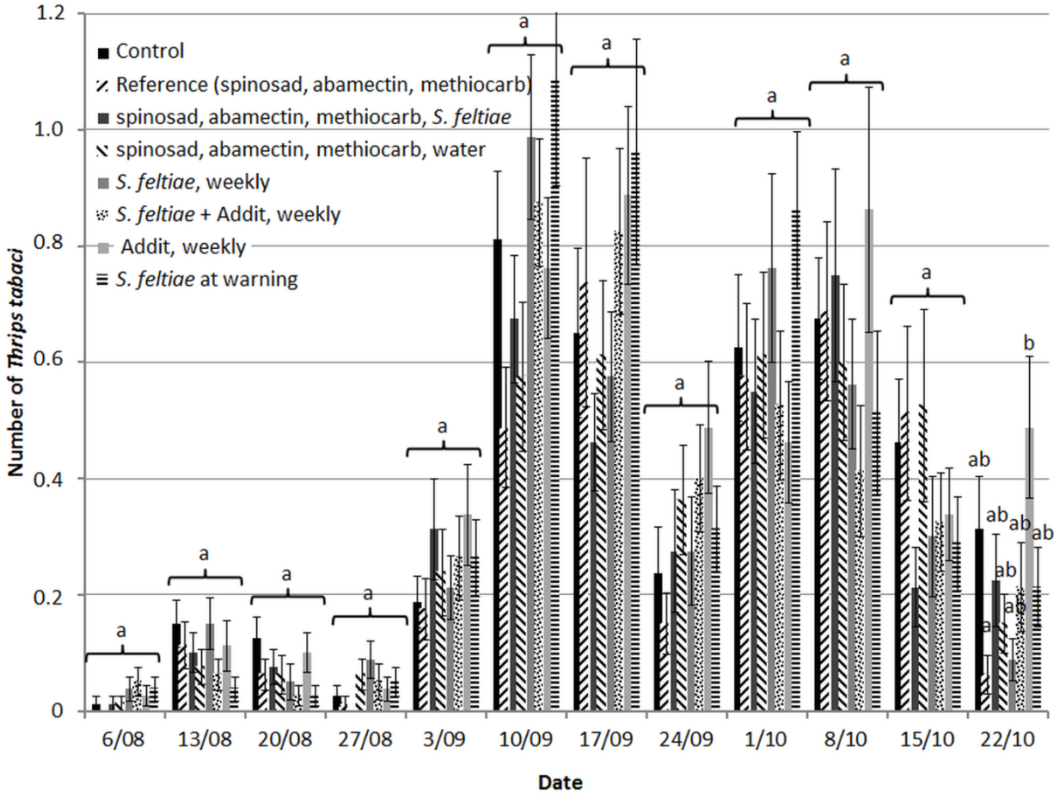

Figure 5. Evolution of the *T. tabaci* population per plant (± SE) in different treatments of the conventional trial. Different letters indicate statistical differences between the number of thrips per plant in different treatments per date (P < 0.05).

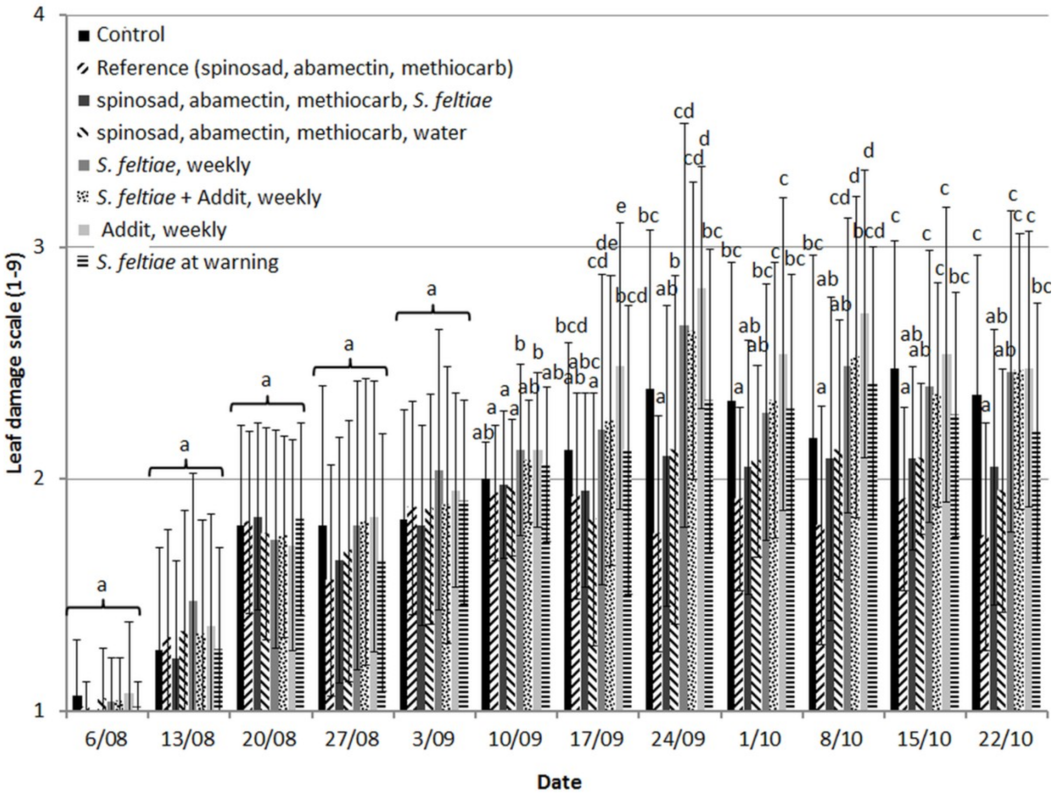

Figure 6. Evolution over time of the leaf damage caused by *T. tabaci* (average leaf damage scale ± SD) in different treatments of the conventional trial. Different letters indicate statistical differences between the average leaf damage in different treatments per date (P < 0.05)

Discussion

The measurements of the concentration before and after all applications in the organic trial, revealed that attention should be paid to possible sedimentation of nematodes in the spray tank. The exceptionally low concentration measured after the spray applications on day 7 was caused by turning off the agitation system of the tank a few minutes before the tank sample was taken (Brusselman *et al.*, 2010). This caused rapid sedimentation of the EPN, and thus lowered the concentration of EPN in the upper layer of the suspension, where the tank sample was taken. The tank sample at the end of the applications on day 22 was taken some time after turning off the agitation system, and here again, the concentration turned out to be significantly lower than before the applications. Although the difference between the concentrations before and after all applications on day 1 and 15 was not significant, a trend towards a lower concentration after spraying was visible. This indicates that the agitation system was barely sufficient to adequately mix the EPN in the tank. Adding xanthan gum would probably have nullified concentration concerns (Beck *et al.*, 2013a). Nonetheless, it was decided not to add xanthan gum, because it also increases the viscosity of the spray suspension, which might prevent a good penetration of the spray suspension into the growing points of the leek leaves. The agitation system did not significantly affect the survival percentage of the IJ of *S. feltiae*, indicating that the pumping system is suitable for use with *S. feltiae*.

Obviously, counting the numbers of surviving thrips is not a good way of estimating the effect of an insecticidal treatment, since the number of thrips is highly variable between individual plants (figures 3 and 5). Moreover, the number of thrips was not significantly affected by spinosad in the organic trial, although leaf damage caused by thrips on spinosad treated plants was significantly lower. Further proof is found in the conventional trial: on all but the last thrips counting day, thrips numbers were statistically equal in all treatments. Besides the high variation between the thrips numbers on individual plants, the most probable cause of the lack of significant differences between thrips numbers in different treatments is migration of adult thrips between plots (Arthurs and Heinz, 2006). Thrips are a highly mobile species. Furthermore, the thrips countings occurred 6 to 7 days after spraying. While the effects of the used pesticides wore off (partially) in the sprayed plots, thrips from neighbouring plots may have recolonized the spinosad treated plots. Thrips mobility may also explain the unexpected peaks and the unexpected lows in the thrips numbers data (data not shown). The evidence in this paragraph points out that counting thrips on leek plants is only useful to monitor the evolution of thrips populations in larger fields (e.g., the entire experimental field with all plots). For that purpose, they might be replaced with more user-friendly blue sticky boards.

Overall, the numbers of thrips were fairly low in both trials. In the organic trial this may have been caused by the cultivar, Antiope, which is fairly resistant against thrips (Dewaele *et al.*, 2014). In the conventional trial, the low numbers may have been caused by a combination of using conventional insecticides on part of the plots and of the migration of thrips out of insecticide-free plots into insecticide-sprayed plots.

Measurements of the leaf damage were much less variable than thrips counts and demonstrated (figures 4 and 6) that EPN treatments were not effective. Only the applications with the band spray boom configuration (C2) had a significant effect on thrips damage, although this effect was minimal, and only showed up on the last sampling date (October 25th). The only treatments showing strong reducing effects on leaf damage were the reference treatment with spinosad in the organic trial, and the treatments including chemical pesticides in the conventional trial. All in all, the results show that foliar sprays of IJ of the selected *S. feltiae* strain are not suitable for controlling the mobile life stages of *T. tabaci* on leek leaves, even in combination with adapted spraying equipment, a surfactant and an attractant.

Temperature was probably not an inhibiting factor for thrips control with EPN, since it is known that *S. feltiae* can infect the larvae of various species between 5 and 28-30 °C, although infectivity is much reduced at both temperature extremes (Hazir *et al.*, 2001; Trdan *et al.*, 2009; Langford *et al.*, 2014). When the thrips population on plants was peaking, between August 16th and September 19th, these extreme temperatures were only surpassed briefly on three occasions: on August 18th (maximum temperature: 33.41 °C), on September 1st (minimum temperature: 4.51°C) and on September 9th (maximum temperature: 28.66 °C). Even if EPN were inactivated permanently by these temperature extremes, which is highly unlikely, the weekly applications of fresh EPN would have circumvented this problem. A lack of humidity should also not have played a major role in the lack of efficiency. Although precipitation between August 30th and September 20th was low, there was no prolonged dry period (figure 2). Moreover, throughout the whole cropping period, water was almost always present in the leek stalks.

The remaining explanation for the poor control of *T. tabaci* by *S. feltiae* is the incompatibility of the species/strain with the leaf-inhabiting stages of *T. tabaci* due to the high activity levels of the latter (in particular the adults), a conclusion that was also drawn for the interaction between *S.* feltiae and *T. palmi* (North *et al.*, 2006). It may, however, still be possible that this EPN species/strain can control the less mobile, soil inhabiting pre-pupae and pupae of *T. tabaci*, as stated by the producer. But given the poor control results obtained with EPN against soil-inhabiting life stages of thrips (see introduction), it is more advisable for future research on using EPN against *T. tabaci* to look for other EPN species or other strains of *S. feltiae* that are more successful at controlling the foliar inhabiting stages of this thrips species, also at lower temperatures. Another route of investigation might be to add a thickening agent to the spray suspension. Previous research (Schroer *et al.*, 2005) proved that a mixture of a surfactant and a polymer thickening agent in the spray suspension reduces the mobility of larvae of *P. xylostella*, and thereby increases control results with *S. carpocapsae*. If such a mixture can also reduce the mobility of *T. tabaci* nymphs and adults, it might improve the chances of *S. feltiae* (and other EPN species) to infect these highly mobile pest insects. It should, however, be noticed that adding a thickening agent to the spray suspension increases its viscosity, and thus its' propensity to flow, which might reduce penetration of the suspension into the cryptic habitat of the thrips. This should however be confirmed by further research.

Although the control results with EPN were outright disappointing, the deposition test revealed that the spray boom with row application kits (C3) produces reasonable coverage of even the most difficult to reach parts of the leek plant. Therefore, this row application technique is perhaps a better alternative for traditional spray booms used for applying fungicides and contact insecticides, two types of pesticides for which coverage is essential.

The conventional trial showed that adding the surfactant Addit to the EPN suspension yielded a (limited) improvement of the deposition of the EPN on the underside of the leek leaves. It would be interesting to examine the effect on deposition of combining the row application technique with adding Addit to the spray tank.

Conclusions

The presented results show that the selected commercial strain of *S. feltiae* was not effective against the foliar inhabiting life stages of *T. tabaci* in leek. Mixing a thrips attractant in the spray suspension and spraying with adapted spray equipment did not improve the efficacy. Compared with the traditional spray boom technique, the row application technique ensured a more even EPN deposition on both the upper side and underside of both old and new leek leaves. This technique offers good potential for improving the applications of fungicides and of contact insecticides in leek.

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