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Control of the spotted leaf miner *Leucoptera scitella* L. in organic fruit growing in Germany

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Abstract

Several aspects of the strategy to control Leucoptera scitella *L. in organic fruit growing were investigated in field trials in the German regions Lake Constance and Lower Elbe, and in semifield trials at the University of Hohenheim. NeemAzal-T/S has a sufficient efficacy to reduce the damage of the first generation and the infestation pressure of a second generation. However, timing of the application is crucial and has to be determined accurately. If the application takes place only 24 h after larval hatch, the efficacy will be considerably reduced. The effect of one application seems to last at least for two weeks. The temperature sum as determined by Gottwald (1996) proved to be useful to give a rough idea about the start of egglaying and of hatching, but in the years 2020 and 2021 proved much too inaccurate to allow a timing of an application as exactly as needed to achieve a good efficacy of NeemAzal®-T/S. The findings regarding the parasitation show high differences in the parasitization rate between the orchards. The most important species,* Pediobus pyrgo *Walker, hatched from overwintering cocoons and from mines in summer.*

Keywords: *Leucoptera scitella*, Neem, timing of applications, efficacy, parasitoids

Introduction

The abundance of *Leucoptera scitella* L. in Southern Germany before the dry summer 2018 was limited only to few orchards in the Lake Constance region. In 2018 and the following years, the pest became more important. In the Lower Elbe region, some organic orchards show a higher infestation since several years. Since the applications of NeemAzal-T/S were not always successful, in 2019 first trials were started to investigate the efficacy of NeemAzal®-T/S for the control of this pest (Al karrat et al., 2020). In the years 2020 and 2021, the trials were continued with the aim to determine the best timing of the application and to elaborate recommendations if and at what time span from the first application a second treatment would be necessary.

Furthermore, the relevance of parasitation and of the climatic conditions for natural control was investigated.

Material and Methods

Efficacy and duration of the effect in field trials: To investigate timing, effect, duration of the efficacy and the required number of applications of NeemAzal®-T/S on the infestation by the leafminer, two field trials **(A and B)** were carried out in 2020 in two organic apple orchards with high infestation levels at Lake Constance in 2019. The trials were arranged in a randomized complete block design with four replications per treatment, (6 rows, 30 trees per row and plot in the field). There were three treatments**: I: Control, II: once treated on 2nd of June and III: Twice treated on 2nd and 23th of June.**

NeemAzal®-T/S 1.5 l/ha and m tree height was applied by the farmer. The control was untreated. In the two middle rows of each plot, 25 branches with varying number of leaves were marked (max. one branch/tree). The number of mines on the leaves was assessed directly before the first application and on June 2nd and 6th, July 4th and 25th post application to determine the effect of NeemAzal®-T/S on the development of leafminer larvae.

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The experiment was repeated in 2021 in four organic apple orchards, of which two were located in the Lake Constance area **(C and D)** and two in the Lower Elbe region of Northern Germany **(E and F)**. In the South, NeemAzal®-T/S was applied once on June 19th. The number of mines was assessed directly before the application and on June 26th and July 10th post application. Mine size was distinguished into three categories: small: < 2 mm, middle: 3-5 mm and full size: > 5 mm).

For trials E and F, mostly the same procedure was followed as in trials A to D. NeemAzal®-T/S was applied twice by the farmers (June 14th and July 5th for trial E; June 15th and July 7th for trial F). The number of mines was assessed on both application dates of NeemAzal®-T/S and on two additional dates (June 24th and July 19th for trial E; June 25th and July 20th for trial F). The mines were distinguished following the same scheme as in trials A to D.

In the regions “Altes Land” and Lower Elbe, the year 2021 started with above average temperatures from January to March By contrast, April and May were colder than long-term average (from 1937 to 2020). In June, when NeemAzal®-T/S was sprayed the first time in our trials, the weather was warmer with an average temperature of 19.7 °C compared to 15.8 °C in the long-term. Moreover, there were only 45.7 mm of precipitation compared to the long-term average of 70.0 mm. The same pattern was observed in July when the second application took place. At this time, the average temperature was 19.9 °C (17.4 °C in the long-term) and there were only 68.9 mm of precipitation (81.0 mm in the long-term).

In the region of Lake Constance, the weather in the first six months of 2020 was warmer than in 2021 and the average temperatures from January to July was 12.2 °C while it was 9.1 °C in 2021. In comparison between the average precipitations from Mai to July, in 2020 the average was 99.1 mm and it was higher in 2021 with 197.4 mm.

For the determination of the effect of NeemAzal-/TS on the development of the larval stage of *L. scitella*, 100 apple leaves were collected in July 2021 from each treatment in the orchards C and D. The leaves were infested with fully developed mines (> 5 mm) with larvae in the last larval stage. The leaves with mines were placed in petri dishes (9 cm) with moistened filter paper and kept in an incubation, maintained at 24 ± 1 ºC and 60-70 % RH. The photoperiod was approximately 16:8 L:D until the adults (moths and parasitoids) emerged.

Timing of the application: To determine the best timing for control application of NeemAzal-T/S against the leafminer, field and semi-field trials were carried out at the University of Hohenheim. In early 2021, overwintering pupae of *L. scitella* were collected and kept at *ca*. 8 C°. Adults were obtained by transferring the pupae at 24 ± 1 °C with a photoperiod of 16 h. For mating, newly emerged adults (both, males and females) were released into a plastic box (20*20*25 cm) covered with nets (0.7 mesh) for 48 h.

I: Field Trials: This experiment was carried out in the garden of the Institute of Phytomedicine at University of Hohenheim. Three treatments were conducted: I: Control, II: application shortly before larval hatch, and III: application shortly after larval hatch.

For each variant three branches with varying number of leaves were marked (max. one branch/ apple tree). On May 8th 2021, each branch was covered with a net (0,7 mesh) and exposed to 10- 12 ovipositing females. After 48 h, the eggs on the underside of the leaves were counted with the aid of a hand-held magnifier and the adults were removed. Development of the eggs has been monitored daily until June 5th 2021.

II: Semi-Field Trials: All tests were carried out under semi-natural conditions by using three small potted apple plants (30-35 cm height) with at least three branches. To obtain the eggs, on June 18th 2021 the potted plants were covered with tightly closing nets (0.7 mesh) and exposed to 10 ovipositing females. After 48 h, the eggs on the underside of the leaves were counted and the females were removed. Development of the eggs has been monitored daily.

Shortly before hatching (June 25th) one potted plant was treated with NeemAzal-/TS 0.3 %, the second one was treated after hatching (June 27th) and the third one left as a control. A hand operated sprayer was used for application. The effect of the treatment was assessed by counting the mines on the leaves 10 days post treatment. The efficacy on the fully developed (large) mines was calculated using the formula of Abbott (1925) comparing the number of the mines between the treatments. The size of the mines was also recorded.

Temperature sums for development of the eggs: To evaluate the temperature sums needed for the development of the leafminer eggs, 35 apple leaves with different numbers of fresh eggs, not older than 24 h, were collected from the field and placed separately in petri dishes (9 cm diam.) lined with moistened filter paper. They were kept in an incubation chamber (24 ± 1 ºC; 60-70 % RH; 16:8 L:D) and monitored until larval hatch The number of hatched and non-hatched eggs as well as the time to larval hatch was recorded.

Parasitization: To determine the parasitization rate of the pupal stage, overwintering pupae of *L. scitella* were collected in spring 2019 in 15 orchards in the region of Lake Constance. Per orchard, 500-600 cocoons were collected, put into plastic boxes (20-25 cocoons each) and kept under laboratory conditions at 24 ± 1 °C with a photoperiod of 16 h until the adults emerged. The infestation rates were determined based on farmers' experiences, information from the extension service and our own observations. The collection was repeated in 2020 and 2021. In the regions "Altes Land" and Lower Elbe Region, the same procedure was followed on 14 orchards in 2020 and 16 orchards in 2021.
In summer, 50-70 apple leaves were collected with fully developed mines with larvae in the last larval stage (> 5 mm) to determine the parasitization rate of *L. scitella* larvae. The collection was conducted in the same apple orchards where the overwintering pupae were collected (both the Lake Constance and Lower Elbe region). Leaves with mines were placed in small jars and kept under laboratory conditions (24 ± 1 °C, 65 -70% RH) until the adults (moths or parasitoids) emerged. The numbers of emerged and non-emerged moths, and parasitoids was counted.

Identification of the parasitoids: **I: Morphological identification:** Specimens were identified and photographed using a ZEISS Stemi DV4 stereomicroscope. A scanning electron microscope (SEM) was used to take photographs of body sections of taxonomic importance, such as the head, antennae, thorax and abdomen. The parasitoids were morphologically identified following keys of Burks (2003), Boucek and Rasplus (1991).
II: Molecular confirmation: Three specimens of each species determined by morphological traits were selected for DNA extraction by using a Qiagen DNA extraction protocol. Universal primers LCO-1490 and HCO-2198 (Folmer et al., 1994) were used for amplification and sequencing of the DNA barcode (Gariepy et al., 2014). The sequence PCR products were verified by gel electrophoresis and cleaned for sequencing with Cycle Pure Kits (C-Line), following the manufacturer's instructions. They was sent for sequencing in two directions using the forward primer to an external service for molecular confirmation was obtained in all samples compared with public sequences available in the GenBank database using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) confirming the taxonomy of all morphologically identified parasitoid specimens.

Results

Efficacy and duration of the effect in field trials

In trial A and B, NeemAzal-T/S was applied for the first time at 2.6.2020 when the first eggs hatched. In the very wet and cold period afterwards these larvae did not develop mines, as the data of the assessment of 12.6.20 shows (Tab. 1). At 23.6.20, new eggs were already partly hatched when NeemAzal-T/S was applied the second time. This resulted in a significant numerical reduction of fully developed leaves. In the plots where NeemAzal-T/S was only applied once at June 2nd 2020, there was still an effect to reduce the development of the larvae hatching at June 23rd 2020, which was significant at July 4th and 25th 2020. However, the effect was lower than in the plots sprayed twice. In trial C and D there is a very high efficacy of the first application and – since the hatching period in 2021 was so short – this application was completely sufficient.

Tab. 1: Effect of NeemAzal on the number of the fully developed mines in field trials (n.f.d.m.) in 2020 and 2021 and degrees of efficacy (ABBOTT) calculated at the end of the first generation.

Trial		Treatments														
		Control					NA1X					NA2X				
		2020														
A	Date	2.6	12.6	23.6	4.7	25.7	2.6	12.6	23.6	4.7	25.7	2.6	12.6	23.6	4.7	25.7
	n.f.d.m**	0.0	0.0	0.03	1.45	2.63	0.0	0.0	0.01	0.58	1.42	0.0	0.0	0.1	0.12	0.34
		a	a	a	a	a	a	a	a	ab	ab	a	a	a	ab	ab
	Efficacy in %						45.9					83.6				
B	n.f.d.m**	0.0	0.0	0.0	0.89	0.26	0.0	0.0	0.0	0.22	0.08	0.0	0.0	0.0	0.08	0.04
		a	a	a	a	a	a	a	a	b	b	a	a	a	b	b
	Efficacy in %						67.7					74.9				

Trial		Control			NA1X		
		2021					
C	Date	19.6	26.6	10.7	19.6	26.6	10.7
	n.f.d.m**	0.0	0.28	1.75	0.0	0.01	0.88
		a	a	a	a	b	b
	Efficacy in %				49.7		
D	n.f.d.m**	0.0	0.14[a]	0.52	0.0	0.0	0.02
		a	a	a	a	b	b
	Efficacy in %				59.5		

Trial		Control				NA1X				NA2X			
		2021											
E	Date	14.6	24.6	5.7	19.7	14.6	24.6	5.7	19.7	14.6	24.6	5.7	19.7
	n.f.d.m**	0.00	0.01	0.33	0.55	0.00	0.01	0.11	0.19	0.00	0.01	0.11	0.16
		a	a	a	a	a	a	b	b	a	a	b	b
	Efficacy in %					65.5				71.8			
F	Date	15.6	25.6	8.7	20.7	15.6	25.6	8.7	20.7	15.6	25.6	8.7	20.7
	n.f.d.m**	0.00	0.06	0.53	1.05	0.00	0.13	0.45	0.86	0.00	0.16	0.37	0.63
		a	a	a	a	a	a	a	ab	a	a	a	b
	Efficacy in %					18.6				40.0			

*In the same row, for all treatments with different letter, the difference between the means is significant **: number of fully developed mines

In trial E, on the last two assessment dates there is a clearly significant difference of the number of fully developed mines to the control of both the one time and the two time application. The efficacy of two applications is higher, but the difference is not significant. In trial F, only the application twice shows a significant difference to the control (Tab. 1).

Effect of weather conditions on the development of the larvae

A low temperature and a high precipitation rate seem to affect the development of the larvae In the field trials, a highly negative effect of cold and humid weather on the larvae directly after hatching was also observed. In the comparison results, the eggs on the apple leaves in the semi-field trials hatched and the larvae developed, whereas the temperature was above 18 °C and the potted apple plants were in a greenhouse.

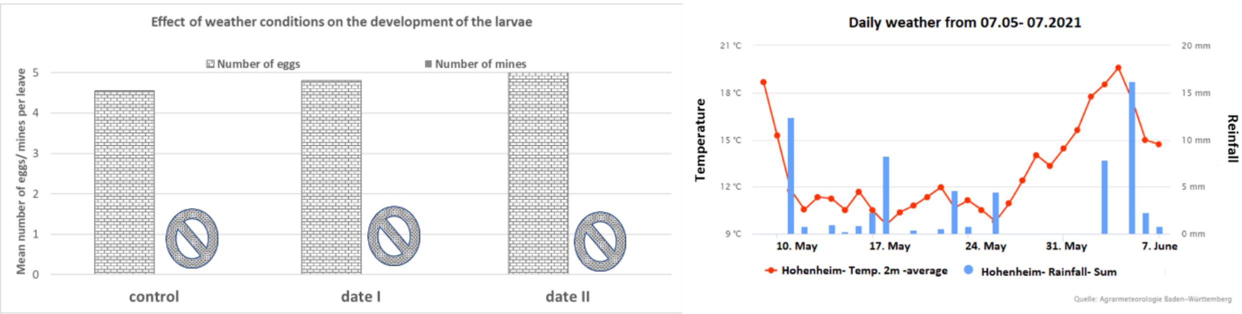

Fig. 1: Effect the weather on the development of the larvae of the leafminer

In the semifield trial (Fig. 1), all larvae died after hatching due to the cold and a highly humid weather (Fig. 1).

Timing of applications

The application of NeemAzal-T/S shortly before larval hatch reduced the number of the fully developed mines (efficacy 88.8 %) compared with the application after larval hatch (efficacy 11.2 %) and the control (Tab. 2).

Tab. 2: Effect of NeemAzal on number and size of mines in the semi-field test, efficacy (ABBOTT) was calculated for the fully developed mines.

Variant	**Mean number of small mines**	**Mean number of middle mines**	**Mean number of big mines**
Applied shortly before larval hatch	0.46	0.14	0.42
Applied shortly after larval hatch	0.63	0.9	2.23
Control	0.48	0.07	2.51
Efficacy % according to Abbott before larval hatch			**88.8 %**
Efficacy % according to Abbott after larval hatch			**11.2 %**

Prognosis of L. scitella *development using temperature sums*

Based on the laboratory experiment results, embryonic developmental time lasted 6.2 days and it needs 105.4 DDs at T_0= 8 ºC and 93 DDs at T_0= 10 ºC until the beginning of larval hatch.

Table 3: Calculation of DDs for reaching the developmental stages of *L. scitella* in 2020 and 2021

Development Stages	DDs according to Gottwald	DDs South		DDs North (trial E)
		2020	2021	2021
Start of oviposition 1st generation	125	117	103	102
Start of larval hatch 1st generation	253	251	307	272
Peak of larval hatch 1st generation	402	295	453	*No data*
Start of oviposition 2nd generation	741	325	637	560
Start of larval hatch 2nd generation	831	*No data*		774

In the field, in the Lower Elbe region, first eggs from the first generation of moths were found at 102 DDs in 2021. The first eggs from which larvae hatched were observed at 272 DDs. For the second generation of moths, start of oviposition was observed at 560 DDs and start of larval hatch at 774 DDs. To calculate the degree days, 8 °C was set as the threshold temperature and 1st March 2021 as the starting date, following the recommendation by Gottwald (1996).

Parasitoids

In the Lake Constance region, the **parasitization rate of the pupal stage** varied among the three years studied. It ranged between 6 % in 2021 and 10 % in 2020 while it was about 9% in 2019. The parasitization rate differed also between the orchards. In average and during these years, the parasitization rate of the overwintering leaf miner cocoons was lower in the orchards with a higher infestation compared with the orchards with a medium and a lower infestation. **Parasitism of** *L. scitella* larvae in summer varied from 4 % in summer 2021 to 23 % in 2020. In 2019 and in 2020 it was beyond 45 % in an orchard where the infestation with the leaf miner was low and strips with flowering plants were present. In the regions of Lower Elbe and "Altes Land", parasitization rates in 2020 were relatively low with an overall average of 1.2 % in the spring generation and 0.3 % in the summer generation. For cocoons collected in March, the parasitization rate was on average higher in orchards with high infestation pressure (3.8 %) than in those with weak infestation pressure (0.6 %). In the summer generation, less than 1 % of parasitoids hatched from the mines of most orchards, except for one orchard with a parasitization rate of 2.8 % and a medium infestation pressure in spring. The highest parasitization rate in 2020 was 8.1 % and was observed in the spring generation.

In 2021, a slight increase in parasitization rates was observed. Although the average parasitization rate of the spring generation was only 4.8 %, relatively high parasitation rates were observed on three orchards with 20.8 %, 14.5 % and 12.7 %. However, with 0.6 % the parasitization rate of the summer generation was again well below the parasitization rate in spring. Again, the average parasitization rate of cocoons collected from orchards with high infestation pressure (5.1 %) was higher than that from orchards with weak infestation pressure (2.8 %). In July, the parasitization rate was again below 1 % for almost all orchards, except three orchards with parasitization rates of 3.7 %, 1.8 % and 1.4 %, all of which had weak infestation pressures in spring.Six parasitoid species from five families emerged from leafminer larvae and pupae were identified. During the winter of the years 2019, 2020 and 2021, in total 1,063 parasitoid specimens were collected in two regions in Germany, including different species of the hymenoptera families Eulophidae and Pteromalidae. More than 86 % of the species belong to the family Eulophidae. *Pediobus pyrgo* (Walker) is the most abundant parasitoid in the two regions attacking the pupal stage, comprising more than 52 % of the individuals found, followed by *Baryscapus spec.* (19 % of the total) and *Chrysocharis pentheus* (Walker) (16 % of the total). The results of surveys of parasitism of the pupal stage of *L. scitella* showed that *P. pyrgo* is the principal parasitoid during the three years studied.

Parasitism of the larvae in summer during the course of the three years study was distributed among several taxa, including 40 % by *Baryscapus spec.,* 29 % by *P. pyrgo* and 31 % by the other species. Comparing the most abundant and dominant parasitoids during the year 2019 shows that *P. pyrgo* was the most abundant parasitoid species, while *Baryscapus spec.* was the dominant parasitoid in 2020 and 2021.

Discussion

NeemAzal®-T/S (NA) has a sufficient efficacy to reduce the damage of the first generation and the infestation pressure for a second generation of *L. scitella. T*he threshold for an economic damage determined by Baufeld & Freyer (1990), is 0.1 – 2.5 mines per leaf for the first generation. If the yield is very high, however, the threshold is reduced to 0.1 mines per leaf. However, this shows that a certain level of infestation can be tolerated for the spotted leafminer. In trial F, the reason for the reduced efficacy against large mines might be that the hatching of the larvae was probably already ongoing when NA was applied. Even in fully developed mines not all larvae developed to adults in the Neem treated plots, thus, the effect on the development of the second generation is probably even higher than the data show. However, the timing of the application is crucial and has to be determined accurately. If the application takes place only 24 h after larval hatch, the efficacy is considerably reduced. The effect of one single application seems to last at least for two weeks. If the hatching period is much longer and the infestation pressure is high, a second application is advisable. In previous studies, it has been proven that there is no effect on eggs (Al karrat et al., 2020). Since the efficacy does not last so long, NA should be applied at a date as near as possible to the first peak of larval hatch. However, it has also to be considered that after the application no rainfall should occur for 6-12 hours to allow the product to be taken up by the leaves. The temperature sums as determined by Gottwald (1996) proved to be useful to give a rough idea about the start of oviposition and of larval hatch But in the years 2020 and 2021 temperature sums proved to be much too inaccurate to allow a timing of the application as exactly as needed for a good efficacy of NeemAzal-T/S. Thus, controls of egg laying and egg development in the field are indispensable for a correct advice regarding the application time. The data regarding the temperature sum needed for the egg development can be used in addition to these controls. The findings regarding the parasitization shows difference in parasitization rate between the orchards. In some orchards, parasitoids seem to play an important role in controlling the infestation pressure, in others not. The most important species, *Pediobus pyrgo*, hatched from the overwintering cocoons and from the mines in summer. Thus, it should further be investigated, if it is possible to transfer parasitoids from orchards with high parasitization rates to orchards where the pest populations are expected to increase. The climate conditions during the hatching period of the larvae play an important role for larval survival. Thus, in years with few precipitation during this period, high infestation rates can be expected.

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