Practice Oriented Results
on Use and Production of
Neem-Ingredients and Pheromones

H. Kleeberg and V. Micheletti
(Eds.)

Proceedings of the 4th Workshop
Bordighera, Italy, Nov. 28th - Dec. 1st 1994

Druck & Graphic, Giessen
1996

Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones

Proceedings of the 4th Workshop; Bordighera, Italy, Nov. 28th - Dec. 1st 1994

Printed by:
Druck & Graphic, 35396 Giessen, Kiesweg 22
1996

Innenteil gedruckt auf 100% Recycling Papier

Compiled and prepared by:
Hubertus Kleeberg and Valerio Micheletti

Distribution:
Trifolio-M GmbH, Sonnenstr. 22, D-35633 Lahnau 2

ISBN: 3-925614-18-4

Printed in Germany
All rights of production, in whole or in part, e.g. in print or by film, radio, television, or any photo-copying are reserved.

CONTENTS

Pheromones

Beneficials

Good afternoon and welcome aboard our meeting!

It is a pleasure to meet participants of all over the world gathered here to talk and discuss on subjects of common interest: how to prevent and avoid damage from dangerous insects by using bio-products capable to control pests without any damage to our environment and to beneficial insects, animals and human beings!

Let's introduce myself - I am Valerio Micheletti a friend of Dr. Kleeeberg. I am here to help him organize this workshop and to help to overcome any language barrier, when needed.

I live in this area where I am running a nursery for subtropical plants. Being reluctant to use poisonous chemicals, when I came to know Prof. Schmutterer and Dr. Kleeberg and their efforts in bio-products I entered in the world of Neem.

In former times, I worked in the Physics of the first microcircuits - well known today as chips for computers: at that time I lived through the dramatic switch from mechanical calculators to electronic computers - I hope now to experience a similar switch from chemical poisons to harmless BIO-PESTICIDES.

Why do we meet in Italy? One reason might be the good climate, and I hope we will have good weather, but mainly because Italy is big in vegetables, orchards and ornamentals and is very interested in the production of crops and flowers free from poisonous residues.

Farmers supported by public programs, University labs and agricultural institutes are already using pheromones, both for trapping and for sexual disorder, as well as beneficial insects like predators, bacteria like *Bacillus thuringiensis* or nematodes.

Neem is under investigation and test. The main problems in converting from chemical pesticides to bio-products are first of all the cost and then the mode of action: Neem-products do not have the knock-down effect of the common pesticides - their effect is slower in time and needs many days before reaching a balanced situation where the damage of the phytophages is reduced to zero or at least to an acceptable level.

Our meeting wants to be informal - you will find in our folder a preliminary schedule, but we can organize it at your will to meet special requirements.

I wish all of you a very pleasant stay here in Bordighera and a profitable workshop.

Bordighera, November 28th 1994 — Valerio Micheletti

Possible uses of Neem in agriculture.

G. Grazzi and L. Rovesti[1]
CNR - Centro di Studio dei Fitofarmaci
via Filippo Re, 8 - Bologna - Italy

Summary

Neem-based formulates were field-tested against several insect pests and on different crops. Good control was obtained for *Leptinotarsa decemlineata* on potato, the sciarids*Lycoriella* sp. on mushrooms and *Bradisia paupera* on primrose and, to lesser an extent, for *Lobesia botrana* on grapes. The results of the trials are briefly commented and some suggestions for further development of this bioinsecticide put forward.

Introduction

Despite the long use in developing countries, only in recent years has neem raised much interest in both Europe and the USA, mainly because of the present orientation towards a sustainable agriculture. In these latter countries neem encounters a harsh competition by chemical insecticides, which generally provide a reliable and standardized level of protection and tend to be cheaper. Neem-based pesticides undoubtedly possess the great advantage over synthetic chemicals of being more environmentally friendly; however, to be accepted by the majority of farmers, their efficacy must be comparable to that of chemicals and they must give consistent results under practical conditions. The availability of standardised formulates with known content of active ingredient(s) is already an important step forward towards these achievements. There is a huge amount of information showing that neem is very active against a number of pests and diseases under laboratory or greenhouse conditions. On the other hand, not so much data are available on field results. However, it is essential that the potential of neem be shown also in the field and in different environments, if it is to become part of our weaponry against plant enemies. For this reason we have focused on the practical use of neem; in particular, to give a wider perspective of its potentialities, we have been comparing it not only with standard chemicals but also with other biocontrol agents. The present paper reports the results of trials carried out in Italy in1994.

---

1 - To whom correspondence should be addressed

Acknowledgements
The valuable technical assistance of Ms. Giulia Toschi and Mr. Nino Adani in the course of the trials and the help of Dr Ilaria Alberti for the statistical analysis of data are gratefully acknowledged. The gift by the companies Ciba-Geigy, Novo Nordisk and Sandoz of some of the pesticides used is also appreciated.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Field tests

***Leptinotarsa decemlineata* (Colorado Potato Beetle).**

Materials & Methods
Location of trial: Castel Maggiore (district of Bologna, Northern Italy)
Crop and Cv: Potato, Cv. Lutetia
Date of plantation: 7.3.94
Treatments:

treatment (a.i.)	rate	n° treats.	date of treats.
Fastac 35 WDG (cypermethrin)	10 g/hl	1	29-5-94
Novodor FC (Btt)	5 l/ha	2	29-5-94/9-6-94
Neem-Azal T (AZA)	50 ppm a.i.	2	29-5-94/9-6-94
Unt. Ctrl.	-	-	-

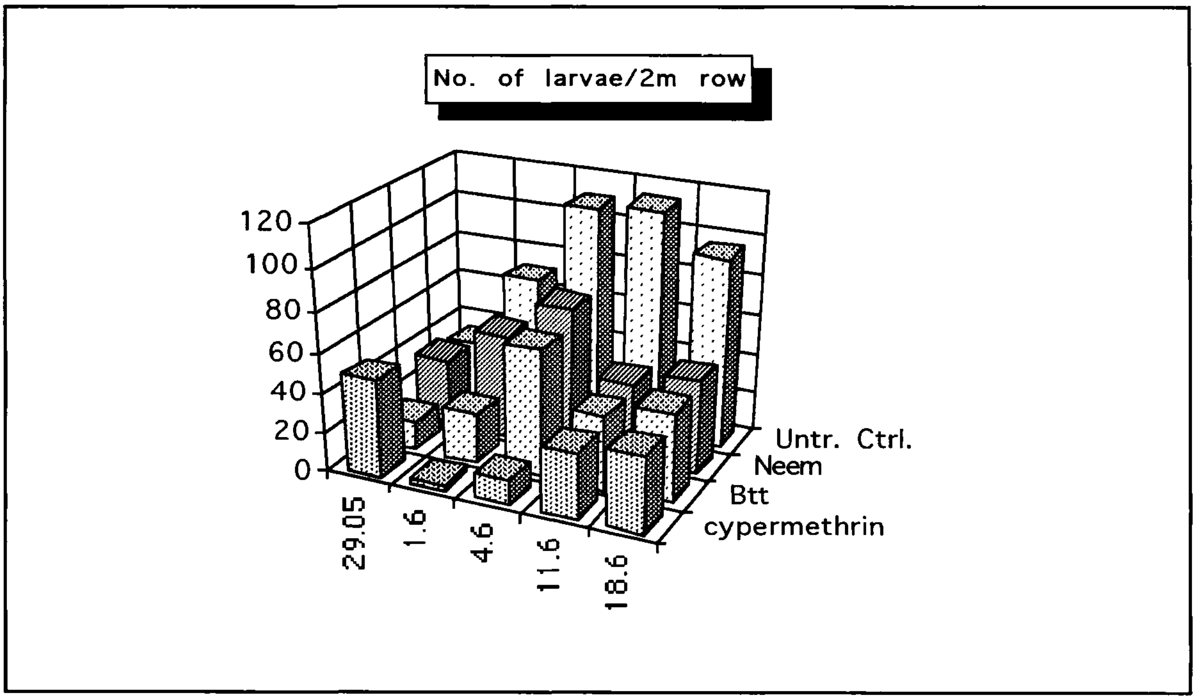
Experimental design: randomized blocks (4 replicates)
Plot size: 30 m^2 (6x5 m)
Water pH: 7.5
Water volume: 8 l/treatment (= 660 l/ha)
Level of infestation: high
Assessment dates: 29.5.94 (pre-treatment), 1.6.94, 4.6.94, 11.6.94, 18.6.94

Results

a) No. of larvae

treatment	n° treats.	No. of larvae/2m row				
		29-5-94	1-6-94	4-6-94	11-6-94	18-6-94
cypermethrin	1	49,0 A°	3,3 B	12,3 B	32,8 B	38,0 A
Btt	2	14,5 A	24,8 A	63,3 A	36,8 B	44,3 A
Neem	2	34,3 A	52,8 A	71,5 A	39,3 B	47,0 A
Unt. Ctrl.	-	31,8 A	70,3 A	109,8 A	113,5 A	94,5 A

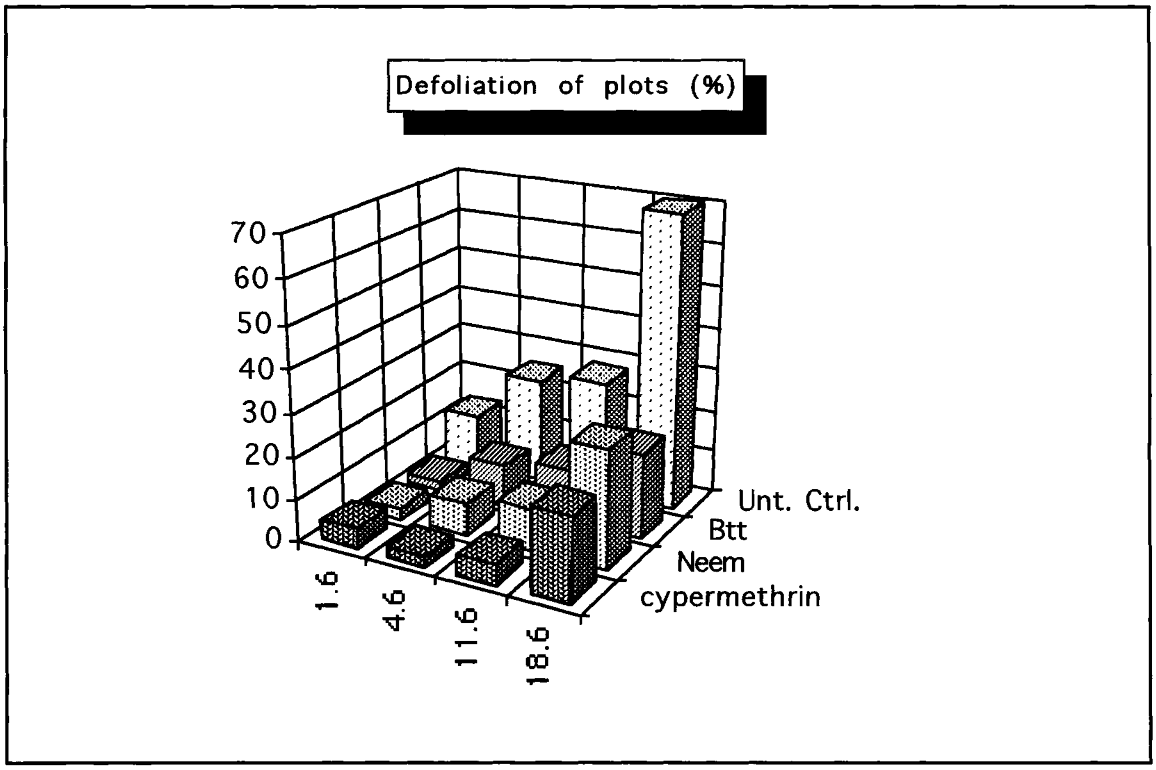
° Values followed by the same letter are not significantly different at P=0.05 (Tuckey's test).



b) Defoliation rate (%) of plots

treatment	n° treats.	defoliation rate			
		1-6-94	4-6-94	11-6-94	18-6-94
cypermethrin	1	5,0 A°	2,5 B	5,0 B	20,0 B
Btt	2	2,5 A	10,0 B	12,5 AB	20,0 B
Neem	2	2,5 A	7,5 B	10,0 AB	27,5 B
Unt. Ctrl.	-	12,5 A	25,0 A	27,5 A	70,0 A

° Values followed by the same letter are not significantly different at P=0.05 (Tuckey's test).



***Cydia molesta* (Oriental Peach Borer)**

Materials & methods
Location of trial: San Martino (District of Ferrara, Northern Italy)
Crop and Cv: Peach, Cv. Stark Red Gold
Training system: palmette
Treatments:

treatments (a.i.)	rate	n° treats.	treats. dates
Neem-Azal T (AZA)	50 ppm a.i.	3	19.6-26.6-2.7
Delfin (Btk)	1 kg/ha	2	19.6 - 29.6
Delfin (Btk)	1 kg/ha	3	19.6-26.6-2.7
Azithion (azinphos m.)	2,5 Kg/ha	1	19-6
Unt. Ctrl.	-	-	-

Experimental design: randomized blocks (4 replicates)
Plot size: 4 plants
pH of water:7.5
Water volume: 2.2 l/plant = 1800 l/ha
Level of infestation: medium
Date of assessment: 17.7.94

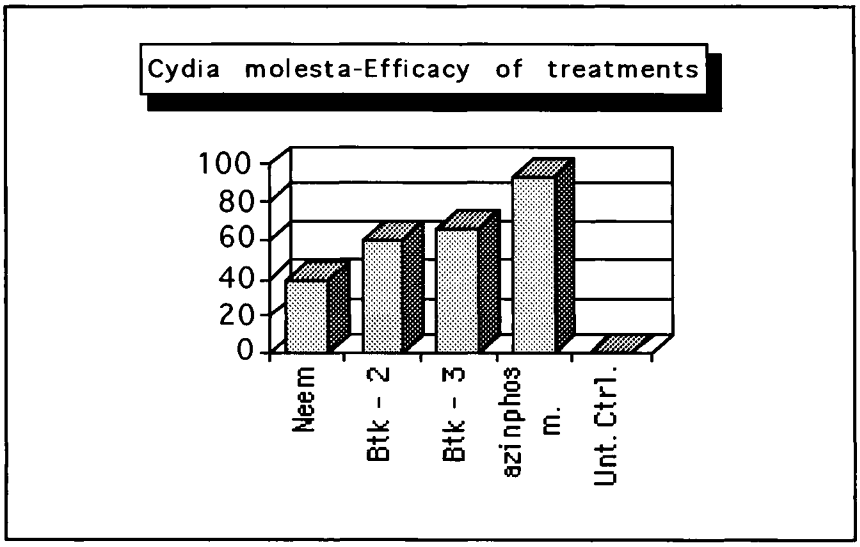
Results

treatment	n° treats.	assessments			efficacy°
		% att. fruits	% att. twigs	damage index*	
Neem	3	7,75	14,75	6,33 BC°	38,95
Btk	2	3,25	15,25	4,16 BC	59,88
Btk	3	2,25	14,5	3,53 C	65,95
azinphos m.	1	0,25	2,5	0,66 D	93,63
Unt. Ctrl.	-	12,75	24	10,37 A	-

° Values followed by the same letter are not significantly different at P=0.05 (Tuckey's test).

$$\text{° Efficacy of treatment} = \frac{\text{damage index in Ctrl.} - \text{damage index in treatment}}{\text{damage index in Ctrl.}} \times 100$$

$$\text{*Damage index} = \frac{(\text{\% attacked twigs/3}) + \text{\% attacked fruits}}{2} \quad \text{(ACTA, 1984)}$$



***Ostrinia nubilalis* (European Corn Borer)**

Materials & Methods
Location of trial: Alfonsine (district of Ravenna, Northern Italy)
Crop and Cv: green bean (*Phaseolus vulgaris*), Cv. Masai
Date of sowing: 18.7.94

Treatments:

treatment (a.i.)	rate (l-kg/ha)	n° treats.	Treatment dates
Delfin (Btk)	1,5	4	20.8-27.8-4.9-11.9
Neem-Azal T/S (AZA)	50 ppm a.i.	4	20.8-27.8-4.9-11.9
Decis (deltamethrin)	0,5	4	20.8-27.8-4.9-11.9
Untreated control	-	-	-

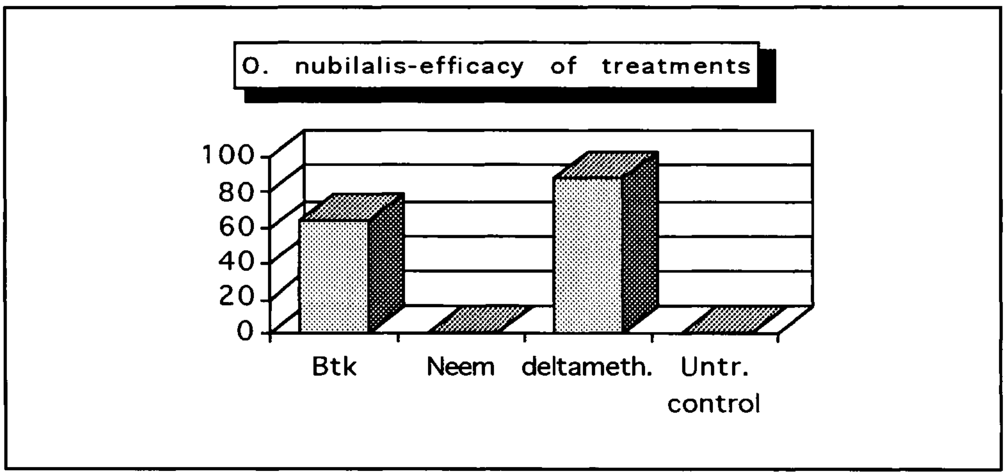
Experimental design: randomized blocks (4 replicates)
Plot size: 30 m^2 (6x5 m)
Water pH: 7.0
Water volume: 12 l/treatment (= 1000 l/ha)
Level of infestation: medium-high
Assessment date: 17.9.94

Results

treatment	n° treats.	weight of pods (25 plants)	% damaged pods	efficacy*
Btk	4	950,3 B°	1,62 B	63,51
Neem	4	1010,0 AB	4,48 A	0
deltamethrin	4	1154,0 A	0,56 C	87,38
Untreated control	-	886,8 B	4,44 A	-

° Values followed by the same letter are not significantly different at P=0.05 (Tuckey's test).

$$\text{* Efficacy of treatment} = \frac{\text{\% damage in Control - \% damage in treatment}}{\text{\% damage in Control}} \times 100$$



***Lobesia botrana* (European Berry Borer)**

Materials & methods
Location of trial: Tebano (district of Forli, Northern Italy)
Crop and Cv: Grapevine, Cv. Trebbiano
Training system: Casarsa
Plantation distances: 3.5x2 m

Treatments:

treatment (a.i.)	rate (kg-l/ha)	n° treats.	treat. schedule	treats. dates
Delfin (Btk)	0,75	2	B	25 June-3 July
Neem-Azal T (AZA)	50 ppm	2	B	25 June-3 July
Ekalux (quinalphos)	1,5	1	A	28 june
Unt. Ctrl.	-	-		-

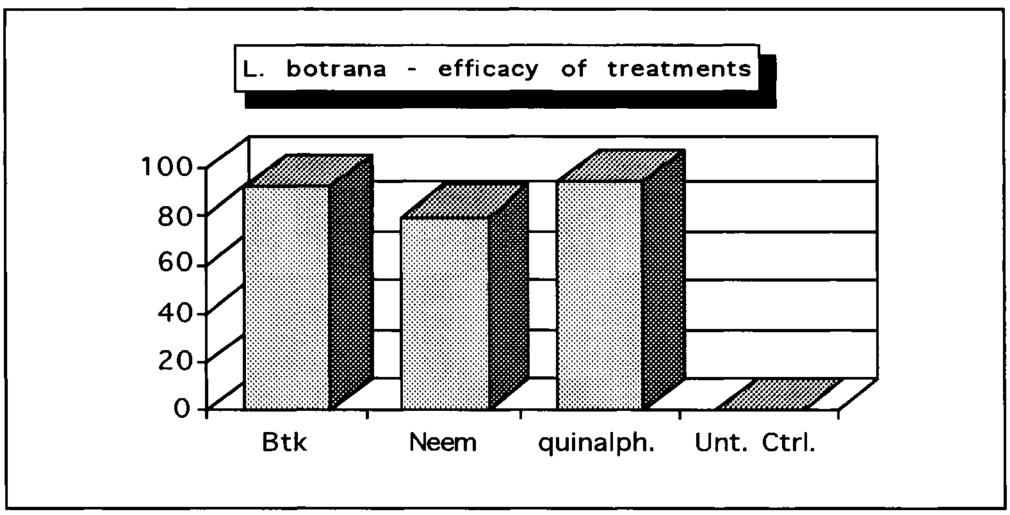
Experimental design: randomized blocks (4 replicates)
plot size: 10 plants
pH of water: 7.5
Volumeof water for treatment: 0.6 l/plant = 1000 l/ha
Level of infestation: medium-high
Date of assessment: 16.7.94

Results

A	B	C	D	E	F
treatment	n° treats.	% of infest. bunches	inf.berries/ bunch	infestation index (DxE)	efficacy of*
Btk	2	10,5	0,27	2,83 BC	92,22
Neem	2	14	0,54	7,56 B	79,27
quinalphos	1	8,5	0,24	2,04 C	94,4
Unt. Ctrl.	-	28,5	1,28	36,48 A	-

° Values followed by the same letter are not significantly different at P=0.05 (Tuckey's test).

$$\text{*Efficacy of treats.} = \frac{\text{infest. index in Ctrl.} - \text{infest. index in treated}}{\text{infest. index in Ctrl.}} \times 100$$



***Mamestra brassicae* (Cabbage Moth)**

Materials & Methods
Location of trial: Savarna (district of Ravenna, Northern Italy)
Crop and Cv: Spinach, Cv. Sporter F1
Date of sowing: 31.8.94

Treatments:

treatment	rate	n° treats.	treats. dates
Neem-Azal T (AZA)	50 ppm	2	3-10 Oct. 1994
Mamestrin (NPV)	3,5 l/ha	2	3-10 Oct. 1994
Delfin (Btk)	0,75 kg/ha	2	3-10 Oct. 1994
chemical standard*	-	2	3-10 Oct. 1994
Unt. Ctrl.	_	-	-

* It was applied the same treatment schedule as recommended by the technical service of the cooperative where the trial was carried out: 1st treat.: Orthofat (acephate) 1.5g/l; 2nd treat.: Decis (deltamethrin) 0.5 ml/l.

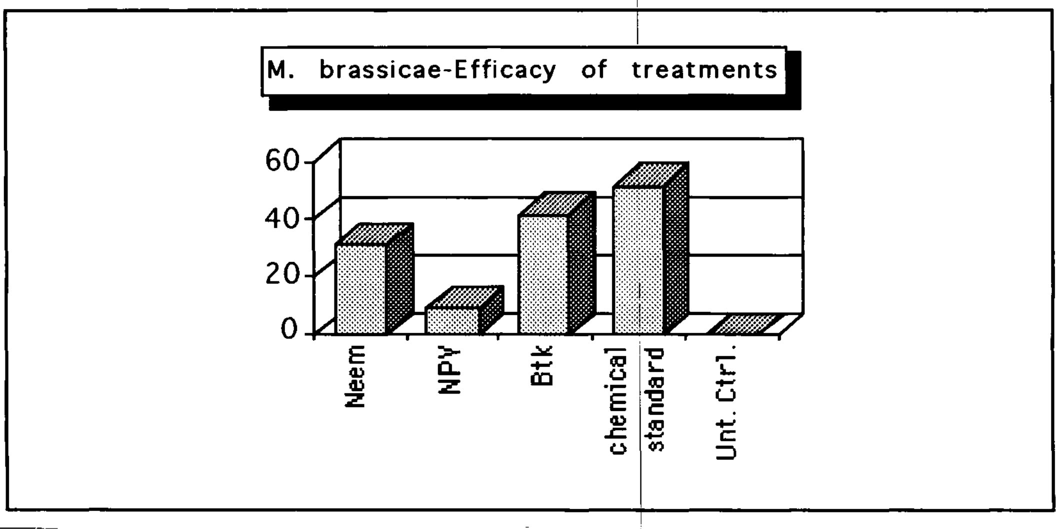
Experimental design: randomized blocks (4 replicates)
Plot size: 15 m^2 (3x5 m)
Water pH: 7.5
Water volume: 5 l/treatment (= 830 l/ha)
Level of infestation: high (artificial)
Other species present: *Agrotis segetum*
Assessment date: 19.10.94

<u>Results</u>

treatment	damage rate (0-5)	weight of 50 plants(g)	% damaged leaves	efficacy*
Neem	1,75	1404 A°	21,11 BC	31,28
NPV	3,5	1593 A	28,04 A	8,72
Btk	2	1735 A	18,03 BC	41,30
chemical standard	1	1659 A	14,68 C	52,21
Untreated control	4,5	1616 A	30,72 A	-

° Values followed by the same letter are not significantly different at P=0.05 (Tuckey's test).

$$\text{* Efficacy of treatment} = \frac{\text{\% damage in Control} - \text{\% damage in treatment}}{\text{\% damage in Control}} \times 100$$



Lycoriella sp. (mushroom sciarid)

Materials & methods

Location of trial: Porcellengo (TV)
Mushroom species: *Agaricus bisporus* ("white button mushroom")
Cultivation system: in shelf-beds

Treatments:

treatment (a.i.)	rate /m2 g form. (g a.i. or J3s)	n° treats.
Armor (cyromazine)	2,5 (0,37)	1
Exhibit (S. feltiae)	- (500.000)	1
Bactimos (Bti)	10 (-)	1
Neem-Azal W (AZA)	2 (0,2)	1
Control	-	-

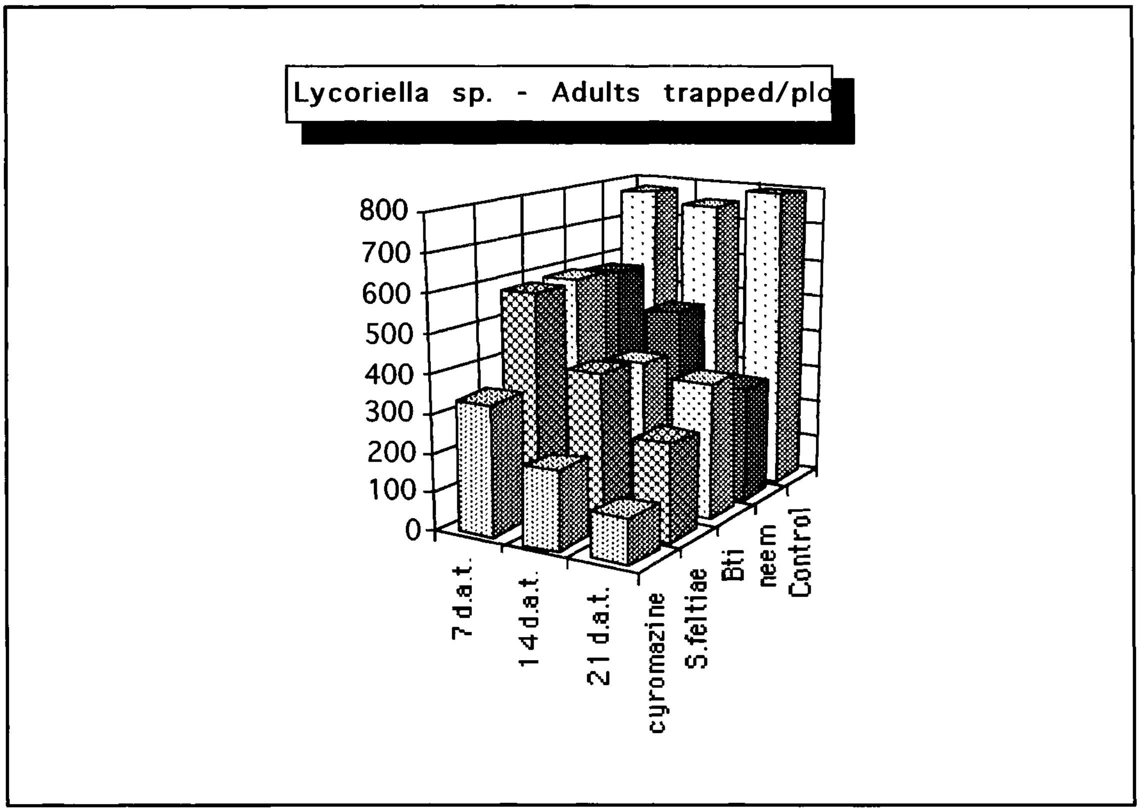
Experimental design: randomized blocks (4 replicates)
plot size: 2 m^2 (=8 m^2/treatment)
pH of substrate: 8
pH of water used for treatment: 7
Water volume applied: 2 l/m^2
Date of treatment: 14.5.94
Level of infestation: high

Results

a) Adults trapped/plot

treatment	sampling date		
	7 d.a.t.	14 d.a.t.	21 d.a.t.
cyromazine	338,5 B	198,0 C	117,5 C
Steinernema feltiae	582,0 AB	394,5 BC	248,3 BC
Bti	584,5 AB	386,8 BC	353,5 B
Neem	576,8 AB	490,8 AB	303,5 BC
Control	778,5 A	749,8 A	794,8 A

* Values followed by the same letter within each sampling are not significantly different at p=0.05 (Tuckey's test).

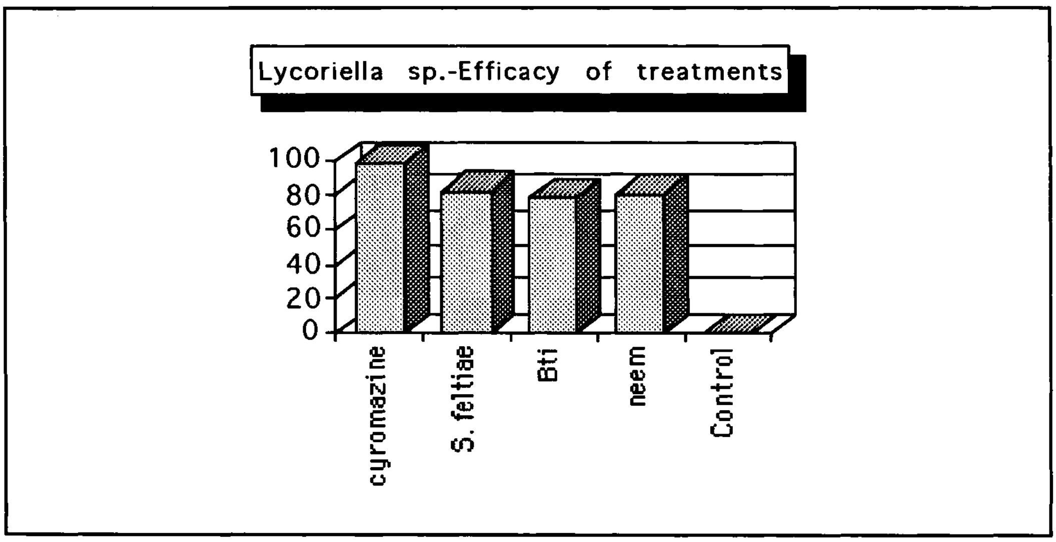


b) Mushroom yield

treatment	Market. mushr. (kg)	waste mushr. (g)	efficacy°
cyromazine	17,40 A*	3,5 B	97,83
Steinernema feltiae	17,51 A	28,3 B	82,57
Bti	18,65 A	37,0 B	78,43
Neem	18,69 A	32,8 B	80,95
Control	17,39 A	160,8 A	-

* Values followed by the same letter within each sampling are not significantly different at p=0.05 (Tuckey's test). No correlation was found for the different treatments between mushroom production and waste.

$$° \text{ Efficacy of treatment} = \frac{\% \text{ damage in Ctrl.} - \% \text{ damage in treatment}}{\% \text{ damage in Ctrl.}} \times 100$$



***Bradysia paupera* (Fungus Gnat)**

Materials & methods

Location of the trial: Gaiarine (district of Treviso, Northern Italy)
Crop and CV.: Primrose (*Primula veris*), Cv. Finale Mix
Cultivation system: in trays (55x33 cm; 336 alveoles/tray)
Date of plantation: 20.7.94

Treatments:

n°	treatment (a.i.)	rate /m2 g form. (g a.i. or J3s)	n° treats.	dates of treats.
1	Trigard 75WP (cyromazine)	0,2 (0,15)	2	8.8.94/4.9.94
2	Exhibit F 27 (S. feltiae)	- (500.000)	2	8.8.94/4.9.94
3	Bactimos (Bti)	10 (-)	2	8.8.94/4.9.94
4	Neem-Azal W (AZA)	2 (0,2)	2	8.8.94/4.9.94
5	Control	-	-	-

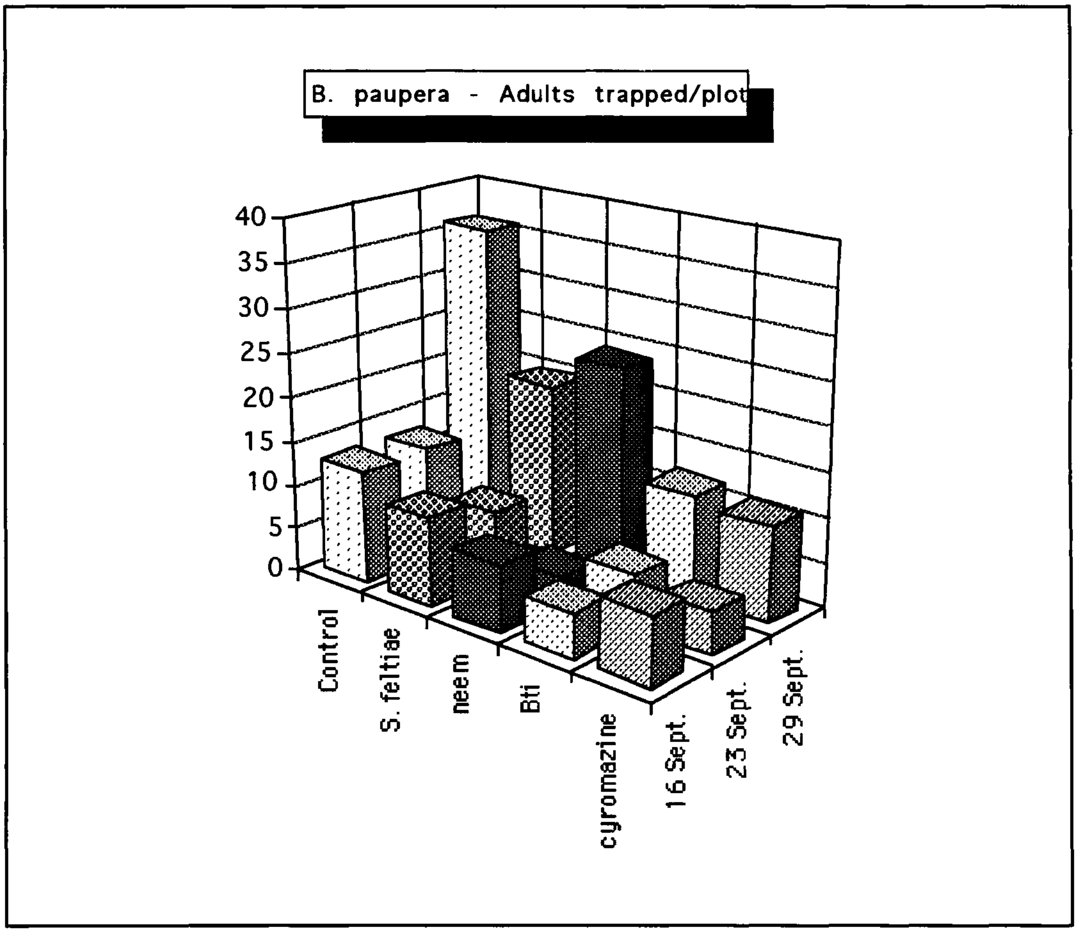
Experimental design: randomized blocks (4 replicates)
plot size: 0.36 m^2 (=1.45 m^2/treatment)
pH of substrate: ±5.7 initially, changing to ±6.3 at the end of cultivation cycle (10 weeks)
pH of water used for treatment: 7.4
Water volume : 2 l/m^2
Level of infestation: medium
Date of assessment: 29.9.94

Results

a) No. of *Bradisia paupera* adults trapped/plot

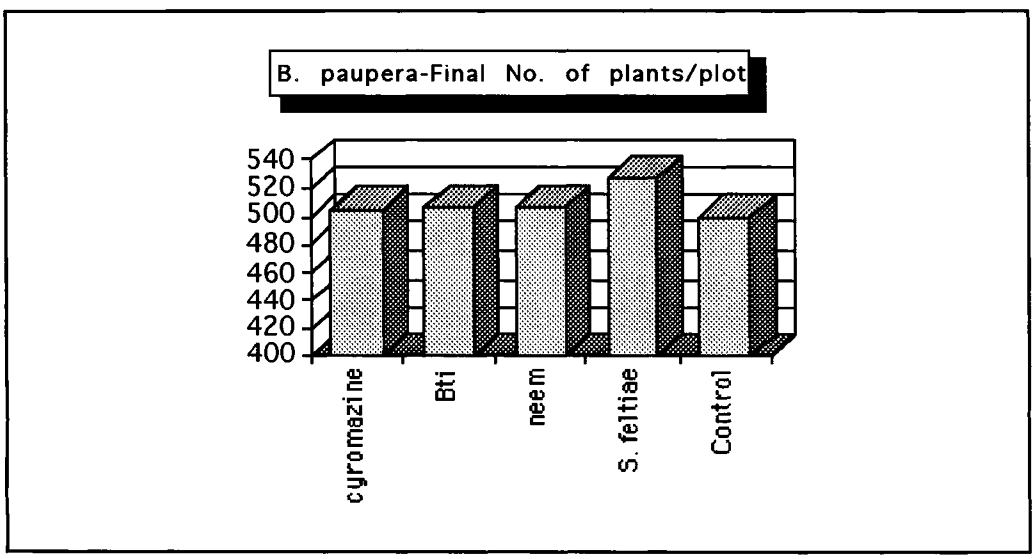
treatment	date of sampling		
	16 Sept.	23 Sept.	29 Sept.
cyromazine	7,75 AB	4,75 B	11,00 C
S. feltiae	10,00 AB	7,75 AB	19,75 BC
Bti	5,00 B	6,00 B	11,75 C
neem	7,50 AB	3,00 B	23,75 B
Control	12,75 A	12,75 A	36,00 A

* Values followed by the same letter within each sampling are not significantly different at p=0.05 (Tuckey's test).



b) Number of plants

treatment	No. of plants	score (1-3 scale)
cyromazine	504,75 B	1,75
S. feltiae	527,50 A	2,25
NN-6038300 (Bti)	507,00 B	2,25
azadirachtin	507,50 B	1,75
Control	500,25 B	1,50



Discussion

The CPB confirmed in our experience to be a good target for neem-based products, which possess a remarkable activity on both larvae and adults of this chrysomelid (Jacobs, 1987; Schmutterer, 1987). The reduction in the number of larvae on the plants was statistically significative only following the second application for both neem and Btt. None of these agents had a knockdown effect comparable to that of the pyrethroid. However, it is very interesting to note that the rate of defoliation of the plots proceeded virtually on the same level for all treatments. This is a clear indication that larvae treated with either neem or Btt, though for completely different mechanisms, do not die immediately but are prevented from causing any further damage. This result is particularly significative considering that on both occasions pesticide treatment were followed by a rainfall within 24-36 hours of application.

Against the OPB neem showed a rather limited activity. This result was expected, as previous studies had shown that this species is not particularly susceptible *in vivo* even under laboratory conditions (Eisenlohr et al;, 1992). It should be pointed out that on twigs neem was as effective as Bt, while its efficacy on the fruits was much lower. Presumably, on peach the systemic properties of neem are sufficient to partially protect the twigs, but not the fruits.

The difficulty for neem to control boring insects was also shown in case of the ECB, *Ostrinia nubilalis,* which nevertheless is a susceptible species under controlled conditions (Ascher, 1990). As a matter of fact, the efficacy against this species was nil, in terms of damaged pods. However, the yield of pods for plants treated with neem and deltamethrin was tendentially higher than in Bt-treated and control plots, though statistically not significative because of the high variability in a few plots . Again, this may indicate a protection limited to the vegetative parts of the plant; this aspect should be further investigated.

Against *Lobesia botrana* on grapes, quinalphos uotperformed all bioinsecticides, in consideration also of the single treatment; nevertheless, the protection level provided by the latter was more than acceptable (at least for grapes destined to wine production). The efficacy of neem was only slightly shorter than that of Bt. This is a very interesting result, if one takes into account also the good activity of neem against downy mildew (Achimu and Schlösser, 1992; Pancaldi and Rovesti, unpublished observations), which constitute the other major problem for this crop.

Against *Mamestra brassicae* (and *Agrotis segetum*) the effect of neem was again on a par with Bt, though there was some inconsistency in the results obtained with different evaluation systems (i.e. visual estimation of plots and analitical calculation of damaged leaves). However, on this particular crop the main problem is given not so much by the damage on leaves as by the presence of larvae at harvest; given the slow activity of neem its use on spinach harvested mechanically is quite unlikely. A previous experience with an untitled neem oil gave negative results (Chianella and Rovesti, 1992), while on brassicas its application appears more promising (Mordu et al;, 1993).

Finally, the control of sciarid flies in both mushrooms and ornamentals appears a very interesting niche market for neem-derived insecticides. On mushrooms neem, *S. feltiae* and Bti significantly reduced the number of adult flies emerging from the compost, and their effect was evident for at least 3 weeks. With regard to mushroom yield no statistical differences were observed between the treatments, but presumably this was mostly because of the patchy emergence of the mushrooms on the beds and the limited size of plots; however, all treatments significantly reduced the amount of mushrooms damaged by sciarid larvae. The number of sciarid was similarly reduced on primrose; in this case neem showed a very good activity, but shorter persistence.

No phytotoxic effects could be observed for neem products in any of the trials.

Conclusions

From the above results it is clear that Neem-derived pesticides have good potential for use against many pests also under practical conditions. With the exception of a few difficult target pests and on particular crops, for which chemical control still seems the only option to guarantee high productions or produces which conform to the present market standards, the use of Neem and other biocontrol agents gives a level of crop protection acceptable from a practical point of view (this would be particularly so where a parallel market for organically-grown products is available). However, it is very important that the evaluation of these new and often innovative products be carried out following appropriate criteria which not necessarily are those used for traditional pesticides (e.g. knockdown effect or mortality of insects), for their real value to be appreciated. In this respect our experience with the CPB (for both neem and Btt) is enlightening, but the same might apply to other situations. In conclusion we can say that Neem does have the requisites to be succesfully used also in industrialised countries. A few problems still remain to be solved, not just for neem but for biocontrol agents in general, before they can really compete with traditional pesticides. Such problems mainly concern a relatively short shelf life and persistence, and a higher price. New technical developments may aid solve some of these constraints, and for neem this goal may be quite close(Immaraju et al., 1994).

References

ACHIMU P., SCHLÖSSER (1992). Effect of neem seed extracts (*Azadirachta indica* A. Juss) against downy mildew (*Plasmopara viticola*) of grapevine. In Kleeberg H. (Ed), "Practice oriented results on use and production of neem-ingredients and pheromones"; proc. of the 1st workshop (Wetzlar, June 19th-20th), 99-107.

ACTA (1974). Controles périodiques en verger: pecher. Brochure ACTA-Lutte Intégréé, Paris, 190 pp.

ASCHER K.R.S. (1990). Neem's insecticidal and acaricidal activity against field crop and orchard pests. In Locke J.C. and Lawson R.H. (Eds) - "Neem's potential in pest management programs"; Proc. USDA Neem Workshop; USDA-ARS, pp. 40-56.

CHIANELLA M., ROVESTI L. (1992). First experiences with neem in Italy and its potential uses in plant protection. In Kleeberg H. (Ed), "Practice oriented results on use and production of neem-ingredients and pheromones"; proc. of the 1st workshop (Wetzlar, June 19th-20th), 57-64.

EISENLOHR U., DOMANGE A.L., LENFANT C., SAUPHANOR B. (1992). Effects of NeemAzal-F on aphids and beneficial insects in peach orchards in France. In Kleeberg H. (Ed), "Practice oriented results on use and production of neem-ingredients and pheromones"; proc. of the 1st workshop (Wetzlar, June 19th-20th), 27-40.

IMMARAJU J., WELLS S., RUGGERO W., NELSON R., SELBY B. (1994). Relative residual activities of azadirachtin, dihydroazadirachtin and tetrahydroazadirachtin. Proceedings BCPC-Pests and Diseases; vol. 1, 53-58.

JACOBSON M. (1986). Neem research and cultivation in the Western hemisphere. In Schmutterer H. and Ascher K.R.S. (Eds.), "Natural pesticides from the neem tree and other tropical plants; proc. 'rd Int. Neem Conf., Nairobi, 10-15 July; 33-44.

MORDU A.J., McKINLAY R.D., DAVIDSON G., HUGHES J. (1993). Azadirachtin and Neem-Azal F in the control of lepidopteran pests of brassicas in Scotland. In Kleeberg H. (Ed), "Practice oriented results on use and production of neem-ingredients and pheromones"; proc. of the 2nd workshop (Wetzlar, Nov. 30th-Dec. 2nd), 87-88.

SCHMUTTERER H. (1986). Fecundity-reducing and sterilizing effects of neem seed kernel extracts in the Colorado potato beetle, *Leptinotarsa decemlineata*. In Schmutterer H. and Ascher K.R.S. (Eds.), "Natural pesticides from the neem tree and other tropical plants; proc. 'rd Int. Neem Conf., Nairobi, 10-15 July; 351-360.

Neem trials in Switzerland 1994

Markus Zuber, Andermatt BIOCONTROL AG, CH-6146 Grossdietwil

Introduction

The fame of an efficient and specific plant seed extract from the tropics has reached the Swiss biological farmers some years ago already.

Finally Andermatt BIOCONTROL AG, a small, but Swiss largest company dealing with and producing goods for biological pest control got the opportunity to start testing the potencies of NeemAzal T in early 1994.

Most of the field trials aiming for registration of NeemAzal T were kindly conducted by scientists of different Swiss federal and private research stations.

To date NeemAzal T will not have a registration for 1995 yet. Some more tests and evaluations about ecotoxicological relevance will have to be carried out. The present results from agriculture and horticulture give us a lot of enthousiasm for continuing our work.

However we are not yet allowed to publish the data of recent field trials in Switzerland before registration. The following list gives a short overview over the 1994 trials and their tendencies.

target insect (- authors)	**tendency**
(++ very good; + good; = no effect; – bad effect)	
rosy apple aphid (*Dysaphis plantaginea*):	
- A. Häseli, FiBL, 4104 Oberwil	++
green apple aphid (*Aphis pomi*):	
- A. Häseli, FiBL, 4104 Oberwil	+
spider mites (*Tetranychus urticae*) on vegetables:	
- I. Wyser & E. Städler, FAW 8820 Wädenswil	+
noctuid larvae on vegetables (*Mamestra brassicae*):	
- I. Wyser & E. Städler, FAW 8820 Wädenswil	+
Colorado potato beetle (*Leptinotarsa decemlineata*):	
- A. Häseli, FiBL, 4104 Oberwil	=
- W. Jossi, FAP Reckenholz, 8046 Zürich	(+)
Oulema sp. on wheat:	
- W. Jossi, FAP Reckenholz, 8046 Zürich	+
Whiteflies (*Trialeurodes vaporariorum*):	
- S. Fischer, RAC Changins, 1260 Nyon	++
effect on *Encarsia formosa*:	
- S. Fischer, RAC Changins, 1260 Nyon	-

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

on mealybugs:

Finally one trial, conducted by S. Stüssi (Andermatt BIOCONTROL AG) can be presented herein.

Mealybugs are a common pest on Citrus and other tropical plants in offices or botanical gardens. One of the newest projects of Andermatt BIOCONTROL AG is to find biological solutions for indoor pest control. On one side beneficial insects are used on the other side soaps or plant extracts like NeemAzal T can be very helpful.

Material & Methods

A lemon tree crowded with mealybugs (*Planococcus citri*) was treated twice (18.6. and 29.6.) with NeemAzal T (0.3%) and Natural (potassium soap; 2%). The effect of Natural alone is supposed to be negligible. In this case it was applied for a better dermal penetration of NeemAzal T.

During the experiment this tree was standing outdoor.In automn it was placed indoor to protect it against cold. The indoor conditions however are not very favorable for this tree because of faint light.

Results & Discussion

Within 4 weeks the population of the mealybugs was reduced drastically.

During the indoor period however the the population has recovered. Later applications of Neem did not reduce the population anymore!

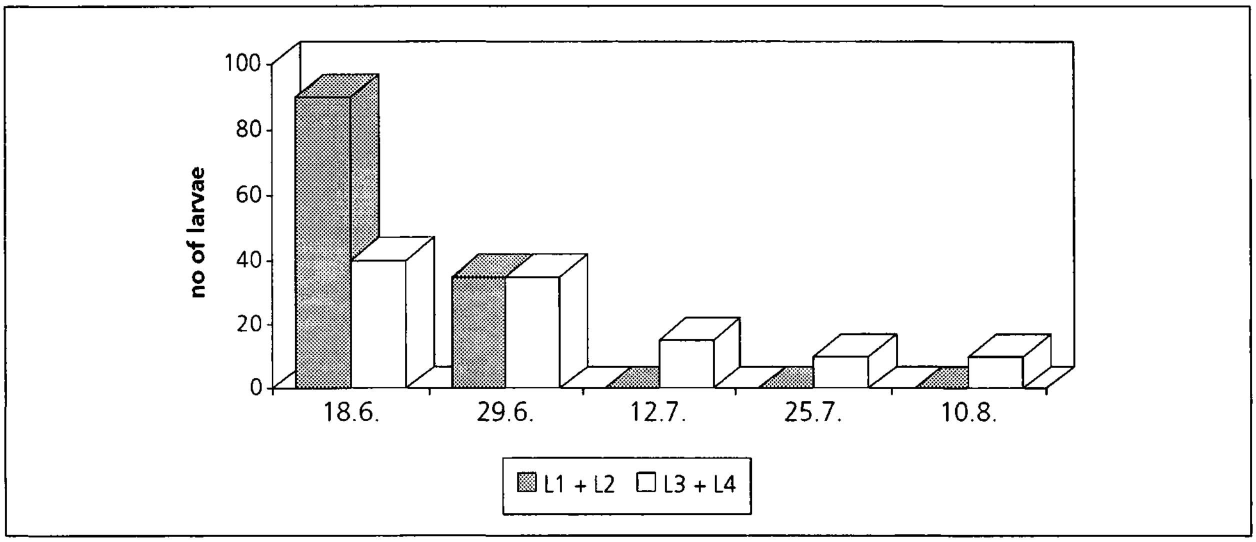


Fig. 1 Development of a mealybug (*Planococcus citri*) population on a citrus tree after two treatments with NeemAzal T 0.5l/ha + Natural 2% (on 18.6. and 29.6.).

Further experiments on mealybugs have to be carried out to be sure about the effects of NeemAzal T and additives on these insects under outdoor and indoor conditions (sunlight effect).

Future perspectives

The first aims for registration of NeemAzal T are directed to the following target insects:

- rosy apple aphid in orchards
- aphids, thrips and spider mites in ornamental plants

A registration against these insects should be possible without further tests about market tolerance.

Acknowledgements

We wish to thank A. Häseli (FiBL), I. Wyser & Dr. E. Städler (FAW), W. Jossi (FAP Reckenholz) and Dr. S. Fischer for the extensive trials mentioned above.

NEEM IN PEST CONTROL : TRENDS AND FUTURE CHALLENGES

A.G. ABRAHAM
NEEM PRODUCTS DIVISION
EID PARRY (INDIA) LTD
MADRAS

I INTRODUCTION:

Neem (Azadirachta indica A Juss) has been revered by Indians for centuries. The knowledge on the remarkable properties of neem tree has been known for years in India and has been used as medicine on one hand and as pesticide on the other (Schmutterer 1995).

For decades in India it has been used to control insect pests in agriculture and storage of food grain. In spite of extensive research and documentation on the efficacy of neem extracts in pest control in early years, it is only in the past 10 years there has been a spurt in the activities to commercialize neem in several countries primarily because of the pressure for user safe, residue free and ecologically friendly pesticides.

The group of azadirachtins in neem has so far proved to be an exceptional model in the research for safer pesticidal sources. Azadirachtin has undoubtedly been determined to be one of the most potent molecules which apart from being an IGR acts on pests on a number of other ways.

The unique features of neem has attracted scientists interest worldwide leading to 4 international conferences .Neem pesticides possess significant advantages over other pesticides although the necessity to emphasize its mode of action and method of providing protection needs greater explanation to the agricultural community, especially in developing agricultural economies.

II NEEM: A VERSATILE TREE:

Neem is one tree wherein every part can be utilized.Neem attracted international attention ever since the report published during early 1960 on the rich bioactive sources of neem extracts and its utility in Agriculture in India. The scientific report by Dr. Pradhan of the Indian Agricultural Research Institute, New Delhi in 1962 sowed the seeds of scientific curiosity of thousands of scientists and intellectuals all over the world. Innumerable publications are available describing the insecticidal properties on diverse pests infesting variety of crops.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

While in India the efficacy spectrum and usage of neem products are continuously increasing, the number of commercial formulations that are also entering the market are also rapidly growing.

While the potential and the need for bioproducts are indisputable the reason to use/shift to such safer pesticides in developed economies is facilitated by Government regulations on residue restrictions, environmental protection, user safety coupled with pressures from the consumers. While the need for increasing agricultural production is gaining momentum in developing economies there is a lack of strong compulsions to change to safer pest control practices .The key elements for neem products that are critical in this scenario are :

a) Governmental support and consumer pressures
b) Quality control and
c) Marketing

The paper attempts to outline some of the trends and future requirements in relation to the above issues for successful use of neem by the farmers.

a) GOVERNMENTAL SUPPORT AND CONSUMER PRESSURES:

Government of India is actively promoting IPM concepts in various crops and to facilitate this, have relaxed the registration requirements for Neem . The knowledge of neem and its application for centuries in medicinal, veterinary and pesticidal uses have also contributed to reducing the registration requirements.

At present, Central Insecticides Board, Government of India have specified that oil based formulations should contain a minimum 0.03% azadirachtin and kernel based formulations a minimum of 0.15% azadirachtin (Parmar 1995). No upper limits have been specified by the authorities.

For neem based formulation regulatory authorities have allowed marketing of the product during provisional registration. The data that is required for provisional registration is product chemistry, acute toxicology, bioefficacy, packaging and labeling. The provisional registration is valid initially for a period of two years with the provision for extension for a further period of two years, during which chronic and sub acute toxicological data as well as multilocation - multiseason trials on crops in which the product is to be marketed has to be generated.

Most of the neem formulations that are currently granted provisional registration in India are 0.03% and 0.15% formulation with the only exception of Parrys NeemAzal-F formulation which contains 5% azadirachtin. Government has made these relaxations to promote neem which places responsibility on the manufacturers to fulfill the conditions laid down. If there is any failure to comply or the authorities feel that the data generated are not genuine they are bound to withdraw the relaxations.

However, the manufacturers feel they would require some assurance that the data generated by them are protected and others cannot apply for a "Me Too" registration using the data. If this assurance is not provided genuine manufacturers are bound to hesitate to submit the data generated. In the interest of neem, the authorities should evolve measures that will provide assurances to the manufacturers in addition to the several relaxations already extended.

b) QUALITY CONTROL :

For any neem formulation to be successful on a long run they will have to provide products that are consistent and effective. While the number of commercial neem pesticides is steadily increasing practical difficulties with neem products is the variation in bioefficacy, aflatoxins besides assurance on shelf life of formulations.

While efficacy of different formulations are bound to vary depending on the extraction and formulation technology it is essential that products that are sold are aflatoxin free as they pose grave danger to the consumers (Ermel & Kleeberg 1995). Guidelines incorporating permitted aflatoxin levels, analytical standards for measurement on azadirachtin and aflatoxins have to be refined/evolved so as to ensure that consumers are provided with quality products .

To ensure aflatoxin free products it is vital for the industry to pay attention to seed collection, procurement and storage facilities besides adopting appropriate steps to improve them.

Mr. Ketker, who is an expert in the neem field in India estimates the neem tree population in India at 25 million (Frances Kanox 1991). The current practice of collection of fruits from these trees is that they are picked after they fall to the ground during the seasonal period by women ,children and unemployed male labour. The earnings from this though small, supplement their income. The fruits are then sold to village level traders who in turn either take it to mundys or directly sell them to end users.

The existing buyers do not demand clean and well dried seeds as most of them are neem oil and neem cake manufacturers. However, with growing number of manufacturers of neem pesticides in the country the demand for the seeds is bound to increase. It is therefore necessary to find innovative ways of increasing the neem tree population and collection of seed. Encouraging farmers to plant neem in denuded or fallow lands will increase number of trees as well as help in social forestation.

The other aspect is to take steps to improve collection, cleaning and storage of seeds at the grass root level. The fact that the active ingredients of neem rapidly degrade if not cleaned, dried and stored is well known. Improvements at village level is possible only if they are educated about the importance & need for carrying out these operations and more importantly obtain better prices for their seed collection efforts.

While this is no easy task and is bound to take considerable time and effort, at Parry we recognize the importance and are working towards this.

c) MARKETING

Neem extracts are primarily recognized for its repellent and antifeedant properties and the IGR activity are not recognized and accepted by the users generally. Scientific literature clearly establishes that neem products rich in active ingredients like azadirachtin and structurally similar compounds have IGR activity and provides consistent and reliable protection from pests. In fact, aza rich neem formulations have been observed to inhibit insect population effectively in view of its strong IGR and other activities. Neem formulations also exhibit slow action compared to chemical pesticides and thus necessitates the need for educating the users on the mode of action and the benefits associated with using neem products.

The expectation of the farmers, the lack of compulsion to use neem products and that they behave differently from synthetic pesticides will have to be borne in mind while evolving a promotional strategy. Promoting the product effectively will necessitate carrying out detailed trials at the field level and to study how the products acts, performs and how economical it is in comparison with the products currently being used by the farmers. This will have to be followed by demonstration trials over a full season of the crop to actually convince the farmers of the benefits.

Lack of specific educational and promotional efforts on neem or promoting neem in the same manner as conventional pesticides is bound to lead to erosion of faith among users.

Keeping this in mind Parry in collaboration with Trifolio have carried out extensive trials on over 60 pests on 21 different crops with good to excellent results in different parts of the world.

NEEM PRODUCT RANGE OF PARRYS

1. NEEMAZAL

NeemAzal is a powdery concentrate of insecticidal active ingredients of neem containing 25 to 40% azadirachtin. NeemAzal can be the base for various commercially viable neem formulations. The product has been granted provisional registration for exports and approval for provisional registration for domestic use is awaited.

2. NEEMAZAL-F

Product has been provisionally registered in India. It is a liquid formulation containing 5% azadirachtin. NeemAzal-F has been commercially launched in India for the control of mites infesting tea and already earned the distinction of being a highly effective neem formulation.

3. NEEMAZAL-T/S

This formulation contains 1% azadirachtin. The application for registration in India will be made shortly.The trials with the product indicates that it is very effective against a wide range of pests.

CONCLUSION

The global potential for neem product as well as other bio pesticide is growing day by day due to concerns for the environment as well as user safety. The Government can play an important role in promoting neem based products and bio pesticides through active IPM programmes as well as by evolving reasonable and appropriate registration requirement for these products. However, the long term success for commercial products will depend entirely on the quality, efficacy as well the marketing ability to change the mind set and convince end users of the benefits and effectiveness of their neem products.

REFERENCES:

1) Ermel K. and Kleeberg H. 1995 Commercial Products, Their Standardisation and problem of Quality control In "The Neem Tree" (Ed H Schmutterer), VCH Publications, Germany PP 375-384

2) Parmar B.S. 1995 Results with commercial neem formulation produced in India. In "The Neem Tree" PP 453-470

3) Schmutterer H. 1995 The Neem Tree, 687 pages, VCH Publications

4) A Report on Study on Neem Seeds by Francis Kanoi Marketing Research 1991 PP 1

NEEM PRACTICE IN COMMERCIAL AGRICULTURE

Valerio Micheletti
Azienda Agricola Persea
18033 Camporosso - Italy

SUMMARY

Neem based formulates were tried in field and in greenhouse on different plants against various insects.
Good control was obtained against Trialeuodes vaporariorum on Solanum muricatum,Myzus per sicae on Lactuca,Liriomyza huidobrensis on Ocimum basilicum. On roses, grown in greenhuose. excellent results were achieved against Tetranychus urticae,whereas against Frankliniella occidentalis and Macrosiphum rosae the efficiency was less satisfactory.

INTRODUCTION

In commercial agriculture to control harmful phytophagous chemical insecticides are used targeted to specific insects. These products are efficient but highly poisonous to beneficial insects and to mammals and humans.
The aim of our experiments was to ascertain the efficiency of Neem formulates as a biological alternative to chemical pesticides.

A serial of trials were done on different plants, ornamentals and vegetables, in different environments, greenhouse and open field .
The results are very satisfactory.A Neem formulate may act efficiently provided its mode of action is well taken in mind : it has not a knock-down effect like a poison has and its efficiency grows stepwise with time.
If the operator looks at the plants short after spray will be surprised to find the larvae stiil there alive and being accustomed to chemicals,his first reaction will be that Neem formulations are inefficient: yes the larvae are there.alive.but they do not feed any more,so that the plants are not damaged.

MATERIALS AND METHODS

The tests were performed by simply replacing chemical pesticides with Neem formulates so that environment,spraying technique and operator's performance were exactly the same in both cases.This was done in order to ascertain the practical efficacy in using Neem products by unskilled operators.

In almost all tests two areas were compared sprayed one with Neem and the other with chemicals.These two areas were physically isolated each-other.No untreated area was used as a "control" whenever the nurseries involved could not efford the risk to contaminate commercial production with dangerous phytophagous.
The efficiency of Neem-azal was determined by observing the elimination of the parasites with comparison with the action of the chemical pesticides.
An efficiency of 100% corresponds to a complete elimination; a lower efficiency means that a certain percentage of insects survived a week after the Neem spray.
The Neem oil formulations,named Neem-Azal T and Neem-Azal T/S , were supplied by the German Co Trifolio ; the formulations were diluted in water to obtain solutions ranging from 5 to 50 ppm of Azadirachtin-A .Spraying was done with standard commercial high volume spraying means .

TRIALS

The following table summarizes the various trials:

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

NEEM PRACTICE IN COMMERCIAL AGRICULTURE

test	*phytophagous*	*plant*	*test area*	*season*	*NEEM spray*			*efficiency*
					type	ppm	interval	
1	Trialeurodes vapor.	Solanum muricatum	greenhouse	Feb-April	Azal-T	5	bimonthly	100%
2	Myzus persicae	Lactuca	open field	summer	Azal-T	15	twice	100%
3	Lyriomyza Huidobrensis	Ocimum basilicum	heated greenhouse	Apr-May	Azal-T/S	5	weekly	100%
4	Tetranychus urticae	Roses	heated greenhouse	Apr-may	Azal-T	50	weekly	100%
5	Frankliniella occidentalis	Roses	heated greenhouse	May-June	Azal-T	50	weekly	80/85%
6	Macrosiphum rosae	Roses	heated greenhouse	June-July	Azal-T	50	weekly	80/85%

1-TRIALEURODES VAPORARIORUM

The test was performed in a cold greenhouse,heated only up to 5°C to prevent freezing,used for growing cuttings of Solanum muricatum (Pepino dulce) a subtropical plant of the Latin-America Andes producing fruits similar to small melons.
The nursery is located on the italian Riviera characterized by a mediterranean climate.The cuttings are grown in the greenhouse from January to April.

Solanum muricatum is subject to infestation from the white fly (Trialeurodes vap.).To control this pest usually is used a chemical product,named Lannate,based on "metomil",at a rate of 200 gr/hl.
Neem-Azal T was diluted in water to get solutions ranging from 5 to 20 ppm of Azadirachtin -A.

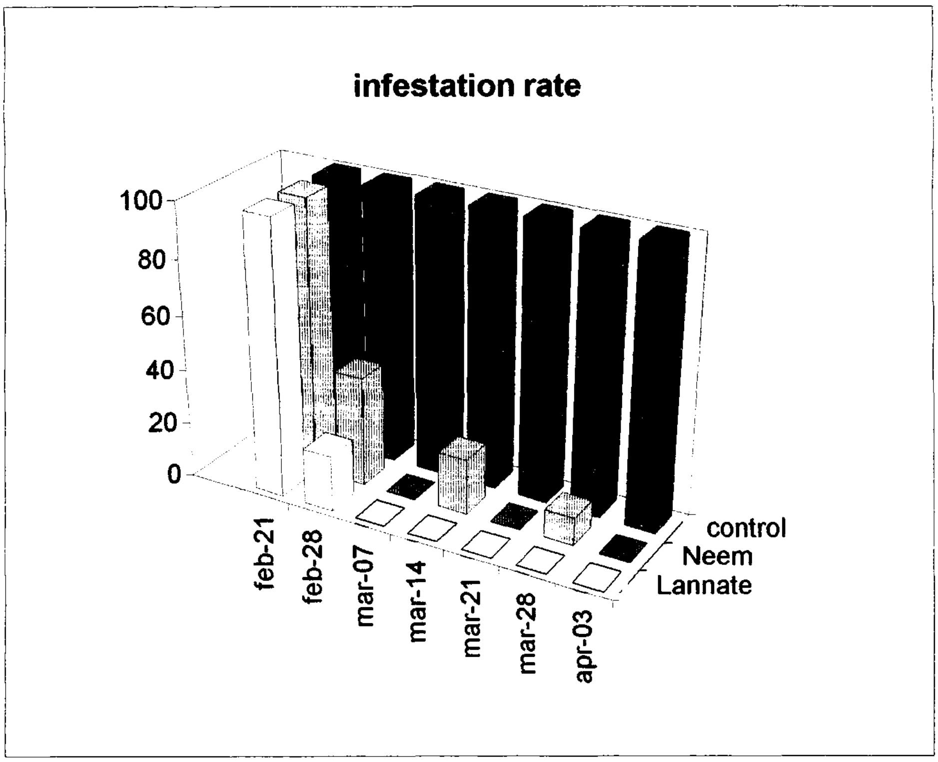
Two trials were made in an area of 60 sq. meters divided in thre zones treated one with the chemical pesticide,the second with Neem formulation and the third-control -with pure water.

A-spring 1993 -cuttings already affected by a strong infestation of white flies were treated as follows:

date	feb-21	feb-28	mar-07	mar-14	mar-21	mar-28	apr-03
Lannate gr/hl	200		200				
Neem-Aza ppmAzad-/	20	5		5		5	

the infestation rate before spraying detected by visual examination was as follows:

date	feb-21	feb-28	mar-07	mar-14	mar-21	mar-28	apr-03
Lannate	100	20	0	0	0	0	0
Neem	100	40	0	20	0	10	0
control	100	100	100	100	100	100	100



Trialeurodes vaporariorum-1993

B-spring 1994-preventive treatment:the same area of last year was treated in advance of the appearance of the infestation:

date	jan-31	feb-7	feb-14	feb-21	feb-28	mar-7	mar-14
Lannate gr/hl 100	100		100				
Neem-Azal T ppm	5		5		5		5
infestation rate							
Lannate	0	0	0	0	0	0	0
Neem	0	0	0	0	0	0	0
control	0	50	100	100	100	100	100

2.MYZUS PERSICAE

The test was made on a field of Lactuca sativa (lettuce) infested by green aphids.
This salad has a growing cycle of 45 days.
The field was sprayed twice at weekly intervals with an aqueous solution of Neem-Azal T at 15 ppm of Azad -A.
The efficiency achieved was 100%.

As a control another field was sprayed with conventional chemicals based on pyretroids.

3.LYRIOMYZA HUIDOBRENSIS

The test was performed on OCIMUM basilicum (sweet basil) a widespread cooking herb used in the mediterranean cuisine.It is sawn directly on the ground in heated greenhouses:the useful cycle between sawing and harvesting is of 4 to 5 weeks.Usually the greenhouse is divided in zones with basilicum at diferrent growing stages so that the production is continous.

The main parasites affecting sweet basil are the leaf miners;in addition the herb is subject occasionally to aphids,red spider mites and thrips.The use of pesticides is subject to strong limitations due to the short growing cycle and the need to avoid toxic residues unacceptable in a food crop.In a continous production the only pesticide allowed is based on pyrethrine.

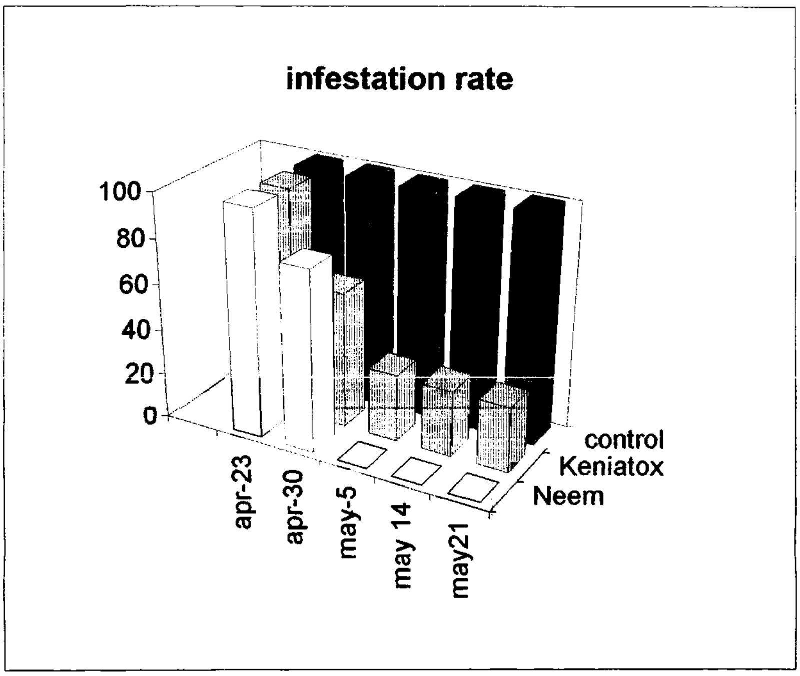
The test with Neem was run in a closed heated greenhouse of 1000 sqare meters with a continous production.After the emerging plants developed 4 leaves an acquesous solution of Neem-Azal T/S at a concentration of 5 ppm was sprayed sistematically once a week until harvesting. All parasites were under control with an efficiency of 100% against Liriomyza huidobrensis.There was no phytotoxic harm to the plants which showed healthy leaves,untouched,bright and dark green,much better looking than those treated with chemical pesticides.

The test field was divided in two area,one treated with Neem,the other with keniatox (based on pyrethrine). This test was repeated four times,from march to july,always achieving the same satisfactory results.

date	1994	apr.23	apr-30	may-5	may 14	may21
Keniatox	gr/hl	100	100	100	100	100
Neem- T/S	ppm	5	5	5	5	5

the infestation rate before spraying detected by visual inspection was as follows:

date	apr-23	apr-30	may-5	may 14	may21
Neem	100	80	0	0	0
Keniatox	100	60	30	30	30
control	100	100	100	100	100



Lyriomyza huidobrensis infestation rate -1994

4.TETRANYCHUS urticae- FRANKLINIELLA occidentalis -MACROSIPHUM rosae.

The test was run in heated greenhouses where roses are grown for cut flowers. The plants are grown directly on the ground : after a resting period from october to January, an intensive fertilizing program is started in February in order to achieve a high volume production of flowers. Due to the high stressed environment with heat and feeding, plants are easily subject to the attack of pests such as Thrips(Frankliniella occidentalis), green aphids (Macrosiphum rosae) and red spider mites (Tetranychus urticae). The infestation reaches its highest peak from mid april to early September :in this period insects are more aggressive and they reproduce at a higher rate.

To control these phytophagous a set of different pesticides is being used -each targeted to a specific insect : the set has to be modified year by year due to the acquired resistance of insects to the specific chemicals.All of the chemicals are highly toxic to animals and humans and some of them ,when widely used,have also phytotoxic effect on the plants.

The area under test was made of a heated greenhouse of 1000 square meters divided in 2 zones,one treated with the Neem-Azal formulation and the other with chemicals;as a control a third small area was sprayed with pure water.

In alla cases Neem-Azal T was used diluted in water to get a spraying solution of 50 ppm Azadirachtin-A

The chemical pesticides were

against Tetranichus urticae : Vertimec, based on an antibiotic fungi
1-st trial period from apr 18 to May 9 ,1994
2-nd trial July 11 toAug 1 ,1994

against Frankliniella occidentalis : Tamaron,based on methamidophos
trial period from May 16 to June 6 ,1994

against Macrosiphum rosae : Hosta Quick ,based on heptenophos
trial period from June 13 to July 4, 1994

The efficiency against spider mites reached 100% in both trials ,whereas with chemicals it was was lower,about80/85%.

Thrips and aphids were more difficult to control with the Neem spray and the efficiency achieved only a limit of 80 to 85%.To get rid of the still active pests the Neem spray was integrated with a chemical pesticide.

Followwing are the spraying programs and the detected infestation rates :

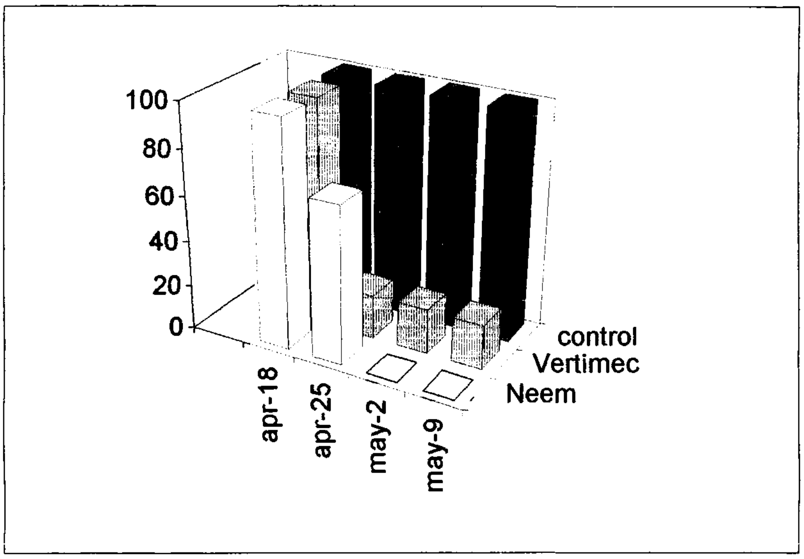
TETRANYCHUS urticae

treatments:

date	apr-18	apr-25	may-2	may-9
Neem-Azal T ppm	50	50	50	50
Vertimec cc/hl	40	40	40	40

infestation rate before spreaying:

date	apr-18	apr-25	may-2	may-9
Neem	100	70	0	0
Vertimec	100	20	20	20
control	100	100	100	100



tetranichus urticae-infestation rate

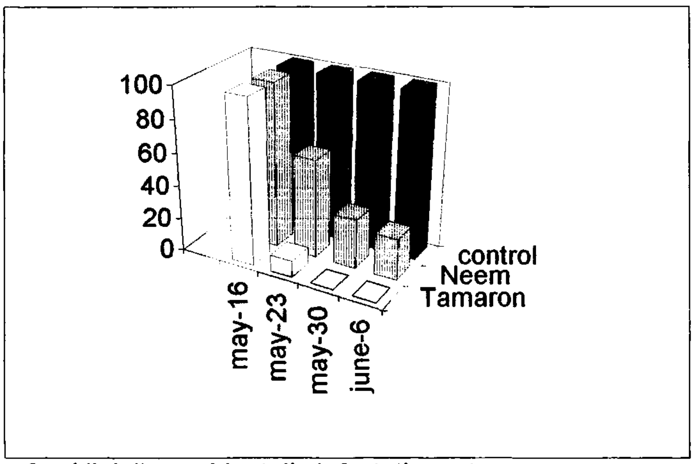
FRANKLINIELLA occidentalis

treatments:

date	may -16	may-23	may-30	june-6
Neem-Azal T ppm	50	50	50	50
Tamaron cc/hl	150	150	150	150

infestation rates

date	may-16	may-23	may-30	june-6
Tamaron	100	10	0	0
Neem	100	60	30	25
control	100	100	100	100



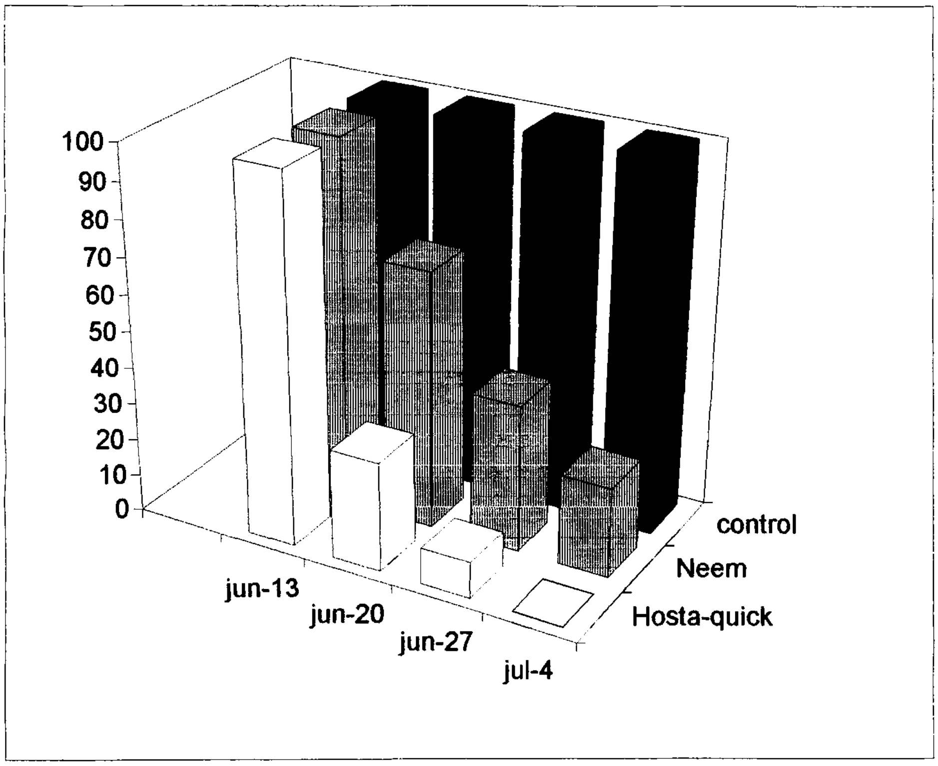
frankliniella occidentalis infestation rate

MACROSIPHUM rosae
treatments

date		June-13	June-20	June-27	July-4
Neem-Azal T	ppm	50	50	50	50
Hosta Quisck	cc/hl	100	100	100	100

infestation rate before spraying:

date	jun-13	jun-20	jun-27	jul-4
Hosta-quick	100	30	10	0
Neem	100	70	40	25
control	100	100	100	100



macrosiphum rosae infestation rate-1994

CONCLUSIONS

Our practice with Neem in commercial agriculture showed that Neem-Azal formulations when used in a preventive way,at the first appearance of the larvae,in a pest free environment have positve permannet effects.When the area is already infested with larvae and adults the action of Neem takes more time to reestablish a pest-free situation :3 to 4 cycles of spraying are required before getting rid of all infestants.

Good efficiency was achieved against Trialeurodes vaporariorum,Myzus persicae and Liriomyza huidobrensis. On roses grown in a heated greenhouse Neem-Azl acted better than a chemical pesticide in controlling Tetranychus urticae. On the contrary it was unable to achieve a 100% control,as required by commercial flower production,against Frankliniella occidentalis and Macrosiphum rosae: in this case the nursery had to integrate Neem sprays with specific chemicals.

ACKNOWLEDGEMENTS

The practical tests of our trials were supported by the valuable assistance of the technical staff of the Cooperativa l'Ortofrutticola of Albenga and by the experience and the accurate work of Mr.Pierfranco Botto,rose grower in Camporosso.

REFERENCES

Ralph E.Webb,Marck A.Hinebaugh,Richard K.Lindquist,Martin Jacobson
Evaluation of Acqueos solution of Neem Sede extract against Liriomyza sativae and L.trifolii-Florist and Nursery crop laboratory ,ARS,USDA,Beltsville ,Maryland -Journal of Entomology 76.357.362 (1983)

Hiram G.Larew,J.J.Knodel-Montz,Ralph Webb,David Warthen
Liriomyza trifolii-Control on Chrysantemum by Neem extract applied to soil J.Econ.Entomology,78.80.84 (1985)

R.K.Lindquist,H.R.Krueger and M.L.Casey
Use f Neem extract as a systemic treatment for Liriomyza trifolii control on greenhouse chrysantemum-The Ohio State University 1986 British Crop protection conference.

J.Kienzle,C.Schultz,M.Straub,A.,Schmidt,B.Weil
Use of Neemproducts for the control of Rosy Apple Aphid(Dysaphis plantaginea passerini) Staatl.Lehr-und Versuchanstalt fuerObst und Weinbau D-74189 Weinsburg Npv.1992 H.Kleeberg proceedings of the 2nd workshop,Wetzlar nov.1992

Frankliniella -neue Erkenntnisse zur Bekaempfung
Ergebnisse aus Gemeinschaftsversuchen der Pflanzenschutzdienste in Frankfurt GbGw 16/1991

F.Mansour,K.R.S.Ascher and N.Omani
Effect of Neem seed kernel extracts on the predacious mite Phytoseiulus persimilis and the phytophagus mite Tetranychus cinnabarinus
Depatment of Entomology,ARO Newe Ya'ar regional experiment station,P.O.Haifa 31999 Israel H.Schmutterer and KRS Ascher proceedungs of the third international Neem conference,Nairobi,Kenya 1o July 1986

H.Kleeberg
Future possibilities of the use of Neem biological and integrated pest management World Neem conference,Bangalore 24-28 Feb 1993

Ergebnisse von NeemAzal-Anwendungen 1994

E. Hummel und H. Kleeberg

Trifolio-M GmbH, Sonnenstr. 22, D-35633 Lahnau

SUMMARY

For practical application in plant protection the formulations NeemAzal-T (5% Azadirachtin A) and NeemAzal-T/S (1% Azadirachtin A) are of interest. Investigations of the efficacy against *Macrosiphum rosae, Aphis sambuci* and *Hyponomeuta padellus* show especially satisfactory results with NeemAzal-T/S.

The comparison of different criteria for assessment in tests with *A. sambuci* reveals problems in quantification due to behavioural peculiarities. The results show that benefitials contribute considerably to the control of the pest in plots treated with NeemAzal-formulation. Especially the comparison of treated and untreated elder bushes proves that the activity of the benefitials alone is not sufficient for the reduction of the pest.

EINLEITUNG

In mehrjähriger Forschungsarbeit wurde ein Extraktionsverfahren zur Isolierung der auf Insekten wirkenden Inhaltsstoffe der Neem-Kerne erarbeitet und schließlich eine optimal erscheinende Formulierung für diesen Wirkstoff gefunden. Wir haben den Wirkstoff **"NeemAzal"** genannt, um an seine Herkunft aus den Kernen des Neem-Baumes, sowie an die wichtigste Wirksubstanzgruppe, die Azadirachtine, zu erinnern.

Die Wirksamkeit von Neem-Wirkstoffen gegenüber saugenden und fraßaktiven Schädlingen (1) ist stark von der Art der Formulierung abhängig. Labor- und Freilanduntersuchungen deuten darauf hin, daß Neem-Wirkstoffe auf unterschiedliche Weise wirken können; dabei kann der Wirkstoff als oberflächlicher Spritzbelag oder nach systemischem Transport innerhalb der Pflanze von den Insekten aufgenommen werden (2,3,4). Aufgrund dieser vielfältigen Aufnahmemöglichkeiten sind detaillierte Untersuchungen zur Optimierung von Anwendungen notwendig, in denen auch die Auswirkungen auf Nutzinsekten registriert werden.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Die optimale Formulierung **NeemAzal-T/S** enthält maximal 4% Wirkstoff, so daß der Gehalt an Azadirachtin A 1% beträgt. Die Wirksamkeit dieser Formulierung wurde an zahlreichen Beispielen überprüft. Dabei sind folgende Effekte besonders wichtig:

1.) Bei Larven beißender Insekten tritt schon wenige Stunden nach der Anwendung ein vollständiger **Fraßstop** ein. Obwohl die wenig aktiven Larven der Schädlinge noch Tage bis Wochen Nützlingen als Beute dienen, stellen sie ihre pflanzenschädigende Wirkung ein. Dies wurde besonders eindrucksvoll an verschiedenen Untersuchungen mit Kartoffelkäfern (5-8), Schwammspinnern und Maikäfern (9) sowie dem Baumwollkapselwurm (10) etc. gezeigt.

2.) Schon nach wenigen Tagen können sich die verschiedenen Larvenstadien von beißenden und saugenden Insekten kaum noch durch Häutung zum nächsten Larvenstadium und schließlich zu Puppen oder Adulten weiterentwickeln. Diesen Effekt bezeichnen wir als "**Häutungshemmung**". In Abhängigkeit vom Schädling und seinem Entwicklungsstadium sowie der Wirkstoffkonzentration kann die Entwicklungsverzögerung nach mehreren Tagen bis Wochen zum Absterben der Schädlinge führen.

3.) Mit relativ geringen NeemAzal-Mengen behandelte Weibchen oder ältere Larven aus denen sich Weibchen entwickeln sind weitgehend unfruchtbar (8). Diese **Unfruchtbarkeit** führt bei zahlreichen beißenden Insekten dazu, daß keine Eier abgelegt werden (1,8), aus denen sich weitere Schädlingsgenerationen entwickeln können. Auch saugende Insekten wie Blattläuse, die sich häufig parthenogenetisch vermehren, bringen keine oder sehr wenige lebensfähige "Junge" zur Welt (2).

4.) Die Untersuchungen deuten darauf hin, daß NeemAzal-Formulierungen keine nennenswerte ovizide Wirkung aufweisen (11).

Aufgrund des beschriebenen insektenspezifischen Wirkmechanismus bezeichnen wir NeemAzal als **Insektistat**.

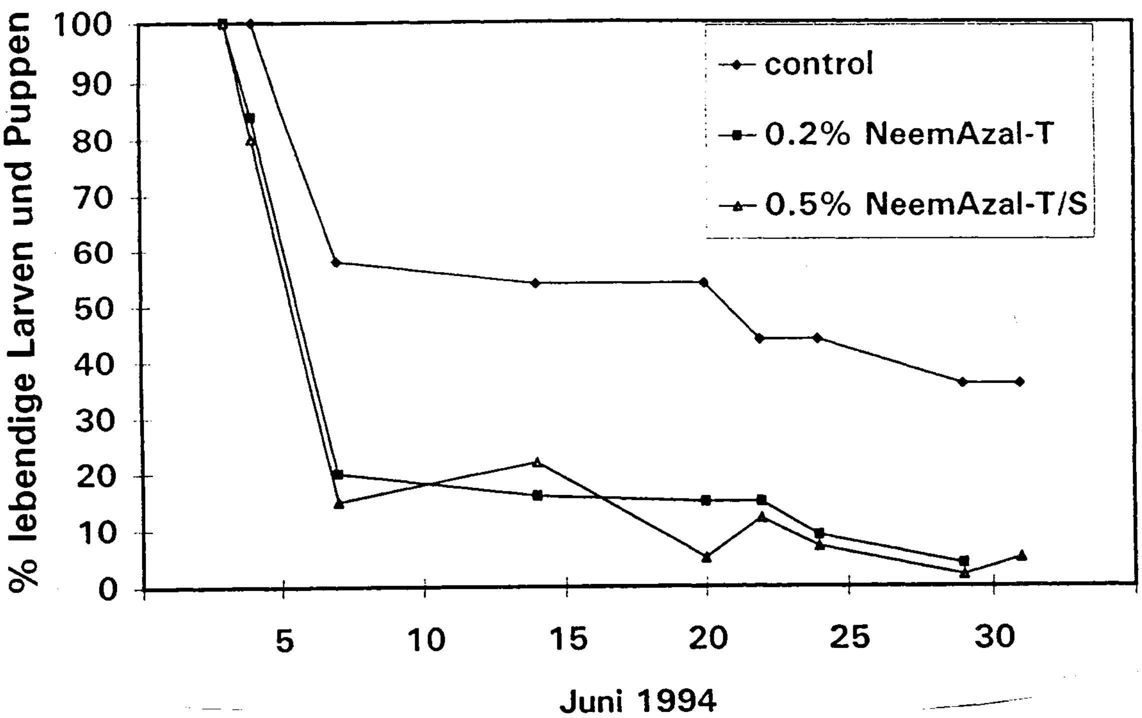


Abbildung 1: Wirkung von NeemAzal-Formulierungen auf die Entwicklung von Larven der Schlehengespinstmotte (*Hyponomeuta padellus*).
Behandlung: 3. Juni; Verpuppung in der Kontrolle: 13. bis 23. Juni.

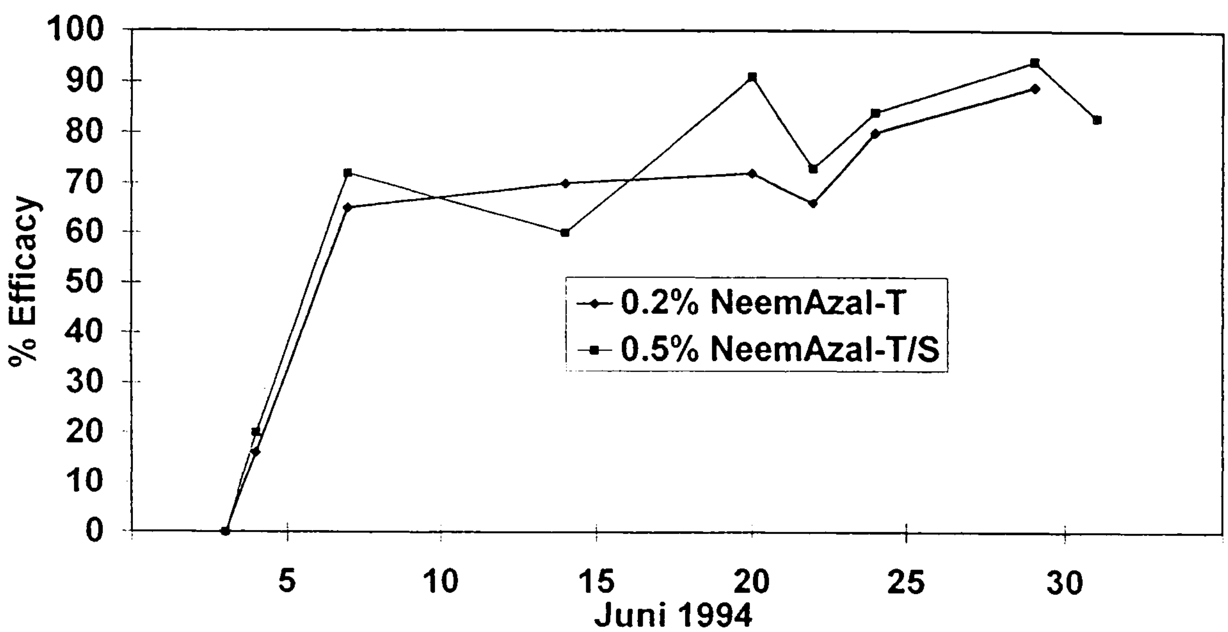


Abbildung 2: Wirkungsgrad der NeemAzal-Behandlung von Larven der Schlehengespinstmotte (*Hyponomeuta padellus*).
Behandlung: 3. Juni; Verpuppung in der Kontrolle: 13. bis 23. Juni.

ERGEBNISSE:

Schlehengespinstmotte (*Hyponomeuta padellus*)

An Hecken und in öffentlichem Grün richtet die **Schlehengespinstmotte** (*Hyponomeuta padellus*) zunehmend mehr Schaden an. Vergleichbar stark befallene, etwa 2 Meter hohe Büsche wurden mit NeemAzal-Formulierung am 3. Juni behandelt. Sowohl in der Kontrolle (Wasser) als auch auf den mit NeemAzal-Formulierung behandelten Büschen ging die Larvenpopulation wegen starker Regenfälle (4.-8. Juni) kurz nach der Behandlung stark zurück (s. Abb.1). Offensichtlich ist jedoch die Abnahme der lebendigen, aber inaktiven Larven auf den behandelten Büschen wesentlich größer als in der Kontrolle. Im Verlauf der Verpuppung (etwa vom 13.-23. Juni) verpuppten sich die Larven in der unbehandelten Kontrolle normal und unter Bildung geordneter Gespinste; insbesondere auf den mit NeemAzal-T/S behandelten Büschen waren einige Larven noch etwa bis zum 30. Juni aktiv. Während des Beobachtungszeitraums trat jedoch auf den behandelten Büschen kaum Fraßschaden auf und die Larven waren deutlich in ihrer Aktivität gestört. Die Berechnung der Wirksamkeit der Behandlung von NeemAzal-Formulierungen unter Berücksichtigung von aktiven und inaktiven Larven bzw. Puppen zeigt zwischen dem 20. und 30. Juni Wirksamkeiten zwischen 70-97% (s. Abb. 2). Die Fraßschäden waren an den behandelten Büschen im Gegensatz zur unbehandelten Kontrolle unwesentlich.

Holunderblattlaus (*Aphis sambuci*)

Zwischen dem 17. und 31. Mai wird eine Zunahme an Blattläusen auf zufällig ausgewählten Zweigen befallener Holunderbüsche in der mit Wasser behandelten Kontrolle registriert (s. Abb. 3). Nach Behandlung mit NeemAzal-T/S nimmt die Anzahl der Blattläuse bis zum 31.5. kontinuierlich ab. Die für den 25.5. und 31.5. berechneten Wirkungsgrade aus diesen Ergebnissen betragen 80 bzw. 100%.

Der Vergleich der Länge der Blattlauskolonien zeigt die größere Wirksamkeit von NeemAzal-T/S gegenüber der Formulierung NeemAzal-T. Obwohl der Wirkstoffgehalt von NeemAzal-T 5-mal größer ist als von NeemAzal-T/S, nimmt die Kolonielänge nach der Behandlung mit 0.5 und 1 % NeemAzal-T/S deutlich stärker ab als bei 0.2 % NeemAzal-T (s. Abb.4).

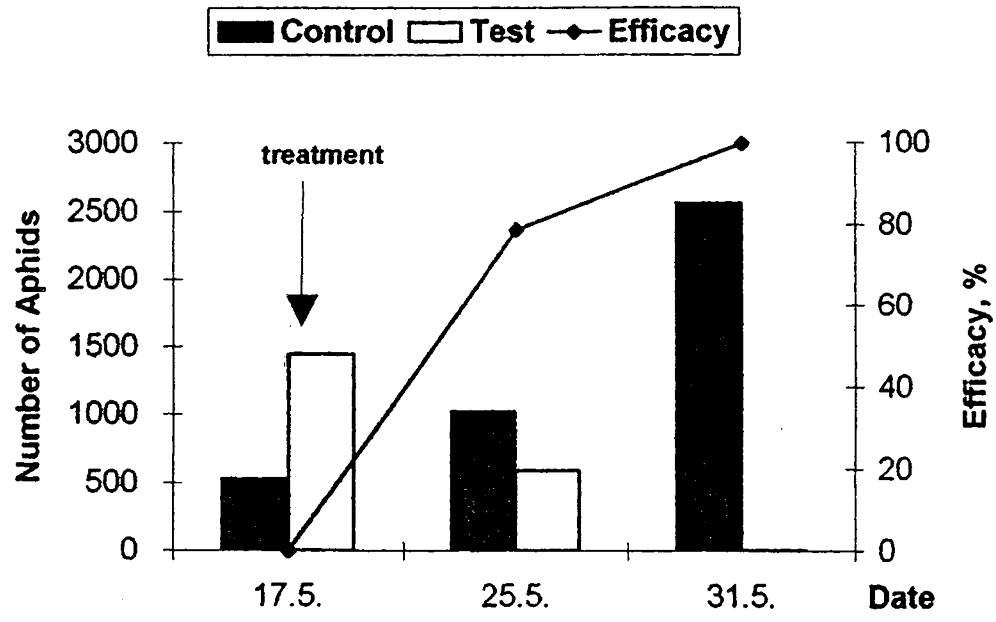


Abbildung 3: Schädlingsbefall und Wirksamkeit nach Behandlung von mit Holunderblattläusen befallenen Holunderbüschen mit 0.5 % NeemAzal-T/S.

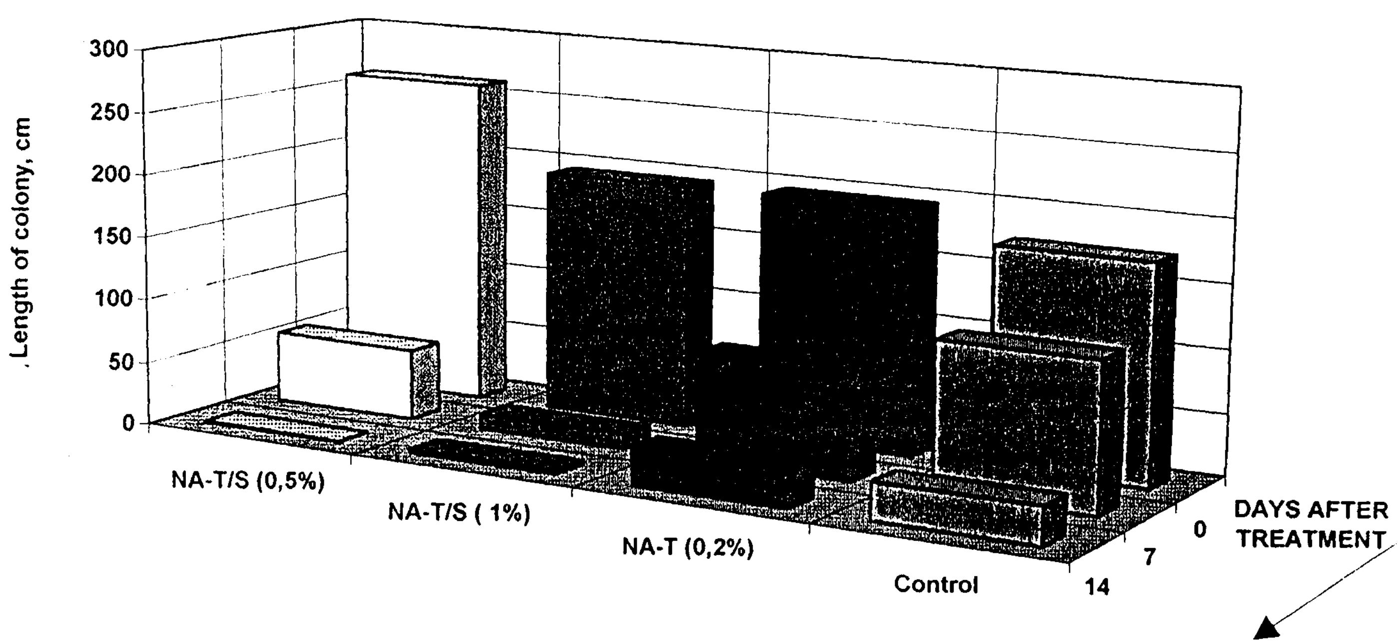


Abbildung 4: Länge von Kolonien der Holunderblattlaus nach Behandlung mit 0.2% NeemAzal-T, 0.5% und 1% NeemAzal-T/S.

Die relativen Längen bzw. Anzahlen ("Wirksamkeiten") der Blattlauskolonien zeigen eine Woche nach Behandlung mit 0.5 % NeemAzal-T/S sogar verschiedene Vorzeichen (s. Abb. 5). Insbesondere bei *A. sambuci* tritt nach Neem-Behandlung eine deutliche Umstrukturierung der Kolonien auf. Die Kolonien werden weniger dicht und zahlreiche Individuen scheinen sich neue Saugstellen zu suchen. Dieser Effekt kann zur Folge haben, daß die tatsächlich vorhandene Anzahl der Kolonien zunimmt. Da sich die Länge der Kolonien erst allmählich verringert, kann leicht eine Fehleinschätzung des Wirkungsgrades erfolgen. Es ist zwar wünschenswert, aber im Freiland praktisch nicht durchführbar, die Anzahl der Blattläuse genau zu bestimmen. In jedem Fall sollte die Koloniengröße und die Kolonienanzahl berücksichtigt werden. In welche Gruppen die Koloniengröße sinnvollerweise eingeteilt wird, hängt auch von der Blattlausart ab.

Die Anzahl der Nützlinge nimmt sowohl auf den Kontrollpflanzen als auch auf den behandelten Büschen vom 17.-25.5. zu (s. Abb. 6). Die darauffolgende Abnahme der Nützlinge auf den behandelten Büschen bis zum 31.5. ist nicht auf das Mittel, sondern auf die Tatsache, daß die Blattlauspopulation während dieses Zeitraums verschwindet zurückzuführen (s. Abb 6). In derartigen Feldversuchen leisten sowohl NeemAzal-T/S als auch Nützlinge einen wichtigen Beitrag zur Schädlingskontrolle; eine Differenzierung ist jedoch im Freiland schwierig.

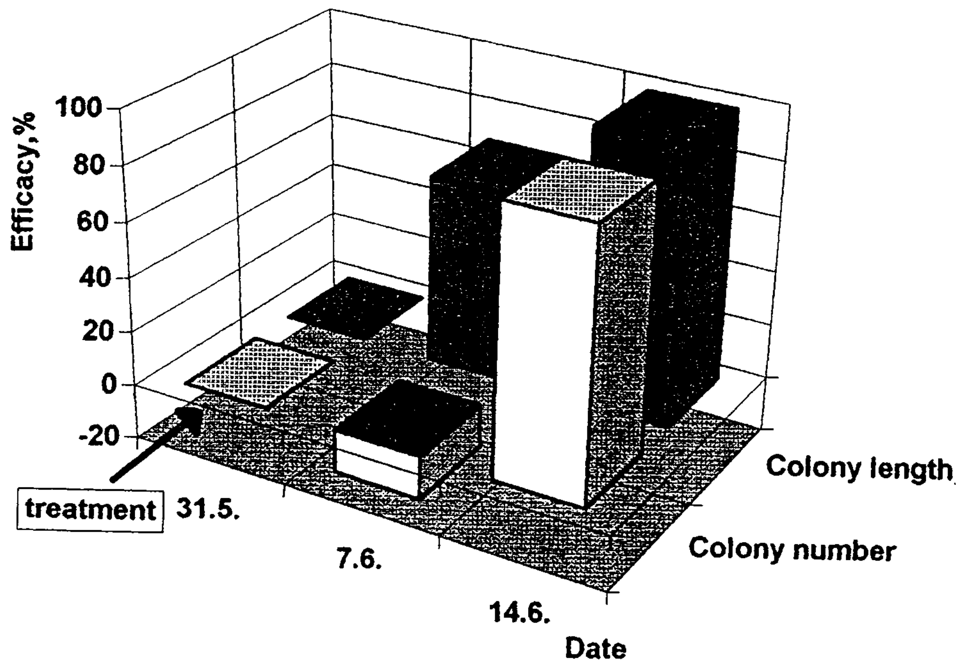


Abbildung 5: Wirksamkeit der Behandlung mit 0.5% NeemAzal-T/S aufgrund der Auswertung der Kolonielänge und der Kolonienzahl.

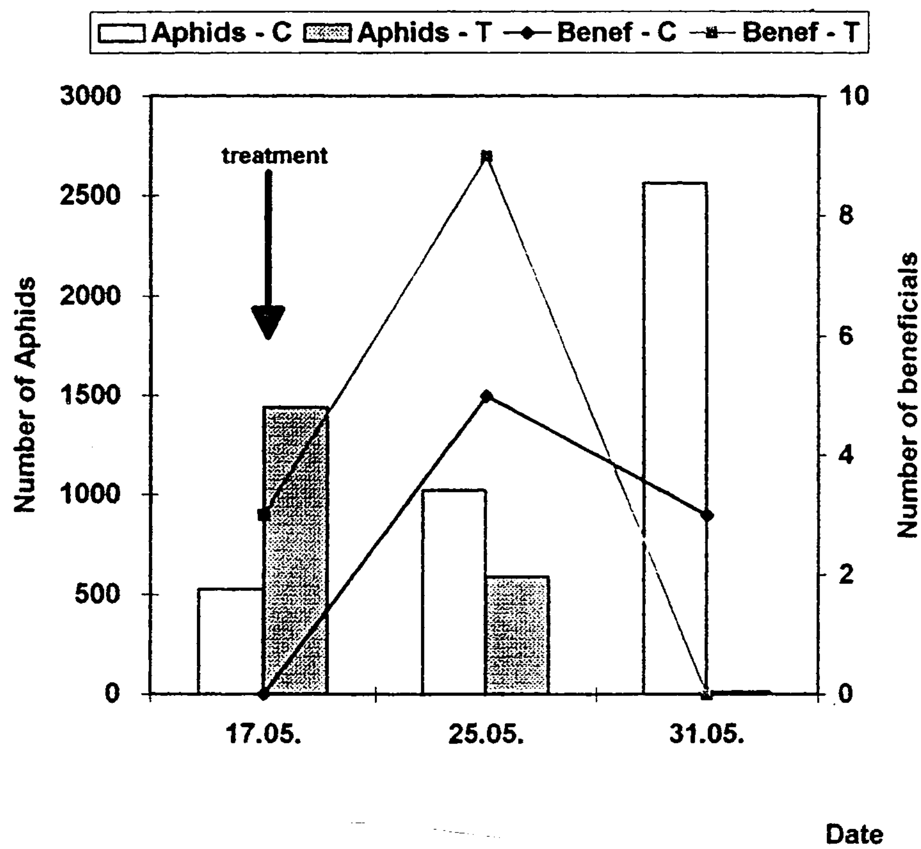


Abbildung 6: Vergleich der Anzahl von Blattläusen und Nützlingen nach Behandlung mit 0.5% NeemAzal-T/S.

Grüne Rosenblattlaus (*Macrosiphum rosae*)

Wie bei *A. sambuci* wurden mit NeemAzal-T/S gute Ergebnisse an von der grünen Rosenblattlaus befallenen Rosen (s. Abb. 7) erhalten. Die Erfahrungen zeigen, daß in diesem Fall eine frühzeitige Behandlung nach Beginn des Zuflugs der Blattläuse besonders wichtig ist. Bei starkem, kontinuierlichem Neubefall scheint eine Wiederholung der Behandlung je nach klimatischen Bedingungen in ca. 1-3-wöchigen Abständen sinnvoll.

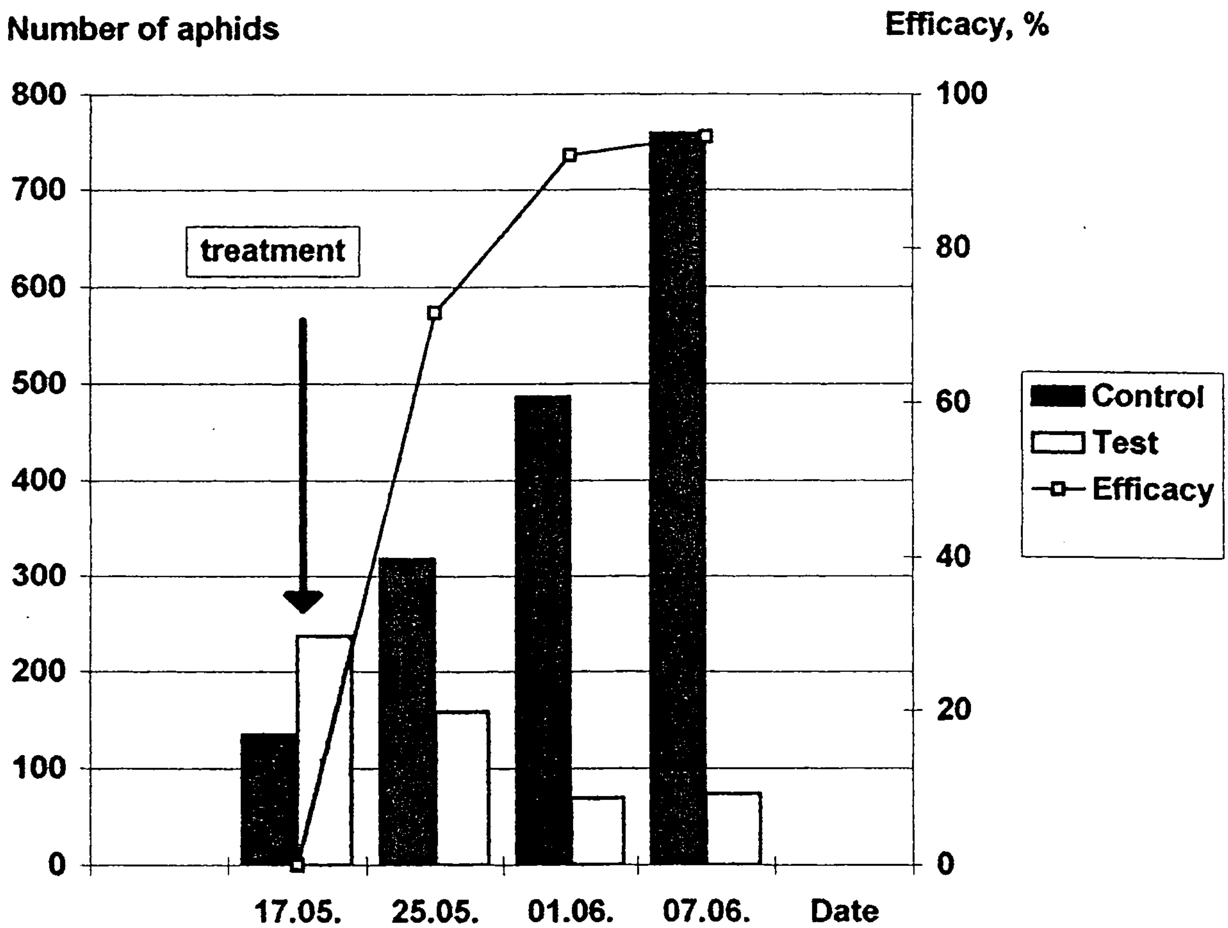


Abbildung 7: Schädlingsbefall und Wirksamkeit nach Behandlung von mit der Grünen Rosenblattlaus befallenen Rosen mit 0.5% NeemAzal-T/S.

ZUSAMMENFASSUNG

Für praktische Neem-Anwendungen im Pflanzenschutz sind die Formulierungen NeemAzal-T (5% Azadirachtin A) und NeemAzal-T/S (1% Azadirachtin A) interessant. Untersuchungen der Wirksamkeit gegenüber der Grünen Rosenblattlaus (*Macrosiphum rosae*), der Holunderblattlaus (*Aphis sambuci*) und der Schlehengespinstmotte (*Hyponomeuta padellus*) zeigen besonders mit NeemAzal-T/S sehr zufriedenstellende Ergebnisse.

Der Vergleich verschiedener Boniturkriterien bei den Versuchen zur Holunderblattlaus macht verhaltensbedingte Probleme bei der Quantifizierung offensichtlich. Die Auswertung der Ergebnisse zeigt, daß Nützlinge in mit NeemAzal-Formulierungen behandelten Anlagen wesentlich zur Kontrolle der Schädlingspopulation beitragen. Insbesondere der Vergleich im Versuch und der unbehandelten Kontrolle beweist jedoch, daß die Aktivität der Nützlinge alleine zur Eingrenzung der Schädlinge bei weitem nicht ausreichend ist.

DANKSAGUNG: Die Autoren danken dem BMBF für finanzielle Unterstützung.

LITERATURNACHWEIS

1.) **Schmutterer H.** (Hrsg.), The Neem Tree, VCH, Weinheim (1995)

2.) **Witt T.**, Kontaktwirkung und systemische Wirkung von NeemAzal-T/S auf die Blattlaus *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), Diplomarbeit, Geisenheim (1994)

3.) **Otto D.**, Sytemic Effects of the Azadirachtin Preparation "NeemAzal-W" on Larvae and Adults of *Leptinotarsa decemlineata*, p. 39 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 3rd Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1994)

4.) **Kleeberg H.**, The NeemAzal-Conception: Test of Systemic Activity, p. 5 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 1st Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1992)

5.) **Hummel E.** Wikung von NeemAzal-Formulierungen auf Kartoffelkäfer *Leptinotarsa decemlineata* im Freiland, Entomologen-Tagung, DGaaE, Göttingen (1995)

6.) **Hummel E.** und Kleeberg H., Anwendungsmöglichkeiten von NeemAzal-Formulierungen gegen Kartoffelkäfer, p. 91 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 2nd Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1993)

7.) **Gottwald R.**, Wirkung von NeemAzal-F auf Larven des Kartoffelkäfers (*Leptinotarsa decemlineata*Say.), p. 37 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 3rd Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1994)

8.) **Otto D.**, Effects of the Azadirachtin Preparation "NeemAzal-F" on Larvae and Adults of *Leptinotarsa decemlineata*, p. 21 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 3rd Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1994)

9.) **Schmutterer H und Nicol C.M.Y.**, Versuche zur gleichzeitigen Verminderung von Schadpopulationen des Waldmaikäfers *Melolontha hippocastani* und des Schwammspinners *Lymantria dispar* durch ein Niemprodukt, Entomologen-Tagung, DGaaE, Göttingen (1995)

10.) **Karelina T.N.** et al., Evaluation of the Biological Activity of NeemAzal and NeemAzal-S Against *Mamestra brassicae, Pieris rapae* and *Heliothis armigera,* p.95 in: Insecticides: Mechanism of Action and Resistance (D. Otto and B. Weber, eds.), Intercept, Andover (1992)

11.) **Hummel E.**, unveröffentlichte Ergebnisse (1995)

EFFECTS OF DIFFERENT NEEMAZAL FORMULATIONS ON *Dysaphis plantaginea*

Ch. Schulz+ and J. Kienzle*
+Kopernikusweg 19, D-74080 Heilbronn
Present address: Universität Hohenheim, Institut für Phytomedizin, Otto Sander Str. 5, D-70599 Stuttgart
*Staatliche Lehr- u. Versuchsanstalt für Wein- und Obstbau, Traubenplatz 5, D-74189 Weinsberg

1 Abstract

In 1994 three field trials concerning the use of neem products for regulation of *Dysaphis plantaginea* were carried out: A field trial with different formulations of NeemAzal, one late application of NeemAzal-T/S on a well developed population of *D. plantaginea* and application of NeemAzal-T in mixture with TELMION under practical field conditions on 23 organic farms.

In the first test all NeemAzal formulations and also Telmion applied on April 16th decreased the population development of *D. plantaginea* significantly. It was shown that it might be possible to reduce the amount of plant oil necessary and that the amount of 30 g/ha Azadirachtin might be better to prevent high infestation rates than half concentration of 15 g/ha.
A late application of NeemAzal-T/S on June 1st lead to a reduction of infestation rate, yet did not statistically reduce the number of colonies which might indicate that it causes direct mortality or reduces fecundity and longevity of the pest.
The application of NeemAzal-T mixed with Telmion by organic fruit growers showed that the product can exert good efficacy if used properly. But more research work has to be done to solve the problem of adequate timing of application if used preventively.

2 Introduction

In recent years studies indicate that neem products cannot only be used for successful control of leaf eating insects but also for the control of certain sap feeding insects such as aphids (LOWERY et al., 1993; LOWERY and ISMAN, 1993).
The rosy apple aphid *Dysaphis plantaginea* (PASS.) is one of the major pests in fruit culture. In intensive organic production systems there is no satisfying way for the control of this aphid. In some years the infestation of this insect can economically threaten the existence of organic farms especially in southwest Germany and Switzerland. Therefore in most years a regulation of this insect is necessary.
The development of *D. plantaginea* starts in early spring with the fundatrix hatching from the egg, laid on two to three year old shoots of apple trees in the fall. The young aphids start sucking near the flower buds causing fruit damage. Later the aphids move to the long shoots causing malformed and retarded shoot growth resulting in a reduction of fruit setting for the next year. Alatae usually start to show up in the middle of June. They move to the summer host *Plantago lanceolata*. In the fall they come back to the apple tree to lay eggs.

The efficacy of soap products which are used for the regulation of *D. plantaginea* according to the EC-regulations for organic farming is often not satisfying. Natural phyrethrum is not advisable since it is a broad spectrum contact insectizide. During the search for an alternative product applicable for organic fruit growers a mixture of neemoil and the formulated rape seed oil product TELMION (HOECHST AG) showed high efficacy in 1992 with a single application during the red bud stage (KIENZLE et al., 1993).

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

In 1993 this results could be proven with mixtures of neemoil and TELMION and also NeemAzal-F and TELMION. The efficacy reached levels comparable to those of commercial insecticides (SCHULZ et al, 1994). Continuing this research work three field trials were carried out in 1994 to work on questions of oil concentration, NeemAzal formulations and application date.

3 Material and Methods

a) NeemAzal formulations and treatments

The field experiment was carried out near Öhringen in a conventional apple orchard (cv.'Jonagold', M 9 planted 1990 in a distance of 3,2 m x 1,2 m) in a randomized complete block design with four replicates per treatment. Each plot consisted of 6 trees with the bordering trees not being used for assessment. Treatments were:

1	NeemAzal-T	(30 g AZA/ha)	
2	NeemAzal-T	(30 g AZA/ha) +	2,0 % TELMION
3	NeemAzal-T	(30 g AZA/ha) +	0,5 % TELMION
4	NeemAzal-T/S	(30 g AZA/ha)	
5	NeemAzal-T/S	(15 g AZA/ha)	
6	TELMION 2 %		
7	Water		

The Azadirachtin content of NeemAzal-T and T/S was 5 %. NeemAzal-T/S is a formulation of NeemAzal-T formulated with a plant oil. The amount of NeemAzal-T and T/S was chosen according to the decision to apply 30 g Azadirachtin per ha (treatment 1 to 4). TELMION is an emulsified rape seed oil used for control of winter eggs of red spider mites. NeemAzal-T (treatment 1) and NeemAzal-T/S (treatment 4 and 5) were heated to 40 °C and were then diluted with a small amount of hot water. For treatment 2 and 3 NeemAzal-T and TELMION were thoroughly mixed before being diluted in tap water to the concentration wanted. The trees were sprayed thoroughly wet with a knapsack sprayer on 16.4.1994 (begin of red bud stage). The air temperature during application time was about 12 °C. At this time no aphids could be found.

Two assessment of the number and the size of the aphid colonies and the occurence of beneficial insects were done on 16.5. and 6.6.1994 on 50 shoots per replication, i.e. 200 shoots per treatment. Since there was only a low infestation rate and the trees were small probably all existing colonies on all test trees were recorded.

For statistical analysis the number of colonies per replication were summarized. The calculation of infestation rate was done for each replication according to BOLLE (1953). Both, the number of colonies and the infestation rate, were subjected to an analysis of variance and separation of means was determined by Tukey's multiple range test.

b) Application date

In the state experiment station at Weinsberg (Heuchlingen) an organic apple orchard was planted in 1980 with eight cultivars for comparison reasons in a randomized complete block design with four replicates. Each replicate consisting of two rows of 8 trees. The cv. "Glockenapfel" was chosen for this experiment. In each replication one row was treated with NeemAzal-T/S (30 g Azadirachtin/ha), the other with water as control treatment. The treatment was done on 1.6. following an assessment of the colonies as described above. Second assessment happened on 17.6. At that time the population was starting to decrease due to alatae leaving for their summer hosts and the effects of beneficial insects.

Assessment and statistical analysis was done as described above.

c) Application on organic farms
23 organic fruit growers used NeemAzal-T in mixture with Telmion on 30 ha. According to results from previous experiments they should use 2 % Telmion for the mixture. The amount of Azadirachtin was also 30 g/ha. The proposed application date was the red bud stage. The fruit growers had to answer an questionaire to document their experience. These results were not evaluated statistically, however this can show tendencies.

4 Results

a) Different NeemAzal formulations

No significant differences (alpha = 0,05) could be found for the number of colonies at the first assessment date. Yet on the second date all neem-treatments showed significantly lower numbers of colonies than control treatment. The effect of the Telmion treatment did not differ significantly from control and neem-treatments (Tab. 1).
There were also differences in terms of the infestation rate. The NeemAzal treatment with the low dose of Azadirachtin (15 g/ha) showed no significant difference to control treatment on the first assessment date. The other treatments with neem formulations and Telmion were significantly better than the water treated control. Until the second assessment date infestation rate remained nearly the same in all neem-treatments and also Telmion. Only the aphid population in the water treatment clearly increased resulting in significant differences between all treatments and control (Tab. 1).

Table 1: Infestation rate (IR) and number of colonies (CN) of *Dysaphis plantaginea* on apple trees (cv. Jonagold) sprayed with different NeemAzal concentrations and formulations on April 16th

Treatment	Azadirachtin mg/l	Telmion ml/l	IR[a] 1. Assessment May 16th	NC[b] 1. Assessment May 16th	IR[a] 2. Assessment June 6th	NC[b] 2. Assessment June 6th
1.) NeemAzal-T	30	-	0,45 b	6 a	0,25 b	4 b
2.) NeemAzal-T	30	20	0,15 b	4 a	1,73 b	8 b
3.) NeemAzal-T	30	5	1,13 b	8 a	0,53 b	7 b
4.) NeemAzal-T/S	30	-	0,23 b	4 a	0,00 b	0 b
5.) NeemAzal-T/S	15	-	1,75 ab	14 a	1,70 b	12 b
6.) TELMION	-	20	1,10 b	8 a	3,63 b	26 ab
7.) Water	-	-	4,20 a	25 a	12,70 a	57 a

Within columns, means followed by a common letter do not differ significantly at the 5 % level (Tukey-Test). Averages of four replications with four test trees per replication.
[a] IR: Infestation rate according to BOLLE (1953)
[b] NC: sum of colononies found on 200 shoots on 16 test trees in four replications

b) Application date

There was no significant difference between the two treatments on the first assessment date, the day of application. 16 days later in both treatments the colony number and infestation rate was reduced which was partly due to beneficial insects and alatae leaving the apple trees for their summer hosts. While the infestation rate decreased significantly in the neem treatment the number of colonies remained uneffected (Tab. 2).

Table 2: Infestation rate (IR) and number of colonies (CN) of *Dysaphis plantaginea* on apple trees (cv. Jonagold) sprayed with NeemAzal-T/S at a concentration of 30 mg/l Azadirachtin on June 1st

Treatment	Azadirachtin mg/l	IR[a] 1. Assessment June 1st	NC[b]	IR[a] 2. Assessment June 17st	NC[b]
1.) NeemAzal-T/S	30	19,0 a	67 a	1,0 b	18 a
2.) Water	-	21,7 a	86 a	12,2 a	46 a

Within columns, means followed by a common letter do not differ significantly at the 5 % level (Tukey-Test). Averages of four replications with six test trees per replication.

[a] IR: Infestation rate according to BOLLE (1953)

[b] NC: sum of colononies found on 200 shoots on 24 test trees in four replications

c) Application on organic farms

20 questionaires from 23 participating farmers could be evaluated.

On seven farms with medium to high infestation rates the proposed mixture applied during the red bud stage showed obvious good results.

The experience of five farms with low to medium infestation could not be evaluated since they had no control treatment, realized great differences in cultivars or had used natural pyrethrum a few days before NeemAzal application.

In the untreated plots in seven orchards hardly any rosy apple aphid infestation was observed this year. For those farms no conclusions can be made. One farmer treated 9 ha after blossomimg proving no satisfying results.

5 Discussion

a) Infestation of *D. plantaginea* was quite low in the orchard of this field trial and the economic threshold in organic fruit growing was not reached. Even under that conditions one early application of different NeemAzal formulations had again a long term effect on the population development of *D. plantaginea*. This was true for Telmion too in this case of a low population development, which is different from previous results under higher infestation rates (SCHULZ et al, 1994). There were no significant differences between the NeemAzal formulations. But there is a slight tendency that 15 g/ha Azadirachtin might be too low for the prevention of higher infestation rates. Since there were no differences between the two Telmion concentration it might be possible to reduce the amount of plant oil and still reach same efficacy. The efficacy of the treatments were not influenced by 20 mm of rainfall one day after application.

b) The results indicates that NeemAzal-T/S might cause direct mortality and influence fecundity and longevity of *D. plantaginea*. This could open the possibility to use NeemAzal-T/S not only before the flowering time since there was also no russeting of the fruits recorded this year on this cultivar.

c) The interest of organic fruit growers in solving the great economic problem caused by *D. plantaginea* was one reason to try to use NeemAzal under practical field conditions on a limited area in accordance with the federal agency for plant protection in Braunschweig. It showed the obvious problem of an early application date as in the case of the red bud stage. NeemAzal-T in mixture with Telmion proved good efficacy under medium to high infestation rates if applied very early, however it showed the problems if applied at a later stage. The question of preventative use of NeemAzal at a time when it is hard to forecast if *D. plantaginea* will cause economic losses has to be worked on.

The research efforts are going to be continued in terms of application date, formulation, concentration, the effects on beneficial insects and other parameters.

6 Zusammenfassung

1994 wurden 3 Versuche zur Regulierung der Mehligen Apfellaus (*Dysaphis plantaginea*) durchgeführt.

In einem Freilandversuch an Apfelbäumen wurde die Wirksamkeit unterschiedlicher NeemAzal Formulierungen überprüft. Die Varianten waren: 1.) NeemAzal-T (30 g Azadirachtin/ha), 2.) NeemAzal-T (30 g AZA/ha) + 2,0 % TELMION, 3.) NeemAzal-T (30 g AZA/ha) + 0,5 % TELMION, 4.) NeemAzal-T/S (30 g AZA/ha), 5.) NeemAzal-T/S (15 g AZA/ha), 6.) TELMION 2 % und 7.) Wasser.
Der Befall war insgesamt gering und lag unterhalb der Schadschwelle im ökologischen Obstbau. Trotzdem traten signifikante Unterschiede zwischen der Wasserkontrolle und den Präparaten auf. Alle NeemAzal-Varianten, allerdings auch die Telmion-Kontrolle, zeigten bei der einmaligen Behandlung zu Beginn des roten Knospenstadiums einen langfristigen Einfluß auf die Blattlauspopulationentwicklung. Tendenziell gab es keine Unterschiede zwischen hoher und geringer Telmionkonzentration als Zusatz zu NeemAzal-T. Zudem scheinen 15 g Azadirachtin bei starkem Befallsdruck zu niedrig dosiert zu sein.

In einem anderen Freilandversuch an der Sorte Glockenapfel wurde NeemAzal-T/S (30 g/ha AZA) auf eine weit entwickelte Population der Mehligen Apfellaus am 1.6.95 appliziert. Nach 16 Tagen zeigte sich ein signifikanter Rückgang der Befallsstärke, nicht jedoch der Kolonienzahl. Die Gründe dafür werden in einer erhöhten Mortalität und eine Verringerung der Fekundität vermutet.

Auf 30 ha wurde von 23 ökologischen Obstbaubetrieben in einem Ringversuch NeemAzal-T in Mischung mit Telmion angewandt, um Erfahrungen mit Wirksamkeit und Anwendbarkeit zu sammeln. 20 Fragebögen konnten zu einer Bewertung herangezogen werden. Bei sieben Betrieben zeigte sich auch in unbehandelten Parzellen kaum Befall, 5 Betriebe können aus verschiedenen Gründen nicht ausgewertet werden. Ein Betrieb setzte auf einer großen Fläche das Präparat in der Nachblüte auf eine bereits entwickelte Blattlauspopulation mit unbefriedigender Wirkung ein. Bei den restlichen sieben Betrieben zeigten sich gute Resultate bei mittlerem bis starken Befall bei Anwendung im roten Knospenstadium und Formulierung mit Telmion.
Die Versuche werden fortgesetzt um Wirksamkeit, Formulierung und Anwendungszeitpunkt zu optimieren und die Wirkungen auf Nützlinge zu untersuchen.

7 References

BOLLE, F. (1953): Über die Beurteilung von pflanzenbaulichen Versuchen. Angew. Botanik **27**, 16-23. In : UNTERSTENHÖFER, G.: Die Grundlagen des Pflanzenschutz-Freilandversuches. Pflanzenschutz-Nachrichten "Bayer" **16**, (1963), 3. 154-155.

KIENZLE, J.; SCHULZ, C.; STRAUB, M.; SCHMITT, A.; WEIL, B. (1993): Use of Neem products the Control of the Rosy Apple Aphid (*Dysaphis plantaginea* Pass.). In: KLEEBERG, H. (1993): Practice oriented results on use and production of neem ingredients: Proceedings of the second workshop. Wetzlar, Nov. 30th - Dec. 2nd 1992. Giessen.

LOWERY, D. T., ISMAN, M. B., BRARD, N. L. (1993): Laboratory and field evaluation of neem for the control of aphids (Homoptera: Aphididae). J. Economic Entom., **86** (3).

LOWERY, D. T. und ISMAN, M. B. (1993): Effects of Extracts from Neem on Aphids (Homoptera: Aphididae) and their Natural Enemies. Proceedings of the World Neem Conf., Bangalore. (i.V.)

SCHULZ, C.; KIENZLE, J.; SCHMITT, A.; WEIL, B. (1994): Experiences with Neem Products for the Control of the Rosy Apple Aphid (*Dysaphis plantaginea* Pass.). In: KLEEBERG, H. (1994): Practice oriented results on use and production of neem ingredients: Proceedings of the third workshop. Wetzlar, 22. - 25. November 1993. Giessen.

Wirkung von NeemAzal-T/S auf die Wickenblattlaus *Megoura viciae* im Labor (1994)

Edmund Hummel und Hubertus Kleeberg
Trifolio-M GmbH, Sonnenstr. 22, D-35633 Lahnau -2

Abstract:
Effects of NeemAzal-T/S on the Vetchaphid *Megoura viciae* in the laboratory (1994)

Laboratory trials show that the formulation NeemAzal-T/S (0,3-0,5%) had very strong effects on the vetchaphid. The preparation (0,5%) is active for almost 2 weeks and the efficacy after direct spray treatment is 94-98%. During the test period the normal development of the aphid population is disturbed and the fecundity of adults is reduced. NeemAzal-T/S shows systemic activity after soil application, however, this mode of application for control of Megoura viciae is not promising for practical purposes.

Einleitung

Versuche zur Schädlingskontrolle mit Extrakten aus den Kernen des indischen Neem-Baumes zeigten in zahlreichen Freiland- und Laboruntersuchungen (1-6) hohe Wirkungsgrade gegenüber Blattläusen. In eigenen Freilandversuchen mit der Neem-Extraktformulierung NeemAzal-T/S gegen die Holunderblattlaus *Aphis sambuci* und Rosenblattlaus *Macrosiphum rosae* wurden sehr positive Ergebnisse erhalten (7). Dabei trugen Nützlinge zu einem beträchtlichen, schwer quantifizierbaren Anteil zur Wirkung bei. Deswegen wurden Laboruntersuchungen mit der Wickenblattlaus *Megoura viciae* durchgeführt, um den Einfluß sowie die Wirkungsweise von NeemAzal-T/S besser kennenzulernen.

Das Ziel dieser Versuche mit NeemAzal-T/S ist:

1). Optimierung der Arbeitskonzentration
2). Ermittlung der Wirkungsdauer
3). Informationen zur Wirkungsweise

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Material und Methoden

Die Versuche wurden mit NeemAzal-T/S (max. 4% Wirkstoff NEEMAZAL; Azadirachtin A-Gehalt: 1%) bei Temperaturen von 22-25°C, einer relativen Luftfeuchte von 60-70% und einer 16stündigen Photoperiode durchgeführt. Wickenblattläuse wurden aus einer parthenogenetischen Dauerzucht aus der Fachhochschule für Gartenbau, Osnabrück entnommen. Die Blattläuse wurden an Ackerbohnenpflanzen in mit Papier abgedeckten Töpfen mit einer Erde-Sand-Mischung in Plexiglaszylindern (21 x 48 cm, mit Gaze bespannt), gezüchtet. Die Pflanzen wurden mit jüngeren Larven (überwiegend L1) infiziert, die mit einem feinen Pinsel auf die Testpflanzen übertragen wurden.. Die Bonituren erfolgten im Abstand von 3 bis 4 Tagen; dabei wurden die Blattläuse nach der Größe in 4 Larvengruppen (L1, L2, L3, und L4) und adulte Tiere (A) unterschieden. Die Behandlung wurde mit einem Handdrucksprühgerät bis zur Tropfnässe durchgeführt. Der Wirkungsgrad wurde nach Henderson-Tilton berechnet.

Im Versuch zur **Optimierung der Arbeitskonzentration** wurden 3 Konzentrationen untersucht: 0,1, 0,3 und 0,5% NeemAzal-T/S. In jeder der 3 Wiederholungen wurden die Pflanzen mit je 50 Blattläusen infiziert (Kontrolle: 80 Tiere). Bei der Bonitur zu diesem Versuch wurde nur zwischen jüngeren (L1 und L2) und älteren (L3 und L4) Larven unterschieden.

Bei der **Ermittlung der Wirkungsdauer** wurde nur eine Konzentration (0,5%) untersucht. Dabei wurden die Pflanzen zuerst behandelt und dann nach 3 bzw. 7 Tagen mit 30 Blattläusen pro Wiederholung infiziert.

Zur **Ermittlung der Wirkungsweise** wurden: 1). Blattläuse auf Filterpapier in Petrischalen behandelt (B-b); die behandelten Blattläuse wurden dann sofort auf unbehandelte Pflanzen (P-u) übertragen oder 2). unbehandelte Blattläuse (B-u) wurden nach Antrocknen des Spritzbelages auf behandelte Pflanzen (P-b) übertragen; die Behandlung wurde mit 0,2 %- bzw. 0,4%-iger Spritzbrühe durchgeführt.

Ergebnisse und Diskussion

1). Optimierung der Arbeitskonzentration

Um den Einfluß von NeemAzal-T/S auf die Entwicklung der Schädlinge abschätzen zu können, wurde das Präparat auf die mit Blattläusen besiedelten Pflanzen in unterschiedlichen Konzentrationen appliziert. Die Wirkung von NeemAzal-T/S ist deutlich konzentrations-abhängig.

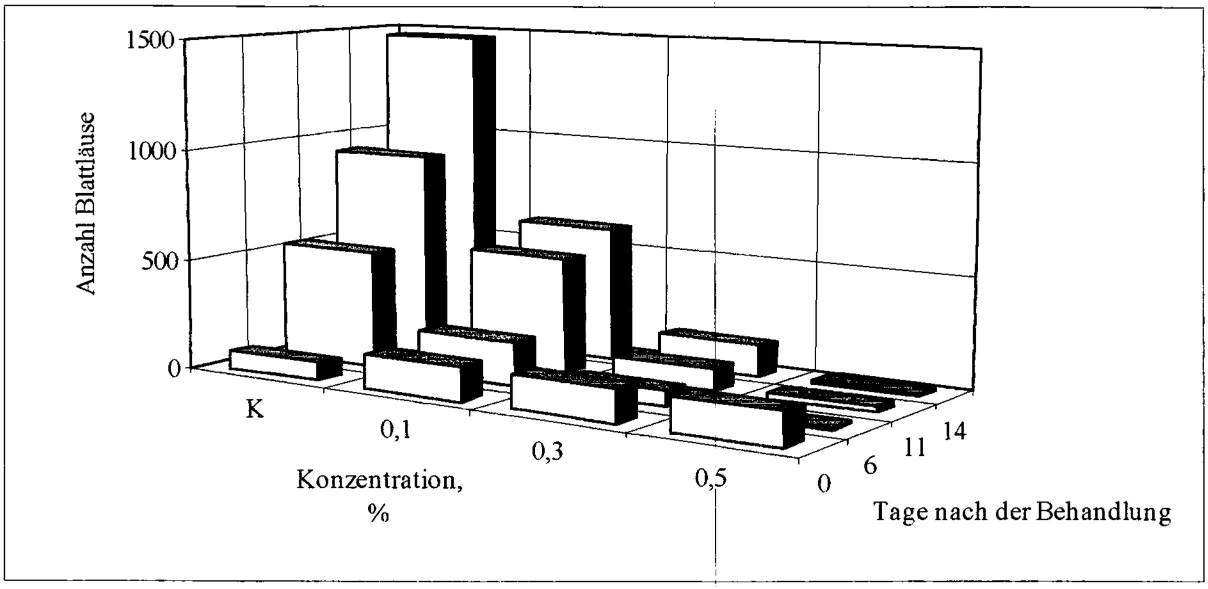


Abbildung 1: Entwicklung der Wickenblattlauspopulation nach Behandlung mit NeemAzal-T/S (0,1-0,5%)

Nur 0.3 bzw. 0.5 %-ige NeemAzal-T/S-Anwendung zeigt eine befriedigend hohe Wirkung (93% bzw. 99%). Während der 2-wöchigen Beobachtungsperiode, die etwa der Entwicklungsdauer von 2 Blattlausgenerationen im Labor entspricht, bricht die Populations-entwicklung mit steigender Anwendungskonzentration zunehmend deutlicher zusammen.

Schon die 0,1%-ige Konzentration beeinflußt die Entwicklung der Blattläuse deutlich. Die Anzahl der Blattläuse nimmt weitaus weniger als in der Kontrolle (K) zu. Der Entwicklungszyklus ist im Vergleich zur Kontrolle deutlich verzögert und die Fertilität ist reduziert; der Wirkungsgrad beträgt bei dieser Anwendung etwa 78 %.

Bei Anwendung von 0,3%-igem NeemAzal-T/S wird die Anzahl der Blattläusen anfänglich reduziert, nach 14 Tagen traten dann verstärk junge Larven auf, die von überlebenden Imagines abgesetzt werden.

Nach der Anwendung 0,5%-iger Lösung bricht die Blattlauspopulation praktisch vollständig nach 6 Tagen zusammen und kann sich nicht mehr aufbauen. Bei dieser Konzentration werden einige adulte Tiere beobachtet, die deformierte Beine, bräunliche Verfärbungen des Abdomens haben oder die sich von ihren Exuvien nicht trennen konnten. Ähnliche Körperverfärbungen wurden auch nach der Anwendung von anderen Neem-Extrakten beobachtet (4, 5).

2). Ermittlung der Wirkungsdauer

Die Wirkungsdauer eines Präparates ist von besonderer Bedeutung, wenn nach der Behandlung Schädlinge erneut zufliegen.

Abbildung 2 zeigt die Blattlausentwicklung von Junglarven, die 3 Tage nach der Applikation auf behandelte Pflanzen übertragen wurden. In dieser Variante ist die Blattlauspopulation in der Folgegeneration (ca. 17 Tage nach der Behandlung) drastisch reduziert und kann sich auch später nicht mehr erholen, weil die wenigen adulten Tiere auch nach 2 Wochen keine oder keine lebensfähigen Larven absetzten. Aus den aufgesetzten Junglarven entwickeln sich nach der Behandlung die älteren Larvenstadien und die Adulten wesentlich langsamer als in der Kontrolle.

In der Variante, in der die Blattläuse nach 7 Tagen auf die behandelten Pflanzen übertragen wurden, kann keine nennenswerte Populationsreduktion festgestellt werden. Die Entwicklung der Larven ist jedoch deutlich verzögert und auch die Fertilität der Weibchen ist niedriger als in der Kontrolle.

Die Ergebnisse deuten also darauf hin, daß auf oder in den Blättern bis zu 7 Tage nach der Behandlung noch Wirkstoff vorhanden ist, der sich insbesondere in einer Entwicklungshemmung und Fertilitätsreduktion bemerkbar macht. Die wirksame Konzentration sinkt jedoch zwischen dem 3. und 7. Tag nach der Anwendung unter ein praktisch relevantes Niveau.

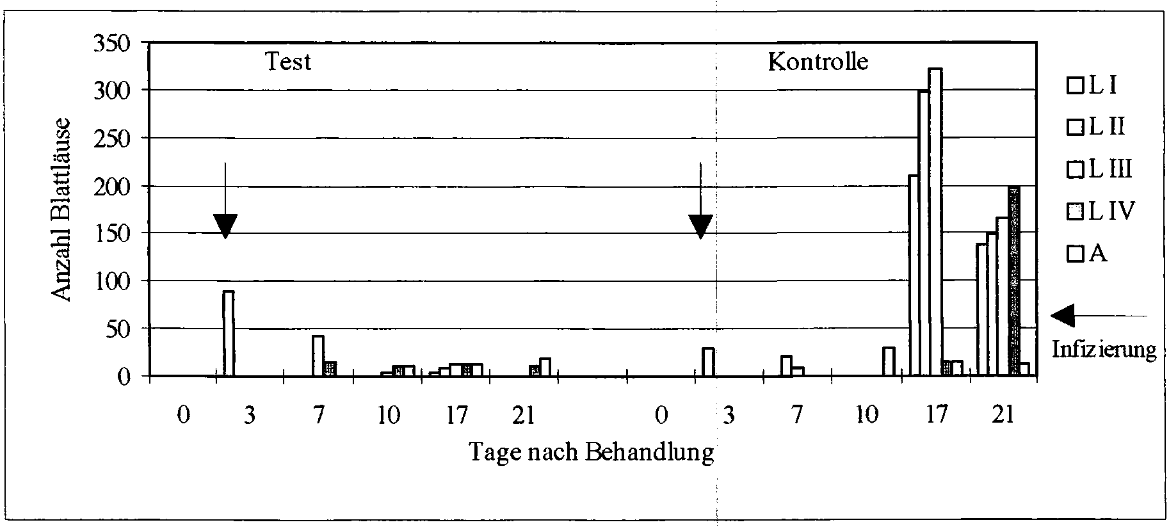


Abbildung 2: Wirkungsdauer von NeemAzal-T/S (0,5%) auf die Wickenblattlauspopulation (Infizierung der Pflanzen 3 Tage nach Behandlung)

3). Ermittlung der Wirkungsweise

Bezüglich der Wirkungsweise von Neem-Inhaltstoffen wurden über direkte Kontakt- sowie systemische Wirkungen nach Blattapplikation berichtet (4, 6).

Bei *M. viciae* zeigt sich bei direkter Behandlung ein deutlicher Unterschied bei 0,2 und 0,4%-iger Spritzbrühe (Abb. 3).

4 Tage nach der Behandlung der Blattläuse (s. Abb. 3: B-b, P-nb, 0.4) mit 0,4%-iger Spritzbrühe sinkt deren Anzahl auf 10-15% und bleibt die ganze Boniturzeit konstant niedrig; bei 0,2%-iger Lösung kann sich die Population nach anfänglicher Reduktion nach 11 Tagen langsam wieder aufbauen. In beiden Varianten, in denen nur die Pflanzen behandelt wurden (Abb. 3, rechter Teil), wird die Anzahl der Blattläuse durch Aufnahme von Wirkstoff innerhalb von 7 Tagen langsam um etwa 80 % reduziert. Die nach 11 Tagen von Adulten abgesetzten Junglarven stellen den Anfang einer neuen Population dar.

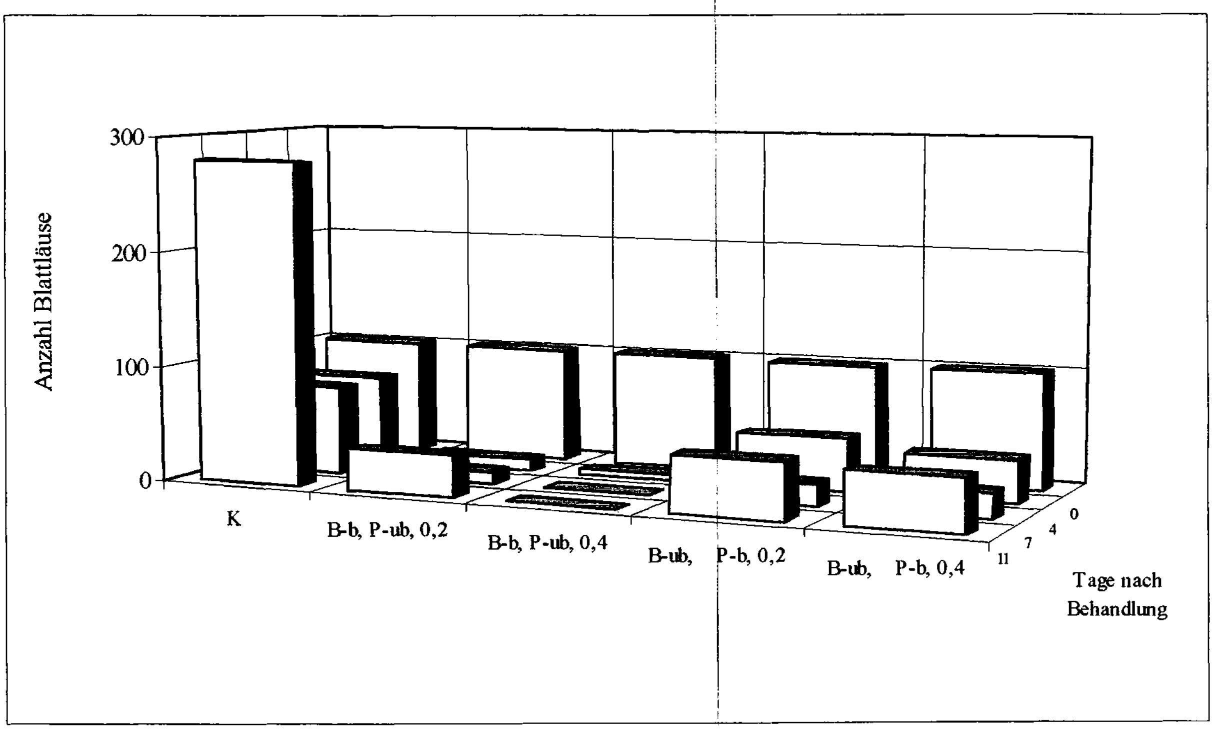


Abbildung 3: Entwicklung der Population der Wickenblattlaus nach Behandlung (b) der Blattläuse (B) bzw. Pflanzen (P) mit 0.2 bzw. 0.4 % NeemAzal-T/S

Zusammenfassung

In Laborversuchen mit NeemAzal-T/S gegen Wickenblattläuse werden hohe Wirkungsgrade, eine Verzögerung des Entwicklungszyklus sowie Fertilitätsreduktion beobachtet. Dabei kontrolliert Neem-Azal-T/S (0,5%) die Schädlingspopulation bei 0.5 %-iger Anwendung für mehr als 2 Wochen; auch bei niedrigeren Konzentrationen (0,1-0,3%) ist der Entwicklungsprozess der Blattläuse gestört.

Das Präparat beeinflußt die Schädlingspopulation auch wenn die Besiedlung von Pflanzen erst 3 bzw. 7 Tage nach der Behandlung (0,5%-ige Spritzbrühe) stattfindet. NeemAzal-T/S wirkt auf die Blattläuse durch direkten Kontakt sowie systemisch durch Saugtätigkeit. Bei topikaler Applikation kontrollieren höhere Konzentrationen (0,4%) die Entwicklung der Schädlinge für wenigsten 11 und niedrigere (0,2%) für etwa 7 Tage.

Danksagung

Die Autoren danken Herrn Prof. Mayr (Fachhochschule für Gartenbau, Osnabrück) für technische und fachliche Unterstützung bei Durchführung der Versuche, Herrn Prof. Schmutterer (Institut für Phytopathologie und Angewandte Zoologie, Gießen) für zahlreiche Diskussionen und dem BMBF für finanzielle Unterstützung

Literatur

1.) Kienzle, J., Schulz, C., Straub, M., Schmitt, A., Weil, B. (1992). Einsatz von Neemprodukten zur Regulierung der Mehligen Apfelblattlaus (*Dysaphis plantaginea*). In: 5. Internationaler Erfahrungsaustausch über Forschungsergebnisse zum Ökologischen Obstbau, Weinsberg.

2.) Lowery, D. T., Isman, M.B., Brard, N.L. (1993). Laboratory and field evaluation of neem for the control of aphids (*Homoptera: Aphididae*). J. Economic Entom., **86** , 864-870.

3.) Schulz, C., Kienzle, J., Schmitt, A., Weil, B. (1993). Neemprodukte zur Regulierung der Mehligen Apfelblattlaus (*Dysaphis plantaginea*). In: 6. Internationaler Erfahrungsaustausch über Forschungsergebnisse zum Ökologischen Obstbau, Fördergemeinschaft ökologischen Obstbau, Weinsberg.

4.) Witt, T. (1994). Kontaktwirkung und systemische Wirkung von NeemAzal-T/S auf die Blattlaus *Acyrthosiphon pisum (Harris)* (Homoptera: Aphididae). Diplomarbeit, Fachhochschule Wiesbaden.

5.) Schauer, M. (1985). Die Wirkung von Nieminhaltsstoffen auf Blattläuse und die Rübenblattwanze. Dissertation Universität Gießen.

6.) Schmutterer, H. (1990). Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann. Rev. Entom., 35, 271-297.

7.) Hummel E. und Kleeberg H (1996) Ergebnisse von NeemAzal-Anwendungen 1994, p. 35 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 4th Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau

Untersuchungen über die Wirkung von Neemformulierungen und anderen Insektiziden auf die Baumwollblattlaus Aphis gossypii Glov. unter Laborbedingungen

Investigations on effects of Neem-formulations and other insecticides on the cotton aphid Aphis gossypii Glov. under laboratory conditions

W. Kühne und D. Chougourou
Universität Leipzig
Studienprogramm Agrarwissenschaften
Wissenschaftsbereich Tropische Landwirtschaft
Arbeitsgruppe Pflanzenschutz und Vorratsschutz
Fichtestraße 28
04275 Leipzig

1. Einleitung

Die Vielzahl von Spritzungen mit konventionellen Insektiziden zur Bekämpfung wichtiger Schadinsekten in Baumwollbeständen hat unter dem Gesichtspunkt unerwünschter Nebenwirkungen in vielen Ländern dazu geführt, nach neuen, alternativen Insektiziden zu suchen. In diesem Zusammhang wurde in jüngster Vergangenheit das Augenmerk mehr und mehr auf Pflanzeninhaltsstoffe gerichtet. Bei diesen Untersuchungen trat neben anderen Pflanzen besonders der Neembaum in den Mittelpunkt des Interesses, da seine Samen den insektiziden Wirkstoff Azadirachtin enthalten. (SCHAUER, 1985; SCHMUTTERER & ASCHER, 1987; ASCHER, 1993).
Im Rahmen einer Forschungsarbeit über die Bekämpfung wichtiger Baumwollschädlinge ist vorgesehen, verschiedene, formulierte Pflanzeninhaltsstoffe und andere mindertoxische Präparate zunächst unter Laborbedingungen zu prüfen. Mit diesen Untersuchchungen, einschließlich späterer Freilandversuche, wird angesterbt, hochtoxische Insektizide durch mindertoxische Insektizide zu ersetzen, um damit eine Verringerung der toxischen Belastung von Baumwollfeldern zu erreichen. Die ersten Untersuchungen wurden mit der Baumwollblattlaus durchgeführt, worüber nachfolgend berichtet werden soll.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

2. Material und Methodik

2.1. Anzucht der Baumwollpflanzen

Die für die Applikation der Prüfsubstanzen notwendigen Baumwollpflanzen wurden unter Gewächshausbedingungen in Blumentöpfen (9 cm ∅, eine Pflanze pro Topf) bei 23-25°C angezogen. Die Sorte hatte die Bezeichnung "Glandless". Fünf bis sechs Wochen nach der Aussaat waren an allen Pflanzen die ersten 2 Laubblätter ausgebildet. In diesem Stadium wurden die Baumwollpflanzen sowohl als Wirtspflanzen für die Zucht der Blattläuse als auch für die Applikation der Testsubstanzen verwendet.

2.2. Haltung der Baumwollblattlaus

Zur Absicherung der erforderlichen Anzahl des Testobjektes war es notwendig, eine Massenzucht der Baumwollblattlaus aufzubauen und über die Testzeit hinweg fortzuführen. Für diesen Zweck wurden jeweils 6-8 Baumwollpflanzen mit Blattläusen bestückt und in Käfigen (50x50x50 cm) unter folgenden Bedingungen gehalten: Temperatur 23-28°C; relative Luftfeuchtigkeit 50-70%; Licht 16 h/Tag. Die Entwicklungsdauer vom ersten Larvenstadium (L_1) bis zum adulten Tier betrug 6-7 Tage. Eine adulte Blattlaus produzierte im Durchschnitt 27 Nachkommen, wobei die Gesamtlebenszeit einer Blattlaus im Durchschnitt bei 19 Tagen lag.

2.3. Insektizide Testsubstanzen

Für die Prüfung der insektiziden/aphiziden Wirksamkeit wurden die nachfolgend aufgeführten Präparate und Mittel ausgewählt.

Tabelle 1: Zusammenstellung der ausgewählten Prüfsubstanzen

Prüfsubstanzen	Wirkstoff	Hersteller/Lieferfirma
NeemAzal-TS	Azadirachtin	Trifolio-M, Lahnau
NeemAzal-T	Azadirachtin	Trifolio-M, Lahnau
NeemAzal-W	Azadirachtin	Trifolio-M, Lahnau
Pyrethrum-Extrakt	Pyrethrine	Delicia, Delitzsch
Pyrethrum-Extrakt + Piperonylbutoxid	Pyrethrine + Stabilisator	Delicia, Delitzsch
Pirimor	Pirimicarb	Celaflor, Ingelheim
Confidor 200 SL	Imidacloprid	Bayer AG, Leverkusen
Confidor 5 GR	Imidacloprid	Bayer AG, Leverkusen
Gaucho 75 WS	Imidacloprid	Bayer AG, Leverkusen
Neudosan	Kaliumsalze natürlicher Fettsäuren	Neudorff, Emmerthal
Telmion	Rapsöl	Hoechst AG, Frankf.
Ammonium-Nitrat-Harnstofflösung (AHL) 1)	(Stickstoff)	Stickstoffwerke, Piesteritz

2.4. Applikationstechnik

Die Ausbringung der Testsubstanzen wurde über folgende Applikationsarten vorgenommen:

- Sprühapplikation mit einem Saug-Druck-Aggregat
- Topikalapplikation über einen Mikroliter-Applikator
- Ausbringung von Granulaten
- Behandlung des Baumwollsaatgutes vor der Aussaat (Beizung)

3. Durchführung der Insektizid-Prüfung

Bei der Sprühapplikation basierte die Prüfung eines jeden Präparates auf einem Versuch bestehend aus 4 Wiederholungen. Die 4 getopften Baumwollpflanzen wurden auf 1 m^2 Sprühfläche gestellt, wobei die gesamte Fläche einschließlich der Pflanzen mit 20 ml/m^2 gleichmäßig besprüht wurde. Die Kontrollpflanzen erhielten die gleiche Sprühbehandlung, allerdings nur mit Was-

1) AHL besitzt keine direkte insektizide Wirkung. Die Bedeutung liegt vorallem in einer gewissen Klebefähigkeit auf den Blättern, wodurch eventuell eine größere Beständigkeit natürlicher Insektizide erreicht werden kann.

ser. Zwei Stunden nach der Applikation wurden an jede Pflanze 10 Blattläuse angesetzt.
Die Topikalapplikation erfolgte in der Weise, daß zunächst jede Pflanze (4 Wiederholungen) mit 10 Blattläusen bestückt wurde. Anschließend erhielt jede Blattlaus auf den Körper (Rücken) 0,1 Mikroliter Testsubstanz bzw. in der Kontrolle die gleiche Menge Wasser appliziert.
Bei der Ausbringung der insektiziden Granulate (Confidor 5 GR) wurden pro Topf zum Samen in die Erde jeweils 0,3 g Granulat gegeben.
Die Behandlung des Baumwollsaatgutes mit dem insektiziden Beizmittel Gaucho 75 WS erfolgte in der Weise, daß 10 g Saatgut mit 1 ml Haftmittel und 0,7 g Beizmittel so lange gemischt wurden, bis jedes Samenkorn von einer roten Schicht umgeben war. Bei der Granulatanwendung und der Saatgutbehandlung wurden die Pflanzen nach der Ausbildung der ersten 2 Laubblätter wöchentlich mit je 10 Blattläusen bestückt.

4. Ergebnisse und Diskussion

Das Kriterium für die Beurteilung der insektiziden Wirksamkeit der Testsubstanzen war sowohl bei der Sprüh- als auch bei der Topikalapplikation die Mortalität, die jeweils nach 24, 48 und 72 h nach dem Ansetzen der Blattläuse festgestellt wurde. Im Fall der Granulatanwendung und der Saatgutbehandlung war die Dauer der Wirkung in Wochen von Interesse, das heißt, die Anzahl von Wochen, in denen die auf den Pflanzen wöchentlich angesetzten Blattläuse abgetötet werden und dadurch blattlausfrei bleiben.
Die mit der Sprüh- und Topikalapplikation der Testsubstanzen erzielten Ergebnisse sind aus den Tabellen 2 und 3 ersichtlich.

Tabelle 2: Ergebnisse der Wirksamkeit der Testsubstanzen auf die Baumwollblattlaus nach Sprühapplikation (Mortalität in %)

Testsubstanzen	Anwendungs-konzentration %	Mortalität in % nach 24	48	72 h
NeemAzal-TS	0,4	8	14	14
NeemAzal-T	0,2	4	8	10
NeemAzal-W	0,2	4	30	48
Pyrethrum-Extrakt	0,2	72	80	80
Pyrethrum-Extrakt + Piperonylbutoxid	0,25	98	100	100
Pirimor	0,05	100	100	100
Confidor 200 SL	0,2	94	100	100
Neudosan	2,0	44	48	50
Telmion	2,0	2	4	8
AHL	16,6	6	6	14

Tabelle 3: Ergebnisse der Wirksamkeit der Testsubstanzen auf die Baumwollblattlaus nach Topikalapplikation (Mortalität in %)

Testsubstanzen	Anwendungs-konzentration %	Mortalität in % nach 24	48	72 h
NeemAzal-TS	0,4	44	52	52
NeemAzal-T	0,2	36	54	54
Pyrethrum-Extrakt	0,2	98	98	98
Pyrethrum-Extrakt + Piperonylbutoxid	0,25	100	100	100
Pirimor	0,05	92	100	100
Confidor 200 SL	0,2	100	100	100
Neudosan	2,0	34	40	40
Telmion	2,0	30	30	30
AHL	16,6	46	46	46

Vergleicht man zunächst die Gesamtheit der Ergebnisse der Tabelle 2 mit denen der Tabelle 3, so wird unverkennbar deutlich, daß durch die direkte Applikation der Präparate auf die Blattläuse (Topikalapplikation) in nahezu allen Fällen weitaus bessere Ergebnisse erzielt wurden. Da sich die Blattläuse haupt-

sächlich an der Unterseite der Blätter aufhalten, wäre es angebracht, zu prüfen, inwieweit in der Praxis durch Behandlung der Blattunterseite bessere Bekämpfungerfolge erreicht werden können. Die beste Wirkung haben sowohl bei der Sprühapplikation als auch bei der Topikalapplikation mit kaum merklichen Unterschieden die Präparate Pyrethrum-Extrakt+Piperonylbutoxid, Confidor 200 SL (ein neues mindertoxisches Insektizid), Pirimor und Pyrethrum-Extrakt gezeigt. Bei den Neemformulierungen (NeemAzal-W wurde nur als Gießmittel angewendet) war die erzielte Mortalität vergleichsweise deutlich geringer. Hier wäre vielleicht eine 2. Behandlung notwendig gewesen, da nach Meinung von Experten (mündliche Mitteilung von Herrn Dr. Kleeberg u.a.) bessere Ergebnisse erst nach einer längeren Einwirkphase erreicht werden können. Weiterhin ist in diesem Zusammenhang von Bedeutung, daß die Wirkung von Neempräparaten nicht nur in der erreichten Mortalität, sondern auch in der physiologischen Beeinträchtigung der Blattläuse gesehen werden muß. Ein dazu durchgeführter Versuch zeigte, daß Blattläuse, die an neembehandelten Baumwollpflanzen 5 Tage gesaugt hatten, nur 20 % der Nachkommen produzierten, als Blattläuse, die an unbehandelten Pflanzen lebten (100 %). Diesem Effekt dürfte bei den Neempräparaten eine grundlegende Bedeutung zukommen, da durch Verringerung der Populationsdichte auch eine Bekämpfung der Blattläuse denkbar wäre. Bei den Präparaten Neudosan, Telmion und AHL lagen die erreichten Mortalitätswerte in der Mehrzahl der Fälle unter 50 %, wobei von diesen Neudosan die bessere Wirkung zeigte. Diesen Präparaten dürfte auf Grund spezieller Eigenschaften vor allem eine gewisse Bedeutung für Kombinationszwecke zur Verbesserung der Wirksamkeit bzw. Beständigkeit von natürlichen Insektiziden zukommen.
Durch die Anwendung von Confidor 5 GR in Granulatform sowie durch die Saatgutbbehandlung mit Gaucho 75 WS konnten die Baumwollpflanzen in beiden Fällen 9 Wochen blattlausfrei gehalten werden.

5. Zusammenfassung

Im Rahmen einer Forschungsarbeit über die Bekämpfung wichtiger Baumwollschädlinge mit alternativen und mindertoxischen Insektiziden wurden erste Versuche mit der Baumwollblattlaus durchgeführt. Von den ausgewählten 12 Präparaten haben sich auf Grund der erzielten Wirkung Pyrethrum-Extrakt + Piperonylbutoxid, Confidor 200 SL, Pirimor und Pyrethrum-Extrakt als die besten erwiesen. Die mit den Neemformulierungen erzielten Ergebnisse waren deutlich geringer, wobei hier allerdings der Wirkung auf physiologische Lebensvorgänge, wie z.B. der Beeinträchtigung der Fertilität der Blattläuse eine wichtige Bedeutung zukommt. Unter dem Gesichtspunkt der Verringerung der toxischen Belastung von Baumwollfeldern einschließlich der gezeigten Wirkung haben sich besonders auch die Anwendung von insektiziden Granulaten sowie die insektizide Saatgutbehandlung als sehr geeignet erwiesen.

6. Literatur

ASCHER, K.R.S.: Nonconventional insecticidal effects of pesticides available from the neem tree Azadirachta indica.
Arch. Insect Biochem. and Physiol. 22, 433-449, 1993

SCHAUER, M.: Die Wirkung von Nieminhaltsstoffen auf Blattläuse und die Rübenblattwanze.
Diss. Univ. Gießen, 1985

SCHMUTTERER, H.; ASCHER, K.R.S.: Natural pesticides from the neem tree and other tropical plants.
Third International Neem Conference 1986, GTZ 1987

Wirkung von NeemAzal-Formulierungen auf Kartoffelkäfer *Leptinotarsa decemlineata* im Freiland

Edmund Hummel
Trifolio-M GmbH, Sonnenstr. 22, D-35633 Lahnau

Abstract: Effect of NeemAzal-Formulations on the Colorado Potato Beetle in the Field.

In the field two formulations: NeemAzal-T (0,05 and 0,2%) and NeemAzal-T/S (0,5 and 1,0%) were tested. The preparations were sprayed after the appearance of the first larvae at June 1st. Both preparations controlled the population of *L. decemlineata* for about 22 days. NeemAzal-T/S exhibited a higher efficacy (82-84%) than NeemAzal-T (42-58%).

Einleitung

Mit verschiedenen Formulierungen des Neem-Wirkstoffes **"NeemAzal"** werden seit etwa 5 Jahren erfolgreich Untersuchungen zur Kontrolle von Kartoffelkäfern durchgeführt (1-12). Mit den Formulierungen NeemAzal-T (5% Azadirachtin A) und NeemAzal-T/S (1% Azadirachtin A) wurden 1994 erste Kleinparzellenversuche durchgeführt. Beide Formulierungen zeigen einen konzentrationsabhängigen Wirkungsgrad gegen Kartoffelkäfer.

Ergebnisse

1994 wurde die ersten Eigelege des Kartoffelkäfers in den Versuchparzellen (je 5 m^2) am 25.Mai gefunden. Der Zeitpunkt für den Einsatz der neuen NeemAzal-Formulierungen wurde mit dem 1. Juni - d.h. beim Schlupf der ersten Larven - sehr früh gewählt. Zu diesem Zeitpunkt befanden sich in den Parzellen auch viele adulte Tiere, die Eier ablegten.

In Abbildung 1 sind die Beiträge der verschiedenen Entwicklungsstadien zum gesamten Befallsverlauf dargestellt. Die Larvenzahlen in den behandelten Parzellen nehmen kontinuierlich ab und die Unterschiede in der Entwicklungdynamik werden erkennbar. Nach Beendigung des Larvenschlupfs (zwischen dem 15. und 20. Juni) befinden sich in der Kontrolparzelle absolut und relativ schon deutlich mehr ältere Larven als in den Versuchsparzellen. Die in den Testparzellen schlüpfenden jungen Larven, entwickeln sich nicht weiter. Die Unterschiede in der Wirkung der beiden NeemAzal-Formulierungen treten am 22. bzw. 29. Juni besonders hervor. Die drastische Abnahme der Kartoffelkäferlarven in den behandelten Varianten ist offensichtlich.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

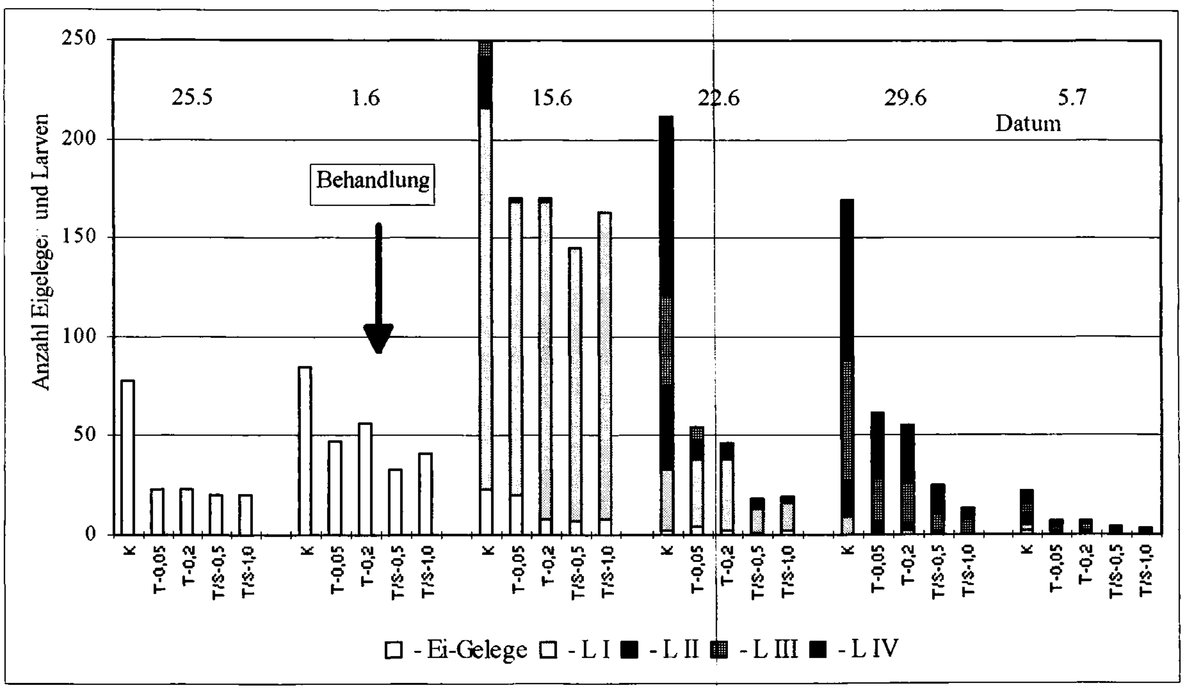


Abb. 1 Entwicklung der Kartoffelkäfer *L. decemlineata* nach der Behandlung mit NeemAzal-Formulierungen (in Absolutzahlen)

Diese relativen Anteile der verschiedenen Larvenstadien werden in der prozentualen Darstellung (Abb. 2) besonders deutlich. Am 22. 6. nimmt der Anteil an L1 in der Reihe Kontrolle < NeemAzal-T < NeemAzal-T/S zu. Dementsprechend werden in der gleichen Reihenfolge geringere Anteile an älteren Larvenstadien gefunden. Die drastischen Reduktion der Gesamtanzahl der Larven und die gegenüber der Kontrolle veränderte Verteilung auf die verschiedenen Stadien, sind auf die entwicklungsstörende Wirkung der Neem-Inhaltsstoffe zurückzuführen.

Berücksichtigt man, daß auch nach der Behandlung am 1.6. noch bis Mitte Juni Junglarven aus neu abgesetzten Eigelegen schlüpfen, so deutet das Ergebnis darauf hin, daß auch nach 2 bis 3 Wochen die Wirkstoffkonzentration auf den Blättern noch genügend hoch ist, um die Entwicklung der frisch geschlüpften Larven zu beeinträchtigen. Erst etwa 4 Wochen nach der Behandlung stellen sich in Kontroll- und Versuchsparzellen wieder ähnliche Verhältnisse im Bezug auf die verschiedenen Larvenstadien ein.. In der mit 1% NeemAzal-T/S behandelten Parzelle sind jedoch auch am 29. 6. nur ältere Larvenstadien zu finden.

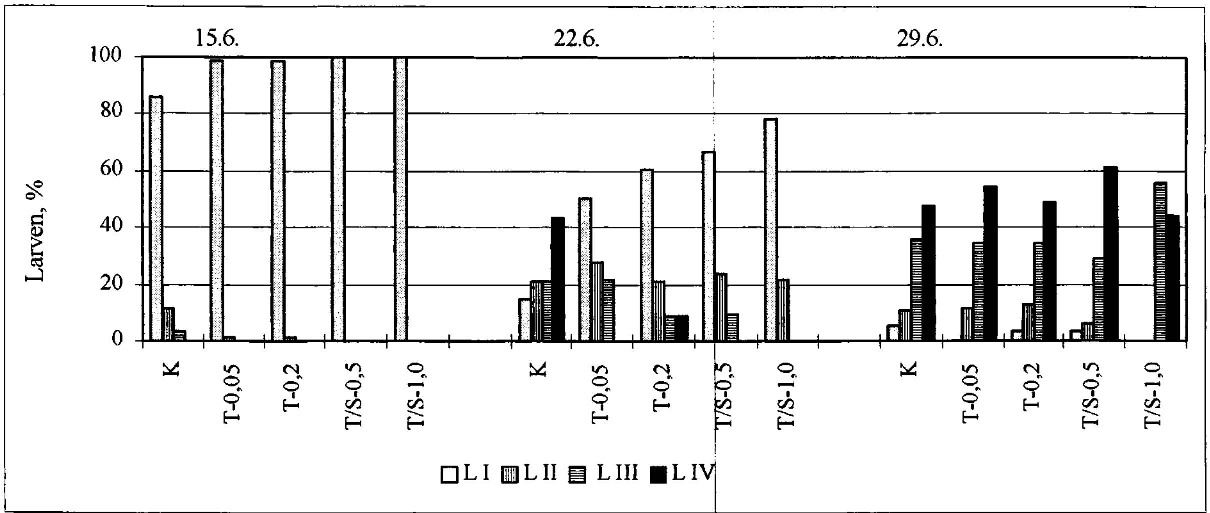


Abb. 2 Entwicklung der Kartoffelkäfer *L. decemlineata* nach Behandlung mit NeemAzal-Formulierungen (prozentual)

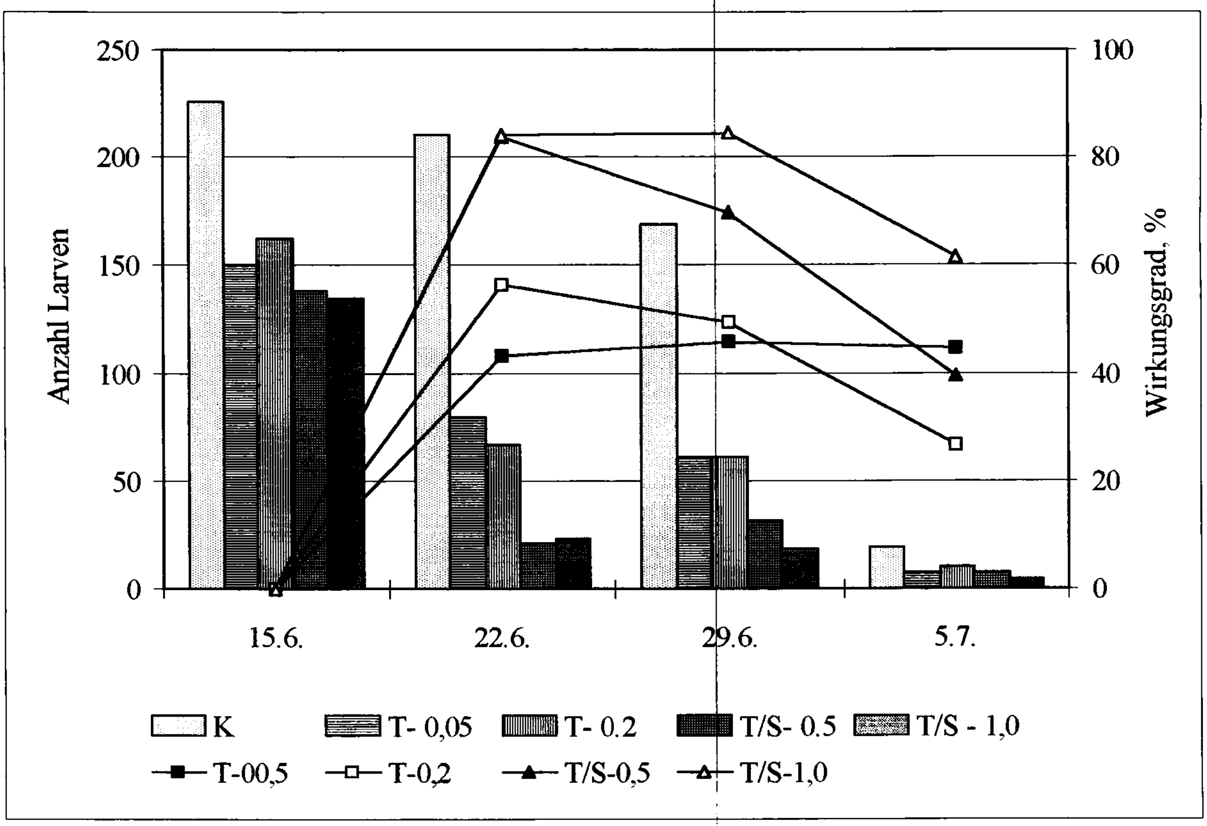


Abb. 3 Wirkung von NeemAzal-Formulierungen auf den Kartoffelkäfer *L. decemlineata*
(□ - Anzahl Larven, —— - Wirkungsgard)

Die Versuchsergebnisse belegen, daß beide NeemAzal-Formulierungen zur Kontrolle des Kartoffelkäfers geeignet sind. Aus praktischen Gründen wurde die Larvenzahl am 15. Juni für die Berechnung der Wirkungsgrade als Bezugswert gewählt. Dies ist auch in Anbetracht der Tatsache sinnvoll, daß Laboruntersuchungen an Eigelegen auf keinerlei oviziden Effekt der NeemAzal-Formulierungen hindeuten.

Nach dem Massenschlupf ergibt die Bonitur am 22. Juni, daß die Formulierung NeemAzal-T/S (0,5 bzw. 1,0 %) fast doppelt so wirksam ist, wie NeemAzal-T (0,05 bzw.0,2%) (Abb. 3). Dieses Ergebnis überrascht zunächst wegen der 5-fach höheren Wirkstoffkonzentration in NeemAzal-T gegenüber NeemAzal-T/S. Auch 29 Tage nach der Behandlung ist der Wirkungsgrad bei NeemAzal-T/S mit 69 bis 84 % noch sehr befriedigend; in der NeemAzal-T-Parzelle bleibt die Effektivität vom 22. Juni bis 5. Juli nahezu unverändert bei etwa 50 %.

Zusammenfassung

Die Ergebnisse deuten an, daß selbst die geringen Mengen an nach 3 bis 4 Wochen auf der Pflanze vorhandenem Neem-Wirkstoff noch ausreichen, um die Entwicklung junger Larven des Kartoffelkäfers zu stören. Insbesondere die Behandlung mit NeemAzal-T/S etwa 1 bis 2 Wochen vor dem Massenschlupf verhindert die Entwicklung der Larven effektiv und langfristig.

Die Versuche mit Kartoffelkäfern, Vertretern besonders fraßaktiver Schädlinge, belegen die Wirksamkeit von NeemAzal-Formulierungen für einen umweltfreundlichen Pflanzenschutz, wobei ist die Formulierung NeemAzal-T/S besonders wirksam. Die physiologische Wirkung der Mittel wird in den untersuchten Formulierungen klar nachweisbar.

Die Ergebnisse lassen den Schluß zu, daß unter den hiesigen klimatischen Bedingungen eine einmalige, frühzeitige Anwendung von NeemAzal-Formulierungen gegen die jungen Larven (L1-L2) des Kartoffelkäfers der ersten Generation, die Entwicklung des Schädlings kontrolliert und den Kartoffelpflanzen einen ausreichenden Schutz für die gesamte Wachstumsperiode bieten kann.

Danksagung

Der Autor dankt dem BMBF für finanzielle Unterstützung bei der Durchführung der Versuche.

Literatur

1.) **Hummel, E.** Erste Erfahrungen mit der Anwendung von NeemAzal-F; S. 17 in: Kleeberg, Practice Oriented Results on Use and Production of Neem-Ingredients. Proc. of the 1st Workshop, Trifolio-M, Lahnau (1992)

2.) **Kovalev, B. et al**. Die Anwendungsmöglichkeit des Präparates NeemAzal-F in GUS-Ländern; S. 23 in: Kleeberg, Practice Oriented Results on Use and Production of Neem-Ingredients. Proc. of the 1st Workshop, Wezlar, Trifolio-M, Lahnau (1992)

3.) **Hummel E.** und Kleeberg H., Anwendungsmöglichkeiten von NeemAzal-Formulierungen gegen Kartoffelkäfer, p. 91 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 2nd Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1993)

4.) **Otto D.**, Effects of the Azadirachtin Preparation "NeemAzal-F" on Larvae and Adults of *Leptinotarsa decemlineata*, p. 21 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 3rd Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1994)

5.) **Otto D.**, Sytemic Effects of the Azadirachtin Preparation "NeemAzal-W" on Larvae and Adults of *Leptinotarsa decemlineata*, p. 39 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 3rd Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1994)

6.) **Otto D**., The Persistence of the Systemic effect of the Azadirachtin Preparation "NeemAzal-W" on Larvae of the Colorado Potato Beetle *Leptinotarsa decemlineata* Say., in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 3rd Workshop (H. Kleeberg and V. Micheletti, eds.), Trifolio-M, Lahnau (1996)

7.) **Gottwald R.**, Wirkung von NeemAzal-F auf Larven des Kartoffelkäfers (*Leptinotarsa decemlineata* Say.), p. 37 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 3rd Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1994)

8.) **Monkos A. et al**. (Hrsg.), in: Informationen für die Pflanzenproduktion, S. 34-45; Heft 14/94; Landesanstalt f. Pflanzenbau Forchheim, Rheinstetten (1994)

9.) **Gottwald R.**, Ergebnisse zum Biopräparat NeemAzal gegen tierische Schadorganismen, in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 3rd Workshop (H. Kleeberg and V. Micheletti, eds.), Trifolio-M, Lahnau (1996)

10.) **Hummel E**. Wikung von NeemAzal-Formulierungen auf Kartoffelkäfer *Leptinotarsa decemlineata* im Freiland, Entomologen-Tagung, DGaaE, Göttingen (1995)

11.) **Pfleiderer, H**. Persönliche Mitteilung (1994)

12.) **Oroumchi, S.** Persönliche Mitteilung (1994)

13.) **Hummel E.**, unveröffentlichte Ergebnisse (1995)

Persistence of Systemic Effects of the Azadirachtin Preparation "NeemAzal W" on Larvae of the Colorado Potato Beetle *Leptinotarsa decemlineata* Say.

D. Otto
Projektgruppe Naturstoffe der Universität Potsdam
Stahnsdorfer Damm 81, D - 14532 Kleinmachnow

H. Kleeberg and M. Henrich
Trifolio-M GmbH Lahnau
Sonnenstraße 22, D - 35633 Lahnau 2

Summary

Feeding L3-instar larvae of the Colorado potato beetle *Leptinotarsa decemlineata* Say. with Azadirachtin infiltrated leaves gave remarkable retarding effects in the development of the larvae. The larvae were fed with cut leaves of potato plants which have grown up in palettes with soil drenched with NeemAzal W solution (100 ppm azadirachtin content, related to 400 g Azadirachtin per hectar). The reactions of the larvae to the systemically infiltrated food differ in a wide scale: As the weakest reaction the growing rate is decreased and the time till pupation is increased. As the heaviest reaction the larvae stop growing and developing totally and die after some days of retardation. These effects can be shown independently of the time interval between drenching the soil with NeemAzal W and cutting the leaves as food (NeemAzal W administering to the soil 3, 6, 9, 12, 15 days ago before taking the leaves). HPLC technique indicates that the Azadirachtin concentration in the cut leaves may be less than 0,1 ppm.

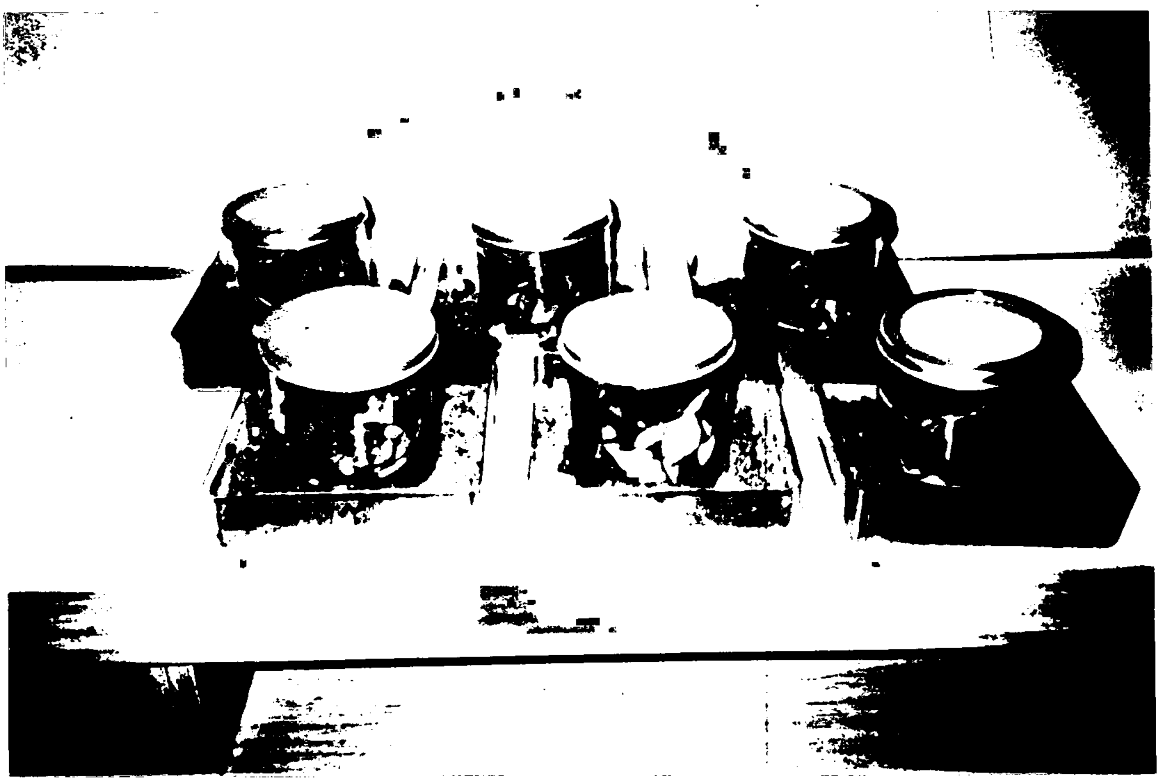
Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Introduction

Reports about the good efficasy of Azadirachtin preparations on the Colorado potato beetle *Leptinotarsa decemlineata* Say. were given already in 1993 on the 3rd Workshop about "Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones" (See the Proceedings of the 3rd and the 4th Workshop). One of these reports dealt with systemic effects of the Azadirachtin preparation NeemAzal W on larvae and adults, feeding on potato plants which were growing in NeemAzal W infiltraded soil (OTTO, 1994). Now a short report will be given about the persistence of this systemic effect. The question is: Is this only a short effect in time due to metabolism and degradtion of the incorporated Azadirachtins, or is ti a long lasting effect.

Figure 1: Palettes for growing up potato plants.
The NeemAzal W solution (100 ppm a.i.) was given into the soil without wetting the leaves.

Figure 2:
Rearing cage in a thermo constant chamber.



Body weight of Larvae

fed with potato plants treated systemically with NeemAzal W different days ago; start with 30 L3-Larvae per variant

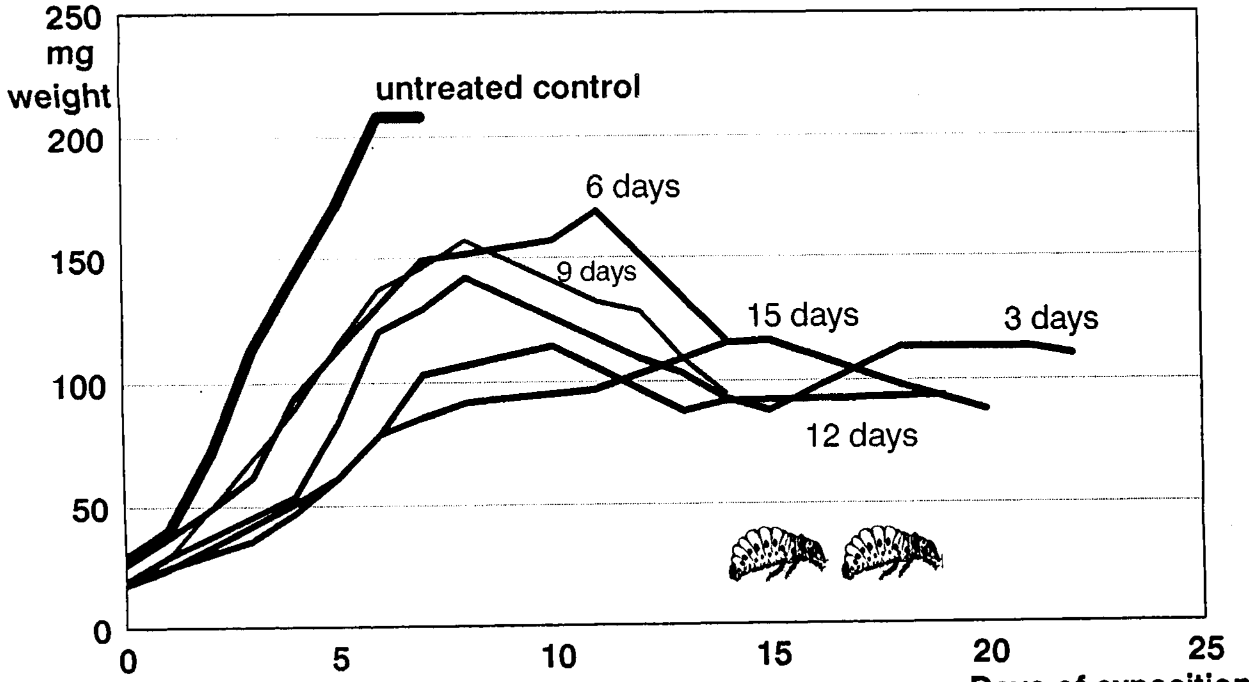


Figure 3

Body weight of Larvae: maximum, **mean**, minimum

start with 30 L3-Larvae per variant

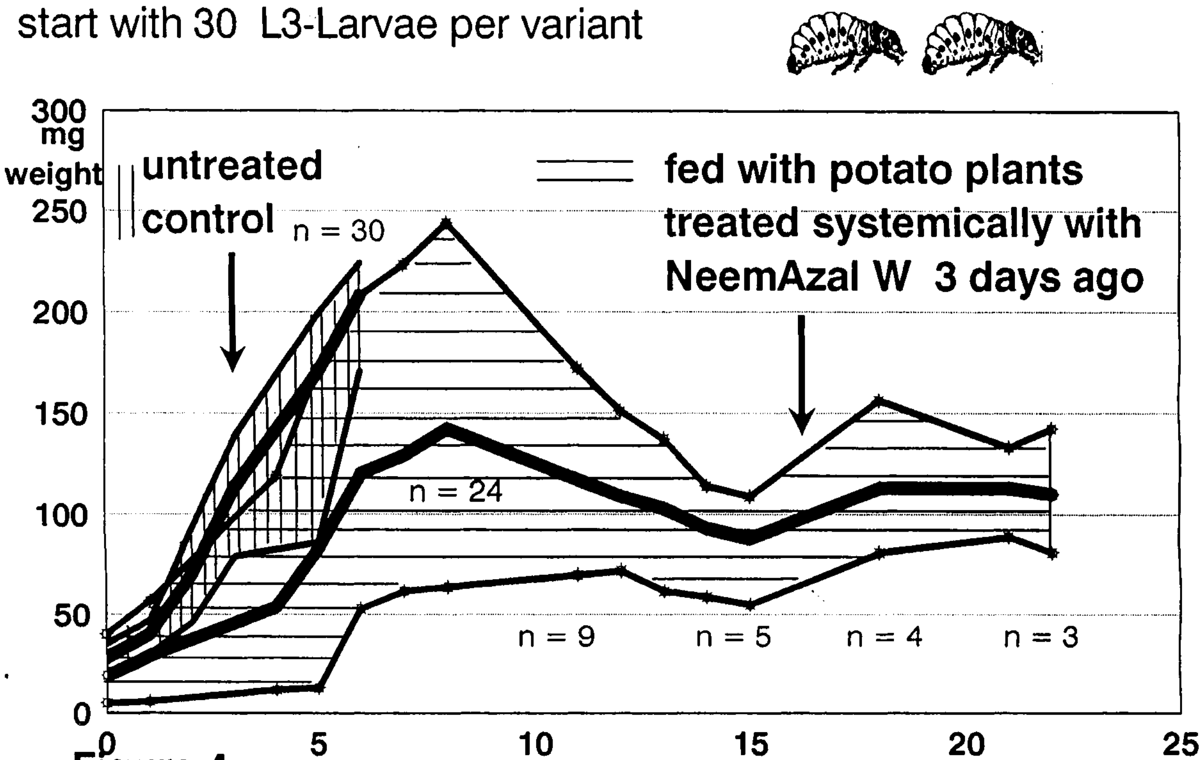
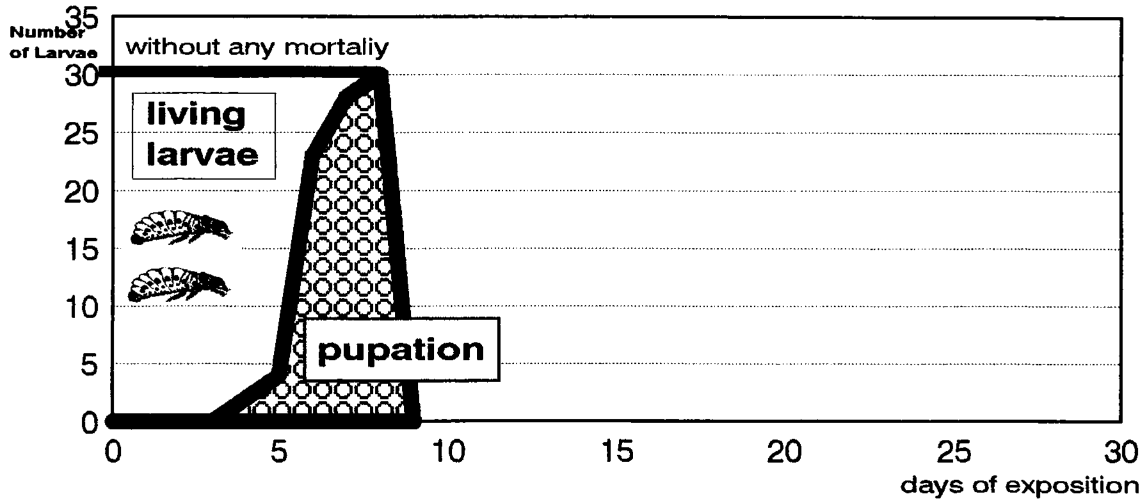
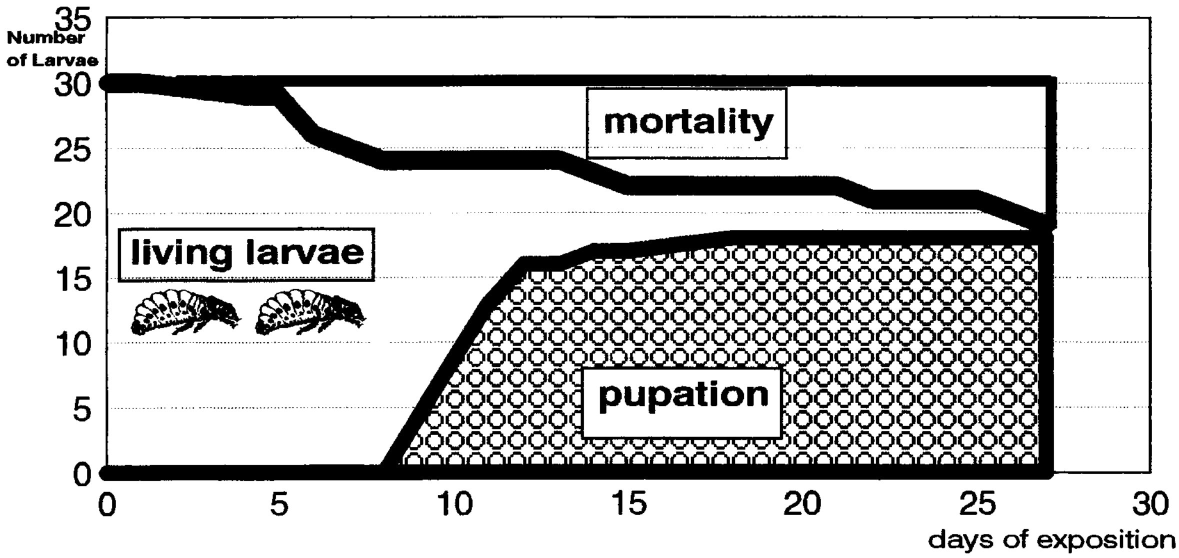


Figure 4

Development of L3-Larve on untreated potato leaves
untreated control



Development of L3-Larve on potato leaves
treated systemically with NeemAzal W
3 days before using as food



Development of L3-Larve on potato leaves
treated systemically with NeemAzal W
15 days before using as food

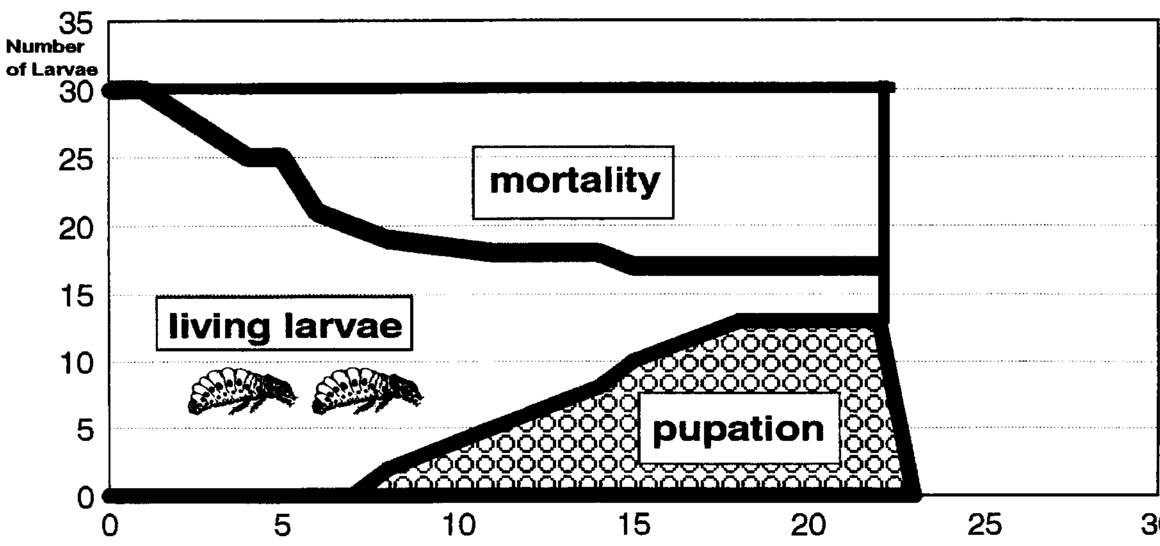


Figure 5

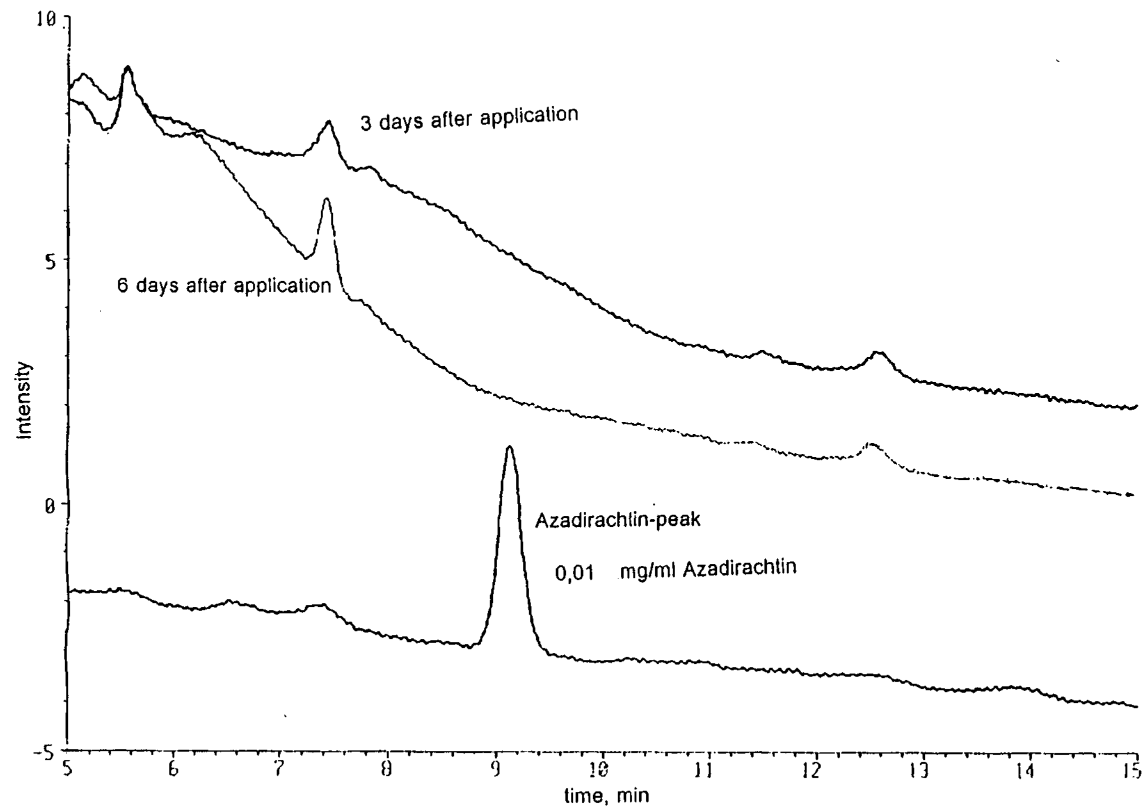


Figure 6: HPLC-chromatogrammes of Azadirachtin and aquatic potato leaves extracts

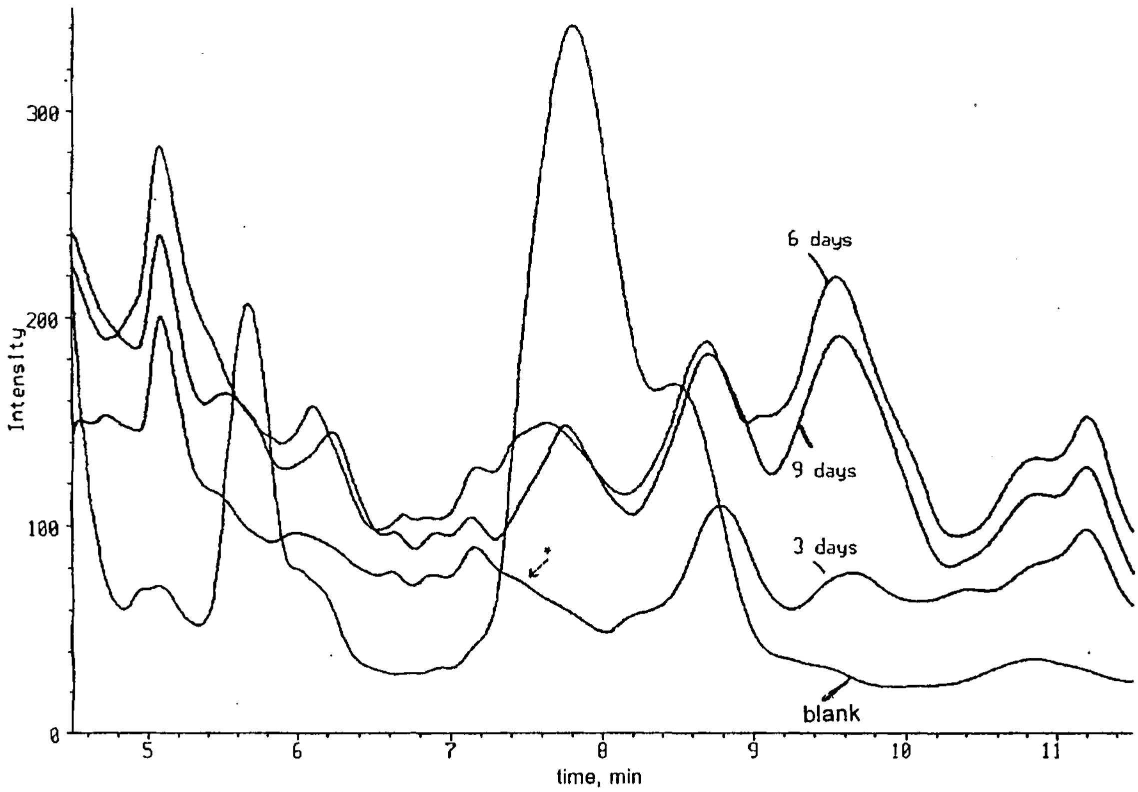


Figure 7: HPLC-chromatogrammes of acetacid ethylether extracts from potato leaves
It could be made evident by additional procedures, that the *-marked shoulder is referring to 0,1ppm Azadirachtin in the 3-day variant

Methods

In palettes of 0,35 x 0,35 cm^2 were growing up 12 potato plants each (sort Adretta) (see Fig.1). The soil in the palettes had a high of about 8-9 cm, the weight of about 5500 to 5800 g. When the plants growed up to about 35 cm, the soil was drenched with 50 ml of a NeemAzal W solution containing 100 ppm active ingredients (Azadirachtines). Concerning the preparation of this solution see OTTO, 1994. By this, the input of Azadirachtins into the soil of each palette mounts up 5 mg. That means a quantity of 400 g a.i. per hectar. After application the soil was repeatly watered with pure water adequate to 1,63 mm rainfall.

3^rd^ instar larvae of the potato beetle were fed with leaves cut from this potato plants at different time distances from Neem administering to the soil. The leaves were cut 3, 6, 9, 12, 15 days after administering, they were put as food to 30 L3-larvae into screen covered cages without bottom, standing over soil, so that old larvae had the opportunity to crawl into the soil for pupation (see Fig.2). The trials were carried out in a climatic constant chamber (22°C, 16 h longday). The development of the larvae were registrated carefully by daily registrating the weight increase, duration to pupation, and mortality. Variant K larvae were fed with untreated leaves.

Besides the biological dates we took probes from potato leaves before and 3, 6, and 9 days after application of Neem Azal W for estimation of the Azadirachtin concentration: the content of Azadirachtines in these probes was analysed in the laboratory of Dr. Kleeberg with HPLC-techniques by Dr. M. Henrich.

Results

Such larvae which were fed with untreated leaves developed from young L3 instar to later L4 instar during 5 to 7 days, their body weight increased during this time from 28 +/- 3,5 mg to 208 +/- 18 mg. None of the 30 larvae died. 4 of the larvae were going to pupation at the 5th day, 24 at day 6, and 2 at day 7 (Fig. 3-5).

Larvae fed with systemically contaminated leaves showed a distinctly delayed growth, a remarkable mortality, and only a part of the population came to pupation as Fig. 3, 4 and 5 show. But the strength of these damaging effects seems not to be dependent from the number of days which lay between drenching the soil and cutting the leaves as food. The differences of dates between the variants are without statistical significance. In this way leaves which were cut 15 days after Neem administration show the same efficasy as leaves which were cut 3 days after Neem application to the soil.

The development of the larvae in the untreated control variant occurs without greater variability, as seen in Fig. 4. By feeding with Azadirachtin containing leaves this picture is changing: There are remarkable differences in the reactions of the larvae to the contaminated leaves. Some larvae feed and develop normally with only a little delay, they get a normal end weight of about 200 mg and they pupate. But an other part of the Larvae fall into stagnation after food uptake during some days. They get a weak and flabby constitution and a brown colour. They stop the development during L4-instar and fail to pupate. The development dates in such a population differ in a wide deviation (as an example see Fig. 4, referring the 3 day variant).

The first important result of these trials is that there is a remarkable systemic activity. The second result is the realization that the injuring effects of the systemically contaminated food is lasting for 15 days and possible more.

To get an information about the real actual content of Azadirachtins in the leaves of the different variants in our trials, we cut leaves at different days after soil drenching for chemical residual analysis. This occurs in the laboratories of the Trifolio company by HPLC-technique. In providing this method, Dr. Henrich was able to detect Azadirachtins in aqueous extracts from potato leaves down to a limit of 1 ppm . In leaves cut from plants in our palette-trials no Azadirachtin was detectable by this method (Fig. 6). . By using acetic ether, or ethanolic solvent and additional procedures a 0,1 ppm concentration of Azadirachtin in the leaves of the 3-day-variant could be detected (Fig. 7). More was not yet to be expected, remembering that the soil in the palette contained only 1ppm Azadirachtins (5mg a.i. were put to more than 5 kg soil !) (see part "Methods").

Mortality of 3rd instar larvae (Trials 1993)

exposed to leaves 1 day and 7 days after drenching the soil with "NeemAzal W"

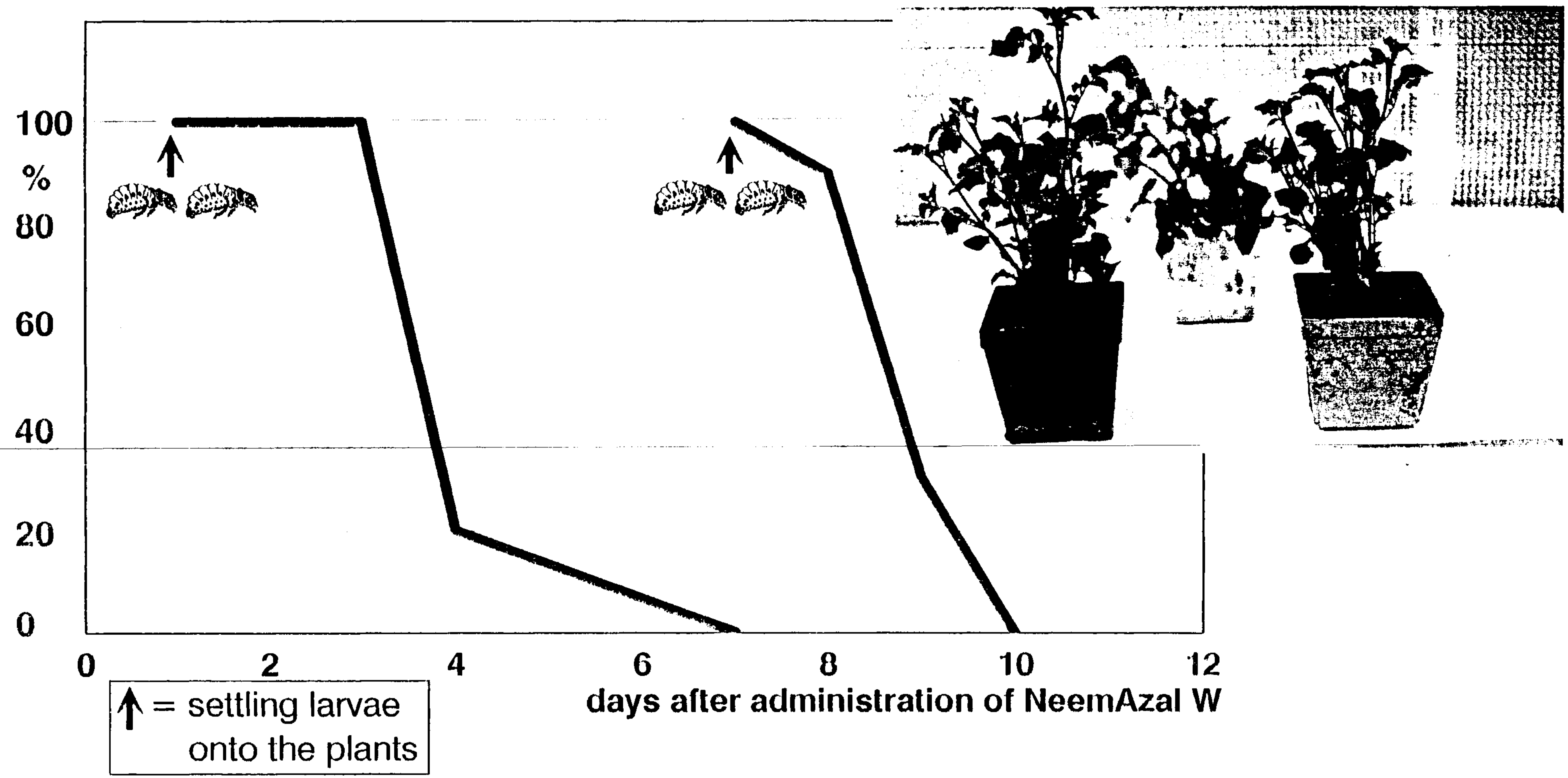


Figure 8

This estimation was not possible in the 6-day and 9-day variant, because peaks of other ingredients were covering the interesting area (Fig. 7).

By this the third important result is the evidence for the very high effectiveness of the active ingredient which is able to injure the insects already in such low concentration.

It may be further possible that the Azadirachtins were converted inside of the plant to other compounds which have also damaging effects to the larvae, but which are not detectable with this HPLC-method, and which are persistent for a long time.

The damages in these experiments in 1994 are not as drastic as those of 1993 (OTTO, 1994) which showed 100 % mortality of L3-instar larvae after feeding contaminated food (Fig.8). The difference may be due to the changed methodical arrangement. 1993 single plants grew up in little flower pots, while 1994 there were 12 plants growing in greater palettes. The concentration of the NeemAzal W solution applied was always the same (100 ppm) and also the quantity of the solution related to the *surface* of the vessel (8 x 8 cm^2 in case of flower pots, 35 x 35 cm^2 in case of palettes; - referred to 400 g a.i.per hectar), but the bigger *volume* of soil in the palettes may be important there. In contrast to the last, the root system of the potato plant in the little flower pot occupies the volumen totally and the uptake of the ingredient may be more sufficiently. But also in 1993 there was no difference in efficasy between food cut 1 day or 7 days after administering the Neem preparation (Fig. 8).

References

KLEEBERG, H.: The NeemAzal Conception: Test of Systemic Activity. in: H. Kleeberg (ed.): Practice Oriented Results on Use and Production of Neem-Ingredients; (Proceedings of the 1st workshop, Wetzlar, 1992), 1992, 5-15

OTTO, D.: Systemic Effects of the Azadirachtin Preparation "NeemAzal W" on Larvae and Adults of *Leptibnotarsa decemlineata*. in: H. Kleeberg (ed.): Practice Oriented Results on Use and Production of Neem-Ingredients; (Proceedings of the 3rd workshop, Wetzlar, 1993), 1994,

Ergebnisse zum Biopräparat NeemAzal gegen tierische Schadorganismen

Reinhold Gottwald
Lehr- und Versuchsanstalt für Integrierten Pflanzenbau e.V. Güterfelde
Berliner Str.; 14532 Güterfelde

Einleitung

Zur Bekämpfung tierischer Schadorganismen stellen Biopräparate eine Alternative zu den herkömmlichen Insektizidmitteln dar. Ihr Einsatz ist zu empfehlen in Gebieten mit Resistenzerscheinungen bei Schadinsekten gegenüber kommerziellen Insektiziden und auf den Flächen, wo aus Gründen toxikologischer Bedenken für den Naturhaushalt eine Anwendung synthetischer Insektizide nicht gestattet ist. Gleichfalls geeignet erscheinen die Bioprodukte für die Applikation im ökologischen Landbau. Als eine zu beachtende biologisch wirksame und umweltfreundliche Substanz zeigt sich der Wirkstoff Azadirachtin, ein Ingrediens des Niembaumes (Azadirachta indica). Bisher sind international in verschiedenen Feldkulturen und gegen eine Vielzahl von Schadorganismen Empfehlungen für erfolgreiche Bekämpfungseffekte bekannt. In einigen Ländern Afrikas, Lateinamerikas, Asiens und den USA kommen verstärkt Niemprodukte zum Einsatz.
In der LVAP Güterfelde wurden, durch die Unterstützung der Firma Trifolio-M in Lahnau, die Versuche 1994 mit NeemAzal-Formulierungen weitergeführt. Die Prüfungen richteten sich wieder mit dem Präparat NeemAzal-F gegen die Larven des Kartoffelkäfers und es erfolgten weiterhin erste Testungen mit dem Präparat NeemAzal-T gegen die Larven des Rothalsigen Getreidehähnchens in der Sommergerste. Beide Niemprodukte unterscheiden sich durch unterschiedliche Formulierungen, wobei der Wirkstoffgehalt von Azadirachtin in beiden Mitteln einen Anteil von 5 % aufweist.

Methodik

Die Daten der Versuchsbedingungen 1994 können der Tabelle 1 entnommen werden. Bedingt durch das späte und verzögerte Auftreten des Kartoffelkäfers erfolgte die Bekämpfung im Juli. Dadurch traten zum Versuchszeitpunkt überwiegend Larven im L_3-Stadium auf. Ein für die Prüfung ausreichender Befall an den Kartoffelstauden konnte für den Versuch genutzt werden. Als Grundlage für die Bonituren dieses Versuches dienten die Prüfmerkmale:

- Anzahl lebender Larven
- Anteil geschädigter Kartoffelstauden

Zum Abschluß des Versuches ermittelten wir je Parzelle mit Hilfe des Klopfkeschers die Anzahl der an 15 Stauden vorkommenden Nutzarthropoden.

Der Befall durch die Larven des Getreidehähnchens zeigte sich Ende Mai/Anfang Juni. Die Applikation zur insektiziden Prüfung der Larven in der Sommergerste erfolgte am 06.06.1994 bei mittlerem Befall.

Die Grundlage für die Bonituren des Getreidehähnchen-Versuches bildeten die Prüfmerkmale:

- Anzahl lebender Larven
- Anzahl geschädigter Fahnenblätter.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Weiterhin konnte je Variante der Kornertrag im Sommergersten-Versuch festgestellt werden. In beiden Versuchen wurde auf phytotoxische Schäden an den Pflanzen geachtet.

Ergebnisse und Diskussion

Kartoffelkäfer (Leptinotarsa decemlineata SAY.)
Die insektizide Prüfung richtete sich aufgrund des späten Applikationstermins gegen alle Larvenstadien, wobei überwiegend Larven im L_3-Stadium vorkamen. Die Larvenmortalität erreichte einen Wert von 82,9 % und lag somit um 7,5 % niedriger als beim Vergleichsmittel (Tab. 2). Damit bestätigt sich das Ergebnis von 1993, daß bei zunehmender Altersresistenz die Mortalität insektizidbedingt abnimmt. Die Fraßschäden an den Kartoffelblättern betrugen 12,6 % und ergaben dennoch eine merkliche Differenz zur unbehandelten Kontrolle von 34,1 %. Phytotoxische Schäden an den Kartoffelstauden traten nicht auf. Die Bewertung der Nutzarthropoden zum Abschluß des Versuches im Juli mit Hilfe des Klopfkeschers ergab eine beachtliche Nützlingsanzahl (Tab. 2). Die höchste Anzahl mit 39 Nützlingen an 15 Stauden kam in der NeemAzal-Variante vor und war somit geringfügig höher als in der unbehandelten Kontrolle. Auch die Variante mit dem nützlingsschonenden Vergleichsmittel Novodor (Wirkstoff Bacillus thuringienis var. tenebrionis) hatte eine bedeutende Nützlingsanzahl. Ein negativer Einfluß auf die Nützlingsfauna wie Spinnen, Raubwanzen, parasitische Wespen, Marienkäfer und Florfliegen war somit durch das Präparat NeemAzal-F nicht nachzuweisen.

Rothalsiges Getreidehähnchen (Oulema melanopus (L.))
Gegen die Larven des Getreidehähnchens wurde das Präparat Neemazal-T in der Sommergerste zum EC-Stadium 45-49 appliziert. Während der Versuchsdauer traten alle Larvenstadien auf. Gegen Ende des Versuches verringerte sich die Populationsdichte in der unbehandelten Kontrolle. Die Anzahl der Käfer war sehr gering und ohne Bedeutung für die Prüfung.

Bei Bewertung der Larvenmortalität, insbesondere zur Abschlußbonitur des Präparates NeemAzal-T, konnte ein hoher Wirkungsgrad von über 96 % nachgewiesen werden (Tab. 3). Unwesentlich war der Unterschied zum Vergleichsmittel. Die durch Larven verursachten Fraßschäden an den Fahnenblättern mit Anteilen von 5 % bei NeemAzal-T und 3 % beim Vergleichsmittel waren gering im Vergleich zur unbehandelten Kontrolle mit 19 % (Tab. 3). Ebenfalls konnten an den Getreidepflanzen keine phytotoxischen Schäden beobachtet werden. Die Ermittlung der Nützlinge durch Kescherfänge zum Abschluß des Versuches ergab eine geringe Anzahl, so daß auf eine Beurteilung verzichtet werden mußte. Der Kornertrag in der NeemAzal-Variante lag im Vergleich zur unbehandelten Kontrolle um 2,50 dt/ha höher. Ähnlich gut war das Ergebnis in der Variante mit dem Vergleichsmittel.

Abschließend ist zuschlußfolgern, daß mit beiden Formulierungen, den Präparaten NeemAzal-F gegen die Larven des Kartoffelkäfers und NeemAzal-T gegen die Larven des Getreidehähnchens, ausreichende Bekämpfungseffekte erzielt werden können. Wirkungsgrade von über 90 % sind bei Anwendung gegen die Junglarven und bei normalen Witterungsbedingungen erreichbar. Ein negativer Einfluß auf die Nützlingsfauna war, wie auch 1993, in beiden Versuchen nicht zu beobachten.
Auf Grund der günstigen ökologischen Eigenschaften verdienen die Niemprodukte für den Einsatz sowohl in der integrierten Produktion als auch im ökologischen Anbau besondere Beachtung.

Tab. 1: Daten der Versuchsbedingungen für die insektizide Prüfung der NeemAzal-Präparate 1994 in Güterfelde

Schadinsekten / Daten	Kartoffelkäfer-Larven	Getreidehähnchen-Larven
Kultur	Kartoffel	Sommergerste
Sorte	Koretta N	Ditta
Mittelkonzentration	0,2	0,2
Brüheaufwand (l/ha)	350	350
Anzahl Wiederholungen	1 (großflächig)	4
Gerät	Hege Parzellenspritze	Hege Parzellenspritze
Druck (bar)	3	3
Düsentyp	Teejet	Teejet
Düsenbohrung (mm)	0,3	0,3
Geschwindigkeit (km/h)	3	3
Boden- und Pfl.-zustand	trocken	trocken
Witterung allgemein	heiter	heiter
Temperatur (°C)	24	14
Luftfeuchtigkeit (%)	45	60
Windgeschwindigkeit (m/s)	0-0,3	0,3-1,3
Boniturtermine (Tage nach d. Appl.)		
1. Bonitur	3	4
2. Bonitur	9	8
3. Bonitur	12	11

Tab. 2: Insektizide Wirkung des Präparates NeemAzal-F gegen Kartoffelkäfer-Larven (L_1 - L_4), Güterfelde 1994

Präparat	Mittlere Anzahl Larven/Staude	Mortalität [1] %	Fraßschäden %	Anzahl Nützlinge/ Kescherprobe
Unbehandelt	10,5	0	46,7	31
NeemAzal-F	1,8	82,9	12,6	39
Vergleichsmittel	1,0	90,4	2,4	22

[1] Wirkungsgrad nach ABBOTT

Tab. 3: Insektizide Wirkung des Präparates NeemAzal-T gegen Getreidehähnchen-Larven, Güterfelde 1994

Präparat	1. Bonitur 10.6.94		2. Bonitur 14.6.94		3. Bonitur 17.6.94		
	Anzahl Larven	Mortalität [1] %	Anzahl Larven	Mortalität [1] %	Anzahl Larven	Mortalität [1] %	Fraßschäden %
Unbehandelt	46	0	58	0	28	0	19
NeemAzal-T	2	95,7	9	84,5	1	96,4	5
Vergleichsmittel	0	100	1	98,3	0	100	3

[1] Wirkungsgrad nach ABBOTT

PRELIMINARY STUDIES ON THE EFFECT OF NEEMAZAL-T AND NEEMAZAL-T/O(1) ON SOME BIOLOGICAL ASPECTS AND SILK PRODUCTION OF MULBERRY SILKWORM *BOMBYX MORI* L.

By

Kelany, I. M.*; A. A. Zannoon, and Shadia M. Omara***
* Plant Protec. Dept., Fac. Agric., Zagazig University
** Seric. Res. Sec., Plant Protec. Instit., Agric. Res. Centre

ABSTRACT

*The biology and silk production of mulberry silkworm, **Bomby mori** L. were studied after feeding the newly moulted 3rd larval instar on mulberry leaves dipped in different concentrations (25, 50 and 75 ppm) of both NeemAzal-T and NeemAzal-T/O formulations.*

Obtained results could be summarized as follows:

1- *All the tested concentrations of both formulations caused highly significant increase in larval mortality rates, and insignificant prolongation in the duration of larval period. In addition, the concentration 75 ppm of NeemAzalT/O only induced highly significant decreases in adult female fecundity.*

2- *The concentration 25 ppm of both extracts induced slight insignificant effects on cocoon and reelable filament indices.*

3- *The concentrations of 50 and 75 ppm of the two extracts decreased significantly the weigth of fresh cocoons and cortices, silk content ratio and length and weight of reeled filament.*

4- *The concentration 75 ppm of the two tested formulations decreased significantly the size of reeled filaments while the other two concentrations caused insignificant effects.*

*Accordingly, it is advisable to keep **B. mori** rearing away from any contamination with such extracts to avoid their adverse effects.*

(1) NeemAzal-T and NeemAzal-T/O are produced by Trifolio-M GmbH Company, Germany

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

INTRODUCTION

Mulberry silkworm, *Bombyx mori* L, is one of the most important economic insects that can play an effective role in increasing national income. Sericulture and the silk industry in general cover a wide range of techniques ranging from agricultural mulberry tree cultivation and biological silkworm rearing to industrial silk production and processing.

Mulberry silkworm is very sensitive to many chemicals and the damage caused by such chemicals steady increase year by year. Contamination with pesticides may be transferred to larvae by different ways such as mulberry trees, rearing rooms, tools, clothes and even the rearers themselves. **Lim et al.** (1990) mentioned that organophosphorus insecticides make a larvae stop feeding and cause trembling and vomiting, while pyrethroids cause the same symptoms moreover the larval body becomes S or U form. Also sulpher compounds make larvae lose appetite and become sluggish.

The neem tree is a common tree and widly distributed in Africa, Asia and Latin America. Compounds of its seed kernels showed some striking effects on a great number of insect species **(Radwanski (1977a & 1977b); Abdul Kareem (1980); Ladd (1980); Redknap (1980); Ali et al. (1983) and Kelany et al. (1991a & 1991b)).**

The use of such components on large scale might be a type of contamination to silkworm larvae as mentioned above. The present work aimed to evaluate the effect of two natural compounds of neem kernel, i. e. NeemAzal-T and NeemAzal-T/O extracts added to mulberry leaves in different concentrations on some biological aspects and silk production of mulberry silkworm, *Bombyx mori* L.

MATERIALS AND METHODS

The present work was carried out in Plant Protection Department, Faculty of Agriculture, Zagazig University during spring Season of 1994.

Three concentrations of each NeemAzal-T (N-T) and NeemAzal-T/O (N-T/O) i. e. 25, 50 and 75 ppm were prepared. Mulberry leaves were dipped for 10 seconds in each concentration and left to dry under room temperature. The control leaves were dipped in tap water.

The silkworm larvae under investigation were obtained from the laboratory culture of *Bombyx mori* L. (Korean Hybrid) reared in Sericulture Research Section in Zagzig. Rearing procedures were carried out under the laboratory conditions of 24-28° C and 70-85% R.H. Newly hatched larvae were provided with suitable amounts of clean fresh mulberry leaves until the end of the second star. Newly moulted third instar larvae were divided into seven groups of 200 larvae each (4 replicates of 50 larvae each). The seven groups were managed as follow:

- Three groups (A) were treated with N-T-concentrations (25, 50 and 75 ppm).
- Another three groups (B) were fed on T-T/O concentrations (25, 50 and 75 ppm).

- The last groups (C) of larvae were fed on mulberry leaves treated with tap water (control).
- Larvae of the seven groups were fed once (the first meal after the second moult) on the treated leaves, then they were fed on clean leaves until the end of their feeding stage.

The following parameters were measured and recorded:

I- Biological measures

1- Duration of larvae stage.
2- Larval mortality rate.
3- Total number of deposited eggs/adult female (fecundity).

II- Productivity measures

1- Cocoon indices:

Thirty fresh cocoons of each treatment and control were collected soon after pupation, then they were cleaned and weighed. After cutting the cocoon and removing the pupae the cortices were also weighed and the cocoon shell ratio was estimated according to **Tanaka (1964)** formula:

$$\text{Cocoon shell ratio} = \frac{\text{Weight of cocoon cortex}}{\text{Weight of fresh cocoon}} \times 100$$

2- Reelable filament characters:

Another 30 cocoons from each treatment as well as control were picked up at random and reeled. The filament length (m) and weight (g) were measured. Size of filament (denier.) was estimated according to **Tanaka (1964)** formula:

$$\text{Size of silk filament (dn)} = \frac{\text{Weight of filament (g)}}{\text{Length of filament (m)}} \times 100$$

Data obtained were statistically analyzed according to **Snedecor and Cochran (1967)** methods.

RESULTS AND DISCUSSION

I - Effect of NeemAzal-T and NeemAzal-T/O on some biological aspects of mulberry silkworm, *B. mori* L.

It is clear from Table (1) that mulberry leaves treated with both N-T and N-T/O formulations affected some biological features of mulberry silkworm. For instance the three used concentrations of both N-T and N-T/O (25, 50 and 75 ppm) caused highly significant increase in larval mortality rates as compared with control larvae but without any significant differences between all treatments. Obtainded results cleared also that the number of deposited eggs/female moth decreased in females developed from larvae fed on mulberry leaves treated with N-T/O at a concentration of 75 ppm. The other two concentrations showed a slight effect as compared with control ones.

On the other hand the duration of larval period was not significantly affected by the two tested extracts.

Table (1): Effect of NeemAzal-T and NeemAzal-T/O on some biological aspects of mulberry silkworm *Bombyx mori* L.

Extract	Concent. (ppm)	Duration (day)	Mortality rate %	No. of deposited eggs/female
N-T	25	36	61.50 a	399 a
	50	36	61.75 a	437 a
	75	35	61.25 a	381 a
N-T/O	25	36	66.00 a	432 a
	50	35	59.00 a	362 a
	75	35	53.25 a	146 b
Control	-	34	10.00 b	400 a
L.S.D. 0.5		-	9.334	84.428
01		-	13.086	118.363

II - Effect of NeemAzal-T and NeemAzal-T/O on silk production of mulberry silkworm, *B. mori* L.

1- Cocoon indices:

Owing to the data in Table (2) the weight of fresh cocoon spun by larvae fed on treated mulberry leaves with all concentrations of both N-T and T-T/O were significantly decreased except those treated with N-T 25 ppm. Moreover, the weight of cocoon cortex was significantly affected by feeding larvae on mulberry leaves treated with 25, 50 and 75 ppm of both N-T and N-T/O.

Obtainde results clear also that feeding larvae on mulberry leaves (treated with N-T and N-T/O) resulted in significant decrease in silk content ratio, while the concentration 25 ppm caused insignificant effect on this parameter when compared with control one.

Table (2): Effect of NeemAzal-T and NeemAzal-T/O on silproduction of mulberry silkworm, *Bombyx mori* L.

Extract	Conc. (ppm)	Cocoon indices			Filament characters		
		Wt. of fresh cocoon (g)	Wt of cortex (g)	silk content ratio (%)	Length (m)	Weight (g)	Size (dm)
N-T	25	1.634 a	0.321 b	19.64 ab	865 ab	0.272 ab	2.83 ab
	50	1.393 b	0.251 c	18.03 b	780 b	0.231 bc	2.66 ab
	75	1.249 b	0.227 c	17.50 b	715 b	0.197 c	2.48 b
N-T/O	25	1.488 b	0.278 b	18.66 ab	830 ab	0.255 b	2.75 ab
	50	1.358 b	0.235 c	17.35 b	340 bc	0.207 c	2.51 ab
	75	1.158 c	0.179 d	15.46 c	645 c	0.171 c	2.38 b
Control	-	1.754 a	0.352 a	20.06 a	970 a	0.319 a	2.96 a
L.S.D. 05		0.1080	0.0218	1.209	105.528	0.0415	0.2438
01		0.1514	0.0305	1.695	147.942	0.0538	0.3418

2- Reelable filament characters:

Data in Table (2) show that both N-T and N-T/O at the concentration of 25 ppm caused insignificant effect on the filament length regarding to control one, while the concentrations 50 and 75 ppm shortened the reelable filament. The weights of reeled silk filament resulted from cocoon spun by larvae fed on mulberry leaves treated with N-T (50 and 75 ppm) and N-T/O (25, 50 and 75 ppm) were significantly decreased compared to that of the control. Concerning the size of silk filament it is clear that N-T and N-T/O at the concentration of 75 ppm decreased significantly the filament size, whereas 25 and 50 ppm of the two extracts showed insignificant effect.

These harmful effects of N-T and N-T/O on the biology, cocoons and filaments may be due to the reduction of food consumed by larvae fed on treated mulberry leaves with the two formulations as it is well known that neem extracts have repellent and deterrent effects **(Ladd, 1980)**.

REFERENCES

Abdul Kareem, A. (1980): Neem as an antifeedant for certain phytophagous insects and bruchid pulses. 1st Int. Neem Conf. W. Germany, June 1980: 223-250.

Ali, S. L.; O. P. Singh and U. S. Misra (1983): Effectivness of plant oils against puls beetles, *Callosobruchus chinensis* Linn. Indian J. Ent.

Kelany, I. M.; M. A. El-Deeb and A. A. El-Fishwawi (1991a): The effect of aqueous neem, *Azadirachta indica* A. Juss, seed kernel extract on the house fly, *Musca domestica* L. Egypt J. Biol. P. Cont. 1 (1): 121-128.

Kelany, I. M.; Shadia M. Omara and Zienab A. Mohamed (1991b): Biological changes of cowpea weevil *Callosobruchus chinesis* (Linn) as influenced by neem seed kernel. Minia J. Agric. Res. & Dev. Vol. 13, No. 2, June (1991).

Ladd, T. L. Jr. (1980): Neem seed extracts as feeding deterrents for the japanese beetles, *Popillia joponica* Proc. 1st Int. Neem Conf. W. Germany: 149-155.

Lim, S. H.; Kim, Y. and S. Lee (1990): Sericulture training manual FAO Bull. 80, Rome, 1990.

Radwanski, S. (1997a): Neem tree 2: Uses and potential uses. Wld. Crops Live stk. 29: 111-113.

Radwanski, S.(1997b): Neem tree 3: Further uses and potential uses. Wld. Crops Live stk. 29: 167-168.

Redknap, R. S. (1980): The uses of crushed neem berries in the control of some insect pests in Gambia. 1st Int. Neem Conf. W. Germany June 1980: 205-214.

Snedecor, G. W. and W. G. Cochran (1967): Statistical methods, Iowa State Univ. Press., Amer. Iowa.

Tanaka, Y. (1964): Sericology, Cent. Silk Board, Bombay, (95):216-220.

Auswirkungen von Niempräparaten auf den Apfelschalenwickler *Adoxophyes orana* F.v.R. (Lep., Tortricidae) und die ihn parasitierende Schlupfwespe *Colpoclypeus florus* Walk. (Hym., Eulophidae)*

Effects of Neem on *Adoxophyes orana* F.v.R. (Lep., Tortricidae) and *Colpoclypeus florus* Walk. (Hym., Eulophidae), an important ectoparasite of leaf rollers

G. Jakob und E. Dickler
Biologische Bundesanstalt für Land- und Forstwirtschaft,
Institut für Pflanzenschutz im Obstbau,
Schwabenheimer Str. 101
D-69221 Dossenheim

In Laborversuchen erwiesen sich Inhaltsstoffe des tropischen Niembaumes *Azadirachta indica* A. Juss. gegen Larven von *A. orana* als hochwirksam. Freilanduntersuchungen sollten Aufschluß über Möglichkeiten des praktischen Einsatzes gegen den Schädling im Apfelanbau geben.
Sowohl die normal üblichen Bekämpfungsmaßnahmen im Frühjahr gegen die 1. Generation als auch Behandlungen gegen die den bedeutendsten Schaden verursachende Sommergeneration zeigten gute Wirksamkeit und erbrachten deutliche Reduktionen der Schäden.
Neben Untersuchungen zu Auswirkungen von verschiedenen Niempräparaten auf Mortalität wurden Prüfungen zur möglichen Beeinträchtigung der Reproduktionsleistung des Wicklers einbezogen. Hierzu erwiesen sich Beobachtungen zum Eiablageverhalten sowie Präparationen des weiblichen Genitalapparates als geeignet.
Bei als Larven behandelten Tieren konnte ein erhöhter Anteil unbefruchteter Eigelege festgestellt werden. Der Grund hierfür ist in erster Linie in der verminderten Fitness und der dadurch geringeren Kopulationsbereitschaft der Männchen zu suchen, was anhand verminderter Spermatophorenanzahlen belegt werden kann.

Desweiteren wurden Auswirkungen von Niem auf verschiedene Lebensstadien des Schalenwicklerparasitoiden *Colpoclypeus florus* bei dessen Entwicklung auf behandelten Wirtsraupen geprüft.
Während die Behandlungen mit Niem auf Lebensdauer und Parasitierungsleistung von adulten Weibchen keinerlei Auswirkungen zeigten, konnten sich Schlupfwespenlarven auf behandelten Schalenwicklerraupen nicht vollständig entwickeln und starben ab.

Jakob G. & Dickler E., Untersuchungen zur Wirksamkeit von Niempräparaten bei der Bekämpfung des Apfelschalenwicklers *Adoxophyes orana* F.v.R.(Lep., Tortricidae), Mitt. Dtsch. Ges. Allg. Angew. Ent., 9, 51-56 (1994)

Jakob G. und Vogt H., Einsatz von Niempräparaten gegen *Adoxophyes orana* F.v.R. und Untersuchungen zu Nebenwirkungen; S. 55 in: Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones, Proc. 3rd Workshop (H. Kleeberg, ed.), Trifolio-M, Lahnau (1994)

* veröffentlicht in Mitteilungen aus der Biologischen Bundesanstalt, Heft 301, 1994

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

CONTROL OF THE GYPSY MOTH, *LYMANTRIA DISPAR* (L.), WITH NEEMAZAL-T IN STANDS OF OAK

CORNELIA M.Y. NICOL* and H. SCHMUTTERER

Institut of Phytopathology a. Applied Zoology, Justus-Liebig-University, Ludwigstr. 23, D-35390 Giessen, Germany.
* Current address: Department of Biological Sciences, Centre of Pest Management, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

ABSTRACT

The gypsy moth, *Lymantria dispar* (L.) (Lep., Lymantriidae), and the cockchafer, *Melolontha hippocastani* F. (Col., Scarabaeidae), showed outbreaks in 1994 in the south of Hesse, Germany. Trials to control both forest pests by application of NeemAzal products in stands of oak were conducted in spring, 1994.

The effects of 1.0 l NeemAzal -T / ha ($NA\text{-}T_{1.0}$) resp. 0.75 l NeemAzal-T / ha combined with 5.0 l Telmion (rape oil formulation) ($NA\text{-}T_{0.75}+R_{5.0}$) on larvae of the gypsy moth is shown. Applications were carried out by helicopter with the ingredients diluted in 50 l water /ha.

Larvae of *L. dispar* collected from the sprayed plots consumed very small amounts of oak leaves, showed a considerable decrease in faeces production and gained hardly any body mass. Their development was inhibited. As a rule, they died 3-4 weeks after spraying as 2nd or 3rd instars. Larvae sampled 22 hours after application of NeemAzal-T with Telmion ($NA\text{-}T_{0.75}+R_{5.0}$) showed 83 %, others sampled 3, 14 and 21 days after treatment 100 % mortality. The treatment of NeemAzal-T with Telmion ($NA\text{-}T_{0.75}+R_{5.0}$) was more effective than solely NeemAzal-T ($NA\text{-}T_{1.0}$).

INTRODUCTION

Up to 1990 high population densities of *Lymantria dispar* (L.) (Lep., Lymantriidae) occurred only in some restricted regions of Germany. To some extent this species was considered to be dying out (WULF *et al.* 1994). But hot and dry summers in 1990-1992 as well as forest management (MINDT 1994, SCHWENKE 1994) caused a severe population increase. In 1994, nearly 80,000 ha of deciduous forests in Germany were infested (WULF *et al.* 1994) and complete defoliation occurred on large areas, mainly on oaks.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

In spring 1994, the gypsy moth as well as the cockchafer *Melolontha hippocastani* F. (Col., Scarabaeidae) showed outbreaks in southern parts of Hesse and South Germany and a severe damage to oaks was expected. In the area of Lampertheim (Hesse) a field study on the simultaneous control of the gypsy moth and of the cockchafer (see SCHMUTTERER and NICOL 1996) in young oak stands in May was conducted. Environmentally tolerable products based on renewable products were used: the neem (*Azadirachta indica*) product NeemAzal-T and Telmion, based on rape oil.

MATERIALS AND METHODS

Besides other treatments (results not shown)

- 1.0 l NeemAzal-T (5% azadirachtin, Trifolio-M) (NA-$T_{1.0}$) per hectar resp.
- 0.75 l NeemAzal-T + 5.0 l Telmion (rape oil formulation, AgrEvo) (NA-$T_{0.75}$+$R_{5.0}$)

were applied with 50 litres water/ha by an helicopter on stands of young oak trees (*Quercus robur*) near Lampertheim at 10th of May 1994 at 7 p.m. The control stand remained untreated. At that time mainly second and some first instar larvae of the gypsy moth occurred. 22 h, 3, 14 and 21 days after application, respectively, larvae were sampled from treated and untreated oaks randomly and brought into a greenhouse at the University of Giessen for further observation. The larvae were put into cages (40 each; 4 replicates) and were fed with fresh, untreated oak branches.

RESULTS

A single application of NeemAzal products affected development and longvity of the gypsy moth larvae severely. In the treated (NA-$T_{0.75}$+$R_{5.0}$) stand the majority of the larvae died 3-4 weeks after application and hardly reached the second or the early third larval instar. These observations coincide with the results obtained in the greenhouse. All larvae died with exception of a few (17 %) sampled 22 h after treatment (Fig. 1). These few had not been affected, and the resulting adults were fertile. Presumably these unaffected larvae did not ingest treated leaves due to ecdysis or to strong antifeedancy.

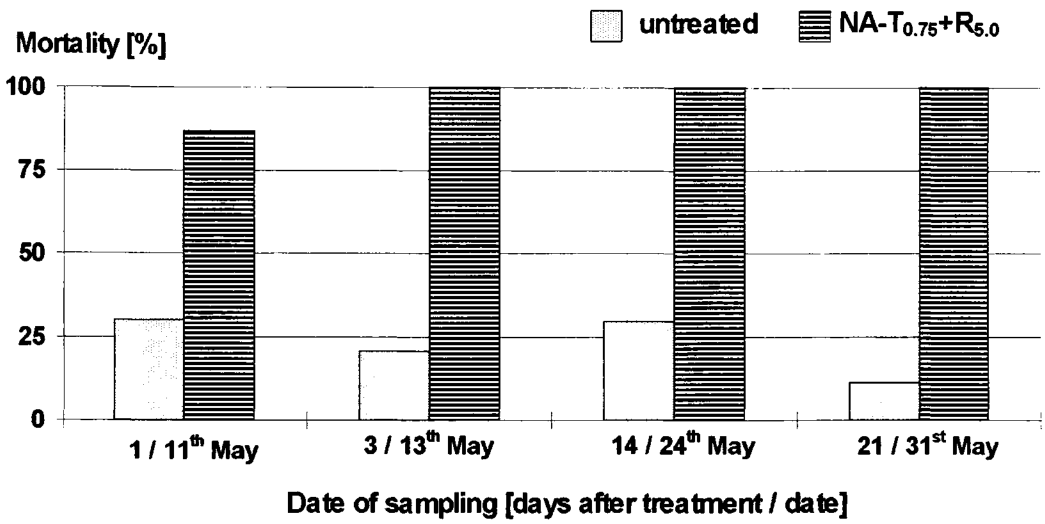


Fig. 1: Mortality of untreated and treated (NA-$T_{0.75}$ + $R_{5.0}$) larvae of *L.dispar* from day of sampling to pupation

The feeding activity of gypsy moth larvae in the treated stands decreased rapidly (second antifeedant effect). Besides evaluations of little damage to leaves this was proved by the very low production of faeces and by hardly any gain in mass (Tab. 1), as compared with the untreated larvae.

Tab. 1: Production of faeces and gain in mass of untreated and treated (NA-$T_{0.75}$+$R_{5.0}$) larvae of *L.dispar* within 24 h

[mg]	untreated	NA-$T_{0.75}$ + $R_{5.0}$
Starting body mass	391,4 $\pm$ 197,9	42,0 $\pm$ 22,7
Faeces (dry mass)	59,2 (n=62)	0,068 (n=20)
Gain in mass	54,7	7,4 ***

Date of sampling: 3 days after treatment; date of weighing: 2 weeks after sampling; *** means are significantly different at $p \leq 0.01$ by U-Test (MANN & WHITNEY).

Moreover, body size of larvae from untreated and treated (NA-$T_{0.75}$+$R_{5.0}$) plots differed about 40 % (sampled 3 days after treatment) and about 50 % (sampled 14 days after treatment), respectively (Tab. 2).

Tab. 2: Body size [mm] of untreated and treated ($NA\text{-}T_{0.75}+R_{5.0}$) larvae of *L.dispar* sampled at different dates

Date of sampling		untreated	$NA\text{-}T_{0.75} + R_{5.0}$	Diff. [%]
3 dat ; 13th May	∅ SD ±	29,16 ± 6,04	12,16*** ± 3,34	41,7
	n	56	31	
14 dat ; 24th May	∅ SD ±	20,40 ± 5,18	10,53*** ± 2,64	51,6
	n	92	150	

Date of measurement: 15 days after treatment; dat: days after treatment; Diff.: differences; *** means are significantly different at $p \leq 0.01$ by U-Test (MANN & WHITNEY).

Besides, body mass of larvae from untreated and treated plots differed significantly (Tab. 1, Tab. 3). Larvae being sampled two weeks after treatment obtained in the average only 23 % ($NA\text{-}T_{1.0}$) resp. 15 % ($NA\text{-}T_{0.75}+T_{5.0}$) of the body mass of the untreated larvae. Their body mass did hardly increase during the further development in the greenhouse (Tab. 3). The treatment with the additive rape oil ($NA\text{-}T_{0.75}+T_{5.0}$) proved to be more effective than solely NeemAzal-T ($NA\text{-}T_{1.0}$), although a lower amount of the active ingredient azadirachtin was applied.

Tab. 3: Body mass [mg] of untreated and treated ($NA\text{-}T_{1.0}$, $NA\text{-}T_{0.75}+R_{5.0}$) larvae of *L.dispar* at different dates

Date of weighing		untreated	$NA\text{-}T_{1.0}$	$NA\text{-}T_{0.75} + R_{5.0}$
15. dat	∅ SD ±	174.54 [a] ± 7.73	40.89 [b] ± 2.55	26.12 [c] ± 1,69
	n	80	80	152
22. dat	∅ SD ±	459.14 [a] ± 302.0?	71.02 [b] ± 63.73	35.49 [c] ± 12.88
	n	77	49	8
27. dat	∅ SD ±	- 1)	178.87 ± 141.83	- 2)
	n		26	

Date of sampling: 14 days after treatment; dat: days after treatment; 1): all pupa ; 2) all dead;
means followed by different letters are significantly different at $p \leq 0{,}01$ by KRUSKAL-WALLIS-Test.

DISCUSSION

A high efficiency of neem kernel extracts on gypsy moth larvae were firstly observed in Germany under laboratory and field conditions by SKATULLA and MEISNER (1975). A severe inhibition of growth and development has been shown by SHAPIRO *et al.* (1994) in the USA. They also found out that addition of neem extract to the gypsy moth polyhedrosis virus led to a faster mortality caused by the virus. *Lymantria monacha* L., closely related to *L.dispar*, showed a high susceptibility to a neem kernel extract as well (BEITZEN-HEINEKE and HOFMANN 1992).

Gypsy moth larvae developing on trees treated with NeemAzal-T are considered to be no longer harmful to their host plants and to ensure a constant supply of food for birds, due to the delayed mortality - in contrast to fast-acting conventional insecticides.

The results presented are promising for the control not only of the gypsy moth. Because of the possibility to control simultaneously outbreaks of *Melolontha hippocastani* (SCHMUTTERER and NICOL1996), these environmentally tolerable products show promising facilities to protect deciduous forests from defoliation.

Acknowledgments: We thank Prof. Dr. Dimitri (Hann. Münden) and his colleagues for organisational support during the investigations. We also thank Prof. Dr. Basedow (JLU Giessen) for useful comments.

References

BEITZEN-HEINEKE, I. and HOFMANN, R. 1992: Versuche zur Wirkung des azadirachtinhaltigen, formulierten Niemsamenextraktes AZT-VR-NR auf *Hylobius abietis* L., *Lymantria monacha* L. and *Drino inconspicua* Meig. Z. Pfl.Krankheiten u. Pfl.Schutz 99, 337-348.

MINDT, G. 1994: Schwammspinnerbekämpfung im Forst. Gesunde Pflanzen 46, 215-220.

SCHWENKE, W. 1994: Über die Grundlagen der Entstehung und Begegnung von Insekten-Massenvermehrungen im Wald. Anz. Schädlingskd. Pflanzenschutz Umweltschutz 67: 120-124.

SCHMUTTERER, H. and NICOL, C.M.Y. 1996: Gleichzeitige Bekämpfung des Waldmaikäfers *Melolontha hippocastani* F. und des Schwammspinners *Lymantria dispar* L. mit NeemAzal T + Telmion im Frühjahr 1994. Mitt. DGaaE 10, in press.

SHAPIRO, M.; ROBERTSON, J.L. and WEBB, R.E. 1994: Effect of neem seed extract upon the gypsy moth (Lepidoptera: Lymantriidae) and its nuclear polyhedrosis virus. J. Econ. Entomol. 87, 356-360.

SKATULLA, K., MEISNER, J. 1975: Laborversuche mit Neemsamenextrakt zur Bekämpfung des Schwammspinners, *Lymantria dispar* L. Anz. Schädlingskd. Pflanzenschutz Umweltschutz 48, 38-40.

WULF, A.; BERENDES, K.H. and WOHLERS, P.W. 1994: Insektenschäden im Forst weiterhin problematisch. Forschungsreport 10, 26-28.

Vielversprechender Behandlungsversuch mit 1%iger Neemsalbe bei kindlicher Scabies*

Dr. med. F.-J. Knust
Abteilung für Kinder-und Jugendmedizin
Ev. Krankenhaus Bethanien Iserlohn GmbH
Hugo-Fuchs-Allee 3
58644 Iserlohn

In der Abteilung für Kinder -und Jugendmedizin des Ev. Krankenhauses Bethanien zu Iserlohn werden seit Anfang 1994 Patienten mit *Pediculosis capitis* mit Neem-Extrakt der Firma Trifolio-M mit recht gutem Erfolg behandelt. Durch diesen Erfolg ermutigt und angeregt durch die Veröffentlichung von Charles (siehe Charles V., Trop. Geogr. Med..Vol 44, ISS 1-2, 1992, Seite 178 bis 181) haben wir einen Behandlungs-versuch mit Neem bei kindlicher Scabies durchgeführt.

Die Behandlung mit o.g. Neem-Extrakt in Form von 3 mal tägl. Vollbädern bzw. 2 mal tägl. Ganzkörperwaschungen zeigte bei einem Patienten keinen therapeutischen Effekt; vielmehr mußte eine Exacerbation der Scabies registriert werden, sodaß wir nach 3-tägiger Behandlung auf die bisherigen konservativen Maßnahmen zurückgreifen mußten (Euraxil Lotio/Crotamiton-Therapie des Säuglings).

Daraufhin setzten wir bei einer 2,4 J. alten Patientin (J.A.) mit ausgeprägter generalisierter Scabies 1%ige Neem-Extrakt Salbe der Firma Trifolio-M ein. Während der Rumpf an vier aufeinanderfolgenden Tagen mit Euraxil Lotio 1 x täglich eingecremt wurde, erfolgte die Applikation von Neem-Salbe im Bereich der gesamten Extremitäten, ebenfalls 1 mal täglich , an vier aufeinanderfolgenden Tagen. Zur Gewährleistung einer ausreichenden Hautatmung wurde der Rumpf jeweils abends gegen 19.00 Uhr und die Extremitäten jeweils morgens gegen 7 Uhr behandelt.

Am Ende der o.g. 4-tägigen Behandlungsdauer war die Patientin von Seiten der Scabies völlig erscheinungsfrei. Es konnte kein therapeutischer Unterschied zwischen Crotamiton und Neembehandelten Hautarealen bezogen auf die Scabieseffloreszenzen ermittelt werden. Als erfreulicher Nebeneffekt der Neem Salben Therapie zeigte die Patientin im Extremitätenbereich eine deutliche Rückläufigkeit der Hautaustrocknung sowie einiger neurodermitischer Effloreszenzen. Dies dürfte auf die regelmäßige Applikation der rückfettenden Neem Salbe bzw. der Salbengrundlage zurückzuführen sein.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Nachzutragen bleibt, daß die Patientin 1 mal tägl. ein "normales" Pflegebad mit pH 5 Eucerin-Waschlotion erhielt.

Wenn es sich auch bei dem o.g. Kasus um eine Einzelfallbeobachtung handelt, so ist der Therapieeffekt jedoch sehr vielversprechend und gibt Anlaß zu der berechtigten Hoffnung, daß in absehbarer Zeit auf die Verwendung von Pyrethroiden und sonstigen Pestiziden bei der Behandlung von Ektoparasiten verzichtet werden kann.

Wir möchten den weiteren Behandlungsversuch mit 1%iger Neem-Extrakt Salbe bei Scabies im Kindesalter dringend empfehlen. Es soll nicht verschwiegen werden, daß zur Zeit sowohl Neem-Extrakt zur Behandlung der *Pediculosis capitis* als aber auch 1%ige Neem-Salbe zur Behandlung der Scabies vom BGA noch nicht zugelassen sind.
Wir empfehlen daher dringend in jedem Einzelfall ein entsprechendes Aufklärungsgespräch mit dem Patienten bzw. dessen Erziehungsberechtigten.

Vor dem Hintergrund der auch in Laienkreisen geführten Diskussion um die Toxizität und Umweltverträglichkeit von Pestiziden war die Einholung einer diesbezüglichen Behandlungsgenehmigung bisher kein Problem.

Control of lice and nits with NeemAzal-FT

M. Schuld & C. Jungen
Institut für Angewandte Zoologie
An der Immenburg 1, D-53121 Bonn

17 street children, at the age from 6 to 14, who live in a home in Chile, were badly infested with lice and nits. So far the controlling of lice was carried out with customary Chilean shampoos. A regular application was not possible because the Shampoo was too expensive, so renewed infestation appeared again. Because of this we put Neem-Extract (manufactured by Trifolio-M GmbH, Sonnenstr. 22, 35633 Lahnau, Germany) to their disposal. This extract contains 4% Azadirachtin A. The manufacturer recommends altogether three treatments: one washing on the first, the third, and tenth day. The child minders rubbed three or four ml of NeemAzal-FT suspension into the children's wet hair and let it work in for about ten minutes.

After the first treatment 12 out of 17 children were already free of lice and nits. The hair of the other 5 children showed a significant reduction of ectoparasites, but they needed a second treatment to eliminate the ectoparasites successfully. Pamela's, Pedro's and Claudia's scalpes showed a slight irritation, which disappeared shortly after the treatment. According to the manufacturer the irritation was based on the formulation of Azadirachtin.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Results:

Children	Hairlength	Infestation	1st treatment	2nd treatment	Comment
Priscila	long	Lice and nits	4ml		Free from lice and nits
Lisa	long	Lice and nits	3ml		Free from lice and nits
Yessica C	short	Lice and nits	3ml		Free from lice and nits
Pamela	long	Lice and nits	4ml	3ml	Free from lice and nits after 2nd treatment
Fernando	short	Nits	3ml	4ml	Free from lice and nits after 2nd treatment
Cristian	very short	Nits	3ml		Free from lice and nits
Petro	short	Nits	3ml	4ml	Free from lice and nits after 2nd treatment
Zuri	long	Nits	3ml		Free from lice and nits
Yessica J.	long	Nits	3ml		Free from nits and lice
Noemi	long	Nits	3ml		Free from nits and lice
Claudia	long	Lice and nits	3ml		Free from nits and lice
Daniela	long	Lice and nits	4ml		Free from nits and lice
Ana	long	Lice and nits	3ml		Free from nits and lice
Carlos	very short	Nits	4ml	4ml	Free from nits and lice after 2nd treatment
Marco	short	Nits	4ml		Free from nits and lice
Xavi	short	Nits	4ml	4ml	Free from nits and lice after 2nd treatment
Felipe	very short	Nits	4ml		Free from nits and lice

Fungicidal activity of commercial neemproducts

STEINHAUER, B.
Institut für Phytopathologie und Angewandte Zoologie
Justus-Liebig-Universität, Bismarckstr. 16, 35390 Gießen, Germany

Abstract: Commercial neem insecticides and other neembased products were used in a platetest and in an in vivo system with cucumber and Sphaerotheca fuliginea to evaluate their fungicidal properties.
Azatin EC and Margosan-O had a strong inhibitory effect on five fungi in the platetest when used at a concentration corresponding to 100 µg azadirachtin/ml. Most other products led to a slight inhibition or even a promotion of fungal growth.
In the in vivo system, a formulated neemoil, Margosan-O and NeemAzal-TS reduced the incidence of S. fuliginea to 0,7 - 3,7 % of the check. A soil application of the products one day before inoculation had no effect on the development of the fungus, indicating they were not systemically translocated.

Introduction

Commercial neem formulations are available from several companies in the USA, India, Thailand and other countries. All these products are designed as insecticides. The azadirachtin content ranges from very low (0,03 %) to rather high (3 - 5 %). In most cases further information on other active ingredients extracted from neem, the amount of neemoil or the formulation additives used is not available.
In these experiments the activity of some commercial neem products on phytopathogenic fungi were tested in laboratory trials on petridishes and in a glass house experiment against powdery mildew of cucumber.

Material and methods

Neemproducts

The following neem products (commercially available insecticides and other neembased materials) were used in the experiments.

Commercial products	Origin	Azadirachtin	Other ingredients
AZATIN EC	Agridyne Inc., USA	3 %	napthalene 4,7 % n-butanol 1,8 % others unknown
Margosan-O	W.R. Grace, USA	0,3 %	unknown
NeemAzal-F	Trifolio-M, Germany	5 %	unknown
NeemAzal-TS	Trifolio-M, Germany	1 %	unknown
NIMBECIDINE	Stanes, India	0,03 %	unknown
NIMEX	Thailand	0,2 %	unknown
Other Neemproducts			
Neemoil Temmen N1	Temmen GmbH, Germany	unknown	85 % neemoil 14,99 % emulsifier 0,01 % antioxidant
NeemAzal powder 5%	Trifolio-M, Germany	5 %	unknown
NeemAzal powder 24%	Trifolio-M, Germany	24 %	unknown

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Laboratory trials on agar plates

For the platetest a glucose-yeast extract agar was prepared with 10 g glucose, 2 g yeast extract and 14 g agar /l deionized water. After autoclaving, the neem products were added to the agar at a temperature of 55° C. The amended agar was poured into petridishes and inoculated with a mycelium covered agar disc (5 mm diameter) from fresh cultures of the test fungi.

The plates were then incubated in the dark at room temperature. The linear growth of the fungi was measured and compared with the growth on control plates with glucose-yeast extract agar.

Fungi in the platetest

Fungus	Isolate no.	Abreviations
Drechslera teres	149	D. t. 149
Fusarium graminearum	151	F. g. 151
Fusarium culmorum	152	F. c. 152
Geotrichum candidum	199	G. c. 199
Alternaria solani	252	A. s. 252
Botrytis cinerea	300	B. c. 300
Pythium ultimum	328	P. u. 328
Pythium inflatum	331	P. i. 331
Colletotrichum gloeosporioides	366	G. g. 366
Botryodiplodia sp.		Botr.

Concentrations of the amendments

The content of azadirachtin was often the only available information of the neem products in the trial. Therefore the amounts of the amendments of the test agar were calculated to give azadirachtin concentrations of 100 and 10 µg/ml. Lower concentrations had to be used where not sufficient product was available.

Product	Azadirachtin µg/ml		Amount/1000 ml agar	
AZATIN EC	100	10	3,3 ml	0,33 ml
Margosan-O	100	10	33,0 ml	3,3 ml
NeemAzal-F	100	10	2,0 ml	0,2 ml
NeemAzal-TS	20	2	2,0 ml	0,2 ml
NIMBECIDINE	10	1	33,0 ml	3,3 ml
NIMEX	100	10	50,0 ml	5,0 ml
NeemAzal 5 %	100		2 g	
NeemAzal 24 %	100		417 mg	
NeemAzal blank			2,0 ml	

Glass house trials with powdery mildew of cucumber

Seeds of cucumber (cv Sensation/Neckarruhm, Hild, Marbach) were sown in 11cm pots in TKS II (4 seeds/pot). After emerging of the seedlings two plants/pot were left for growing. When the second leaf had appeared the plants were kept in that stage by removing the growth point.

The upper leaf surfaces were sprayed with the test solutions or 10 ml of the solutions were applied to the soil. For each treatment four pots with two plants were used.

One day after the treatment the plants were inoculated by spraying a conidial suspension (3000 conidia/ml) of the powdery mildew fungus. Disease assessment was done 12 days after inoculation by counting the pustules per leaf.

Results

The diameter of the cultures was measured three days after inoculation and the growth of the fungi on amended agar compared with the growth of the check (Tab. 1, Tab. 2).

Tab. 1: Linear growth of fungi on glucose-yeast extract agar amended with neem products in percent relative to the check 3 dpi., first trial.

Fungus	NeemAzal-F 100 μg/ml azadirachtin	NeemAzal-F Aza 10	Azatin EC Aza 100	Azatin EC Aza 10	Margosan-O Aza 100	Margosan-O Aza 10
D. t. 149	16,67	16,67	0	0	0	16,67
F. c. 152	15,49	22,54	0	26,76	32,36	95,77
A. s. 252	14,63	17,07	0	36,59	20,73	62,20
P. u. 328	30,59	65,88	0	49,71	0	94,12
P. i. 331	3,33	9,41	0	0,78	0	78,43

Tab. 2a: Linear growth of fungi on glucose yeast extract agar amended with neem products in percent relative to the check 3 dpi., second trial.

Fungus	NeemAzal-TS Aza 20	NeemAzal-TS Aza 2	NeemAzal-TS blank	NeemAzal 5 % Aza 100	NeemAzal 24 % Aza 100
D. t. 149	38,8	52,5	43,3	20,0	25,0
F. g. 151	90,9	101,3	98,7	26,0	59,7
F. c. 152	163,0	148,2	130,9	94,4	83,3
G. c. 199	102,3	109,3	114,7	104,7	95,4
A. s. 252	89,9	97,8	101,9	58,4	60,7
B. c. 300	58,7	70,7	71,0	55,4	48,9
P. u. 328	69,7	97,6	92,9	0,0	4,9
P. i. 331	108,6	127,8	115,0	80,8	90,2
C. g. 366	80,6	97,0	71,6	67,2	71,6
Botr.	102,3	109,3	114,7	104,7	95,4

Tab. 2b:

Fungus	NIMEX Aza 100	NIMEX Aza 10	NIMBECIDINE Aza 100	NIMBECIDINE Aza 100
D. t. 149	0,0	32,5	0,0	35,0
F. g. 151	14,3	64,9	66,2	85,7
F. c. 152	57,4	116,7	127,8	185,2
G. c. 199	90,7	104,7	120,9	123,3
A. s. 252	15,7	175,3	50,6	70,8
B. c. 300	6,5	77,2	66,3	75,0
P. u. 328	0,0	68,5	0,0	65,5
P. i. 331	41,6	70,6	51,8	103,5
C. g. 366	19,4	59,7	73,1	86,6
Botr.	90,7	104,7	120,9	123,3

Tab. 3: Glass house trial with powdery mildew of cucumber, number of pustules/leaf and percent relative to check

Treatment	Azadirachtin μg/ml	Pustules/leaf (average)	% rel. to check
Check	-	34,0	100
Neemoil N1, 1 %, sprayed	?	1,3	3,7
NeemAzal-TS sprayed	20	0,8	2,2
NeemAzal-TS soil application	20	32,6	95,8
Azatin EC, sprayed	100	19,3	56,8
Azatin EC, soil application.	100	39,3	115,4
Margosan-O, sprayed	100	0,3	0,7
Margosan-O, soil application.	100	30,4	89,3

Discussion

Platetest

The test fungi differed strongly in their sensitivity to the amendments, while *Pythium ultimum* was inhibited by most of the treatments at high concentrations. Other fungi, like *Fusarium culmorum* and the isolate of *Botryodiplodia,* were even promoted in their growth.

NeemAzal-F inhibited the growth of five fungi to 30-3 % of the check at a concentration corresponding to 100 μg azadirachtin/ml. NeemAzal-TS did not strongly inhibit the growth of the fungi except *D. teres* at a concentration corresponding to 20 μg azadirachtin/ml. The effect was similar when the NeemAzal-TS blank was used for amendment.

NeemAzal powder (5% and 24%) inhibited the growth of *D. teres, F. graminearum, B. cinerea* and *P. ultimum.* NeemAzal powder is the nonformulated mixture of active ingredients extracted from neemseeds.

Azatin EC had a strong effect on the fungi and completely inhibited their growth at a concentration corresponding to 100 μg azadirachtin/ml. A "blank" containing 4,7 % naphthalene and 1,8 % n-butanol did not affect fungal growth (data not shown).

Good effects were also obtained with Margosan-O at the high concentration.

Nimex inhibited the growth of most fungi to less than 20 % of the check at the higher concentration.

Nimbecidine completely inhibited the growth of the fungi *D. teres* and *P. ultimum* at a concentration corresponding to 10 μg azadirachtin/ml, while others were promoted in their growth.

The results indicate that the concentration of azadirachtin is not a usefull marker for the fungicidal properties of a neem formulation. Other not yet identified ingredients must have a stronger influence on fungi. Many formulations contain neemoil which has fungicidal properties as well. Finaly the formulation additives can have an effect on the fungi.

In vivo test with powdery mildew of cucumber

Spraying the leaves with Neemoil N1, NeemAzal-TS or Margosan-O almost completely inhibited the development of the powdery mildew, while Azatin EC reduced the pustule number to only 56,8 % of the check. Similar as in the plate test the effect on development of powdery mildew was not correlated with the concentration of azadirachtin in the spraying solution. The strong influence on the development of mildew pustules by Neemoil N1 and Margosan-O (which contains larger amounts of neemoil) can be partly attributed to the film forming effect of oils that has been shown by several authors (HÄBERLE, 1993; HÄBERLE & SCHLÖSSER, 1993; HORST & KAWAMOTO, 1992).

Soil application of the commercial products had no effect on the number of pustules, systemic effects could not be observed.

Literature

HÄBERLE, R. 1993: Prüfung verschiedener Produkte zur alternativen Bekämpfung von *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll., dem Erreger des Echten Mehltaus an Gurke. Diplomarbeit, Gießen, Germany

HÄBERLE, R. & E. SCHLÖSSER (1993): Protective and curative effects of Telmion on *Sphaerotheca fuliginea* on cucumber. Med. Fac. Landbouww. Univ. Gent **58**, 1461-1467

HORST, R. K. & S. O. KAWAMOTO (1992): Effect of Sodium Bicarbonate and Oils on the Control of Powdery Mildew and Black Spot of roses. Plant Dis. **76**, 247-251.

Effects of neemoils on powdery mildew of cucumber

STEINHAUER, B. and R. HÄBERLE, Institut für Phytopathologie und Angewandte Zoologie, Justus-Liebig-Universität, Bismarckstr. 16, 35390 Gießen, Germany

Abstract: *Cucumber plants were inoculated with conidial suspensions of Sphaerotheca fuliginea and treated with formulated neemoils, protectively (one day before inoculation) or curatively (six days after inoculation). Pustule numbers were significantly reduced in all treatments, protective sprays being more effective. In curative treatments with low oil concentrations, differences in the quality of the oils became visible. The cloudy oil N2 proved to be most effective against powdery mildew. HPLC-analysis of the oils showed, that intensively coloured and cloudy oils were rich in ingredients, while clear oils produced only few small peaks.*

Introduction

Powdery mildew is the most important disease of cucumber grown in greenhouses and occurs worldwide. The disease is caused by *Sphaerotheca fuliginea* and *Erysiphe cichoracearum*, *S. fuliginea* being predominant under greenhouse conditions. For field cultivation resistant cultivars are available and a chemical treatment is normally not necessary (CRÜGER 1991).
Under greenhouse conditions with lower light intensity and higher temperatures, treatments with fungicides are essential, especially because resistant cultivars are not available (SITTERLY 1978).
Because of the high selection pressure, *S. fuliginea* has developed resistance against several fungicides. Resistance has been found against Carbendazim, Dimethirimol, Pyrazophos and Benomyl. A decreasing sensitivity against the ergosterol biosynthesis inhibitors Fenarimol, Imazalil and Triforine has been reported (KOOISTRA et al. 1972, DEKKER & GIELINK 1979, SCHEPERS 1983/1984). This has caused an intensive search for alternative treatments against powdery mildew of cucumber.
In the present investigation the effect of formulated neemoils on *S. fuliginea* was studied.

Material and methods

Plants

Seeds of cucumber cv Sensation/Neckarruhm (Hild, Marbach) were sown in TKS 2 in 11 cm pots. When the second leaf had appeared the growth point of the plants was removed to keep them in that growth stage. Two plants per pot were left for the experiments.

Source of inoculum and inoculation

Inoculum for the trials was obtained from three to six weeks old cucumber plants grown in a greenhouse chamber at 22-27° C and 12h light, 12h dark, which had been inoculated with *S. fuliginea*. Inoculum from fresh pustules was collected by removing the conidia with a brush and water. Conidial suspensions were adjusted to 3000 conidia/ml and sprayed on the upper leaf surface of the test plants with a hand sprayer.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Treatment
The upper leaf surfaces of the testplants were sprayed with suspensions of formulated neemoils in water (0.5 %, 1 % and 2 %) with a handsprayer. The treatments were made either one day before inoculation (protective) or six days after inoculation (curative). The neemoils were of various origins and have been formulated by the Temmen GmbH, Hattersheim, Germany. The formulated neemoils had the following composition.

Neemoil	85.00 %
Emulsifier	14.99 %
Antioxidant	0.01 %

The following six formulated oils were used.

TI	orange yellow,	cloudy
N1	orange yellow,	cloudy
N2	light brown,	very cloudy, parts settle down
N6	orange yellow,	clear
N11	light yellow,	clear

Assessment
Plants were kept under greenhouse conditions (see above) and the pustules were counted 12 days after inoculation.

HPLC
An aliquot of 100 mg of each oil was extracted once with 2 ml of methanol in an ultrasonic bath at 40° C for 10 min. The extracts were centrifuged at 4500 g for 10 min and 20 µl of the clear supernatant analysed by HPLC.
Samples were separated on a RP18 column (Merck Superspher 100, 4 µm, 125 x 4 mm with precolumn 4 x 4 mm) at 40° C and a flow rate of 1 ml/min.

Gradient:		30 % methylcyanid	10 min
	to	40 % methylcyanid	20 min
		40 % methylcyanid	20 min
	to	30 % methylcyanid	5 min

The eluent was monitored at 225 nm. Peaks were identified by their retention times in comparison to available standards, namely azadirachtin A, salanin, desacetylsalanin, nimbin and desacetylnimbin which were obtained from the Trifolio-M GmbH, Lahnau, Germany. The concentrations of the five compounds in the oil samples were calculated on the basis of calibration curves of the standards.

Results

Inoculation trials with formulated oils
Plants were treated with the neemoils one day before inoculation (tab. 1) or six days after inoculation (tab. 2).

Tab. 1: Effects of preinoculation treatments with Neem-Oils against *Sphaerotheca fuliginea* on cucumber (application - 1d)

Neem-Oil	concentration	pustules/leaf*		% efficacy	
		1	2	1	2
N1	0,5 %	10,2 bcde	2,5 c	88,7	94,9
	1,0 %	10,0 bcde	2,1 c	88,9	95,8
	2,0 %	5,2 cde	1,2 c	94,2	97,6
N2	0,5 %	13,1 bcde	4,4 c	85,5	91,1
	1,0 %	9,1 bcde	2,3 c	89,9	95,4
	2,0 %	3,4 e	0,9 c	96,2	98,2
N6	0,5 %	14,9 bc	3,8 c	83,5	92,3
	1,0 %	13,8 bcd	4,3 c	84,7	91,3
	2,0 %	4,8 de	1,2 c	94,7	97,5
N11	0,5 %	11,0 bcde	6,9 bc	87,8	86,1
	1,0 %	6,2 bcde	2,5 c	93,1	95,2
	2,0 %	4,5 de	1,7 c	95,0	96,6
T I	0,5 %	16,0 b	15,2 b	82,3	69,3
	1,0 %	15,1 bc	2,3 c	83,3	95,2
	2,0 %	7,0 bcde	2,6 c	92,3	94,7
untreated check		90,4 a	49,5 a	-	-

* same letter means no significant difference in the Tukey-test (P = 0,05)

Tab. 2: Effects of postinoculation treatments with Neem-Oils against *Sphaerotheca fuliginea* on cucumber (application + 6d)

Neem-Oil	concentration	pustules/leaf*		% efficacy	
		1	2	1	2
N1	0,5 %	51,2 b	17,8 bcd	43,4	64,0
	1,0 %	19,8 defg	5,3 de	78,1	89,3
	2,0 %	2,6 g	2,2 e	97,1	95,6
N2	0,5 %	8,1 fg	4,8 de	91,1	90,3
	1,0 %	3,1 g	3,4 e	96,6	93,1
	2,0 %	4,1 g	1,7 e	95,5	96,6
N6	0,5 %	39,6 bc	21,5 bc	56,2	56,6
	1,0 %	32,1 cd	29,4 b	64,5	40,6
	2,0 %	6,5 fg	7,0 de	92,8	85,9
N11	0,5 %	38,1 bc	24,8 b	57,9	49,9
	1,0 %	26,7 cde	9,0 cde	70,5	81,8
	2,0 %	10,1 efg	1,4 e	88,8	97,2
T I	0,5 %	55,4 b	17,8 bcd	38,7	64,0
	1,0 %	24,5 cdef	10,8 cde	72,9	78,2
	2,0 %	3,9 g	1,2 e	95,7	97,2
untreated check		90,4 a	49,5 a	-	-

* same letter means no significant difference in the Tukey-test (P = 0,05)

In both cases the pustule numbers on all treated plants were significantly reduced as compared to the untreated check. A protective treatment was generally more effective, than a curative treatment. Even at the lowest oil concentration (0.5 %) the pustule number was reduced by more than 80 % (except oil T I in experiment 2) by the preinoculation treatment, while the efficacy was below 65 % (except oil N2) after postinoculation treatment.
At the highest concentration of 2 % all oils had an efficacy of near 90 % or higher irrespective of the time of application.
In the postinoculation treatments with low concentrations it was possible to differentiate the oils. A treatment with N2 reduced the pustule number by more than 90 %. The other oils had an efficacy between 38.7 % and 64 % .

Inoculation trials with formulation additives
The oils were formulated with 14.99 % emulsifier and 0.01 % antioxidant. To check their effect plants were treated with the appropriate concentrations of the blank formulation one day before and six days after the inoculation (tab. 3).

Tab. 3: Effects of pre- and postinoculation treatments with blank formulation against *Sphaerotheca fuliginea* on cucumber

application	concentration	pustules/leaf*		% efficacy	
- or + in days	blank formulation	1	2	1	2
- 1d	0,075 %	28,3 bcd	35,7 b	42,8	50,0
	0,15 %	18,7 cd	21,5 bc	62,2	70,0
	0,3 %	9,5 d	13,6 c	80,8	81,0
+ 6d	0,075 %	74,0 a	82,0 a	- 49,5	- 14,5
	0,15 %	51,7 ab	74,6 a	0,0	0,0
	0,3 %	41,6 bc	68,1 a	0,0	0,0
untreated check		49,5 b	71,6 a	-	-

*** same letter means no significant difference in the Tukey-test (P = 0,05)**

In the preinoculation treatments the pustule number was significantly reduced by concentrations between 0.075 and 0.3 % of the formulation additives as compared to the untreated check. In the postinoculation treatment the blank formulation had no significant effect on the pustule number, at the lowest concentration the number of pustules was even increased.

Pustules
After protective treatments the pustules were 7-7.5 mm in diameter which was comparable to the untreated check, the shape of the pustules was circular. After curative treatments the pustule size was reduced to 2-3 mm, with N1 and N2 sometimes to only 1.5 mm, some pustules were not circular.

HPLC-analysis of the oils

Analysis by HPLC showed great differences between the oils (tab. 4). The brown and very cloudy oil N2 had the highest concentrations of desacetylnimbin, desacetylsalanin, nimbin and salanin. In the clear oil N11 none of the five standards was detectable. The detectable azadirachtin concentrations were generally quite low (maximum 0.02 %), in three oils (NI, N6, N11) no azadirachtin was detectable.

Tab. 4: Concentrations of five standards in the neemoils in µg/ml

Oil	Azadirachtin	Desacetyl-nimbin	Desacetyl-salanin	Nimbin	Salanin
TI	n.d.	186.83	274.74	569.93	1878.23
N1	220.85	665.65	639.25	1177.06	3337.89
N2	68.75	1446.85	1362.9	1849.47	13170.29
N6	n.d.	n.d.	135.37	n.d.	1512.66
N11	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. not detected

By a comparison of the chromatograms (fig. 1) the following general observations could be made: N2 contains many compounds and these in high concentrations. N6 and N11 do only have a small number of compounds, the concentrations are low. TI and N1 contain several compounds at a medium concentration.

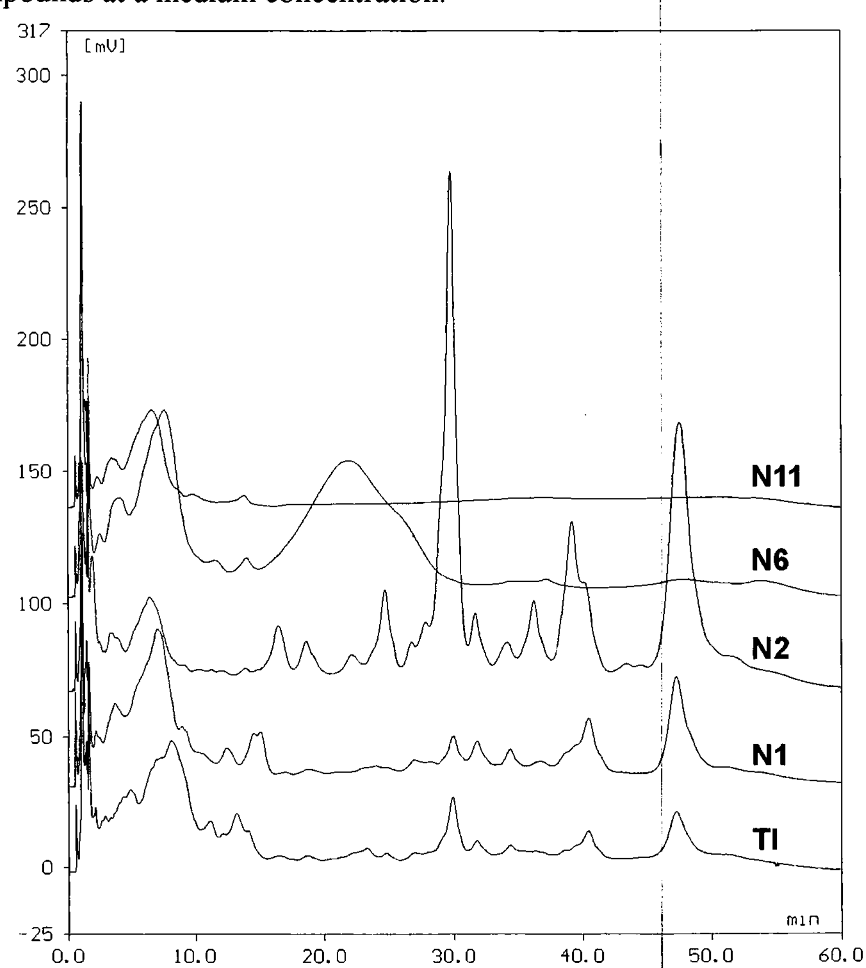


Fig. 1: Overlay of the Chromatogramms of the five neemoils

A detailed view of the chromatogram of N2 (fig. 2) shows about 10 detectable compounds between azadirachtin and Desacetylnimbin that can not be identified so far. The largest Peak has a retention time of 29.77 min and is also detectable in NI and N1, but at much lower concentrations.

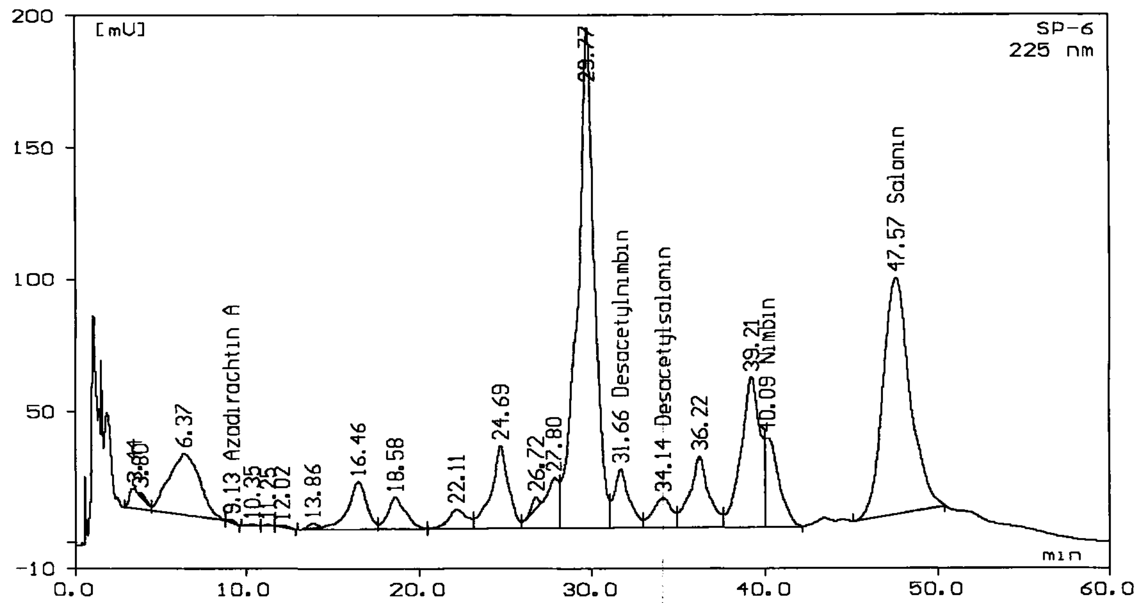


Fig. 2: Chromatogram of Neemoil N2

On the contrary the oil N11 contains only few compounds with a retention time shorter 10 min and these are at low concentrations (fig. 3).

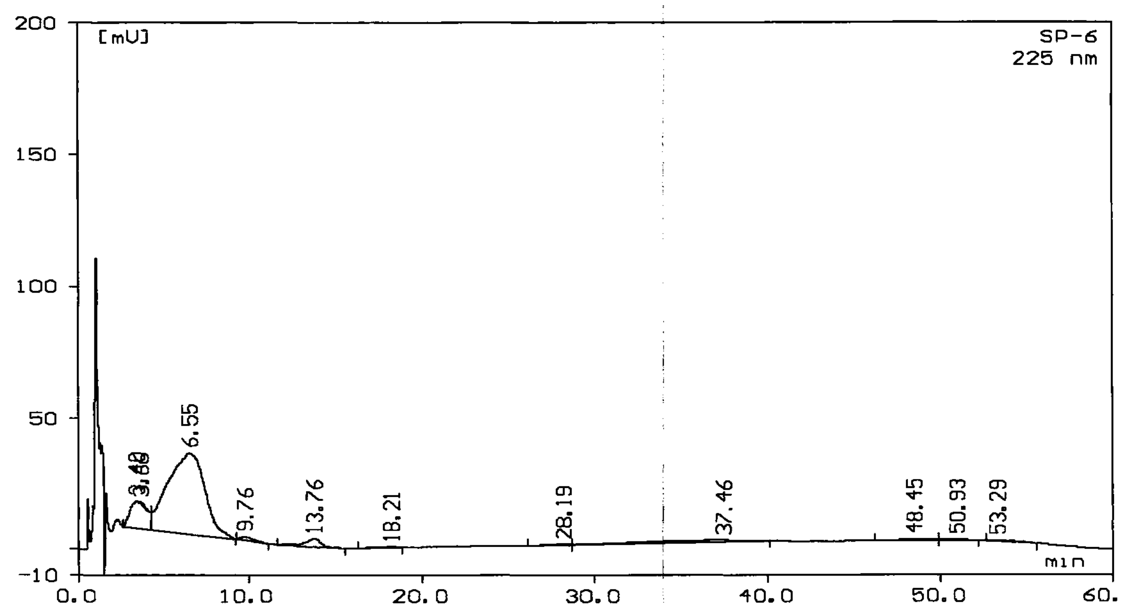


Fig. 3: Chromatogram of neemoil N11

Discussion

Preinoculation treatments of cucumber with formulated neemoils had a good efficacy against powdery mildew. At a concentration of 0.5 % oil the pustule number was reduced by 69.3 - 94.9 %. At the highest concentration used (2 %) the efficacy was 91.5 - 98.2 %. No great differences could be observed between the oils, namely at the concentrations 1 % and 2 %. The treatment with formulation additives alone reduced the pustule number by 42.8 - 81 %, depending on the concentration.

In the postinoculation treatments the efficacy of the oils ranged from 38.7 - 91.1 % and from 85.9 - 97.2% at the 0.5 % and 2 % concentrations, respectively. No significant difference could be observed in the efficacy at the 2.0 % treatment. In the 0.5 % treatment the best control could be achieved with N2. Treatment with the blank formulation had no effect on the pustule number.

To explain these differences two modes of action can be proposed: In the preinoculation treatment the oil component, irrespective of neem ingredients, and the formulation additives form a barrier on the cucumber leaves. Most of the conidia on the leaf can not develop, only few pustules are formed. The pustules produced are of normal size. This is in line with observations from HÄBERLE (1993) and HÄBERLE & SCHLÖSSER (1993), who used Telmion, a formulated rapeseed oil developed by the Temmen GmbH, Hattersheim, Germany, containing the same formulation additives, against powdery mildew of cucumber. In a preinoculation treatment the efficacy was 77.8 and 97.25 % at concentrations of 0.6 and 2.0 %, respectively. The pustule size was slightly reduced. In postinoculation treatments the efficacy was lower, namely at the lower concentrations.

The oil component, irrespective of neem ingredients, and the formulation additives seem to be most important for a protection against powdery mildew in the very sensitive stage of conidia germination, and explains why there were no great differences in the efficacy of the oils.

In the postinoculation treatments with the low neemoil concentrations the oils differed in their efficacy. N2, the oil with the highest contents of azadirachta compounds, was most effective.

At that stage the powdery mildew fungus had already established itself on the leaves, sometimes young developing pustules were seen. Compounds with fungicidal activity in the neemoils can stop further development of the mildew pustules. The pustules that still develop are smaller in size and the form is not circular.

Several authors have described fungicidal effects of Neemkernelextracts. ACHIMU & SCHLÖSSER (1992) used aqueous neem seed extracts to control *Plasmopara viticola* on grapevine. The sporulating area was reduced by nearly 100 % with a preinoculation treatment 2 - 14 days before inoculation and by more than 90 % with a curative treatment up to three days after inoculation. HÄBERLE & SCHLÖSSER (1993 b) could reduce the pustule number *of S. fuliginea* on cucumber by 80 - 93 % by curative treatments with aqueous neem seed extacts. ROVESTI et al. (1992) reduced the affected leaf surface with *S. fuliginea*, *Erysiphe graminis* f. sp. *tritici* and *E. graminis* f. sp. *hordei* to 6.8 %, 0 % and 25 % respectively by a postinoculation treatment (four days after inoculation) with aqueous neem seed extract. *Phytophthora infestans*, *Cercospora beticola* and *Septoria apiicola* were not affected by a treatment one day before inoculation.

STEINHAUER (1993) purified a fungicidal compound from neem kernels, which was effective against several fungi in plate tests. The compound has not yet been identified but is probably one of several fungicidal priciples in the neem kernels.

The curative effect of formulated neemoils against powdery mildew of cucumber is most important for a practical use of these oils in greenhouses. It seems to be possible to control developing powdery mildew epidemics at a stage where first pustules become visible. The recommended concentration should be 1 % formulated neemoil. Concerning the quality of the oils only a preliminary evaluation is possible. The oils vary in their colour, cloudynes and content of azadirachta compounds. It can be proposed, that clear, most probably solvent extracted and further refined oils contain less of antimycotic compounds and are for that

reason less suitable for curative control measures. The dark and cloudy oils, most probably pressed and not further refined, contain more ingredients at a higher concentration. They are distinguished by a good curative effect against powdery mildew of cucumber even at rather low concentrations.
No clear statements can be made concerning the effect of an unknown oil, because the fungicidal principles are still under investigation.

References

ACHIMU, P. & E. SCHLÖSSER (1992): Effect of neem seed extract (*Azadirachta indica* A Juss) against downy mildew (*Plasmopara viticola*) of grapevine. Med. Fac. Landbouww. Univ. Gent **57**, 423-431

CRÜGER, G. (1991): Pflanzenschutz im Gemüsebau. Ulmer Verlag, Stuttgart

DEKKER, J. & A. J. GIELINK (1979): Decreased sensitivity to pyrazophos of cucumber and gherkin mildew. Neth. J. Pl. Path. **85**, 137-142

HÄBERLE, R. 1993: Prüfung verschiedener Produkte zur alternativen Bekämpfung von *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll., dem Erreger des Echten Mehltaus an Gurke. Diplomarbeit, Gießen, Germany

HÄBERLE, R. & E. SCHLÖSSER (1993): Protective and curative effects of Telmion on *Sphaerotheca fuliginea* on cucumber. Med. Fac. Landbouww. Univ. Gent **58**, 1461-1467

HÄBERLE, R. & E. SCHLÖSSER (1993): Protective and curative effects of neem seed extracts against *Sphaerotheca fuliginea* on cucumber. In: Kleeberg, H. (ed), Proc. of the 2nd workshop "Practice oriented results on use and production of neem-ingredients and pheromones", Wetzlar, Germany

KOOISTRA, T., W. H. A. SLOOT, & W. H. JANSEN (1972): Resistentie van komkommer-meeldauw (*Sphaerotheca fuliginea*) tegen Benlate, Topsin M en Bavistin. Gewasbescherming **3**, 121-125

ROVESTI, L., S. DI MARCO & D. PANCALDI (1992): Effect of neem kernel extract on some phytopathogenic fungi under greenhouse conditions. Z. PflKrankh. PflSchutz. **99**, 293-296

SCHEPERS, H. T. A. M. (1983): Decreased sensitivity of *Sphaerotheca fuliginea* to fungicides which inhibit ergosterol biosynthesis. Neth. J. Pl. Path. **89**, 185-187

SCHEPERS, H. T. A. M. (1984): Consequences of resistance to fungicides for the control of cucumber powdery mildew. Med. Fac. Landbouww. Rijksuniv. Gent **49**, 139-141

SITTERLY, W. R. (1978): Powdery mildews of cucurbits. In: Spencer, D. M. (ed.), The powdery mildews. Academic Press, London, New York, San Francisco

STEINHAUER B. (1993): Antimycotic activity of a methanolic exract from *Azadirachta indica* A. Juss. Proc. 4th Int. Neem Conference, Bangalore, India (in print)

A Short Report on Cultivation and Use of Neem in Nicaragua 1987 to 1994

Carlos Sanchéz, Copinim
Anne Katharina Gruber, Proyecto Insecticidas Botanicos Nim, Nicaragua

1. The planting of the tree

Up to the year 1994 about 500.000 Neem trees have been planted in Nicaragua in different ways: as monocultural plantations (3 ha to 200 ha), in agro-forestry systems (alley cropping), in and around pastures and as scattered trees in villages or along the roads. The optimal spacing is form 6 x 6 m to 8 x 10 m, depending on soil fertility and purpose of use. In larger monocultural plantations rows of other high growing tree species are necessary as a windbreak to protect younger trees against breaking of branches and canopies and protecting elder ones from the loss of flowers. During the first three years intercropping with annual cultures is common (sesame, corn, beans etc.).

The Nicaraguan National Forest Agency improves planting of Neem in reforestation areas, but there are several problems not yet completely investigated about this recently imported tree species: its competitiveness, its roll as an insect host plant etc.

2. Maintenance and Harvesting of Neem Trees

The main problems for Neem trees are: competitive associated vegetation and fire. Therefore the Neem plantations are surrounded by broad fire protection belts and twice a year the associated vegetation is cut down by menpower or by machines, leaving the organic material as mulch. During the first years lower branches of the trees are cut to favour one single strong trunk, allowing lateral branching form 1.5 m upwards in order to develop a voluminous canopy.

In Nicaragua trees are flowering from March to April. Fruits are ripening in June. Harvesting of the yellow ripe and the green but physiological ripe fruit is done by hand in one single step ladders and cutting those branches growing steeply above 3.5 m hight at a specific point near some well developed lateral branching.

Average fruit yield in monocultural plantations of six year old trees has reached now 15 kg per tree and year. Single trees had a yield of 30 to 50 kg. The whole harvest of Neem fruits in Nicaragua in 1994 has been 120 t of fruit.

Peasants need at least 3 to 5 well developed trees, giving a total yield of 80 kg of fruit, what means 12 kg of dried seed for the preparation of 300 l of Neem water extract, sufficient for one application on 1 ha of annual crop.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

3. Artisanal and Semi-industrial Processing of Fruit and Seed

The Neem Project developed a semi-industrial method to produce simple but marketable Neem insecticide products and a manual (artisanal) method of processing Neem fruit for those peasants who have some trees of their own and would like to prepare their own home-made Neem insecticide.

This method consists of the following steps:

1. Daily collection of ripe fruits under the trees.
2. Peeling of fruits by hand.
3. Washing the seeds.
4. Drying seeds under sunlight for 4 to 5 hours (1 step).
5. Final drying of the seeds in the shadow (10 to 15 days).
6. Storage of dried seed in 20 kg bags (dark, dry, environmental temperature and rel. humidity less than 80%).
7. Quality control of fungal contamination (internal control of samples of 100 seeds per 20 kg or bag). 1 to 2% of contamination is tolerable.
8. Grinding seeds in a regulable corn mill, just when pest control becomes necessary.
9. Soaking and stirring ground Neem seed in water (40 g per liter) during 6 to 12 hours.
10. Immediate application using all types of sprayers.

The semi-industrial method consists of the following steps:

A: HUMID PROCESS

1. Harvesting yellow and green fruits.
2. Selecting the green fruits and spreading them in the shadow for ripening.
3. Peeling (depulping) ripe and ripened fruits after one night (12 hours) fermentation in plastic bags or other closed vessels.
4. Washing depulped seeds in a semi-automatical water recycling pump-system.
5. Rapid drying under sun on wire netting riddles for 3 to 4 hours, turning the seeds once or twice.
6. Final drying in the shadow (roof) either on the same riddles for 12 to 15 days depending on climatic conditions or in a special drying system with counter current flow of dried air through slowly moving seeds.
7. Controlling residual humidity by moisture testers as usually used for corn and other grains. Less than 9% humidity is sufficient for unproblematic storage of seeds as raw material for the.insecticide production under dry and dark conditions with environmental temperature and relative air humidity of semi-arid regions of Nicaragua.
8. Storage for one year.

B: DRY PROCESS

9. Decorticating seeds (breaking and removing the "shell") after six weeks of storage.
10. Quality control of fungi-contamination, selecting contaminated seeds by hand.
11. Quality control of azadirachtin content (sending samples to Germany, Trifolio-M GmbH).

12. Grinding Neem seeds in a hammer mill to get the first marketable product: registerd as **NIM 20** for water extracts.
13. Pressing Neem seed to get the second marketable produkt: Neem oil, formulated with 20% of emulsificators, registered as **ACEITE DE NIM CE 80** for application in water.
14. Grinding the residue of the pressing step (cake) to get the third product: ground Neem cake, registered as **NIM 25**, suitable for water extracts but also for incorporation into the soil and for seed coating.
15. Stirring the very fine residual sediment of the pressed oil together with some oil to get a fourth product called **PASTA NIM,** suitable for healing of wounds of cattle and other domestic animals.
16. Extracting ground Neem seed in a pilote plant (french type of parfume extractor) in two steps applying first hexan to get the azadirachtin free oil fraction and then ethanol. The ethanol extract is formulated with only 1% of emulsifiers and still contains some oil. Azadirachtin content is standardized at the level of 4000 ppm. This is called **NIM 4000**.

17. Pulverizing the last extraction residue to get an organic Neem by-product that can be used as a fertilizer called ABONO NIM.

These last three products are still in the process of evaluation and registration.

4. Use of Neem Products in insect pests and nematode control

Many laboratory and field trials have been carried out in Nicaragua since 1983 and the actual Neem products are recommended for the control of the following pests:

1. **NIM 20** (ground Neem seed without the "shell")
concentration: 15, 18 or 20 g per l of water in crops and up to 30 g per l in control of ectoparasites of cattle.
against: larvae of Lepidoptera feeding on leaves
leaf feeding beatles (Coleoptera)
leaf miners (Diptera and Lepidoptera)
fruit and stem borers (Lepidoptera and Coleoptera)
aphids (Homoptera)
nymphs of bugs (Heteroptera)
ectoparasites (ticks) of domestic animals.

Ground Neem seed can be mixed at a ratio 1:3 with saw dust or sand and applied directly to vegetative cones of maiz plants.

2. **NIM 25** (ground seed cake)
concentration: 20, 23, 25 or 30 g per l of water in case of water extracts;
incorporation: 1.5 to 2.5 kg per 1000 m^2 of soil.
against: same spectrum of insect pests as NIM 20 in form of water extracts and nematodes.

3. **ACEITE DE NIM CE 80** (formulated Neem oil)
concentration: 2 ml per l of water in seed beds, 4 to 10 ml per l of water in fields, depending on the degree of infestation.
against: white flies and other sucking insects (Homoptera and Heteroptera).

4. **PASTA NIM** (stired cremy mixture of sediments and oil)
   application: small amounts covering wounds and introducing to infested wounds.

5. **NIM 4000** (formulated ethanol extract)
   concentration: 6 to 6.5 cc per l of water.
   against: same spectrum of insect pests as NIM 20

6. **ABONO NIM (pure organic residue of extraction steps)**
   application: 50 to 75 kg per ha depending on soil necessities.

All NIM products for insect pest control are tested if compatible with other biological methods for plant protection and can be used togehter with B.t. or NPV products as well as with liberations of natural ennemies like *Trichogramma sp*.

The last field trials have been performed against the african citrus leaf-miner (*Phyllocnistis citrella*) and the pineapple fruit-borer (*Thecla basilides*) with very good results.

CURRENT STATUS OF SYNTHETIC PHEROMONE USE IN INTEGRATED PEST MANAGEMENT IN PEACH ORCHARDS IN ITALY

Fabio MOLINARI
Istituto di Entomologia e Patologia vegetale. Facoltà di Agraria.
Università Cattolica del Sacro Cuore di Milano - Sede di Piacenza

Summary - An overview is presented on the application status of synthetic pheromones in the control of peach pests in some of the most important cultivation areas: in northern Italy, Emilia Romagna and Piemonte and in southern Italy, Calabria.
Monitoring by pheromone traps for *Cydia molesta* (Busck) and *Anarsia lineatella* Zeller, adopted on a large scale, has made a significant reduction in chemical applications during the last 15 years; in Emilia Romagna the I.P.M. Regional Project involved, in 1994, about 3000 orchards for more than 6500 hectares, that is 19% of regional peach orchards.
C. molesta is also controlled by means of mating disruption has proved to be a valuable means for controlling, after application on more than 3000 hectares in 1992.
Studies on synthetic pheromone of diaspidid *Pseudaulacaspis pentagona* (Targ.) and *Quadraspidiotus perniciosus* (Comst.) have been carried out to assess its possible use in IPM.
Trimedlure, a parapheromone for tephritid flies, is used for monitoring *Ceratitis capitata* Wied., to determine the starting moment of the hazardous period for peach damage in southern areas.

Introduction

Synthetic pheromones have found an important place in pest control in stone fruit orchards: coordinate programmes, in which the presence of well trained technicians who can rely upon research Institutes allow a large diffusion of Integrated Pest Management.

The Institute of Entomology of Piacenza has given, since the mid seventies, its scientific support to several programmes of Integrated Pest Management in some of the main fruit-growing areas in Italy.

In Emilia Romagna, the regional Project for Integrated Pest Management involved, in 1994, more than 6500 hectars peach orchards (19% of peach surface in the region).

In Piemonte, a fruit-grower association, Asprofrut, coordinates the activity of technicians on some 3500 hectares peach orchards.

In Calabria, agricultural Cooperative OSAS in Castrovillari, province of Cosenza, has about 1000 hectars peach orchards.

The pheromones of most of the major pests of peach (Tab. 1) have been identified. The most widespread use of these substances is monitoring adult flight catching males of some lepidoptera in sticky traps, and recently mating disruption has been applied to control *Cydia molesta* and *Anarsia lineatella* on a large scale; attractants for Rhynchota and Diptera have been tested as well.

Tab. 1 - Peach pests of major interest in Italy

- *Taeniothrips meridionalis* Priesner
- *Myzus persicae* (Sulzer)
- *Pseudaulacaspis pentagona* (Targioni Tozzetti)
- *Quadraspidiotus perniciosus* (Comstock)
- *Anarsia lineatella* Zeller
- *Cydia molesta* (Busck)
- *Ceratitis capitata* Wiedmann

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Monitoring

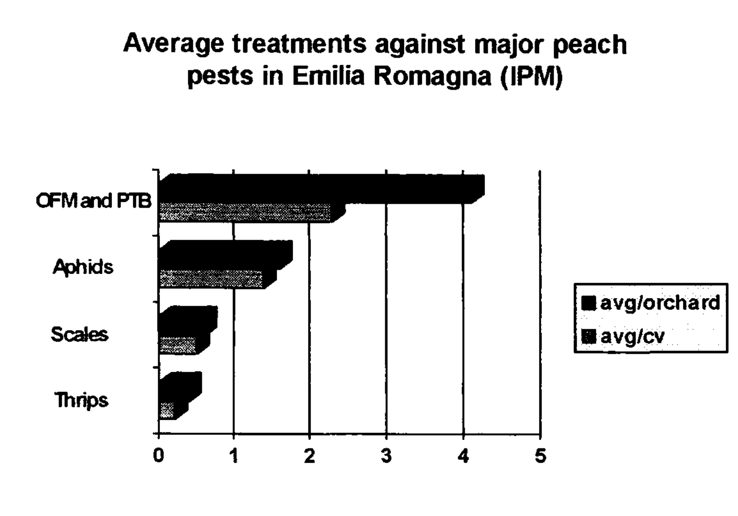


Fig. 1 - (1992) Average number of treatments on major pests per peach orchard and on the different cultivar in Emilia Romagna in farms in which IPM strategies are applied.

Oriental Fruit Moth (*Cydia molesta* (Busck)) and Peach twig borer (*Anarsia lineatella* Zeller) are the stone-fruit pests in the fight against which synthetic pheromones have determined the most striking changes.

Monitoring by pheromone traps for *Cydia molesta* (Busck) and *Anarsia lineatella* Zeller. Male catches give reliable information on population density, especially for the former species.

Field trials and laboratory data indicate a reliability of trap catches in estimating actual population, particularly for *C. molesta*; a less sound relation has been seen for *A. lineatella*.

After these remarks economic thresholds based on male captures have been proposed, that have been largely tested within IPM programmes. For *C. molesta*, an economic threshold of 10 males per trap per week, using 2-3 traps per orchard, is adopted. Treatments against *A. lineatella* are applied when an average of 7 males per trap per week are recorded, or when 5 males per trap are caught for two weeks in succession. The lower threshold for *A. lineatella* has its reasons both in the preference of Anarsia larvae for fruits, and in the poor trustworthiness of trap catches: the behaviour of *A. lineatella* males in the presence of pheromone are not as deeply studied as *C. molesta*: finally, monitoring is less reliable.

Sexual pheromones of two scale insects, the diaspidid *Pseudaulacaspis pentagona* (Targ.) and *Comstockaspis perniciosa* (Comst.) have been the object of researches to assess the possibility of their utilization in Integrated pest management (Cravedi and Molinari, 1988; Cravedi and Mazzoni, 1993 and 1994).

Synthetic pheromone of *Pseudaulacaspis pentagona* (Targioni Tozzetti): *(Z)-3,9-dimethyl-6-isopropenyl- 3,9-decadien-1-yl propanoato* (WPS I) has been used for baiting traps of different models. The pheromone used has been synthetized by the *Istituto Donegani* in Novara (Italy). Wing traps and tent trap have been tested, baited with different doses of pheromone.

The preliminary results indicate that tent traps are to be preferred, as the checking is easier than for wing traps. The attractiveness of the trap increases with the dose up to 50 µg, the no significant increase can be observed. Males are lured to the trap at great distance (200 m).

The infestation coefficient based on winter samplings shows a good relationship with the number of males caught during the first activity period.

In Piedmont 2 flight periods have been observed, while in southern Italy and in northern areas with a warmer climate, the peaks of adult activity are 3.

Pheromone traps can be considered a valuable tool for assessing population level and a basis for choosing control strategies.

The parapheromone *trimedlure* is used to attract adult Mediterranean fruit fly *Ceratitis capitata* Wied., in southern Italy: the aim is to determine the starting moment of the hazardous period for peach damage in southern areas.

Mating disruption

Mating disruption technique has been evaluated since 1988 and several practical aspects of application on peach were pointed out (Molinari and Cravedi, 1988, 1989) in 1990 specific dispensers were commercialy available in Italy, and the suface treated increased up to 3000 hectares peach orchards in 1992. Commercially available dispensers were RAK 5-6, for Oriental fruit moth and peach twig borer, Elios Pesco and Ceckmate for oriental fruit moth.

Peach growing areas in Italy can differ greatly from one another for environmental, agronomic and phytopatological conditions; such differences are to be considered when applying pheromones for mating disruption.

In some orchards in Emilia-Romagna, where pest population was particularly high, as much as 60-80 mg pheromone per hectare per hour was necessary to avoid damage (Molinari and Cravedi, 1989, 1992; Pari *et al.*, 1990).

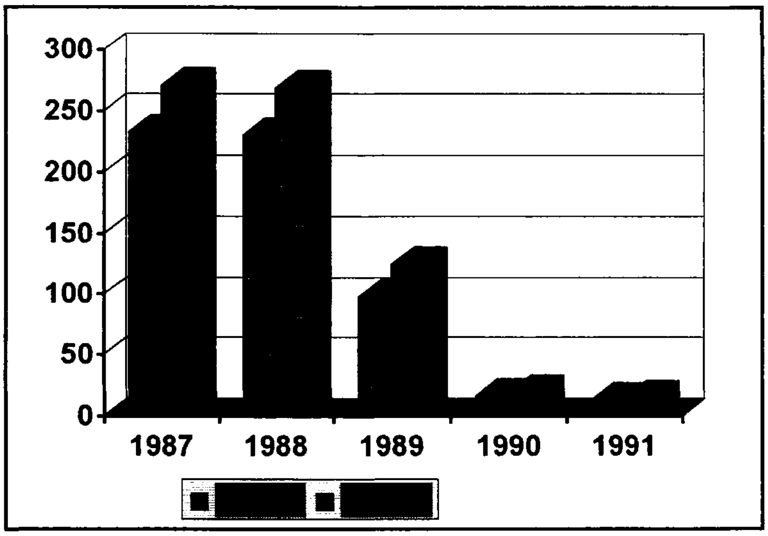


Fig. 2 - Verzuolo (CN). Actual amount of synthetic pheromone of *C. molesta* released (g/hectar) during a trial (after 120 and 180 days)

In many other cases lower amounts of pheromone were required. A progressive reduction of the release rate has been tried in peach orchards were no damage had been detected after two years of mating disruption application: in 1991 and 1992 only a small number of dispensers were applied on the border trees (Fig. 2) (Molinari and Cravedi, 1992). The amount of pheromone can be strongly reduced according to the pest population level; in pluriennal applications a significant reduction of the application cost can be achieved.

In Piedmont, a single district near Saluzzo, in the province of Cuneo, including 172 peach orchards on 300 hectares, was treated for the first time in 1990 with pheromones for mating disruption (Cravedi *et al.*, 1991). At harvest, fruit damage in 160 out of 172 orchards, did not exceed 0,5%.

After the good results obtained in this first year, the experience was repeated on a wider area in 1991 and nel 1992 (Galliano e Vittone, 1993).

Literature

CRAVEDI P., MAZZONI E., 1993 - Response of *Pseudaulacaspis pentagona* (Targioni-Tozzetti) to sexual pheromone. OILB/SROP Groupe de Travail "Protection Intégrée en Verger", Compte-rendu de la Reunion du Sous-groupe "Pecher", Rimini 4-5 Septembre 1992, 4-7.

CRAVEDI P., MAZZONI E., 1994 - Il feromone sessuale di *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Homoptera: Diaspididae). Esperienze di monitoraggio. Atti del Convegno MAF "Innovazioni e Prospettive nella difesa fitosanitaria", Ferrara 24-25/10/94, 143-146.

CRAVEDI P., MOLINARI F., 1988a - Impiego del feromone sessuale di sintesi per la cattura dei maschi di *Pseudaulacaspis pentagona* (Targioni Tozzetti) (Homoptera, Diaspididae). - Atti XV Congr. naz. ital. Ent., L'Aquila, 13-17 giugno 1988: 933-938.

CRAVEDI P., MOLINARI F., 1988b - Efficacité de le pheromone de synthese de *Pseudaulacaspis pentagona*. - Proceedings of the working group "Integrated protection in fruit orchards". Sub group "Peach orchards", Valence 31th august - 2nd september 1988. WPRS Bulletin 1988/XI/7:24-25.

CRAVEDI P., MOLINARI F. 1988c. "Efficacité de la phéromone de synthèse de Pseudaulacaspis pentagona". *WPRS Bulletin*, XI/7:24-25

CRAVEDI P., MOLINARI F., ARZONE A., ALMA A., GALLIANO A. 1991. "Applicazione sperimentale su base comprensoriale del metodo della confusione sessuale contro *Cydia molesta* (Busck) su pesco. Inf. Fitopat. 12:27-31.

CRAVEDI P., MOLINARI F., GUARINO F. and COSENTINI F.. 1992. "Esperienze di applicazione del metodo della confusione contro Cydia molesta (Busck) in pescheti della Calabria." Atti Giornate Fitopatologiche, Copanello (CZ) 21-24 aprile 1992 1:115-122.

GALLIANO A., VITTONE G., 1993 - Application experience of the mating disruption method on a territorial scale. Bulletin OILB srop, Sous-Groupe "Pêcher", 16 (4): 22-24.

MAZZONI E., CRAVEDI P., 1995 - Sensilli antennali del maschio di *Pseudaulacaspis pentagona* (Targioni-Tozzetti) (Homoptera: Diaspididae). Atti XVII Congresso nazionale italiano di Entomologia, Udine 13-18/6/94, 351-352.

MOLINARI F. and CRAVEDI P. 1988a. "Esperienze sul metodo della confusione nella lotta contro *Grapholita molesta* Busck (Lepidoptera, Tortricidae)." Atti XV Congr. naz. ital. Ent., L'Aquila 1988 :965-972.

MOLINARI F. and CRAVEDI P. 1988b. "Synthetic pheromone and control of *Grapholita molesta* and *Anarsia lineatella*". *WPRS Bulletin*, XI/7:39-40

MOLINARI F. and CRAVEDI P. 1989. "Applicazione dei feromoni con il metodo della confusione contro *Cydia molesta* (Busck)(Lepidoptera Tortricidae) e prove preliminari su *Anarsia lineatella* Zell. (Lepidoptera Gelechiidae)." Boll. Zool. agr. Bachic. Ser. II, 21:163-182.

MOLINARI F. and CRAVEDI P. 1990. "Applicazione dei feromoni secondo il metodo della confusione nella lotta contro *Cydia molesta* (Busck)(Lepidoptera Tortricidae) in Piemonte." Redia, 73:381-395.

MOLINARI F. and CRAVEDI P. 1992a. "Application of mating disruption method in peach orchards in Italy." Working group "Use of pheromones and other semiochemicals in integrated control" 31 august-3 september 1992, San Michele all'Adige (Italy) IOBC/WPRS Bulletin, 1992/15/5: 52-55.

MOLINARI F. and CRAVEDI P. 1992b. "The use of pheromones for the control of *Cydia molesta* (Busck) and *Anarsia lineatella* Zell. in Italy". *Acta Phytopatologica et Entomologica Hungarica* 27 (1-4): 443-447.

NICCOLI A., SACCHETTI P. and LUPI E. 1990. "Il metodo della confusione nel controllo di *Cydia molesta* (Busk) e *Anarsia lineatella* Zell. in un pescheto della Toscana." Redia

PARI P., SPADA G., GARAFFONI M., GUARDIGNI P., CANESTRALE R., MINGUZZI R., RAVAIOLI M., CARLI G., 1990 - Il metodo della confusione sessuale nella difesa contro *Cydia molesta* Busk. ed *Anarsia lineatella* Z. nei pescheti dell'Emilia Romagna. Inf.tore fitopatol., 10: 35-42.

REGIONE EMILIA-ROMAGNA, 1992. Lotta Integrata. Supplemento al n.3 di Agricoltura. 36 pp

ROTUNDO G. and VIGGIANI G. 1989. "Esperienze sul controllo dell'Anarsia e della Tignola orientale con il metodo della confusione sessuale." L'Informatore Agrario 40:67-68.

Reduced Orientation Ability of DIABROTICA BEETLES in Illinois maize fields permeated with the plant kairomone p-methoxy-Phenylethanol and the sex pheromones 10-Methyl-tridecane-2-one

Hans E. Hummel(1) and
Robert L. Metcalf (2)

(1) Institute of Phytopathology and Applied Zoology, Biological and Biotechnical Plant Protection, Justus-Liebig-University,Ludwigstrasse 21 D-35390 Gießen- Germany-

(2) Department of Entomology, University of Illinois, 215 Morrill Hall, 505 S. Goodwin, Urbana, Illinois 61801 - USA-

Agenda:

Introduction : The need for action with novel IPM approaches

Corn rootworm beetles (Diabrotica) as corn and cucumber pests with annual losses exceeding 1 billion US-Dollars

Plant parakairomones active in Diabrotica

Materials and Methods:

p-Methoxy-Phenylethanol (MPE) as a plant parakairomone
10-Methyl-Tridecane-2-one as a sex pheromone of D. undecimpunctata howardi

The cylindrical sticky cardboard trap and the semiochemical attraction source

The orientation disruption scheme and its consequences

Results :

Orientation disruption by MPE in D. barberi and D.virgifera virgifera

Mating disruption by 10-methyl-tridecane-2one in D.undecimpunctata howardi

Conclusions:

Ecology and economy

Significance for future corn rootworm management

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

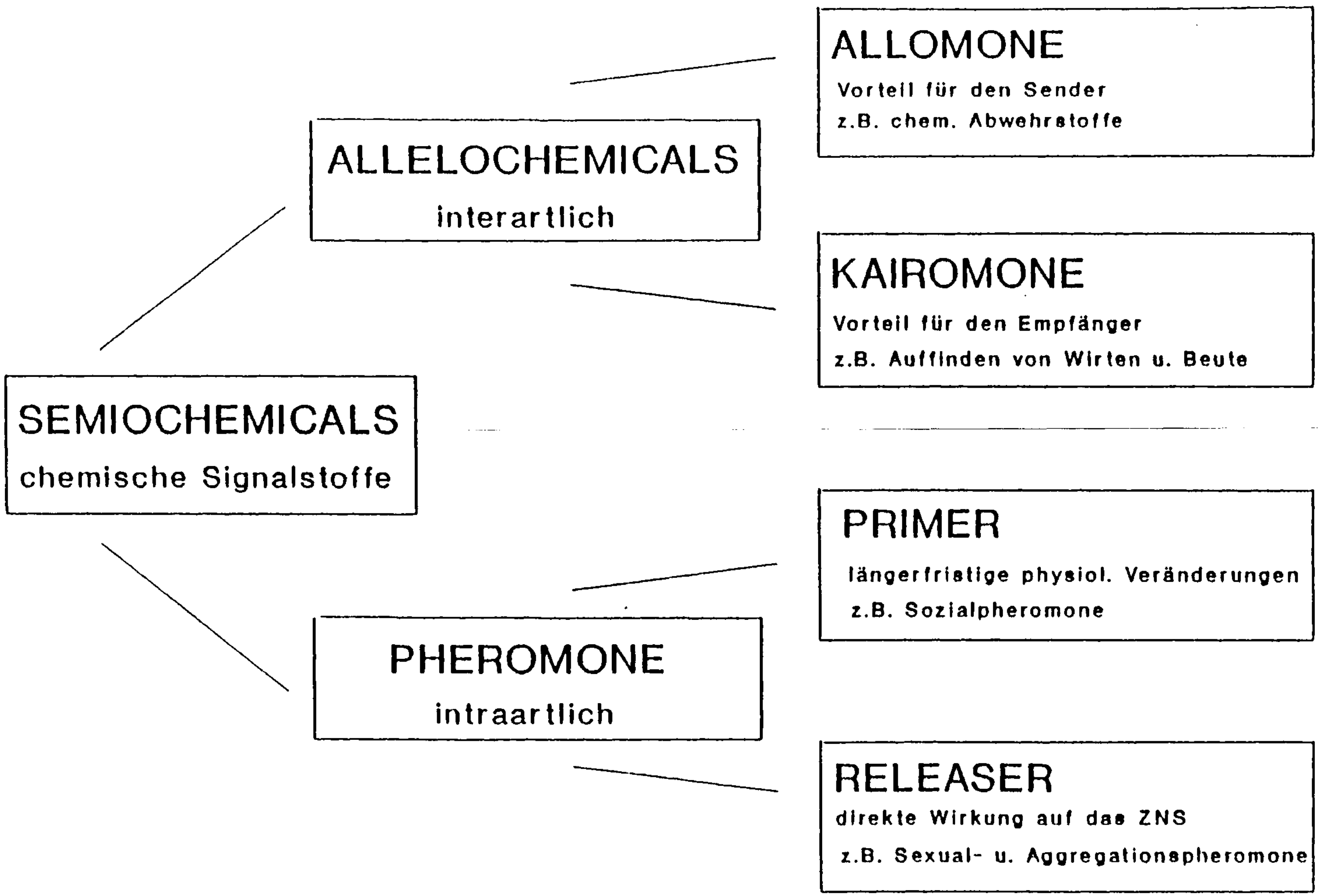


Abb.1: Hierarchie der chemischen Signalstoffe

(taken from R. Plarre (1992))

Definition of the term "kairomone" :

Kairomones are substances emitted by one species that modify the behavior of a different species to the benefit of the receiving species.

Def. by E.F.Tinsworth, chapter 35 in:
R.L.Ridgway et al.(1990) eds.,
Behavior-Modifying Chemicals for Insect Management. Applications of Pheromones and other Attractants,
M.Dekker, New York and Basel.

Table Volatile Plant Kairomones Attractive to Insects.

Insect	Kairomone	Source	Mol. Wt.	Bp(°C)	Reference
Diabrotica virgifera (western corn rootworm)	indole	*Cucurbita* blossom	117	253	Andersen & Metcalf (1986)
	estragole		148	216	
	β-ionone		192	ca.260	Lampman & Metcalf (1988)
Diabrotica barberi (northern corn rootworm)	eugenol		164	254	Ladd et al. (1983)
	isoeugenol		164	266	Metcalf & Lampman (1989c)
	cinnamyl alcohol		134	250	
Diabrotica cristata	eugenol		164	254	Yaro et al. (1987)
	isoeugenol		164	266	Lampman & Metcalf (1988)
	cinnamyl alcohol		134	250	
Diabrotica undecimpunctata (southern corn rootworm)	cinnamaldehyde	*Cucurbita* blossom	132	246	Lampman et al. (1987)
Acalymma vittatum (striped cucumber beetle)	indole	*Cucurbita* blossom	117	253	Andersen & Metcalf (1986) Lewis et al. (1990)

Lit.: R.L.Metcalf and E.R.Metcalf (1992). Plant Kairomones in Insect Ecology and Control. Chapman and Hall, New York and London. p.8 and 9

Table : *Cucurbita maxima* blossom volatiles and the limits of response (LR) by *Diabrotica* spp. on sticky traps.

Blossom volatile	LR (mg) *D. barberi*	*D. cristata*	*D. u. howardi*	*D. v. virgifera*
1,4-dimethoxybenzene	>100	>100	>100	>100
1,2,4-trimethoxybenzene	>100	>100	100	>100
benzyl alcohol	>100	>100	>100	>100
benzaldehyde	100	100	100	100
phenethanol	10–30		100	>100
phenylacetaldehyde	>100		10–30	100
4-methoxybenzyl alcohol	>100		>100	>100
4-methoxybenzaldehyde	>100		>100	>100
indole	>100	100	100	1.0
cinnamyl alcohol	1.0	3–10	10–30	100
cinnamaldehyde	100	10–30	0.3	30–100
α-ionone	>100	>100	>100	>100
β-ionone	100	100	100	1.0
nerolidol	>100		>100	>100
4-methoxycinnamaldehyde*	100	30–100	>100	0.03
4-methoxyphenethanol*	0.1	1–3	>100	100

* not identified as a blossom volatile, therefore termed "parakairomone "

Lit.: R.L.Metcalf and E.R.Metcalf (1992). Plant Kairomones in Insect Ecology and Control. Chapman and Hall, New York and London. p.84

PLANT KAIROMONES IN INSECT ECOLOGY AND CONTROL

Table . . Attractivity of Methoxyphenethanols to Adult Corn Rootworms*

Lure	1 day sticky trap catch—100 mg (mean ± S.D.)[1] *D. barberi*
$C_6H_5CH_2CH_2OH$	12.2 ± 5.5a
4-$CH_3OC_6H_4CH_2CH_2OH$	60.3 ± 26.8b
3-$CH_3OC_6H_4CH_2CH_2OH$	7.2 ± 3.4a
2-$CH_3OC_6H_4CH_2CH_2OH$	13.5 ± 9.3a
$C_6H_5CH{=}CHCH_2OH$	50.7 ± 50.9b
4-$CH_3OC_6H_4CH{=}CHCH_2OH$	5.2 ± 3.1a
control	4.7 ± 3.9a

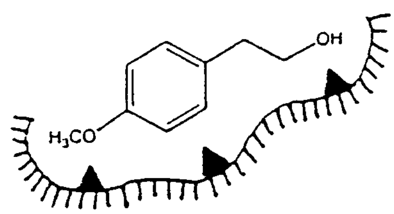
[1] means followed by different letters are significantly different ($P \leq 0.05$).

* Data from Metcalf & Lampman (1991).

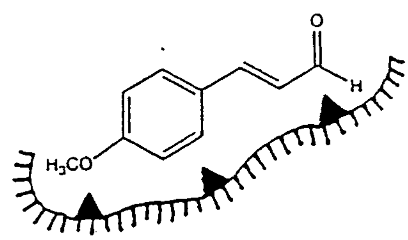
Lit.: R.L.Metcalf and E.R.Metcalf (1992). Plant Kairomones in Insect Ecology and Control. Chapman and Hall, New York and London. p. 94

MAPPING THE ACTIVE SITES FOR KAIROMONE RECEPTORS

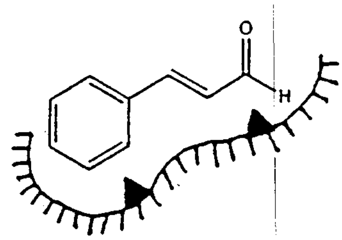
IN DIABROTICITE ROOTWORM BEETLES



D. barberi



D. v. vigifera



D. u. howardi

Figure : Maps of suggested receptor site interactions of *Diabrotica barberi* with 4-methoxyphenethanol, *D. v. virgifera* with 4-methoxycinnamaldehyde, and *D. u. howardi* with cinnamaldehyde.

Lit.: R.L.Metcalf and E.R.Metcalf (1992).Plant Kairomones in Insect Ecology and Control. Chapman and Hall, New York and London. p.95

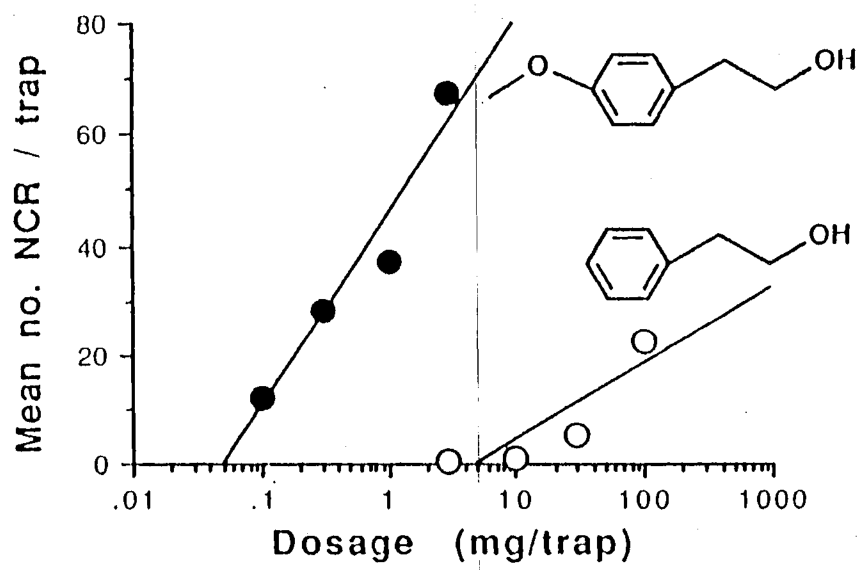
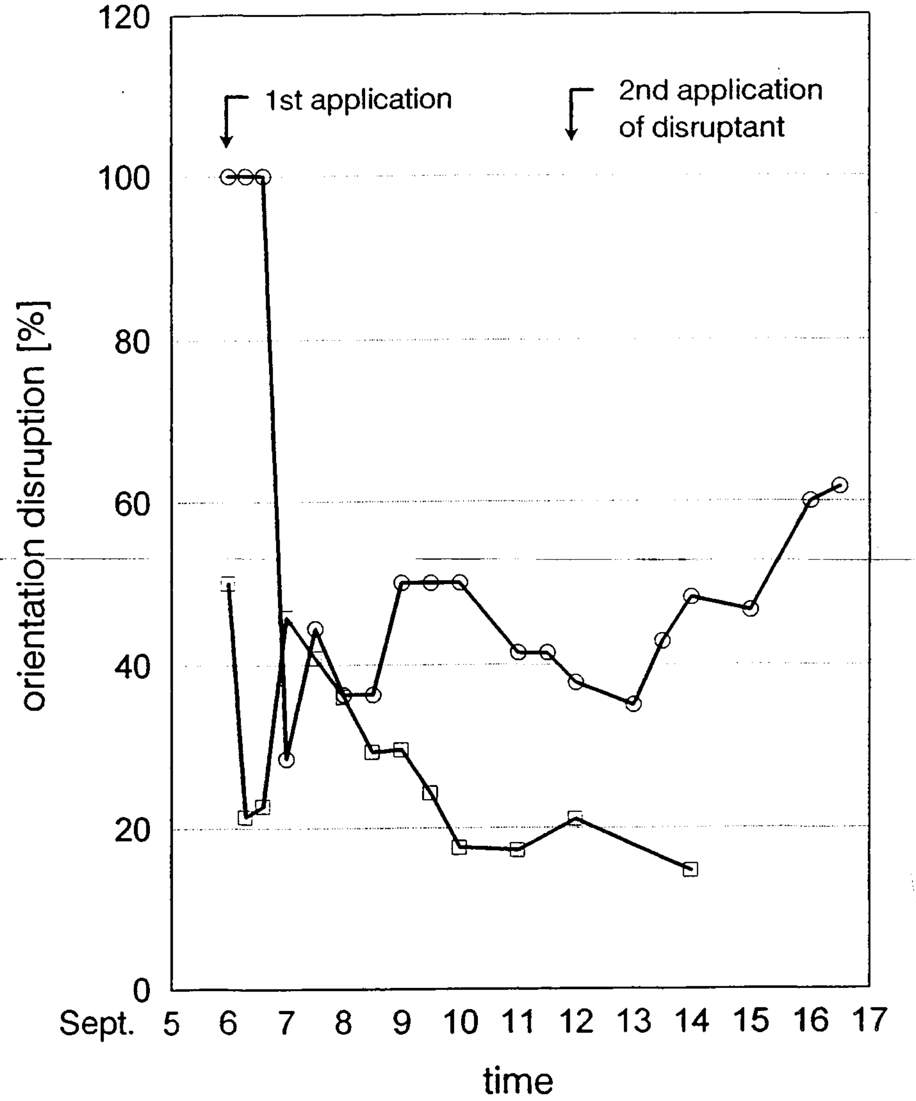


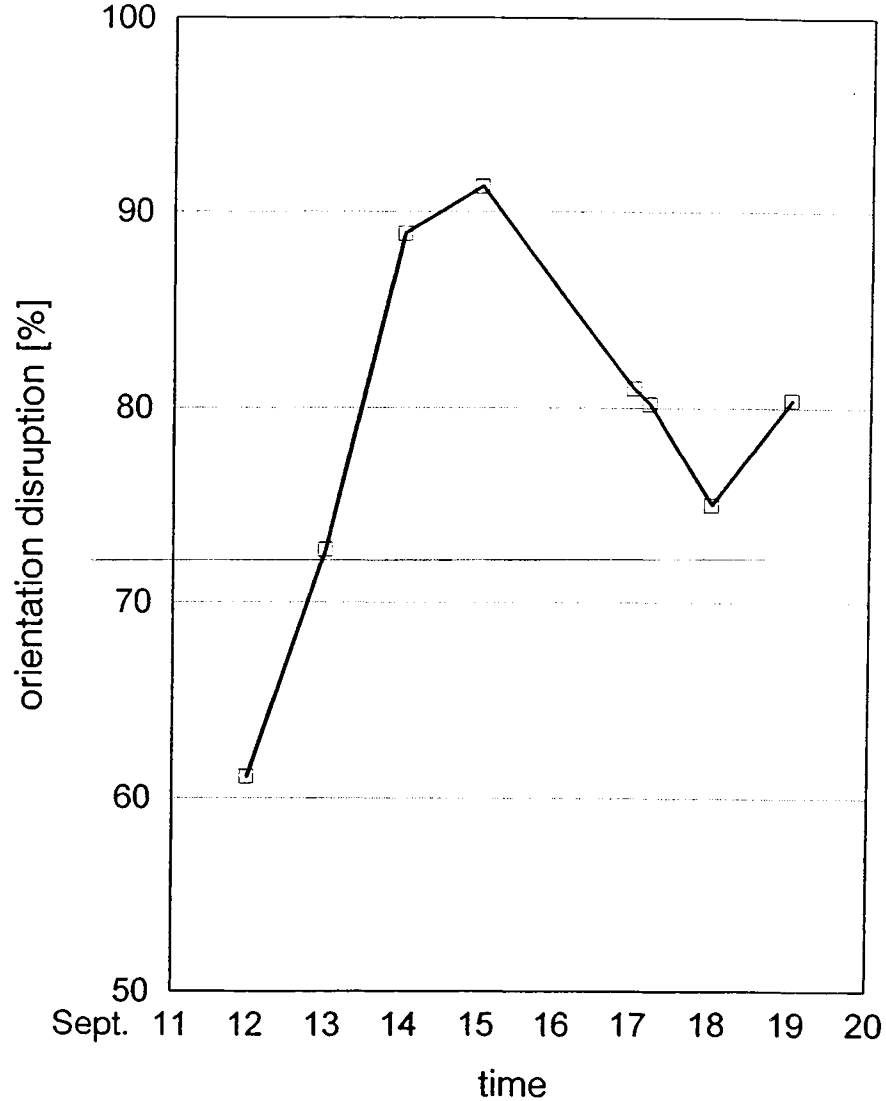
Figure : Attraction of *D. barberi* (NCR) adults to cylindrical sticky traps baited with varying doses of phenethanol and 4-methoxyphenethanol. Reprinted with permission from Metcalf & Lampman 1991.

Lit.: R.L.Metcalf and E.R.Metcalf (1992). Plant Kairomones in Insect Ecology and Control. Chapman and Hall, New York and London. p. 93

Orientation disruption effects on <u>Diabrotica barberi</u> and <u>D. virgifera virgifera</u> in Illinois mayze field by MPE - Sept. 94



Orientation disruption effects on <u>Diabrotica undecimpunctata howardi</u>, University of Illinois, South Farm, Urbana, Ill. USA - Sept 94



Further Evidence for the Presence of a Female Sex Pheromone in the Colorado Potato Beetle *Leptinotarsa decemlineata* Say. and its Biological Characterization

A video documentation

D. Otto
Projektgruppe Naturstoffe der Universität Potsdam
Stahnsdorfer Damm 81, D - 14532 Kleinmachnow

Summary

An appetency male of *Leptinotarsa decemlineata* meeting a partner beetle distinguishes between male and female by perceiving a chemical sign on the surface of the female's body and elytren by frequently touching it with the antennae and the maxillar palpae. As a contact aphrodisiaca this pheromon excites and stimulates the male, releasing the further steps in copulation behaviour. It can be taken away totally by organic solvents. Glass dummies, covered with ethanolic extract from fertile females, excite and stimulate the males too. Young females do not yet bear the pheromone, it will be secreted since ovaries come to function. Fresh dead females and isolated female's elytrae are also carrier of the exciting pheromone. A test method by using cut off elytrae was developed. The stimulating efficasy of isolated elytrae decreases during 24 hours.

The female sex pheromon has exciting but no attracting properties.

Females being sterilized by NeemAzal F lack to produce eggs, vitellogenin proteins and also sex pheromone.

Introduction

In middle-europe normaly the pest *Leptinotarsa decemlineata* Say. has one generation per year. During summer beetles and larvae are consuming potato leaves. In late summer the old 4th instar larvae are crawling into the soil for pupating. Already 2-3 weeks later appear the young beetles which start to feed potato leaves to get a food-supply for the wintertime. After that they again crawl into the soil for hibernation. In late spring the beetles appear again and start to feed the young potato shoots. During this they become fertile, they copulate and the females lay their eggs in form of patches with up to about 40 eggs onto the leaves. The egglaying period is lasting during nearly 2 month, and the total amount of eggs per female is about 600 up to 800. The females will be mated several times and the polygame males are ready to copulate again day for day.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

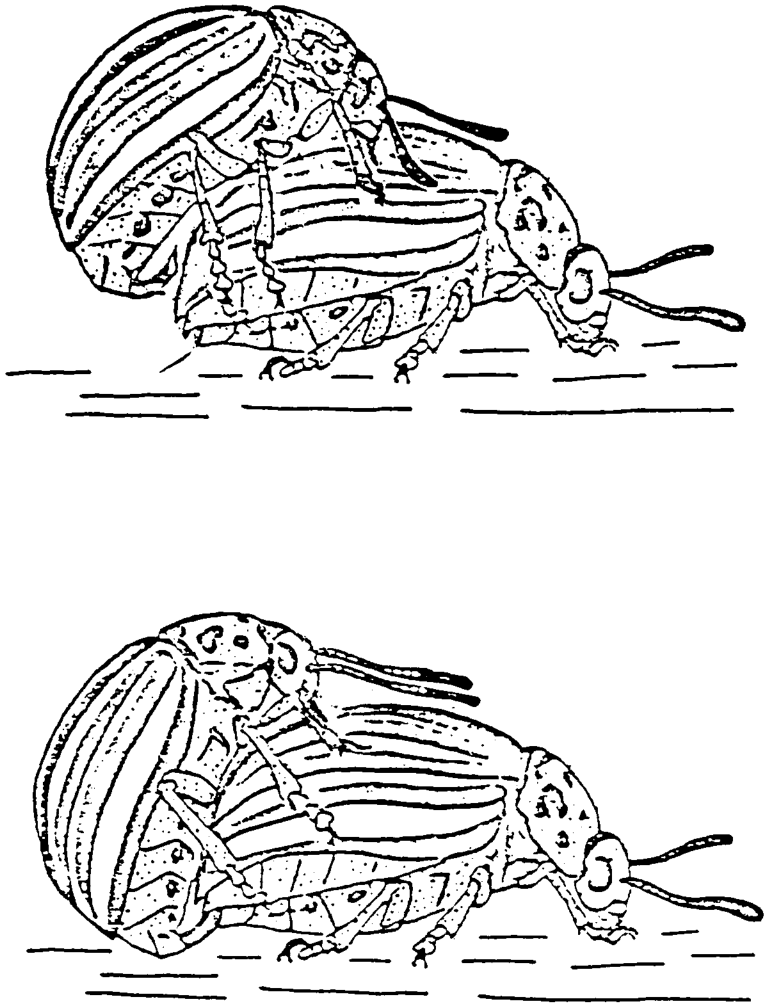
Some certain aspects of the sexual behavior of the Colorado potato beetle are described by THIBOUT (1982) and SCENTESI (1985). In 1979 LEVINSON et al. brought first evidence, that there may be a female sex pheromone involved in the copulating behavior. We took up and continued this observations and experiments to learn something more about this pheromone.

Laboratory observations

A male which is willing to copulate shows an appetence behavior, that means it shows searching activities to meet a female. It discover the presence of a potato beetle by visual signs in a short distance of only about 1...1,5 cm and turn to this beetle without distinction whether it is a female or a male. In contact with the beetle the appetency male tips its antennae to the surface of the partners elytrae. If the partner is a male, the searching male turns off, going away. But if the partner is a female, the antennae find a sign which brings the male in visible excitation. Riding up it mounts the female's back in a quick action, drumming frequently and incessantly with the antennae and also with the tips of the maxillar palpae to the female's elytrae. In this way excited, the male brings out its aedeagus and slides backward in the position for copulation (Fig.:1a,b). When mating is finished after 5...15 minutes, the male usually remains for some time in a resting position on the female's back ("guardening behavior", SCENTESI, 1985).

An appetency male is not able to detect a female as a female from a distance. Even few millimeters hinder from perceiving the female. The female's signal is not alluring and attracting, it is only exciting.

Males which were isolated from females during several days show a strong appetence behavior. They display a striking search activity. Meeting and contacting a partner, they immediatly mount the back and try to copulate, without examinating the kind of sex of the partner. Only somewhat later they notice the error, if they are riding on a male, and they abandon it. This behavior indicates, that the female sex pheromone is not the only releaser for the mating behavior of the male. The profile and form of the elytrae for instance may act with it more or less in a complex.



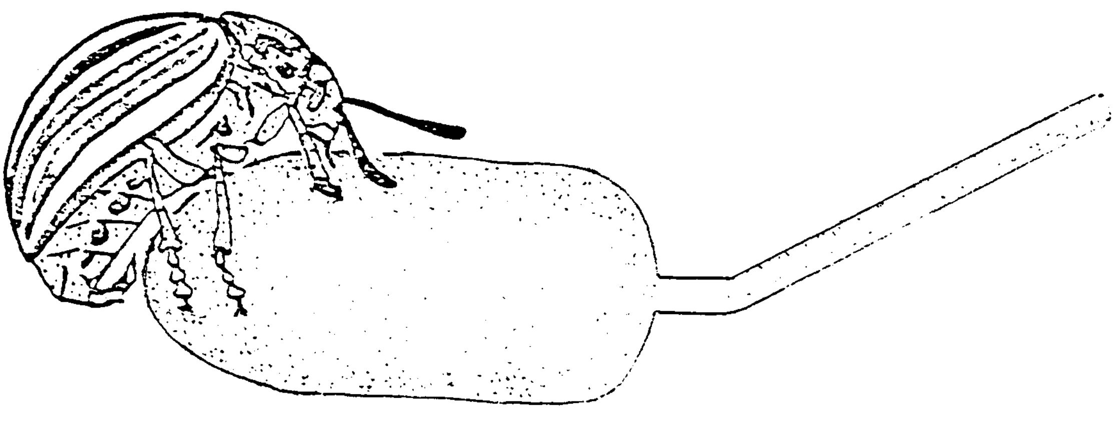


Fig. 1a, b : Two typical steps in the copulation behaviour of the Colorado potato beetle. (drawn by using a pattern in THIBOUT, 1982)

Fig. 1 c : A male becomes excited and display copulation behaviour when contacting a glass dummy covered with ethanolic pheromone extract. (Fashion and Preparation of the dummy related to hints by T. Jermy and W. Francke, see text)

The Reaction of the strong appetency males is in line with the main knowledge of the ethological science: To realize an instinctive action it is necessary that there is a physiological appetence in the actor and a releasing factor in the environment. The weaker the appetence of the actor, the stronger must the releasing factor be; and the stronger the appetence, the more the animals are comming to action already in a weak exciting situation. - This behavior is an exception, referring to strong appetency males.

Normally the male examinates the partner by touching its elytrae with the antennae and maxillar palpae to decide between female and male, before it is going to copulate or to abandon it. The female's sign excites the male and releases the further steps in copulation behavior.

Laboratory experiments

The prove of a female sex pheromon

In the following experiments we used fertile males older than 10 days after isolating them from females during 20 to 24 hours. They were put in portions of 5 individuals in glas dishes (Petri dishes of 9,5 cm diameter).

If the males meet 5 fertile females in this vessel, after few minutes 5 pairs in copulation can be observed.

If in the dish are 5 dead females which were killed immediately before by diethylether or by -18 °C, the males recognize the dead females as sexual partner without restriction. Drumming with antennae and maxillar palpes on the surface of the female's elytrae they become excited, clinging to the female's back, erecting and introducing the aediagus like in a normal copulation. That means, the female's sex sign is not referring to an acute activity of a living female, but can be detected also in fresh dead ones.

The female's sex sign can be extracted from dead females by ethylalcohol and other organic solvents. In such way treated females have lost their exiting and stimulating properties. Males which meet an extracted female leave it after short examination without switching on a copulation behaviour.

Contaminating a dummy made of glass with a ethylalcoholic extract, the dummy get exciting properties for appetency males. Like on a female the male touches vehemently and frequently the glass dummy's surface by antennae and maxillar palpi. In high exciting state the male graspes to the dummy with all its legs and attempes intromission of its aedeagus (Fig. 1 c). It shows quite the same behaviour as during copulation with a real female. This is the evidence that the female's signal is of chemical nature and means it is a sex pheromone.

A short hint for such a reaction with a dummy has given already LEVINSON et al. 1979, but without any particular points. T. Jermy, Budapest, used in 1983 dummies which were pieces of a rough glass rod, sized nearly to the body size of a beetle (unpublished, personally information by W. Francke, Hamburg). Respecting this information we used such glass dummies too, fastened to a thin glass handle (Fig. 1 c). To prepare the extract, we filled a small vessel with 500 µl pure ethanol and dipped subsequently 6 or 12 females for 1 minute each in this bath. The solution dryes slowly in the open air, a slight yellow residue coveres the rough surface of the glass dummy.

Experiments with isolated elytrae

Dead females of which the elytrae were removed are still able to excite the males too, but the males must fail to climb the female's back and to copulate.

On the other hand also the isolated elytrae of fertile females are exciting for males. This led to work out a special biotest ("elytrae-test", see Fig.2). In the middle of a Petri dish we mounted 10 pairs of cut off elytrae. Into this arena 10 males were put, after having been isolated from females during 20 to 24 hours. The males searching for partners meet one another and examine one another, but do not become sexually excited. Only when they meet and come in touch with the patch of female's elytrae they start to receive the stimuli by their frequently swaying antennae and palpi. With excited movements they crawl in the pile of elytrae and try to grasp something for mounting.

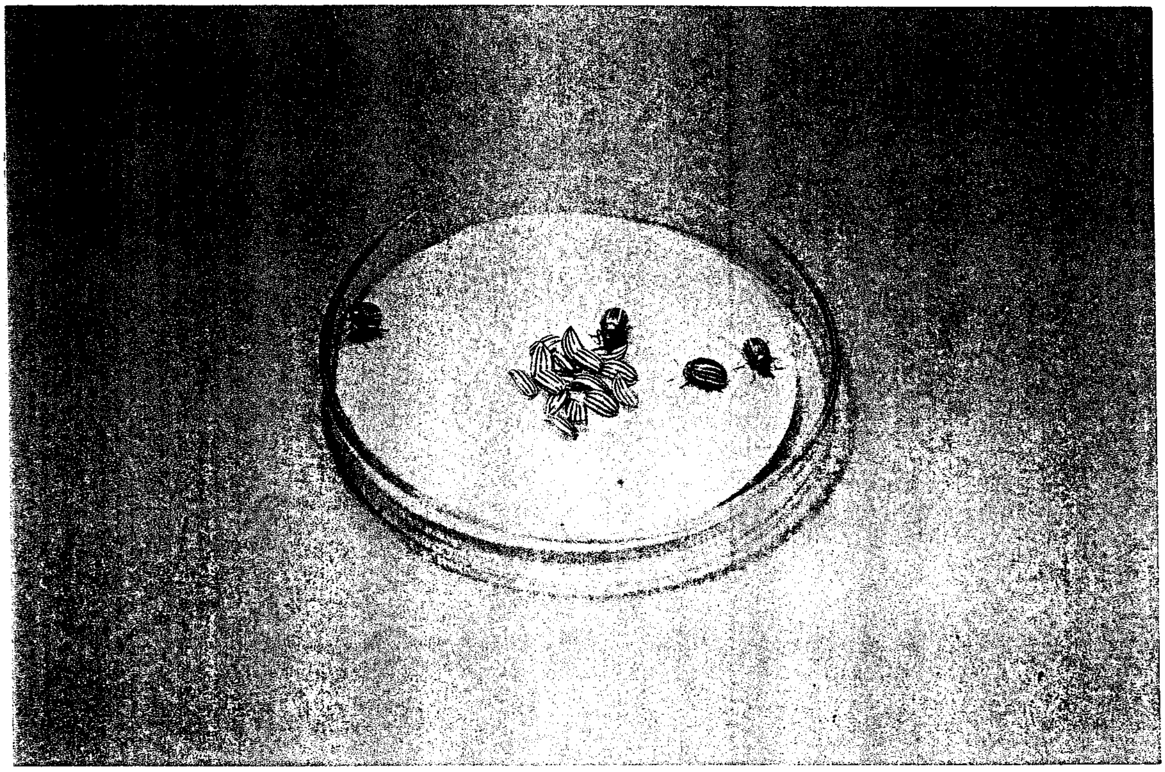


Figure 2: The "Elytrae-Test"

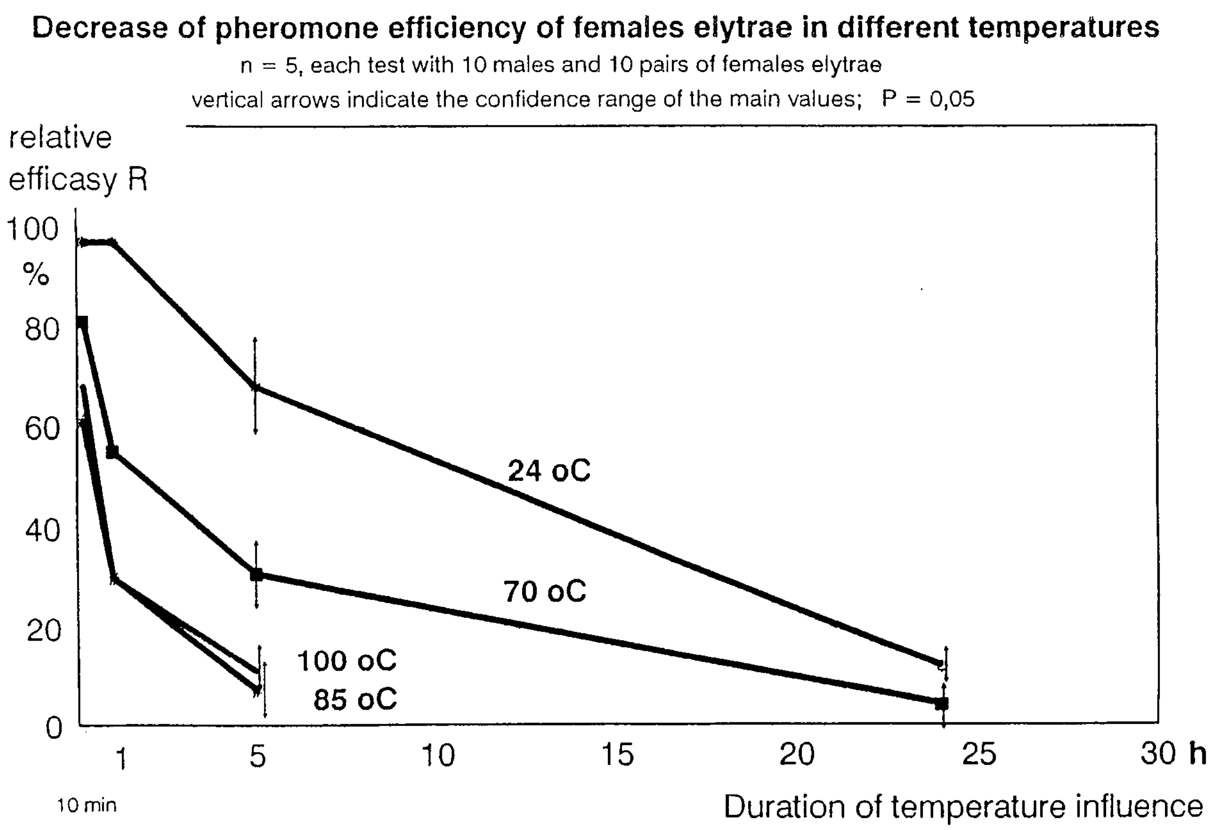


Figure 3

To have a bonitur of the strength of male's reaction we decide 3 grades (notes) of intensity:

Note 1 : - normal reaction to fertile females: Drumming the elytral surface lastingly and intensively with antennae and palpae. Excited crawling between the elytrae and attemps to grasp one of them

Note 0,5 : - Meeting the patch of elytrae the male examines with attention the elytrae by repeated touching and drumming with antennae and palpae. But the male enters not the excited steps as in bonitur 1, but leave the object after some time of examination.

Note 0 : - Meeting the elytrae and contacting them with the antennae the male turnes off and abandonnes the object without extended stay.

After one male has contacted the batch of elytrae, the bonitur note of its reaction will be registrated, and it will be removed out of the test dish. In this way at the end of the test we have available 10 bonitur notes from the 10 test males which enable us to calculate a main note; written as percent value (= main note x 100) we get a parameter **R** for the intensity of the reaction, that means of the exciting power of the elytrae: If all 10 males get note 1, R becomes 100 %. Is none of the 10 males is reacting (note 0), the value of R becomes 0 %. Between these two corner values the concrete test values will lay.

Using this elytrae-test we got the following results:

1. Elytrae of fertile femals: R = 92 - 100 %
2. Elytrae of fertile females after extraction with organic solvents: R = 0 %

   Solvents like acetone, bencene, pentane, chloroforme, ethanole extract the pheromone totally.

3. Elytrae of fertile males: R = 0 %
4. Elytrae of young, not yet fertile females: R = 0 %
5. Fresh cut elytrae of fertile females: R = 92 - 100 %
   - after 5 hours laying in open air: R = 58 - 78 %
   - after 24 hours laying in open air: R = 8 - 15 %
   - after 24 hours laying in a closed vessel: R = 35 - 55 %

   (See Fig 3 and 4)

The results of point 5 concerning the decrease of exciting power during 24 hours indicate, that the pheromone is not stable in the cut elytrae. It decreases either by volatility or by decomposition. In the living female it will be permanently reproduced and re-incorporated into the elytrae. It seems that the decrease is due to evaporation, because it is more slowly in closed vessels, and absence of oxigen (in argon-atmosphere) and light do not stop the decrease (Fig.3 and 4).

The failure of female sex pheromone in yuong, not yet fertile females

Immediately after hatching from the pupae the young females are not exciting to males. The males are not going to copulate with young femals. In such young femals the ovariols are not yet developed. The femals have to take up food (as a fertilization feeding) during about 6 to 10 days, then in the hemolymphe the proteins vitellogenin 1 and 2 occur to be incorporated as vittelin 1 and 2 into the growing eggs (Peferoen and De Loof, 1986), now the female's abdomen is swelling. Not before these events the females become exciting for sex activities of the males. Electrophoretically isolated vitellogenins from the hemolymphe and vitellins from eggs however are not exciting for males, as we could show in related investigations.

In an additional experiment we have sterilized one day old femals by feeding them during 24 hours with potato leaves which were contaminated with a **NeemAzal F** solution (Trifolio-M GmbH Lahnau, 100 ppm Azadirachtin in the solution). After this the beetles get untreated food.

These females failed to become fertile. In their hemolymphe did not appear Vitellogenins and there was no development of eggs. And they also failed to develop the sex pheromone in a normal extent.

At the age of ten days 15 these femals were testet by contacting them to 10 males each. We noticed the exciting parameter R % as

R = 0; 0; 0; 0; 5; 5; 5; 10; 10; 10; 15; 25; 25; 30; 40 %.

Main of 15 x 10 notes: **R = 12 %**

These females had a diminished vitality at all, 13 of them died at day 14.

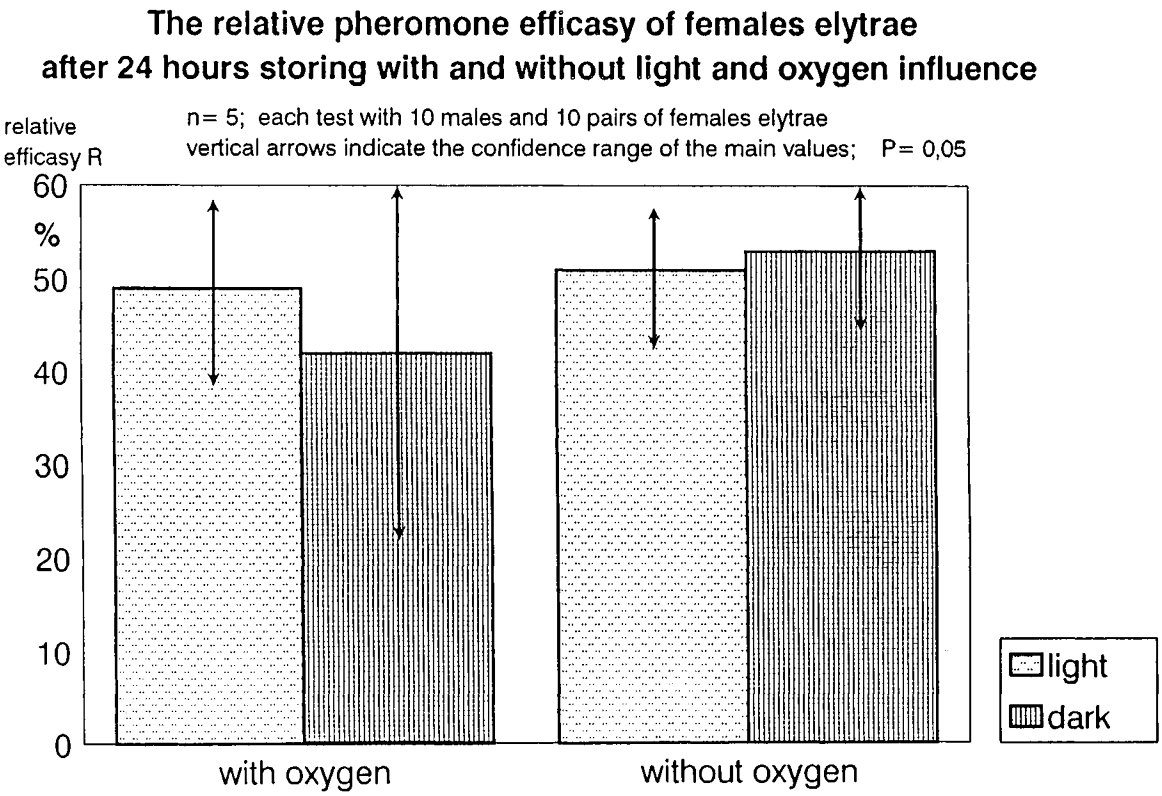


Figure 4

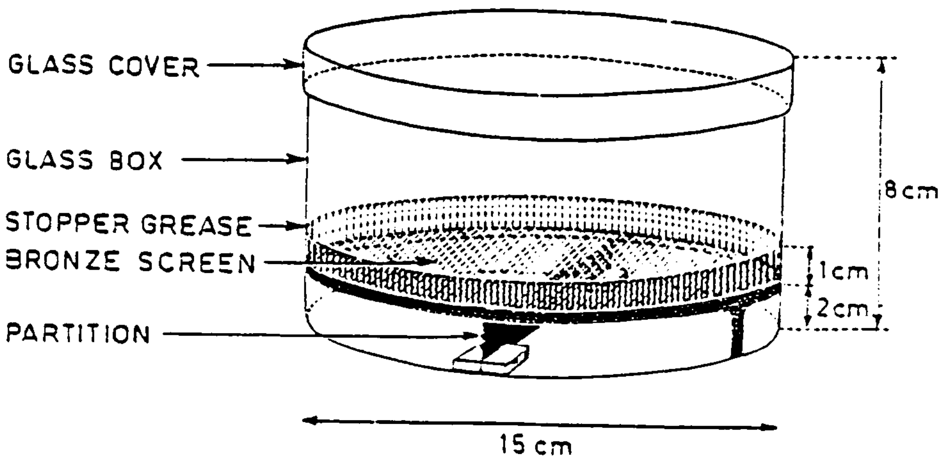


Figure 5: Two-choice olfactometer (from BONGERS, 1970)

Experiments with an olfactometer

As already discussed above, the males can not detect the female's pheromone from a distance, but only when touching the females. However there are some references and hints, that pheromone molecules evaporate into the air and that air born molecules can excite males. Evaporation may be the matter of the loss of exciting power of cut off elytrae. A positive reaction in an electro antennogramme preparation (EAG), like LEVINSON et al.(1979) have reported, presuppose that the Air stream flowing over female's elytrae will be loaded with pheromone molecules. Levinson et al.(1979) reported also about some results of olfactometer experiments. The males were walking on a porous surface in an arena, they stay more often in such parts which are few millimeters above females or female extracts. However, this reaction is very weak (factor 2,5 over the extract of 100 females).

We have repeated such olfactometer tests with similar results, using an equipment similar to that which BONGERS (1970) has described for food detection experiments with the potato beetle (Fig. 5). A petri dish (15 cm diameter) was divided in two halves by a glass partition. One half was settled with 5 living or 5 dead females or with 20 pairs of elytrae. The petri dish was covered with a screen and filter paper and surrounded with a glas cylinder in which the test males were put. To prevent deranging stimuli from the environment the equipment in total was surrounded by a dark paper cylinder with an open top side for observation and for elucidation exactly from the top. Moreover the total equipment was turned at 45 degree every 15 seconds. In every case only 2 to 5 males were allowed to walk in the test arena, they were observed during 5 minutes and after every 15 second it was noticed whether they stay in the control half or in the pheromone half. Only such males were included to the protocol which were activ moving during testing time.

Above of the females, the scent of the pheromone brings the males to examine and probe the air by swaying their antennae for some seconds before the walk was continued. Because that, the males can be observed a little bit more often in the pheromone half of the arena. The difference will be evident only by using a large amount of observations (n = 280 or 680 per variant). The statistical significance will be evaluated by the non parametric sign-test (LORENZ, 1992).

In a control test with none pheromone in each half we got the following result:

Number of observations:	680
expected values:	340 : 340
confidence range (P = 0,05):	314 : 366
test results:	**334 : 346**

That means, the values are still distributed inside of the confidence range, the distribution is accidentally.

The results with pheromone baited olfactometer halfes showed Tab. 1 and Fig. 6. The males reside at least only a little bit, yet even significantly more often (longer) in the baited half then in the control half. These results have no high evidence.

Table 1:

Bait	number of observations	No. of males in the pheromone baited half: 1:1-value	upper confidence limit (P = 0,05)		value observed
1	640	320	341	<	354
2	280	140	154	<	161
3	280	140	154	<	165

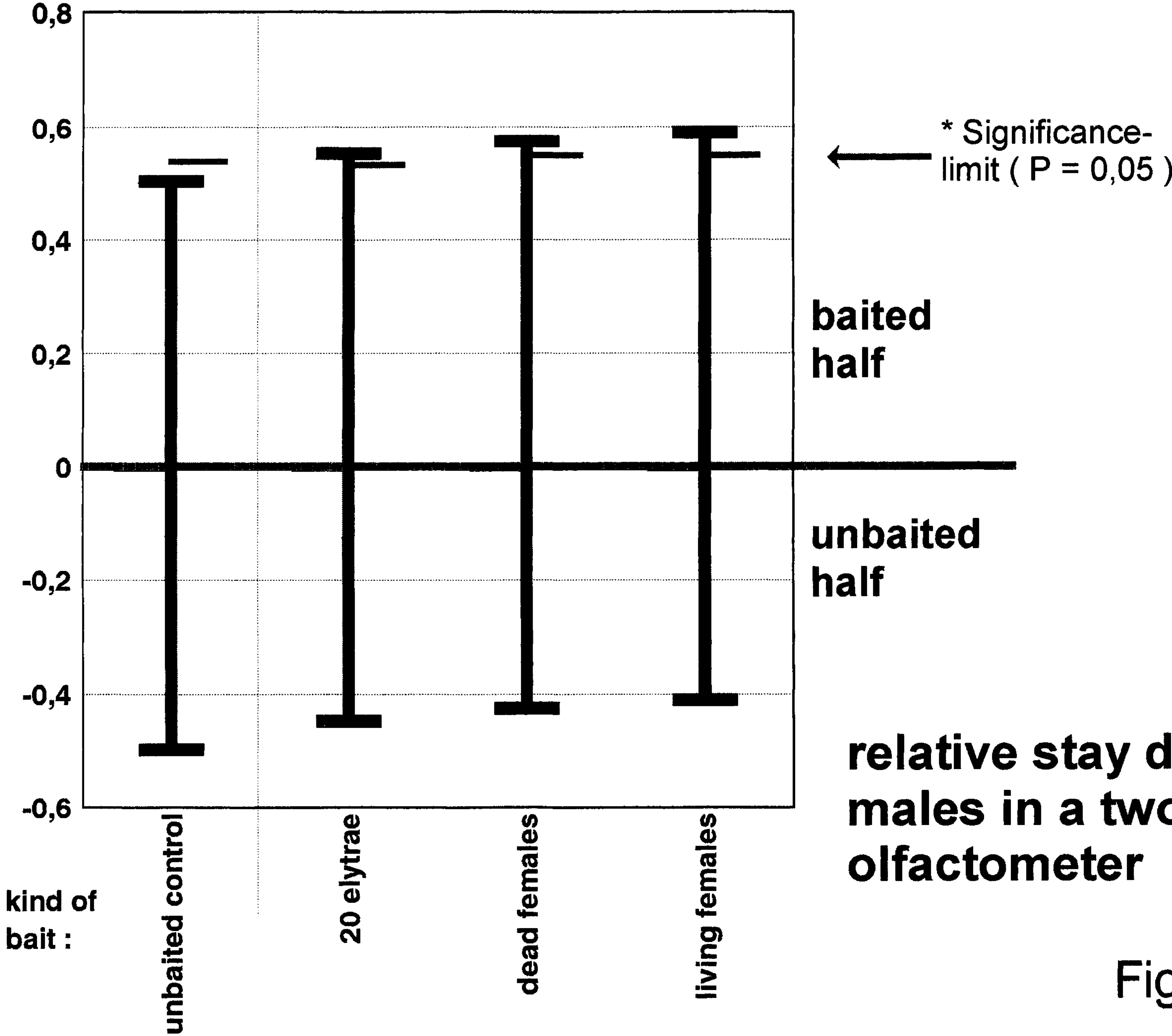
[bait 1 = 20 pairs female elytrae]
[bait 2 = 5 dead females]
[bait 3 = 5 living females]

Conclusions

The female sex pheromone of the Colorado potato beetle serves the male to recognize a touched female as a sex partner and to release the further steps of the copulation behavior. In this way the pheromone is an aphrodisiaca, not an attractive lure. The pheromon can be removed from the female and transfered to an glass dummy by ethylalcohol. There are some circumstances that the pheromon has a weak evaporation.

Discussion

In the coleopterean family of the Chrysomelidae (to which the Colorado potato beetle belongs) only in the genus *Diabrotica* female sex pheromones are known up to day (CUTHBERT and REID, 1964; BALL and CHAUDHURY, 1973; SCHWARZ, JACOBSON and CUTHBERT, 1971; GUSS 1976). The chemical structures of these pheromones are identified by GUSS et al. (1982, 1983, 1985).
These pheromones attract the males from a distance of 15 m and more. Synthetic pheromones could be testet successfully in practice oriented experiments for observation and desorientation by HUMMEL (1989).
In case of the Colorado potato beetle such kinds of pheromone application may not be possible.



relative stay duration of males in a two-choice olfactometer

Figure 6

References

BALL, H.J.; CHAUDHURY, M.F.B.: A Sex Attractant of the Estern Corn Rootworm. J. Econ. Entom. **66**, 1973, 1051-1053

BONGERS, W.: Aspects of host-plant relationship of the Colorado beetle. Meded.Landbouwhogeschool Wageningen **70-10**, 1970, 77 S.

CUTHBERT, F.P.; REID, W.J.: Studies of Sex Attractant of Banded Cucumber Beetle. J. Econ. Entom. **57**, 1964, 247-250

GUSS, P.L.: The Sex Pheromone of the Western Corn Rootworm (*Diabrotica virgifera*). Environmental Entomology **5**, 1976, 219-223

GUSS, P.L.; CARNEY, R.L.; SONNET, P.E.; TUMLINSON, J.H.: Stereospecific Sex Attractant for *Diabrotica cristata* (Harris)(Coeleoptera, Chrysomelidae). Environ. Entomol. **12**, 1983a, 1296-1297

GUSS, P.L.; TUMLINSON, J.H.; SONNET, P.E.; MCLAUGHLIN, J.R.: Identification of a female-produced sex pheromone from the Southern corn rootworm *Diabrotica undecimpunctata howardi* Barber. J. Chem. Ecol. **9**, 1983b, 1363-1375

GUSS, P.L.; SONNET, P.E.; CARNEY, R.L.; TUMLINSON, J.H.; WILKIN,P.J.: Response of Northern corn rootworm *Diabrotica barberi* Smith and Lawrence to stereo-isomers of 8-methyl-2-decylpropanoate. J. Chem. Ecol. **11**, 1985, 24-26

GUSS, P.L.; TUMLINSON, J.H.; SONNET, P.E.; PROVEAUX, A.T.: Identification of a female-produced sex pheromone of the Western corn rootworm. J. Chem. Ecol. **8**, 1982, 545-556

HUMMEL, H.E.: Natural products as biotechnical weapons towards the future pest management of *Diabrotica* beetles. Med. Fac. Landbouww. Rijksuniv. Gent 54/3a, 1989, 945-954

LEVINSON, H.Z.; LEVINSON, A.R.; JEN, T.L.: Sex Recognition by a Pheromone in the Colorado Beetle. Naturwiss. **66**, 1979, 472-473

LORENZ, R.L.: Grundbegriffe der Biometrie, 3.Auflage, 1992, Gustav Fischer Verlag Stuttgart Jena New York.

PEFEROEN, M.; DE LOOF, A.: Synthesis of vitellogenic and non-vitellogenic yolk proteins by the fat body and the ovary of *Leptinotarsa decemlineata*. Comp. Biochem. Physiol. **83B**, 1986, 251-254

SCHWARZ, M.; JACOBSON, M.; CUTHBERT,F.P.: Chemical Studies of the Sex Attractant of the Banded Cucumber Beetle. J. Econ. Entom. **64**, 1971, 769-770

SZENTESI, A.: Behavioral Aspects of Female Guarding and Inter-male Conflict in the Colorado Potato Beetle. Proc. Symp. on the Colorado Potato Beetle XVIIth International Congress of Entomology (Ed.: Ferro, D.N.; Voss,R.H.); Univ. Massachusetts at Amherst Research Bulletin **704**, 1985, 127-137

THIBOUT, E.: Le compartement sexuel du doryphore, *Leptinotarsa decemlineata* Say. et son possible controle par l'hormone juvenile et les corps allates. Behav. **80**, 1982, 199-217

APPLICATION OF ENTOMOPHAGES IN GREENHOUSES IN RUSSIA

S.S.Izhevsky, A.K.Akhatov
(Moscow,Russia)

Since many years and on a large scale have entomophagous insects been applied in greenhouses in Russia. Before the disintegration of the USSR, Ministry of Agriculture adopted a decision on obligatory transition to the biological control in the sphere of plant protection in greenhouses. Every large greenhouse complex had then a newly-organized laboratory producing means of biological control: entomophages, acariphages, microbiological preparations. And, though it was not a 100 per cent success, a significant development of the biological method was still achieved. The last few years showed that the tendency to substitute pesticides for entomophages is not only determined by ecology and hygiene, but also by economy - the cost of modern chemical means of plant protection is too high.

Who and when applies entomophages?

At present the major volume of the cultures, grown in greenhouses in Russia - vegetables, greens and flowers - is produced by large greenhouse complexes, that have been in the majority of cases reorganized into joint-stock companies from state enterprizes. Such complexes exist in almost all large cities of Russia. Many of these have got laboratories producing means of biological control. As a rule, the volume of production in these laboratories covers only the needs of the complex itself. It is common practice, that the specialists from the laboratory apply the entomophages within their complex. Only the largest laboratories produce entomophages and microbiological means of plant protection in such quantities that they can sell them to other greenhouse complexes and to private persons as well. There are no independent large firms in Russia yet, that specialise in production of biological means of plant protection.

What entomophages are applied?
Against what pests are they applied?

In the greenhouses in Russia entomophages are applied against pests of cucumbers, tomatos, pepper, onions, salad, as well as of a number of flowers: roses, chrysanthemums, carnations. The basic pests and the entomophages, most often used against them are practically the same as in other European countries (*Table 1*).

How are entomophages applied?

The strategy of application of beneficial insects and mites in greenhouses in Russia is similar to the one in Western Europe. The means of biological control are applied within IPM systems. Several methods used of entomophages application are used (*Table 2*):

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

- preventive releases (entomophages are released before the pest achieves threshold population number);
- creation of artificial entomophagous reservations;
- flood releases (mass releases of entomophages when plants are densely occupied by the pest.

Very often the application of entomophages is combined with pesticides application or application of microbiological preparations. There can be different tactics of application of one and the same entomophagous insect against different species of pests. This depends on biological and behaviouristic specificity of target species. For example, various aphid species have different migration capabilities and, in connection with this, they require different periods of time to spread over the greenhouse out of the primary location. M.euphorbiae, for example, forms dense colonies on plants but due to the small number of alate female, its distribution is of a "spot" character. At the same time, *70-80%* of A.gossypii colonies consist of alate female and nimphs that spread quickly all over the greenhouse. Hence,- *2-3* colonizations of A.aphidimyza larva and cocoons against M.euphorbiae will be quite enough, whereas to control A.gossypii multiple colonization of A.aphidimyza both to the spots of concentration and all over the greenhouse is required.

In Russia there have been worked out quality standards for a number of entomophages which, in particular include the data on the weight and volume of the biological material. For example, 1 g of clean mummies of Encarsia formosa must have *30.000* specimen in it; the share of viable insects should be *90%. 1 g* of Lysiphlebus testaceipes mummies should have *5.000* specimen in it (viability - *90%*); *1.000* pupa of Aphidoletes aphidimyza must have the volume of *6 ml*, have *90%* of viability and sex ratio *0,4-0,6*.

What entomophages are investigated now in Russia?

The search of new active entomophagous insects is conducted now in Russia by many organizations specialised in scientific and applied sciences. Recently some effective species that can be used in greenhouses were found in different regions of Russia (*Table 3*).

LIST OF ENTOMOPHAGES AND ACARIPHAGES APPLIED IN GREENHOUSES IN RUSSIA

Entomophages	Target species
ACARI	
Ambliseius mckenziei Sch.et Pr.	Thrips tabaci Lind.
Phytoseiulus persimilis A.-H.	Tetranichidae
INSECTA	
Aphelinidae (Hym.)	
Encarsia formosa Gah.	Trialeurodes vaporariorum Westw.
Aphididae (Hym.)	
Aphidius matricariae Hal.	Aphis gossypii Glover
"	Myzodes (Myzus) persicae Sulz.
Lysiphlebus testaceipes Cres.	A. gossypii
Lysiphlebus fabarum M.	Aulacorthum solani Kalt.
Braconidae (Hym.)	
Dacnusa sibirica Telenga	Liriomyza bryoniae Kalt.
Eulophidae (Hym.)	
Digliphus isaeae Walk.	L.bryoniae
Miridae (Hem.)	
Macrolophus nubilus H.- S.	T. vaporariorum
Cecidomyiidae (Dip.)	
Aphidoletes aphidimyza Rond.	A. gossypii
"	Macrosiphum euphorbiae Thom.
"	M.persicae
Coccinellidae (Col.)	
Cycloneda limbifer Casey	A. gossypii
"	M. persicae
Chrysopidae (Neur.)	
Chrysopa carnea Steph.	Aphididae
Hemerobiidae (Neur.)	
Micromus angulatus Steph.	M. euphorbiae

TABLE 2

ENTOMOPHAGES APPLICATION METHODS

Entomophages	Pests	Culture	Rate of release		Intervals between of releases (days)
A.matricariae	M.persicae	pepper	1:5-1:30		5-7
"	"	flowers	2-5/m^2	*[5]	
A.aphidimyza	A.gossipii	cucumber	1:5	*[1]	5-7
"	M.euphorbiae	tomato	1:1-3:2	*[2]	5-7
"	"	"	1:2	*[3]	5-7
Ch.carnea	A.gossypii	cucumber	1:50-1:20	*[1]*[6]	
"	M.persicae	pepper	"		
C.limbifer	A.gossypii	tomato	1:5 -1:10	*[1]	
D.sibirica	L.brioniae	tomato	1:15		
D.isaeae	L.brioniae	"	"		
E.formosa	T.vaporariorum	cucumber	1:5		8-10
"	"	tomato	1:10		8-10
"	"	egg-plant	1:20		8-10
"	"	"	20-30/m^2	*[7]	
"	"	"	50-60/m^2	*[8]	8-10
L.testaceipes	A.gossypii	pepper	2-5/m^2	*[5]	
"	"	flowers	"		
"	"	cucumber	10/m^2		
L.fabarum	A.gossypii	cucumber	1:20	*[1] *[3]	
M.angulatus	M.euphirbiae	tomato	1:-1:10	*[1]	
"	"	verdure	"		
"	"	roses	1:3	*[4]	
M.occidentalis	Tetranichidae	roses			
M.nubilis	T.vaporariorum	cucumber	0,5-2,5/m^2		
Ph.persimilis	Tetranichidae	cucumber	80-130/m^2	*[9]	6-8

*[1] colonization of larva
*[2] distribution of cocoon over greenhouse
*[3] imago release
*[4] distribution of eggs over greenhouse
*[5] release before mass aphids emergence
*[6] number of A.gossypii does not exceed 100 per one pepper plant or 250-300 M.persicae per one pepper plant
*[7] release on seedlings before 5-7 days for planting
*[8] release on a greenhouses
*[9] serious infestation by tetranichids - 250-350/m^2

ENTOMOPHAGES PROSPECTIVE FOR APPLICATION IN GREENHOUSES

Entomophages	Target species	Origin
Aphidius gyphuensis Ahmead	M. persicae	Sotchi
Aphidoletes aphidimyza Rond.	Aphididae	Primorije
Chrysopa perla L.	Aphididae	Sotchi
Coccinella septempunctata L.	Aphididae	Sotchi
Diglyphus minocus Walk.	Liriomyza spp	Tatarstan
Encarsia partenopea Masi.	T.vaporariorum	Moscow
Leis dimidiata Fabr.	Aphididae	China
Lysiphlebus fabarum M.	A. gossypii	Sotchi
"	Aulacorthum solani	Sotchi
Lysiphlebus japonica	A. gossypii	Primorije
Lysiphlebus fritzmulleri Mack.	A. gossypii	Šishinev
	"	Kazakhstan
	"	Kemerovo
Opius pallipes Wesm.	Liriomyza spp	Moscow
Orius spp.	Frankliniella occidentalis	Moscow
Propylaea quatuordecimpunctata L.	Aphididae	Sotchi
Hippodamia tredecimpunctata L.	Aphididae	Sotchi

Aphidius gyphuensis Ahmead

Very effective against Myzus persicae (*97%*)

Aphidius matricariae Hal.

Possible infestation by hyperparasitoids: Alloxysta mulensis, Aphidencyrtus aphidivorus, Pachyneuron aphidius.

Aphidoletes aphidimyza Rond

One larva paralizes 45-50 preys (50 % of them are eaten). An offspring of one pair in the first generation may destroy 1500-2000 aphids. Optimal development conditions for Aphis fabae Scop. are 25 C,70% H,16-h day). Development cycle of one generation is 24 days; only 4-5 days of which makes prey stadium. Fecundity - average 100 (max. 400). Oviposition longevity -10 days. A female deposits 70% of eggs on the 3-4-th day of its life. Far-eastern population has its higher effectiveness at 25-26 C and H 80-90%.

Chrysopa perla L.

Polyphage. Among preys are aphids, acari, whiteflies. Imago and larva - predators. A female eats up to 46 A.gossypii specimen per day. Cannibalizm is very low. Eurybiotic: life optimum - 15-30 C, H 30-95%. Norm of release on a cucumber is 1:5 (against Aphis fabae Scop.).

Coccinella septempunctata L.

Effectivenes on sweet pepper against aphids (release larva 1-2 instars (1:10) is never lower 80%). However, imago have a strong tendency to leave the greenhousess (migration activity is very high).

Cycloneda limbifer Cas.

A female longevity - 1,5 - 2,0 months. Fecundity - up to 900. When prey number is 500 aphids per plant effective in correlation 1:25; when the number is higher - 1000 aphids - effective correlation is 1:5 - 1:10.

Dacnusa sibirica Telenga

Widely spread in the European part of Russia. Parasitoids of Liriomyza spp Pupa can be preserved up to 6 months at 5°C.

Diglyphus isaeae Walk.

Common in the European part of Russia. Infestation of L.bryoniae population is 9-14% in field stations.

Diglyphus minicus Walk.

Common in the natural station in the European part of Russia in combination with Dacnusa. Infestation of Liriomyza larvae may reach 90%. By autumn usually actively replaces Dacnusa everywhere.

Encarsia (Trichoporus) partenopea Masi.

Common in Palaearctic. Not so specific as E.formosa. Among hosts are T.vaporariorum, Bemisia tabaci Effectivenes is the same as that of E.formosa: in greenhouses on cucumber - 75%, on tomato - 85%. Hibernates successfully out of greenhouses in central Russia. Ecologically plastic. Tolerant to temperature variations from 12 to 45 C, H 40-90%

Hippodamia tredecimpunctata L.

Successfully reproduces and spreads inside greenhouses. Has a high search capability. It is released in greenhouses at the larva stage (1-2 instars) against M.persicae and M.euphorbiae. Correlation 1:1 gives 80% effectivenes. Activity is sharply decreased at the temperature higher than 25 C.

Leis dimidiata Fabr.

Polyphagous predator introduced from China. The larva of the 2 instars are recommended for application. Effectiveness against A.gossypii on cucumber (predator-prey correlation 1:5 and 1:10) is 99,9%, on sweet pepper the effectiveness is 96,2% (the correlation is 1:90). Also effective on flowers if the correlation is 1:200. Gives no effect on roses. Leis is reared on Schizaphis graminum Bond. Has no diapause. Average fecundity 2000. Development optimum 20-25 C. Development cycle 20-25 days.

Lysiphlebus fabarum Marshall

Widely spread in the south of Sibiria; also in Moscow region. Polyphagous. Highly effective on cucumber against A.gossypii. Parthenogenesis is characteristic. Fecundity - up to 150. It takes one generation 8-9 days to develop. It is not demanding regarding air humidity - optimal development at 40% of humidity. Has a highly developed search ability. It is reared on Aphis fabae (on Vicia faba). Applied at the stage of mummies; correlation 1:20.

Lysiphlebus japonica

Widely spread in Primorye (Far East of Russia). When released in greenhouses infests to up 95% of A.gossypii.

Lysiphlebus fritzmulleri Mack.

Widely spread in Russia, Moldavia, Kazakhstan. Polyphagous. Kishinev population (Moldavia) in greenhouses infests 100% of A.gossypii. In Kazakhstan common on alfalfa, infesting Aphis fabae. Develops at 18-35 C, H 40-60%. Fecundity 40-50.

Macrolophus nubulis H.-S.

Polyphagous predator (cannibalism is registered when reared in mass). When the the older instars or up to 40 imago. The larvae of the predator can develop at 13 C (tolerate temperature increase up to 42 C), regardless air humidity. In laboratory the predator successfully develops on crioconserved eggs of Sitotroga; development temps are not decreased, as well as voracity and search ability. In greenhouses can be released on cucumbers at early stages - right after the final planting of the plants, i.e. before the pests appear. The predator keeps its number by feeding on honeydew and some species of soil invertebrates. Recommended release norm - up to 2.5 thousand specimen on 1000 sq.m. Most active in the middle and upper layer of cucumbers.

Metaseiulus occidentalis (Nesbitt)

Applied on roses against Tetranichidae. The methods of rearing are similar to those of Ph.persimilis. It is less demanding, however, to air humidity.

Micromus angulatus Steph.

Aphidophagous insect. Fecundity - up to 2000. Cannibalism is not strongly expressed. Especially effective against M.euphorbiae and M.persicae on tomato and salad, as there it has got no competitors. Apart from aphids feeds on pollen and nectar. Develops at temperature 15-35 C (optimum 18-22 C),H 80-90%. Recommended release of larvae of 1-2 instars (the larvae can migrate within the range of 10-15 m). Application at egg stage is possible. When larvae are released, the correlation of the larva of the 1th instars and preys - 1:5, of the 2d instars - 1:10; eggs - 1:3. Effective at the average prey density (500 aphids on a plant). Effectiveness after a single release (correlation 1:3) achieves 96,5%. In case 5 and more thousand aphids on one plant - two successive releases are required.

Opius pallipes Wesm.

Larva-pupa endoparasitoid of Liriomyza spp. Preferentially infests the larva of the 2d instars. Develops in the host's pupa. 73-77% infestations of L.bryoniae in field stations were registered. Development optimum 25-30 C. Development duration then is 10-14 days. Fecundity 59-67 eggs. Sex correlation 1:1. By release correlation 1:30 effectiveness is 41-74%.

Propylaea quatuordecimpunctata L.

Polyphagous predator. Prefers aphids and thrips. Develops and spreads successfully in greenhouses. Actively deposits eggs even at low density of the prey. Imago average life longevity - 65 days. Larva stage - 7-8 days. Egg deposition period lasts 30-80 days. Fecundity 270-360. Optimal development conditions - 24-25 C, H 70-80%. In these conditions 80% of larva emerge from the eggs. It is recommended for application at the larva stage of the 1-2 instars on cucumbers and pepper; correlation 1:10. Against high aphid number (up to 200 specimen on a plant) imago releases are recommended in correlation 1:25. The additional release is recommended in 1 week. Prefers the upper layer of the plants.

EFFECT OF NEEMAZAL ON BENEFICIAL INSECTS

Eu.G.Khorkhordin, M.K.Mironova

Moscow, Russia

Chemical control of pests has become difficult and problematic in some regions on account of an increasing resistance of many injurious mite and insect species to the classic pesticides. Continuous or heavy usage of some pesticides has created serious problems arising from contamination of biosphere, toxicity to human beings, fishs, predators and parasites. Such natural control agents against pests as Neem-extract preparations, in all probability, may be considered as one of the most important components of IPM programms. However, for true evaluation of preparation complex effect great care must be exercised in its side effects. Tests of preparation influence on beneficial insects and mites are one of necessary branches of this work.

MATERIAL AND METHODS

The special laboratory sprayer has been constructed for uniform dispersion of the preparation on experimental surface. The amount of the preparation which was dispersed all over the experimental area was adequate to the mean expenditure rate recommended by firm-producer. During the treatment period the aerozol uniformly coats the internal surface of the experimental chamber, the dishs for insects and the covers. Treated dishs were dried during 5 minutes, then insects were placed into dishs. The methods were analogous in all cases, exepting experiments with mites. In this test mites were placed on the disks of the beans leaves, these disks

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

were placed in experimental chamber for treatment. 20 females of mites were on each disk, 5 dishs in each variant, 400 specimens were used at all. The control was treated by water. During experiments the temperature was maintaned at 22-25°C and RH about 70%. The alive and dead specimens were registered every day or every several days, depending on the tested species. All trials were made in 5 replications with 10-20 specimens in each.

RESULTS

The effect of Neemazal was studied on the representatives of four insect orders and one species of mites.

Coleoptera

Adults of three species coccinellids (Coccinellidae) were tested in our trials. There are:

1. Coccinella septempunctata L. It is widely dispersed entomophagous insect which is used for biological control of many pests. Beetles of natural population were used in our tests.

2. Cryptolaemus montrouzieri Muls., predator of many coccids species. The entomophagous was importated in many countries, where is used for biological control of coccids and mealybugs.

3. Nephus reunioni Fursch., predator of mealybugs, is considered a promising agent of biological control.

Inasmuch as preparation did not decrease survival of C.septempunctata during some first days, the test duration was longer than others. Durable observations for beetles revael quite high resistance C.septempunctata to Neemazal (Fig.1). Result of statistic estimation shows that the dynamics of beetles mortality slightly differed from the control variant only at initial period.

Then the mortality dynamics had similar character in all variants and statistically significant differences were not found.

Similar results were received in tests, where coccinellids C.montrouzieri and N.reunioni were used. On this account we give data only about C.septempunctata.

Hemiptera

Podisus maculiventris Say, one of the well known members of the family Pentatomidae was used in our trials. This species is a predator of Colorado potato beetle and is reared in our laboratory.

Estimation of resistance level of 2-nd instar larvae P.maculiventris to Neemazal shows that larvae seem not be influenced. All specimens were alive during trials and their further development was normal.

Hymenoptera

Lysiphlebus testaceipes Cres. (Aphidiidae), a parasite of some aphids, also may be consider as the resistive species. As shows Figure 2, the influence of Neemazal on the emergence and mortality of imago L.testaceipes was negligible. The difference between results in the control and in the test was statistically insignificant. The emergence dynamics and mortality was practically the same in all variants. Similar results were received with the treatment of adults L.testaceipes.

Leptomastidea abnormis Gir. (Encyrtidae), known as effective parasite of mealybugs, belongs to most Neemazal sensitive species. The calculation according to Abbot`s formula (with the control correction) showed that the mortality of the adult parasites on fifth day of experiment could reach 90 and 100 per cent in the variants with 0,5% and 1,0% concentrations of Neemazal. Results of this trial are presented at Fiure 3.

Fig.1 Effect of NEEMAZAL on Coccinella septempunctata

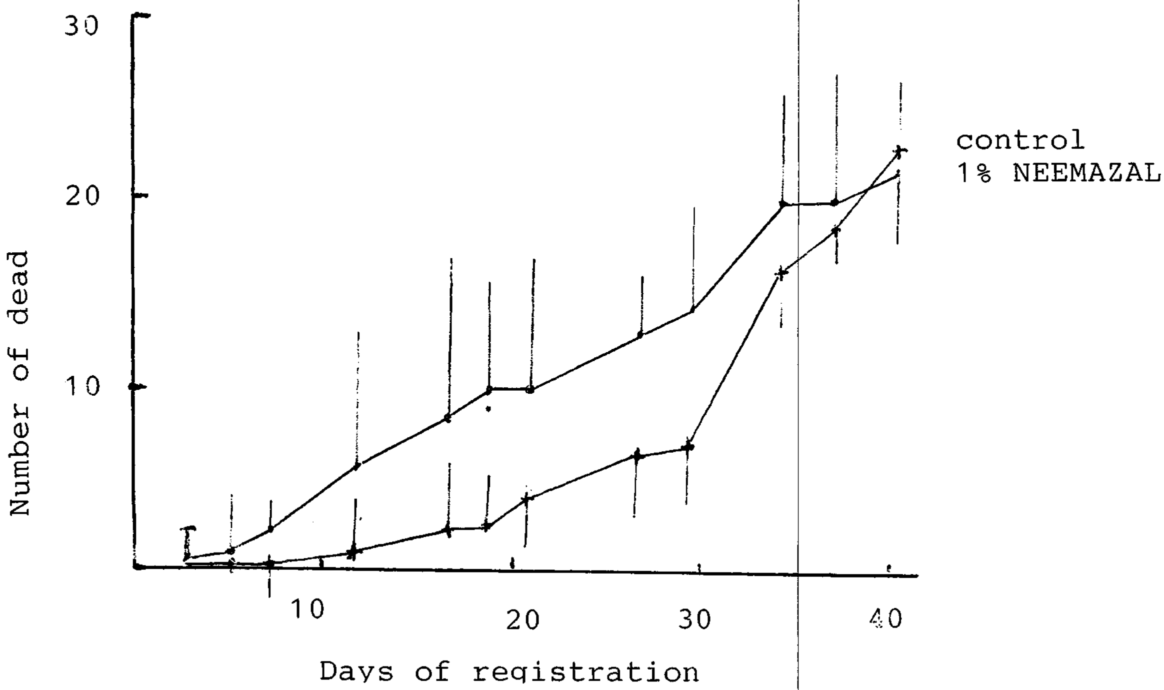
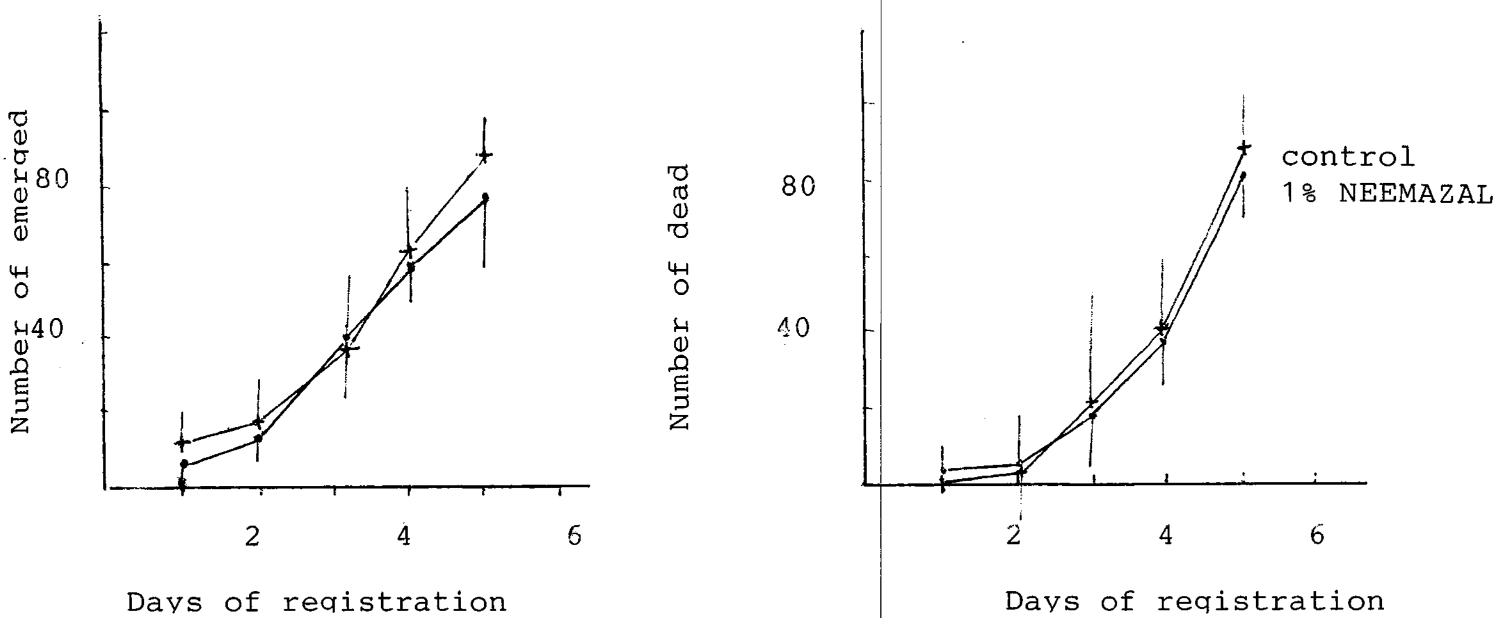


Fig.2 Effect of NEEMAZAL on Lysiphlebus testaceipes



Neuroptera

Chrysopa carnea Steph. (Chrysopidae). It is the common predator, which is widely used in greenhouses against aphids. In laboratory is reared on the eggs of Sitotroga cerealella.

In the preliminary tests with Ch.carnea specific effect of Neemazal was found. It was expressed in the decrease of the feeding activity and the inhibition of the development of treated larvae in comparison with control. Such complex effect of Neemazal requires additional investigations and another estimation methods.

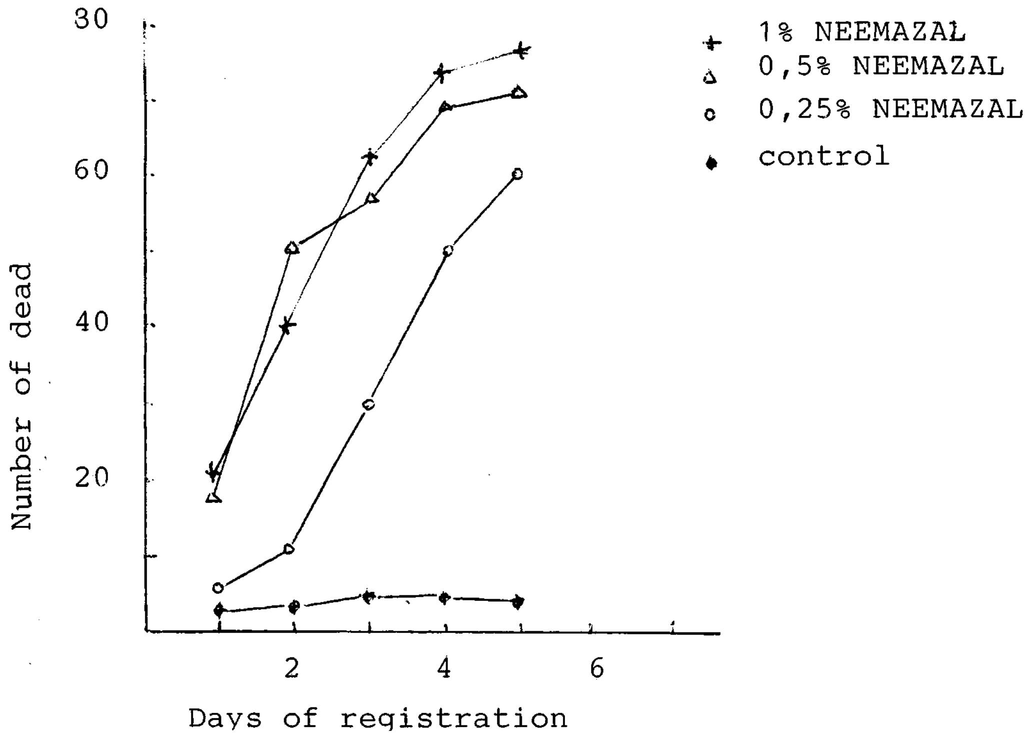
Acari

Amblyseius finlandicus Oudem. (Phytoseiidae). It is widely distributed predator of tetranychid mites, especially Schizotetranychus pruni. This species is evaluated as a perspective mean of biological control of spider mites on fruit cultures. The females of A.finlandicus from Krasnodar region were used in experiments. Mortality dynamics of predatory mites (Fig.4) shows that maximal effect of preparation was expressed on the third day, then was more weak. Besides, the effect of Neemazal was almost proportional to used concentration and this distinct response of test species was only in trials with A.finlandicus.

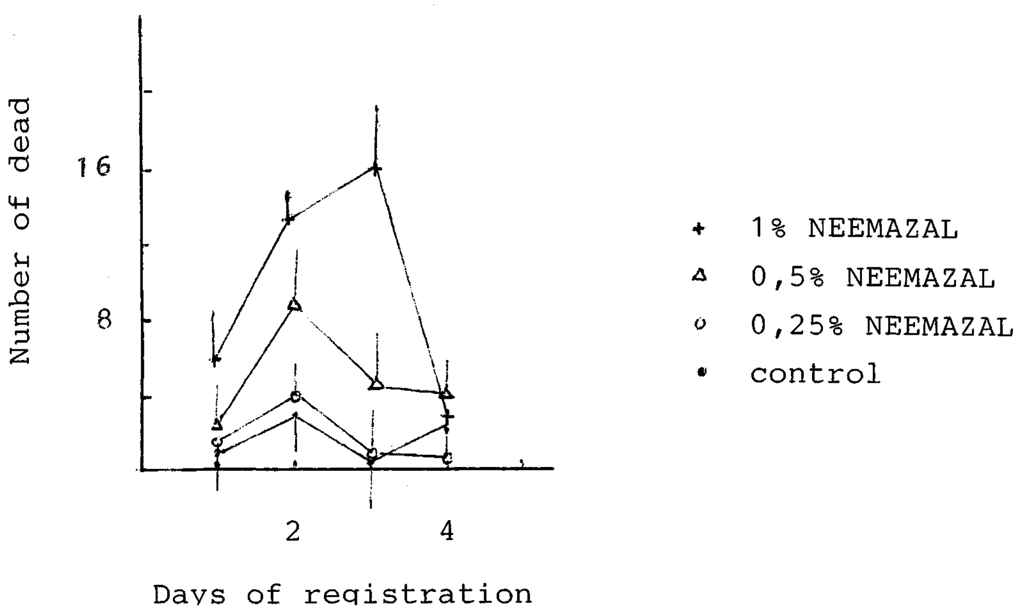
SUMMARY

The effect of various concentrations of Neemazal -FT on adult and larvae of 8 species entomophagous invertebrate was studied in the laboratory. The results of experiments demonstrate that effect of Neemazal on tested species was faint or was not pronounced at all. On the basis of investigations it could be supposed that entomophagous insects from the orders Hemiptera and Coleoptera are more resistive to Neemazal than parasitic Hymeno-

Fiq.3 Effect of NEEMAZAL on Leptomastidea abnormis



Fuq.4 Effect of NEEMAZAL on Amblyseius finlandicus



ptera. Predatory mite Amblyseius finlandicus occupies the intermediate position.It is possible to use in IPM programms Neemazal preparations in combination with tested species, which have been displayed the high level of resistance to this biopesticide.

Impact of NeemAzal on the arthropod fauna in an organic apple orchard

Kienzle, J.; Zebitz, C.P.W.
University of Hohenheim, FG Angewandte Entomologie, Otto Sander Str. 5, D-75093 Stuttgart.

Abstract

Faunistic characters of an organic apple orchard treated with NeemAzal after the flowering and a similar untreated orchard are compared. No striking differences between the orchards could be observed. Since Neemazal was applicated when aphid population was already increasing, population was not controlled in time. Thus, the occurrence of predators in the aphid colonies could be observed in both orchards. The number of predators was similar in both orchards. However, the predator/prey relation was closer in the untreated orchard. This may be due to the higher aphid population in the treated orchard or to a minor attractiveness of the aphids treated with NeemAzal to the predators.
This data may give a first indication, that NeemAzal probabely has minor side effects on the arthropod fauna in the apple orchard than the common botanical insecticides as pyrethrum or rotenon.

Introduction

Within the last decade, the knowledge about the insecticidal properties of neem increased also in the European countries. Recently, in fruit growing it showed good effects in the control of *Dysaphis plantaginea* Pass., an important pest in apple orchards which caused serious damage during the last years. In organic fruit growing, the control with products based on soap is not very effective (BAER, 1993). Products based on natural pyrethrum revealed often no sufficient effect in aphid control but considerable side effects on the whole arthropod fauna. Thus, detailed informations about the side effects are very important for the estimation of NeemAzal as a possible solution for aphid control in organic fruit growing.
KAETHNER (1991) could observe no effects of neem preparations on *Coccinella septempunctata* L. and *Chrysopa carnea* Steph.. VOGT (1993) could confirm these results on *C. carnea* under field conditions. However, in laboratory, a certain long term effect could be found.

Little is known yet about the impact of NeemAzal on the arthropod fauna of the apple orchard if applicated under field conditions in the whole orchard. In 1994, first time NeemAzal was applicated in organic orchards for aphid control.
Faunistic studies were done during this year in several organic orchards. Since one of these orchards was sprayed with NeemAzal after the flowering, the comparison with a similar orchard in the same region may give first orientative data about the side effects of this treatment on the arthropod fauna.

Practice Oriented Results on Use and Production of
Neem Ingredients and Pheromones IV
H. Kleeberg & V. Micheletti (eds.)
Copyright 1996 by Trifolio-M GmbH

Methods

Both orchards are situated in the region of Lake Constance. The untreated orchard is more distant from the lake, however with similar conditions as regards pest and antagonist pressure.

Table 1: Characteristics of the two orchards

	treated orchard	untreated orchard
variety	Idared	Gloster
rootstock	M 9	M 9
mulching system	frequent mulching	frequent mulching
spray program	copper, sulphur, granulosis virus	copper, sulphur, granulosis virus
surroundings	orchards, meadows, little hedge (distant ca. 20 m)	orchards, meadows

In both orchards beating trap samples (100 beats) were done at five dates during 1994. The development of the aphid population and the occurrence of predators in the colonies was assessed by control of 1000 randomly selected shoots per orchard.

NeemAzal T (600 ml/ha = 30 mg Azadirachtin/ha) was applicated together with 7 l/ha of TELMION (formulated rape seed oil) at 17.5.1994 in the treated orchard. This orchard shows every year a very high aphid infestation. Since NeemAzal in this orchard was applicated after the flowering, aphids were not controlled in time. Thus, in June aphid population was very high in this orchard.

The arthropod species abundance and composition was similar in the two orchards (table 2) with only slight differences. *Orius spec.* shows a higher population in the untreated orchard. In this orchard the population of spider mites was considerably higher during this period.

The aphid population was higher in the treated orchard than in the untreated orchard (fig. 1). At the end of June population broke down in both orchards due to emigration of the aphid and the attack of beneficials, especially *Adalia bipunctata*.
At 25.5.94, when aphid infestation was increasing but the temperatures were still low, predators were found only occasionally in both orchards. At 14.6.1994, when aphid populations were completely developed, a high number of predators were found in the colonies.
The numbers of predators on 1000 shoots was similar in both orchards (fig. 2). Calculating the predator/prey relation (predator per aphid colony) it is better in the untreated orchard (fig. 3).

Tab. 1: Results of the beating trap samples (100 beats) (number of individuals treated orchard/untreated orchard). Treatment 17.5.1994

Family/species	Sample date 27.4.	10.5.	25.5.	14.6.	14.7.
Thomisidae	9/22	15/11	11/27	4/6	9/3
Typhlocyba frogatti Baker	2/0	1/4	0/11	1/5	9/4
Phyllobius oblongus L.	35/49	68/63	55/65	1/0	
Orius sp., adults	0/5	11/8	12/31	0/2	20/4
Orius sp., larvae				4/4	2/0
Miridae, adults		0/1	0/3	2/2	5/5
Miridae, larvae			4/3	1/1	1/0
Coccinella septempunctata L.	3/4	17/4	1/0		
Adalia bipunctata L.	0/1		0/3	1/5	
Chrysopa carnea Steph, adults	4/0	0/3		2/1	

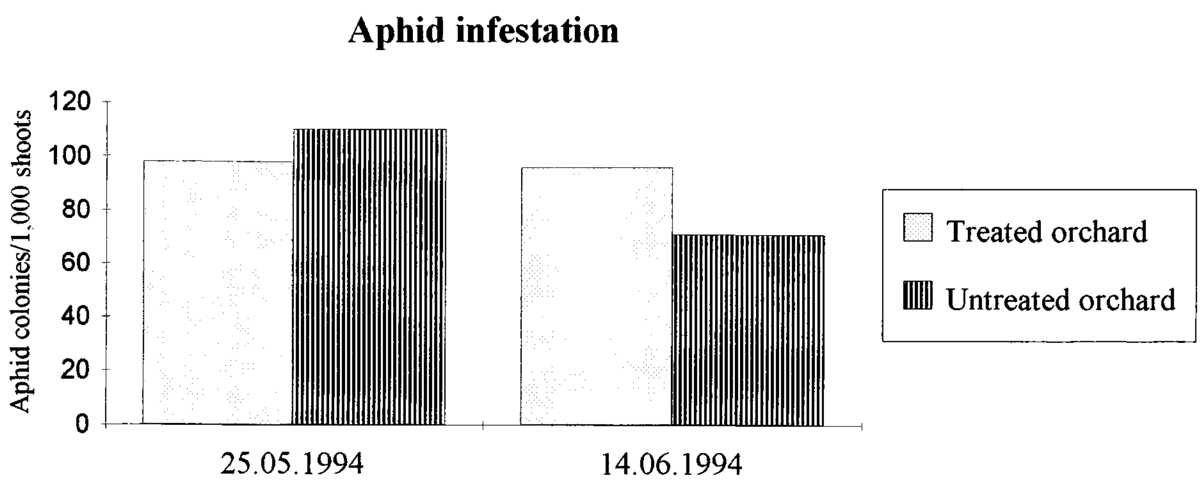


Fig.1: Aphid infestation in the orchards

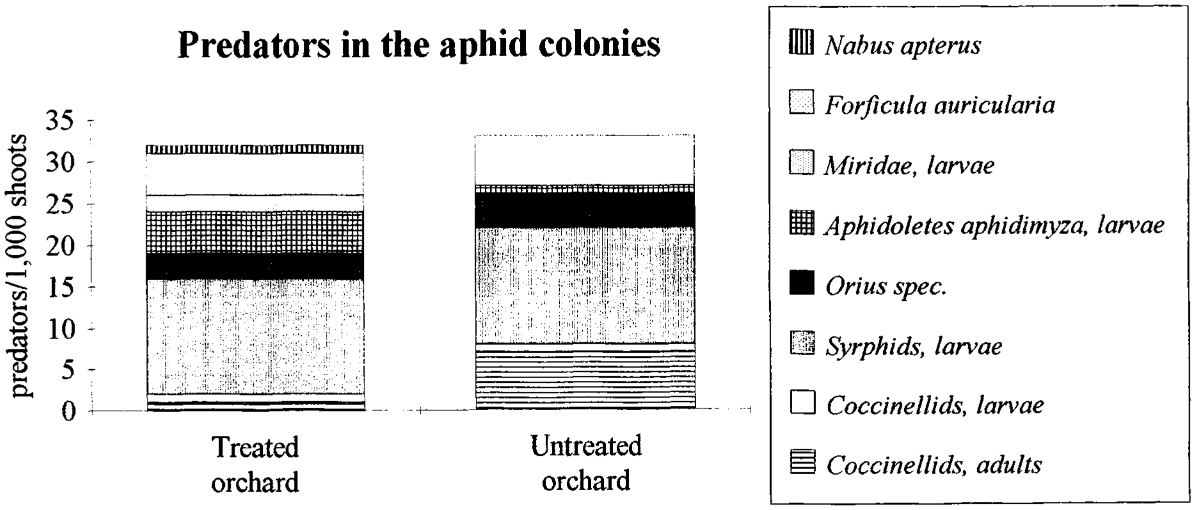


Fig. 2: Abundance of predators in the two orchards on 14.6.1994

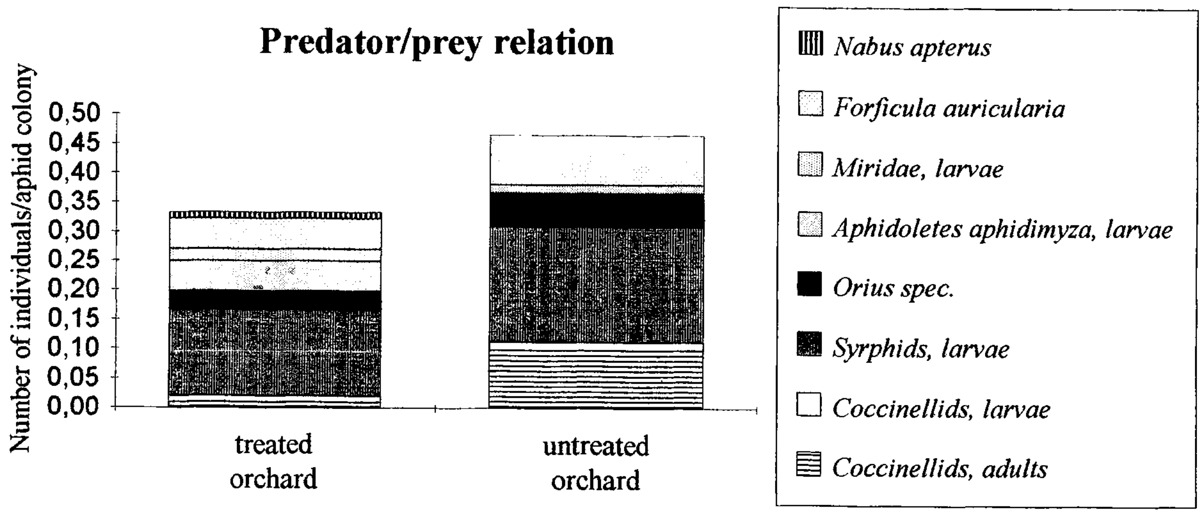


Fig. 3: Predator/prey relation on 14.6.1994

Conclusions

The arthropod species composition in the treated orchard was as expected in a normal organic orchard and very similar to the untreated one. The differences between the orchards are slight and can be attributed to different location and surroundings. The higher abundance of *Orius spec.* is probably due to the higher population of spider mites in the untreated orchard in summer. No conspicous decrease of the number of species and individuals could be observed neither after the treatment nor in the consecutive period.
Since NeemAzal was applicated after the flowering when aphid population was already increasing, the efficacy was not sufficient for their control. Thus, the effect of a NeemAzal treatment on aphid predators could also be observed. Predators occurred in similar number in the orchard. The higher abundance of predators per aphid colony relation six weeks after the treatment in the untreated orchard may be due to the lower aphid population in this orchard or to a possible minor attractiveness of the Neem treated aphids for the predators.

The methods allowed no assessment effects on fertility, fecundity and fitness of the arthropods which should be elucidated in the future. Thus, side effects of NeemAzal certainly cannot be ecluded. Considering these data, however, it can be assumed that NeemAzal might cause less disturbance of arthropod fauna and ecological equilibrium in the apple orchard than the common botanical insecticides as pyrethrum or rotenone.
Further studies will be done on this subject in the next years.

Literature

BAER, M.; KIENZLE, J.; ZEBITZ, C.P.W. (1993): Wirkung von Kaliseife auf Apfelblattläuse. In: 6. Internationaler Erfahrungsaustausch zum Ökologischen Ostbau, Weinsberg.

KAETHNER, M. (1991): Keine Nebenwirkungen von Neempräparaten auf die aphidophagen Prädatoren *C. carnea* und *C.septempunctata.* In: Anz. Schädlingskde, Pflanzenschutz, Umweltschutz **64**, 97-99.

VOGT, H. und JAKOB, G. (1993): Einsatz von Neempräparaten gegen A.orana F.v.R und Untersuchungen zu Nebenwirkungen. In: 6. Int. Erfahrungsaustausch zum ökologischen Obstbau, Weinsberg.