Promising field and semi field results forcherry fruit fly control using Neem

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Abstract

The European cherry fruit fly, Rhagoletis cerasi *(Diptera: Tephritidae), is the major pest species in cherry orchards throughout Europe. Adequate control measures are lacking to date, especially in organic fruit growing. In our prior studies the proof has been given for neem (a.i. azadirachtins) to interfere with ovary development and impede successful reproduction in* R. cerasi*. In fruit fly control under field conditions, however, it usually failed. To our belief the latter is mainly founded in the unsuitable layout of these field trials. Three main conditions have to be fulfilled to apply neem successfully under field conditions: First, females have to be detained from immigrating from the control to the treated area. Second, the application of neem products has to be started together with the first detections of flies on Yellow Traps. Third, the active ingredient has to be present in the field constantly. Taking these precautions into account, we here present semi field and field trials we carried out with neem in bait spray and semi field trials with cover spray. The tested baits and the cover spray contained NeemAzal-T which is an oil-free formulation. Beforehand, we compared acceptances of bait containing either the latter formulation or, the already licenced oily formulation NeemAzal-T/S under laboratory conditions.*

In laboratory studies R. cerasi *preferred the oil-free formulation in bait. In semi field and field trials infestation rates were significantly reduced by all bait treatments as well as the cover spray. Infestation in the treated trees was below the economic threshold. The outcome of the study reveals the high efficacy of neem under exclusion of immigration of mature females and is essential in decision making regarding preconditions of orchards necessary for a successful application of neem products for cherry fruit fly control.*

Keywords

GF 120, *Rhagoletis indifferens*, *Rhagoletis cingulata*, Azadirachtin, field trial design

Introduction

Cherry growers in Europe have one main problem in order to produce marketable fruits: The infestation with larvae of the cherry fruit fly, *R. cerasi*. Its control still relies on the use of the systemic insecticide Dimethoate, but its use becomes increasingly restricted. A withdrawal from the market due to the EU wide reduction program for broad spectrum insecticides can be suspected. Furthermore Dimethoate is not an option for organically managed orchards. Against this background there is a high need for new environment-friendly control strategies. In this study we focused on the use of bait sprays. Bait has to be ingested by the target insect and therefore contains phagostimulants like proteins and sugar (Yee & Chapman 2005, Mangan *et al*. 2006). Together with the spatially restricted application, bait sprays reduce application rates of insecticides and are more selective as compared to cover sprays.

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A good example for the successful use of bait sprays against tephritids is the use of the spinosad containing GF-120™ Naturalyte Fruit Fly Bait (Dow Agrosciences, Indianapolis, Indiana, USA) (Burns *et al*. 2001, Yee 2007, Thistlewood *et al*. 2010). In our study, two different neem formulations were incorporated into bait. Whilst we have proven in prior studies the principal effectiveness of both formulations, namely NeemAzal-T and NeemAzal-T/S, in laboratory and semi-field they usually failed in our field experiments (Vogt 2009, Kleeberg & Vogt, 2010). We suspect that immigration of mature females is the main reason for the gap between efficacy in the lab or semi field and under field conditions.

In the present study we first tested the influence of the insecticide formulation on bait acceptance. Afterwards we carried out semi field trials to prove the principal efficacy of neem under environmental influences like precipitation and UV-light. Furthermore we developed and tested a field trial setup suitable to evaluate the efficacy of neem bait under exclusion of immigration of mature females.

Material and Methods

Study insects and baits

The insects derived from collections of the previous year, where pupae were first stored under room conditions (20 to 25°C) and subsequently transferred to the cold room (3 to 5°C) for at least 5 months. Post-diapause development was allowed in a climate chamber (25 ± 0.5°C / 18 ± 0.5°C, RH 65 ± 5 %, photo period light : dark16:8 h, 4 to 6 klux) (Köppler *et al.* 2009). .

The baits used in experiments contained the oily insecticide formulation NeemAzal-T/S® or the oil free formulation NeemAzal-T® (products of the Trifolio-M GmbH, Lahnau, Germany. Note that NeemAzal-T is not yet marketed). Furthermore baits contained sugar and yeast in the relation 400:1, the bioemulgator Ledophil® (Handelsvertretung Ledophil, Traude Klose, 99439 Büttelsted) to enhance rainfastness, and water. The abbreviations used in this paper are summarized in Table 1. Note that the baits NATS 1% and NAT 0.2% are comparable concerning their insecticide content.

Table 1: Component abbreviations used in the paper.

Component	Abbreviation
1% NeemAzal-T/S in bait (resultant ai = 0.0001%)	NATS 1%
0.2% NeemAzal-T in bait (resultant ai = 0.0001%)	NAT 0.2%
1% NeemAzal-T in bait (resultant ai = 0.0005%)	NAT 1%
0.2% NeemAzal-T + 98% water (resultant ai = 0.0001%)	NATcover

Experimental setups

Laboratory

No choice and choice experiments were carried out in the climate chamber (conditions as described above) in order to investigate the acceptance of different baits by the study insects. For both treatments we used white plastic boxes (6 x 11 x 11 cm) with transparent lid and a water reservoir in the center. In case of no choice tests, one hole of approximately 1.5 x 2.5 cm was cut in the center of the lid and three drops (each 10 µl) of respective bait were presented on a rectangular cover slip placed on the hole. Droplets were dried for 24h in the climate chamber and afterwards 5 female flies, starved for about 17h were introduced into the box. Flies were up to 7 days of age. The cover slip with the dried out bait was weighed before and after the experiment and the difference in weight

was calculated. For all baits, a control of the same setup without flies was run and weight differences in the treatment were corrected for the mean weight difference in the control. If only one bait was offered at a time (no choice design), experiments were carried out from approximately 10 am of day 1 to 10 am at day 2, i.e. 24h. In choice tests the same boxes were used, now with two holes cut centrically at opposite sides of the lid, where two different baits were presented at a time. This experiment was carried out with pre-fed flies for three days from 10 am at day 1 to 10 am at day 4, i.e. 62h. As in treatment 1, weight differences were calculated and corrected for mean values of a control, carried out simultaneously. In each experiment at least 4 replicates of the control were carried out per bait. In the choice experiment comparing NAT 0.2% vs. NAT 1% one replicate was excluded because flies escaped.

Semi field and field trials

Both were carried out in Dossenheim in the experimental orchards of the institute (Baden-Württemberg, north of Heidelberg) (latitude: 49°27.02'; longitude: 8°40.48').

Semi field

Single caged trees (cages approximately 9 m^3; cherry varieties 'Hedelfinger' and 'Kordia') were treated with 30 ml of a respective bait spray once a week for four weeks. Bait was sprayed on three dispersed spots of the treetop. Within the first 3.5 weeks, young flies (<4 days) were introduced into the cages twice a week, totaling 33 males and 30 females per cage. Starting with the first introduction of flies one application of bait or cover spray (cf. Table 1) was carried out once a week for 5 weeks with 40 ml of bait or 1.5 l of cover spray applied each time. Bait was applied using an air pressure driven color sprayer, for cover spray a knapsack sprayer was used. Two controls were carried out, one matching the conditions in bait sprays with weekly blank bait applications (blank bait control), one without treatment (untreated control). All fruits were collected at the 15.06.2011 and a random sample of 400 fruits per tree was examined.

Field trial

The sweet cherry orchard used for the trial was, planted in 2007 with the cherry variety 'Regina'. The orchard is 0.52 ha in size and has 9 rows with 33 trees with a planting distance of 5 x 3.5 m. The control and the treated area consisted each in 165 cherry trees. Between both areas, we installed a chemical barrier consistent in three cherry tree rows, that were treated weekly with 0.1% Pirimor and a not yet marketed pyrethroid bait. In the treated area we applied 40 ml NAT1% per tree using a knapsack sprayer. The application dates were 10.05, 17.05, 25.05, 03.06, 09.06.2011. Because Yellow Trap captures indicated a very low cherry fruit fly population that year, young flies (<4 days) were released centrally in both areas. In total about 160 males and 180 females were released in the control and the treated area, respectively. Infestation rates were calculated by defining 10 blocks à 4 trees centrally in both areas. From every block a sample of 200 fruits was examined, with 50 fruits randomly collected per tree. Samples were taken at the 24.06.2011.

Statistical analyses

Because the assumptions for parametric tests were not always met we used non-parametric tests in all experiments. In all statistical analyses the significance level was $\alpha=0.05$.

In laboratory experiments the Kruskal-Wallis test was applied. In case more than two baits were compared, approximate p-levels were estimated using the Kruskal-Wallis test and subsequently the Nemenyi-Damico-Wolfe-Dunn test (Hollander & Wolfe 1999) was carried out to ascertain multiple significance levels.

In semi field trials we tested if infestation rates of NATcover < untreated control and if NAT 1% and/or NAT 0.2% < blank bait control and if untreated control < blank bait control. These comparisons were chosen, because it was expected that all treatments reduce infestation rate and that the application of blank bait may increase infestation rates. The analyses were carried out using the Wilcoxon Exact Test with subsequent Holm correction for the familywise error rate.

In the field trial the Kruskal-Wallis-Test was carried out to compare infestation rates.

Results

Under laboratory conditions, significantly more NAT 0.2% than NAT 1% was fed in no choice and choice experiments (Table 2). No significant difference was found between NAT 0.2% and NATS 1% when comparing feeding amounts under no choice conditions, but when having a choice, females fed significantly higher amounts of NAT 0.2% (Table 2). In semi field trials, fruit infestation was significantly reduced in all treatments against the respective control, but there was no significant difference between both controls (Figure 1). Under field conditions application of NAT 1% significantly reduced infestation from about 9% in the control to 0.5% in the treated area.

Table 2: Comparison of the amount of bait fed by females (n=5) in one day supplied with only one bait (No choice design) and in three days supplied with two baits simultaneously (Choice design), respectively. Mean values, given with SD, show differences of bait weight after the experiment as compared to before, corrected for the weight loss of the respective bait in a control (n≥4) without insects. In all cases the Kruskal-Wallis-Test was carried out. Significant p-values are given in bold.

Experimental design	Bait	Mean ± SD (mg)	N	P
No choice	NAT 0.2%	2.6 ± 0.4	8	**< 0.001**
	NAT 1%	1.1 ± 0.3	8	
Choice	NAT 0.2%	4.8 ± 0.7	15	**< 0.001**
	NAT 1%	0.2 ± 0.4	15	
No choice	NAT 0.2%	2.8 ± 0.8	9	0.389
	NATS 1%	2.1 ± 0.8	9	
Choice	NAT 0.2%	3.5 ± 1.8	16	**0.001**
	NATS 1%	1.3 ± 0.1	16	

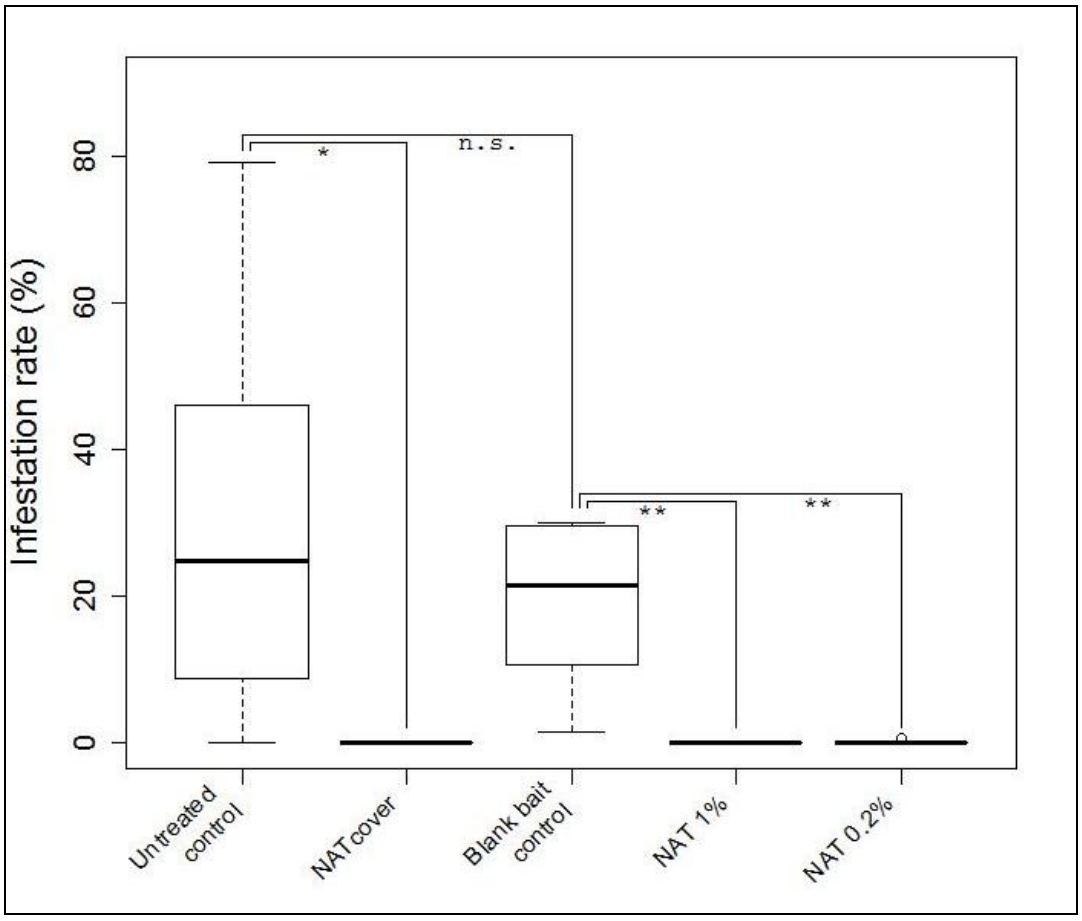

Figure 1: Boxplots of infestation rates in semi field trials with 5 replicates per treatment and control. Connecting lines show which treatments and controls were compared statistically (cf. methods) and if the effect was significant (** = $p < 0.01$, * = $p < 0.1$, n.s. = not significant).

Discussion

Bait mixtures containing neem in different formulations and concentrations were tested for their efficacy in cherry fruit fly control in laboratory, semi-field and field assays. Based on preliminary work (Vogt 2009, Kleeberg & Vogt, 2010) and especially due to our results presented here from 2011, we can state that neem bait has high potential for control of *R. cerasi.* Oil-free formulations are most promising because they are less repellent. From an efficacy point of view there is no difference in semi field trials if bait or a cover spray is applied. However the cover spray experiment has to be seen as a first trial to estimate its principal efficacy. The application rate with 3 ml insecticide formulation per tree is 7.5 times higher than with bait spray. Before initiating field trials using cover spray it remains to be investigated if reduced rates of such a spray remain effective. An additional finding of the study is that *R. cerasi* is able to survive and to cause infestations on caged trees with or without blank bait application. In fact we even found the highest infestation rate at an untreated tree with about 80% infested cherries. Hence flies encounter all resources for surviving and reproduction on cherry trees, presumably for the most part by grazing on leaves (Yee 2008).

For successful control, females should be provided with bait consistently after emergence (i.e. treatments should start with the first sight of flies on Yellow Traps), because neem affects ripening of ovaries and consequently egg production as well as fertility of eggs. An alternative tactic may be the pretreatment with an effective contact insecticide such as SpruzitNeu® (Neudorff GmbH KG, Emmerthal, Germany) within an adequate time span

after first sight of flies, in order to reduce initial population pressure in the orchard, followed by neem treatments. Up to date, weekly applications of neem are recommended with additional treatments after high precipitation events. Furthermore immigration of females has to be obviated. Our field results show that if the mentioned prerequisites are complied, neem products can effectively control *R. cerasi* in cherry orchards. Generally their use is most promising in isolated orchards or if collectively coordinated treatments of adjacent cherry orchards are carried out.

Due to the similar foraging and as neem seems to have similar impact on other tephritids (De Ilio, 1999) it is well possible that our results can be carried over to other pests such as the olive fruit fly (*Bactrocera olea*) and the Mediterranean fruit fly (*Ceratitis capitata*). Mediterranean areas with low summer precipitations are advantageous for bait application whereas long growing periods of fruits and occurrence of multivoltine pest species will increase seasonal work load and costs. Corresponding field trials would be of high interest in order to assess the possibilities of an elevated use of this environment friendly control method.

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