Control of Adoxophyes orana F.v.R. with entomopathogens and NeemAzal-T/S: first approach for optimal combination strategies

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Abstract

The application of NeemAzal-T/S, a product based on Neem extracts and plant oil, for aphid control in apple orchards shows promising side effects on *Adoxophyes orana*. However, the efficacy of the recommended dosage of 3 l/ha at red bud stage is not enough for a satisfying control of *A. orana* orchards with medium and high pest pressure. Field trials were carried out to introduce these side effects in combination strategies with *Bacillus thuringiensis* or CAPEX 2 (Granulovirus) for efficient control of this pest.

Keywords

Adoxophyes orana; Lepidoptera, Tortricidae; NeemAzal-T/S; *Bacillus thuringiensis*, Capex 2; Granulovirus; combination strategies; apple orchards; biological plant protection;

Introduction

The summer fruit tortrix moth *Adoxophyes orana* F.v.R. is known as a serious pest in pome fruits in Central Europe. In organic farming, two registered products can be used for the control of this pest: CAPEX 2 (specific Granulovirus) and *Bacillus thuringiensis* (Bt). In practise the reduction of high populations with CAPEX 2 gave varying results with a sometimes unacceptable efficiency (HÖHN et al. 1998). The results of application of Bt were not satisfying, too (VAN THE GEEST 1971). The application of Capex has a long term effect on the population of *A. orana* (SHIGA et al. 1973; SATO et al. 1986; ANDERMATT 1989). However, long term effects, until now, have not been considered in control strategies. NeemAzal-T/S (NA), a product based on Neem extracts and plant oil, is widely used for aphid control at red bud stage in organic apple orchards at the concentration of 3 l/ha. Higher doses (5 l/ha) of Neem showed good effects against *A. orana* (JACOB 1996). Such concentrations, however, are not applicable for reasons of registration and cost. Nevertheless, the treatment of 3 l/ha NA shows interesting side effects on *A. orana* that might be used in combination strategies with the aim to improve the efficacy and to reduce the risk of failure of treatments with entomopathogens. The aim of our research was to find out if and how the side effects of the aphid treatment with NA (3 l/ha normal treatment; 2 l/ha splitted treatment) could be combined in the most efficient and economic way with Bt (trials **A**) or CAPEX 2 (trials **B**).

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Material and Method

Trees were treated with a knapsack sprayer (Solo 12l) (trials A: combination of Bt and NA) or a tractor (trial B: combination of Capex 2 and NA) when most larvae were in the 3rd larval instar. One to three days after the first application, little gauze cages were placed on the tip of shoots infested with larvae. Air and rain could penetrate these cages but larvae could not escape. Some plots were sprayed a second time 1 - 2 weeks later. Three to four weeks later cages where opened and the dead or alive larvae, pupae, adults or parasitoids were assessed. Alive larvae and pupae were put separately in small boxes and fed with leaves in the laboratory (temp. 20 °C; 16 h L / 8 h D) until they died, pupated or hatched as an adult.

In preliminary laboratory trials (Kumpmann, unpublished) different *Bt-* products were screened on their efficacy against larvae of *A. orana*. Delfin (*Bt kurstaki*) gave the best results, but since it is not registered in Germany for fruit growing, it was not used in these trials. Dipel (*Bt kurstaki*) and XenTari (*Bt aizawai*) showed nearly the same efficacy. XenTari (Bt) was chosen for all these field trials because the additional efficacy of *Bt aizawai* against Noctuidae is an advantage for the fruit grower.

A-1: Bt- NA test 1 The experiment was laid out as a randomised block design with 4 replications per treatment in an orchard in Rheinland Pfalz (cv. Idared) in spring. The plots were treated on April 8th 00 with NA in concentration of 2 l/ha or 3 l/ha or Bt 0,4 kg/ha or 0,6 kg/ha alone or in combination (Nr 2-8), the combination NA 2 l/ha and Bt 0,4 kg/ha twice (Nr 8 and 9). One further plot of "Elstar" neighbouring the orchard was treated with Bt 0,4 kg/ha (Nr 10). A second application with 0,6 kg/ha Bt was done in the treatments Nr 8 and Nr 10 on April 20th 00 (tab. 2).

A-2: Bt- NA test 2 (I and II) For the first part of this trial in the experimental station of the University of Hohenheim in summer, a orchard (cv. Golden Delicious) was divided into 4 plots and treated on June 29th 00 with 0,4 kg/ha Bt alone (Nr 2) or in combination with 1 l/ha (Nr 3) or 2 l/ha (Nr 4) NA. A second application with 1 kg/ha Bt for all plots (excluding the control) was done at Juli 6th 00. For the second part, a "Jonagold" - orchard was divided into three plots and treated on June 26th 00 with 2 l/ha NA alone (Nr 2) or in combination with 0,4 kg/ha Bt (Nr 3). A second application with 0,6 kg/ha Bt was done on July 5th 00 in all treatments (excluding the control) (tab. 3).

A-3: Bt- NA test 3 This trial took place in the experimental station of the university of Hohenheim with one replications in each of the cultivars "Elstar", "Golden Delicious" and "Boskoop". In each replication the 5 plots were randomised. The trees were treated on April 30th 01 with 2 l/ha NA alone (Nr 2) or in combination with 0,4 kg/ha Bt (Nr 4). A second application with 0,8 kg/ha Bt in the treatments 3, 4 and 5 was done on May 5th 01 (tab. 4).

B. Trials to improve the efficacy of Capex by combination with NeemAzal-T/S In these trials, the orchards of 3 locations ("Kipp", "ST", "P1" and "P2") were divided into 2 parts, one treated with Capex, the other treated with Capex and NA (tab. 1) in spring, in each trial, after the application gauze cages were put on shoots infested with larvae in order to get the mortality of these larvae. Additionally, in the trial "Kipp" the population development was recorded by assessing the number of larvae on the shoots on April 26th 99, July 9th 99 and July 21st 99 (1000 to 2000 shoots / plot) and the damaged apples on the Sept 2nd 99 (1000 apples / plot) in rows where no larvae were confined.

Tab. 1: trials B: to improve the efficacy of Capex by combination with NeemAzal-T/S in field trials: experimental data

Location	Region	Capex - treatment		NeemAzal-T/S – treatment	
		date	conc. (ml/ha)	date	conc. (l/ha)
Kipp	Lake Constance	28.4.99 /4.5.99	100[1] / 100[1]	28.4.99	3
ST	South Tyrol	15.4.00 /21.4.00	50 / 50	21.4.00	3
P 1	Dresden	18.4.00 /25.4.00	100[1] / 100[1]	25.4.00	3
P 2	Dresden	18.4.00 /25.4.00	50 / 30	25.4.00	3

[1] equivalent to 100% of the recommended application rate

For all trials with confined larvae two degrees of efficacy (E) were calculated:
The efficacy of the treatment on the larvae (according Schneider-Orelli (UNTERSTENHÖFER 1963)):

E_{mort} = (% Mort (treatment) - % Mort (control)) / (100 - % Mort (control))

which considers only the mortality of larvae and pupae caused by the treatment. And the efficacy of the treatment on the system which considers also the mortality caused by parasitism (according Abbott (UNTERSTENHÖFER 1963))

E_{sys} = (% survived adults (control) - % surv. adults (treatment)) / (% surv. adults (control))

In trials with only one replication (A-2) the data where tested employing the Chi-square-test. In trials with three or four replications (A-1; A-3) analysis of variance with subsequent Tukey - test served as statistical tests.

Results and Discussion

A Combinations of NeemAzal-T/S and *Bacillus thuringiensis*

The mortality of larvae and pupae of *A. orana* in all treatments of trial A-1 (tab. 2) was very low showing a range between 15 and 54 %. A reason may have been the unusual low temperature after the first application (a daily mean of 7.5 – 9.5 °C on 7 out of 8 days), and additionally an extremely windy location of the orchard. Since both products, NA and Bt, are stomach poisons they have to be taken up with food, which depends highly on temperature. Thus, very low temperatures lead to an extremely low feeding activity and, in consequence, low efficacy rates.

In trial A-1 (tab. 2), depending on the sprayed concentrations, the combination of NA and Bt at the same time leads to a lower or higher efficacy compared with the application of one product alone. This corresponds to results of many laboratory trials (Kumpmann, unpublished). Both, NA and Bt, cause a feeding inhibition in the larvae depending on the amount of intake (JAKOB 1996; KARIYA 1978). The higher the concentration of one product, the faster the larvae stops feeding and the smaller is the amount of intake of the other product. Therefore, together with the temperature, the concentrations of the products are the reasons for a better or worse efficacy of the combined application of NA and Bt.

Tab. 2: Effect of different treatments with NeemAzal-T/S (**NA**) and XenTari (*Bacillus thuringiensis,* **Bt**) on larvae of *Adoxophyes orana*: Results of **trial A-1**

Nr	Treatment 1.spray / 2.spray	N	Mort larvae	Mort pupae	Mort total	E_{mort}	para. larvae	adult Para.	Adults	E_{sys}
1	Control	99	10	5	15 a[1]		2	2	83 a[1]	
2	NA 2 l / none	118	18	13	31 ab[1]	18	3	3	66 ab[1]	20
3	NA 3 l / none	115	32	19	52 b[1]	43	2	2	47 b[1]	43
4	Bt 0,4 kg / none	108	26	5	31 ab[1]	18	1	1	68 ab[1]	17
5	Bt 0,6 kg / none	108	35	2	37 ab[1]	26	0	0	62 ab[1]	25
6	NA 3 l + Bt 0,4kg / none	117	34	6	41 b[1]	30	0	0	59 ab[1]	28
7	NA 3 l + Bt 0,6kg / none	123	27	8	35 ab[1]	24	1	1	64 ab[1]	23
8	NA 2 l + Bt 0,4kg/ Bt 0,6 kg	106	51	3	54 b[1]	45	3	3	43 b[1]	48
9	NA 2 l + Bt 0,4kg / none	112	31	5	36 ab[1]	24	5	5	59 ab[1]	29
10	Bt 0,4 kg / Bt 0,6 kg	104	30	5	35	24	2	1	63	24

N = total number of Larvae; E = Efficacy (ABBOTT); E_{Sys}= efficacy on the system; E_{mort}= efficacy of the treatment; all data except N in %; [1] Means within a column followed by the same letter are not significantly different (alpha < 0,05, Tukey's multiple range test)

Tab. 3: Effect of different treatments with NeemAzal-T/S (**NA**) and XenTari (*Bacillus thuringiensis,* **Bt**) on larvae of *Adoxophyes orana*: Results of **trial A-2 (I and II)**

Nr	Treatment 1.spray / 2.spray	N	Mort larvae	Mort pupae	Mort total	E_{mort}	para. larvae	adult Para	Adults	E_{sys}
I-1	Control	168	11	2	13 a[1]		11	10	76 a[1]	
I-2	Bt 0,4kg / Bt 1 kg	112	72	1	73 b[1]	69	3	2	24 b	68
I-3	NA 1 l + Bt 0,4 kg / Bt 1 kg	185	71	6	77 b[1]	74	1	1	22 b	72
I-4	NA 2 l + Bt 0,4 kg / Bt 1 kg	133	83	6	89 c[1]	88	4	2	7 c	91
II-1	Control	76	18	5	24 a[1]		20	18	57 a[1]	
II-2	NA 2 l / 0,6 kg Bt	153	70	8	78 b[1]	71	3	2	14 b[1]	76
II-3	NA 2 l+Bt 0,4 kg/ 0,6 kg Bt	173	61	12	73 b[1]	64	3	2	18 b[1]	68

N = total number of Larvae; E = Efficacy (ABBOTT); E_{Sys}= efficacy on the system; E_{mort}= efficacy of the treatment; all data except N in %; [1] Means within a column followed by the same letter are not significantly different (alpha < 0,05, Chi^2 test)

Tab. 4: Effect of different treatments with NeemAzal-T/S (**NA**) and XenTari (*Bacillus thuringiensis,* **Bt**) on larvae of *Adoxophyes orana*: Results of **trial A-3**

Nr	Treatment 1.spray / 2.spray	N	Mort larvae	Mort pupae	Mort total	E_{mort}	para. larvae	adult Para	Adults	E_{sys}
1	Control	117	11	5	16 a[1]		24	20	60 a[1]	
2	NA 2 l / none	108	32	22	54 b[1]	45	14	9	35 ab[1]	42
3	none / Bt 0,8 kg	117	72	7	78 c[1]	74	5	4	17 bc[1]	72
4	NA 2 l / Bt 0,8 kg	146	83	8	91 c[1]	90	4	4	4 c[1]	94
5	NA 2 l + Bt 0,4 kg/ Bt 0,8 kg	106	92	1	93 c[1]	92	1	1	7 c[1]	88

N = total number of Larvae; E = Efficacy (ABBOTT); E_{Sys}= efficacy on the system; E_{mort}= efficacy of the treatment; all data except N in %; [1] Means within a column followed by the same letter are not significantly different (alpha < 0,05 Chi^2-test)

The successive treatment of 0,6 - 1,0 kg/ha Bt following an application of NA 2 l/ ha alone or in combination with 0,4 kg Bt showed promising results. The efficacy was higher than Bt applied alone two times (tab.2 and 3: trial A-1 and 2(I)) or in combination with only 1 l/ha NA (tab. 3: trial A-2(I)). No significant differences of efficacy was observed in these trials between the treatments where in the first application NA 2 l/ha was sprayed alone or in combination with 0,4 kg Bt (tab. 3 and 4: trial A-2(II) and A-3).

The very high efficacy of the second application of Bt alone in trial A-3 is probably due to the very high temperature after spraying (6 days a daily mean of 16-18°C and no rain). The additional shadow of the net cages on the leaves had probably no effect on efficacy. *A. orana*-larvae are killed mainly by the UV-stabile Bt crystals, the UV-instabile spores are not required (VAN DER GEEST 1981). But due to a slightly different feeding behaviour of the larvae because of a different micro-climate in the cages the uptake of Bt might have been increased.

Reasons for the promising results of the successive treatment with Bt may be that in the time between the applications the intake of NA is not (NA sprayed alone) or not very strongly (NA sprayed with a small amount of Bt) stopped by the rapid feeding inhibition of Bt. About 1-2 weeks after the first NA-application the feeding activity of sublethal harmed larvae increase again and the larvae are therefore able to take up the Bt of the second application. There might be a combination effect that larvae with a sublethal intake of NA are more susceptible to the Bt crystal. The reason could be a combination effect of sublethal NA and sublethal Bt in the midgut of the larvae. The endotoxin of Bt causes swelled and ruptured cells in the midgut of Lepidoptera (KRIEG 1986), but after uptake of sublethal doses the midgut tissue recovers because newly regenerated cells take the place of the damaged ones. Neem may cause an inhibition of mitosis in the epithelium cells of the larval midgut. Besides that, after the intake of Neem midgut muscles of some insects become swollen and disrupt in a dose and time depending manner (MORDUE & BLACKWELL 1993).

B Trials to improve the efficacy of Capex by combination with NeemAzal-T/S

In case of a low efficacy of 50-60% of Capex alone (because of a late application date or low concentrations) the addition of NA increased the efficacy at a rate of 30 -43% (tab. 5). In case of high larval mortality of 90% or more for Capex alone the addition of NA showed no further increase.

Tab. 5: Trials to improve the efficacy of Capex 2 by combination with NeemAzal-T/S on larvae of *A. orana* on different locations: Results of the caged larvae of **trials B**

Location	Efficacy (SO*) of the additional treatment with NeemAzal-T/S	Mortality (Capex)		Mortality (Capex+Neem)
Kipp	30%	57%	➔	81%
ST	43%	54%	➔	94%
P 1	6%	93%	➔	99%
P 2	0%	91%	➔	91%

* Schneider-Orelli (UNTERSTENHÖFER 1963), the Capex-plot was used asˆcontrol¤, the combination-plot asˆtreatment¤

In the trial on locationˆKipp¤, the increase of the efficacy in the combination assessed by counting the larvae on the shoots (tab. 6) was higher than the improvement of the larval mortality found in the net cages (tab.5). Both, NA and Capex, have a direct mortality on the larvae and pupae and a long term effect on the population of *A. orana*: After a treatment of Capex in spring, virus-infected larvae can be found also in the following generations (SHIGA et al. 1973; SATO et al. 1986; ANDERMATT 1989). Treatments with the recommended concentration of Capex in spring lead to 8 - 30% virus-infected larvae of *A. orana* in the summer generation (KIENZLE et al. in press; KUMPMANN, unpublished). Adults treated with a sublethal dose of NA as larvae show reduced fitness and fertility (JACOB 1996). Assessing the larvae on shoots, both, the short and the long term effect of the products where observed.

Tab. 6: Trial to improve the efficacy of Capex by combination with NeemAzal-T/S on larvae of *A. orana* on location ¤Kipp¤: Results of assessed shoots / apples of trials B

	Capex	Capex + Neem	Efficacy (HT*) of the additional treatment with NeemAzal-T/S
Larvae/shoot 26.4.99	1,1 %	2,5 %	
Larvae/shoot 9.7.99	1,2 %	1,3 %	52 %
Larvae/shoot 21.7.99	4,5 %	2,4 %	77 %

*HT = Henderson-Tillton (UNTERSTENHÖFER 1963)

The amount of damaged fruits (tab. 6) was lower in the combination (0,4 %) than in the plot only treated with Capex (1,0 %) but general to low for interpretation.

Conclusions

Further trials are necessary to confirm these results. Based on the available data, possible recommendations for the control of higher populations of *A. orana* for practise might be: Combine the application at red bud stage of 2 or 3 l/ha NA against aphids with 40% of the recommended doses of Bt only when winter moth is a problem. Apply 80 - 100% of Bt depending on the temperature 5 - 10 days after the first treatment when the feeding activity of the larvae increases again. Since long and short term effects have to be considered, the evaluation of the effects of Capex and NA on *A. orana* is very difficult. An additional NA application improves a low efficacy of Capex and therefore reduces the risk of failure. Further trials are necessary to see whether an application with NA allows the reduction of the Capex concentration.

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