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# Control of the apple sawfly *Hoplocampa testudinea* Klug in organic fruit growing and possible side effects of control strategies on *Aphelinus mali* Haldeman and other beneficial insects

## Regulierung der Apfelsägewespe im Ökologischen Obstbau und Nebenwirkungen der Strategien auf die Blutlauszehrwespe

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### Abstract

The effect of Quassia extract on eggs and larvae of the apple sawfly *Hoplocampa testudinea* was studied. The efficacy of this extract is mainly due to an oral toxicity to the neonate sawfly larvae. The main active ingredients, Quassin and Neoquassin, were tested separately. Wheras Quassin has a considerable efficacy also on older larvae, Neoquassin is less efficient in this case. While Quassin and Neoquassin are found in different Quassia sources in varying relations to each other and have different efficacy, they have to be considered separately in the definition of extract quality by the content of active ingredients. These findings mean, that the "egg maturity" is not important for application date. Nevertheless, the application must take place before the larvae hatch. It was shown that low rates of Quassin (4-6 g/ha) can show very good results in the field, in other cases the rates necessary for good efficacy are much higher. This corresponds to farmers experience. Several factors as application technique and the condition of the blossom must be taken in consideration and will be object of further studies.
The side effects of Quassin, Neoquassin and Quassia extract on *Aphelinus mali* and other beneficial arthropods were tested. Quassia is harmless to all organisms tested.


**Keywords:** *Hoplocampa testudinea*, Quassia, Quassin, Neoquassin, *Aphelinus mali*

### Introduction

Traditionally, in organic fruit growing the apple sawfly *Hoplocampa testudinea* Klug is controlled by the use of extracts of Quassia wood. The good efficacy of such preparations was shown in 1986 by BLOKSMA (1994), NOACK (personal communication, 1992 and 2001) and HOEHN et al. (1996). Nevertheless, there was always some uncertainty about the best application date and the number of applications necessary in years with a prolonged hatching period of the larvae and high infestation. Really high infestations, however, were rather uncommon in the organic orchards. This situation changend in 1999 and 2000 when the apple sawfly in most parts of Germany caused striking damage. The problems seemed to be due mostly to the quality of the Quassia wood (KIENZLE et al., 2002). The main substances cited in literature as active ingredients of Quassia are Quassin and Neoquassin (Dou et al., 1996; Hager 1977 in Wichtl 1997) which are found in different Quassia wood sources in varying ratios to each other (from 1: 0,7 to 1 : 2,7).


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To develop quality criteria for Quassia wood based on the active ingredients, it is important to understand whether the effect of Quassin and Neoquassin on the apple sawfly and the non-target organisms is comparable or not. If yes, they could be summarized together, if not, each substance has to be considered separately.
The recommendations for the application date of Quassia are dissenting: There are reccomendations to spray meeting the stage “eyes of the larva visible”, others favoured the application time when the eggs become opaque. Thus, for the exact determination of the best application date it should be tested which “egg maturity” stage is most sensitive to Quassia.
Furthermore, the side effect of Quassia on beneficial organisms, especially on *Aphelinus mali* Haldeman, one of the most important beneficial arthropods in organic orchards, should be tested.

## Material and Methods

For the laboratory trials with eggs and neonate larvae (Trial 1) apple blossoms with eggs of the apple sawfly were collected in untreated orchards. The pure substances Quassin and Neoquassin or the raw extract were applied by an air brush until runoff. Not earlier than 12 hours after application of these substances to the blossoms containing the eggs, the eggs were transferred to untreated blossoms. Within this time the eggs could adsorb liquid containing the active ingredients. The transferred eggs were put on the receptacle of the new blossom and covered with a little piece of moisted filter paper to prevent desiccation. In each treatment, 40 blossoms were used. The peduncle of each blossom was put in an Eppendorf tube with pierced cap filled with water placed in a base of plaster and kept in a little transparent plastic box. Several parameters were monitored from which the feeding signs on the blossoms resulted to be the most important parameter. Three classes of feeding signs were distinguished: Complete feeding gallery, short “stopped” feeding gallery and stings (small superficial feeding signs). For the calculation of the efficacy, a weighted average was calculated using the equation: 1 x complete galleries + 0.5 x “stopped galleries” + 0.1 x stings. With these averages, for the comparison of Quassin and Neoquassin an “efficacy” (ABBOTT) of Quassin in confront to Neoquassin was calculated adopting Neoquassin as “control”. For statistical analysis only the complete galleries were considered using contingency tables ($\alpha = 0.01$). For the trials with older larvae fruits with primary or secondary infestation were collected in the field. The larvae were obtained by careful dissection of the galleries and placed on treated fruits. The fruits were picked in untreated orchards, the application and the experimental design were similar as for the neonate larvae.
In trial 2, 3 replications with 20, 11 and 18 larvae per replication were used, in trial 3 for each treatment 30 larvae were assessed. Both, the pure substances and the quassia extract with a defined content of quassin were prepared by Trifolio-M GmbH.
The field trials took place at different locations in different regions of Germany in 2002 and 2003. The trials had a randomised block design with 4 replications per treatment (6-12 trees per replication) in organic orchards. In most cases (except Ahrweiler 2002), the substances were applied with a knapsack sprayer. The primary and the secondary infestation were assessed at two different times, controlling 50 fruit clusters in each replication for infested fruits.
For the test of the side effects on *A. mali*, the test substances were diluted in deionised water for application at a volume rate of 600 L/ha. Adult *A. mali* regardless of sex, and of the age of 36-48 h, were used in the test. The parasitoids were reared at BBA Dossenheim. The exposure cages for the test of contact toxicity consisted of two treated square glass plates (6 cm) and an untreated acrylic glass ring in between held together with two rubber bands. The acrylic glass ring had four drilled holes, 0.9 cm in diameter. The exposure cage stood in vertical position. The opening at the top was to provide the fructose solution in an Eppendorf tube (0.5 ml, the tip was cut off) with cotton wool. The two holes (left and right side) for ventilation were covered with nylon netting to prevent the wasps from escaping.

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One of these openings was connected to an aquarium pump system. Each pump (200 l/h) aerated six cages. The ventilation system prevented a build-up of pesticide vapour within the exposure cages.
The opening at the bottom was used for the introduction of the wasps. It was plugged with a cork stopper during the test. For the test of oral toxicity the test units were the same as described above, modified by feeding the wasps with active ingredient diluted in a 25 % fructose solution.
The test was carried out with six replicates, containing five adult wasps of mixed sex each. Wasps were exposed to the dried residues / treated fructose over a period of 48 hours. The two glass plates from each test unit were treated on one side using a laboratory sprayer (Potter Precision Laboratory Tower, Burkard, U.K.). Before application, spray patterns were checked visually for uniformity. Within approximately one hour of application the test units were assembled and five wasps were placed in each exposure cage. The wasps were transferred carefully with a small air pump. As soon as the deposit had dried, the wasps were introduced. After the wasps were transferred into the exposure cages, the test units were placed in an environmental chamber and connected to the ventilation system. After 48 hours the test units were examined for wasp mortality using a dissecting microscope. A study was considered invalid if the mean mortality rate in the control was above 12.5 %.

## Results

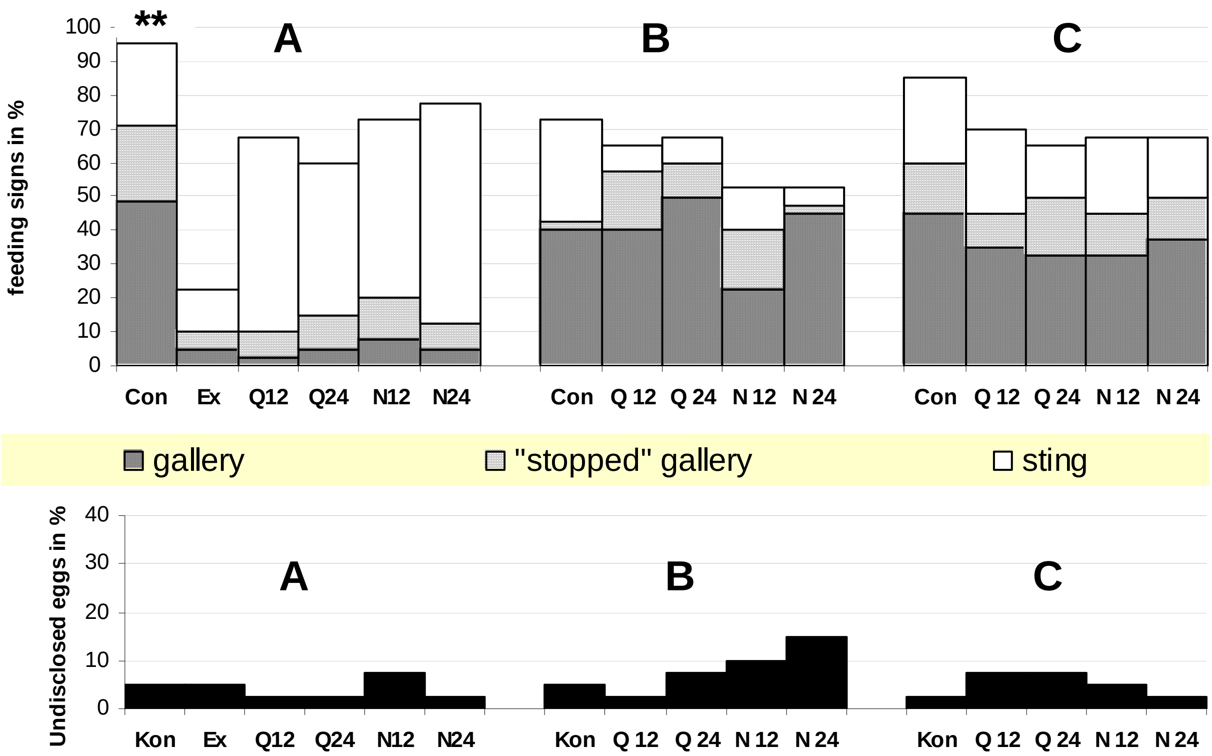


Figure 1: Effect of Quassin (Q) and Neoquassin (N) and Quassia extract (Ex) in different rates (12 and 24g/ha) on eggs and larvae of the apple sawfly when untreated eggs were transferred on treated blossoms (A) or treated eggs in stadium “hyaline” (B) or “eyes of the larva visible” (C) were transferred on untreated blossoms: Feeding signs on the blossom and number of undisclosed eggs.

There was only a negligible effect of Quassia ingredients on sawfly eggs. The larvae, however, were very sensitive to both ingredients as neonatae (figure 1). The only significant difference between the variants in figure 1 could be shown between the control and the treatments in trial A. The older the larvae, the lower is the sensitivity to Neoquassin and the higher is the difference between Quassin and Neoquassin (Table 1).

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The effect of Quassin on the older larvae was confirmed by observations in the field, that Quassia had also an effect on secondary infestation.

Table 1: Efficacy (ABBOTT) of both active ingredients and „efficacy“ of Quassin in confront to Neoquassin on neonate and older larvae in laboratory trials with treated blossoms or fruits

| Trial | Larvae | g/ha ca. | Efficacy (ABBOTT) in % Quassin | Efficacy (ABBOTT) in % Neoquassin | "Efficacy" (ABBOTT) in % Quassin in confront to Neoquassin |
|---|---|---|---|---|---|
| 1 | neonatae | 1.5 | 71.9 | 61.0 | 17.8 |
| | | 3 | 75.1 | 71.1 | 5.7 |
| | | 6 | 91.6 | 63.0 | 45.3 |
| | | 12 | 80.7 | 69.5 | 16.2 |
| | | 18 | 76.7 | 75.5 | 1.6 |
| 2 | at migration to 2nd fruit | 6 | 68.6 | 31.4 | 54.3 |
| | | 12 | 84.3 | 39.9 | 73.8 |
| | | 24 | 89.9 | 50.3 | 79.8 |
| 3 | soon after migration to 2nd fruit | 6 | 20.6 | 0.3 | 20.3 |
| | | 12 | 41.6 | 18.1 | 28.7 |

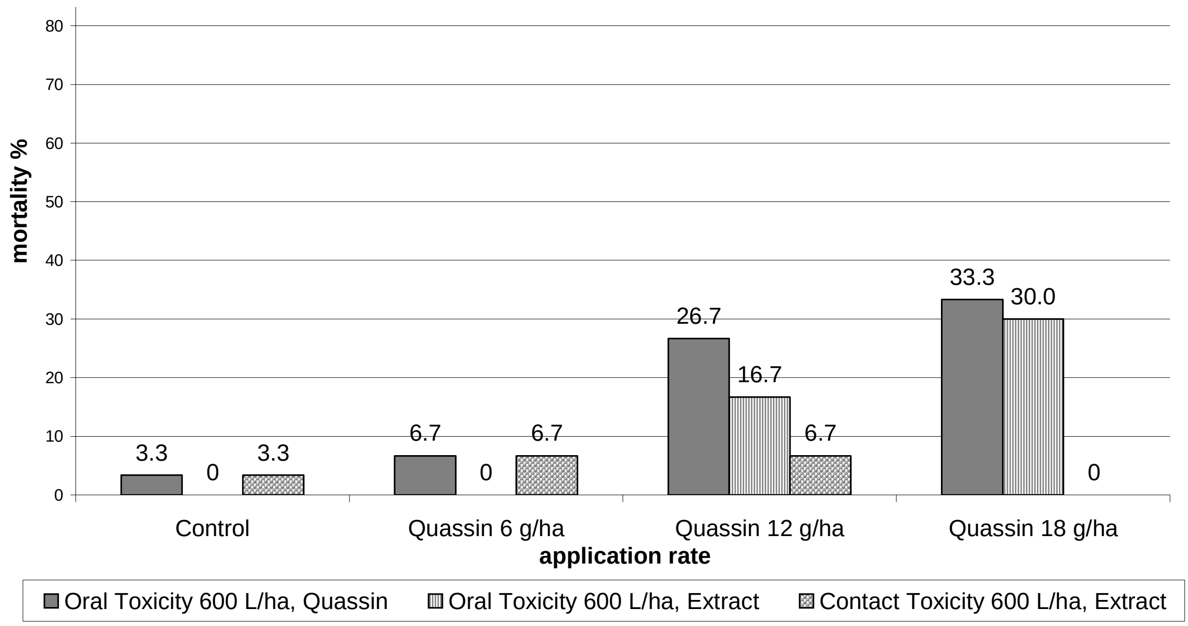


Figure 2: Side effects of Quassin (active ingredient) and Quassia extract on *A. mali*

In the laboratory, for the neonate larvae, a dose-effect-curve could not be determined because the efficacy was very good also at the lowest concentration applied (table 1). In the field, the results are varying, too (table 2). This variation, however, is not correlated with the infestation rate of the untreated control.

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Table 2: Efficacy (ABBOTT) and infestation in control in different field trials

| Locality | Year | Quassin g/ha | Efficacy of the treatment in % | | Infested fruits on 100 clusters in untreated control | |
|---|---|---|---|---|---|---|
| | | | Primary infestation | Secondary infestation | Primary infestation | Secondary infestation |
| Lake Constance I | 2002 | 8 | 66.3 | 91.5 | 43.0 | 41.0 |
| | | 12 | 80.2 | 98.8 | | |
| | | 18 | 87.2 | 91.5 | | |
| Lake Constance II | 2002 | 8 | 81.8 | 90.7 | 33.0 | 37.5 |
| | | 12 | 87.9 | 92.0 | | |
| | | 18 | 93.9 | 97.3 | | |
| Lake Constance III | 2002 | 8 | 65.7 | 82.0 | 105.0 | 100.0 |
| | | 12 | 73.3 | 82.5 | | |
| Ahrweiler | 2002 | 6 | 84.0 | 95.7 | 9.5 | 8.4 |
| | | 12 | 92.1 | 96.8 | | |
| | | 18 | 97.4 | 96.2 | | |
| Jork | 2002 | 6 | 30.4 | 70.0 | 34.5 | 65.0 |
| | | 12 | 37.7 | 66.9 | | |
| | | 18 | 65.2 | 91.5 | | |
| Lake Constance | 2003 | 4 | 92.9 | 100.0 | 70.5 | 28.5 |
| | | 12 | 97.2 | 98.2 | | |
| | | 18 | 93.6 | 100.0 | | |
| Jork | 2003 | 4 | 90.7 | * | 64.0 | |
| | | 6 | 81.3 | * | | |

** Secondary infestation not assessed because of high fruit drop*

At recommended field rate (12 g/ha) Quassin and Quassia extract were harmless to *Aphelinus mali, Forficula auricularia*, *Aphidius rhopalosiphi, Coccinella septempunctata and Chrysoperla carnea.* The results obtained for the main test organism *A. mali* are given in figure 2. Neoquassin did not show any effect.

## Discussion

According to these results, the question which embryonic stage of the sawfly larva in the egg is the most sensitive to Quassia treatments can be answered very simply concluding that it is not at all important that the eggs have contact with Quassia. On the other hand, it is very important that the neonate larvae ingest Quassia directly after hatching before they start to produce galleries. Thus, only primary damage can be controlled.

Tests conducted of YAMADA (2004) demonstrated that the most important mode of action of Quassia on the larvae is by ingestion and not by contact.

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Regarding te application time and frequency, this means, that Quassia must be applied before larval hatch. Some results indicate that Quassia is not washed easily off by precipitations. Thus, it does not seem very important for a good efficacy to carry out the treatment as close as possible to larval hatch. In contrast, it is of main importance that the active ingredients are placed in such a way, that the larvae can ingest them easily: possibly on the receptacle of the blossom. Thus, actually the interest is focused towards best application technique and the optimal shape and condition of the blossom (open or closed, fresh or fading). Nevertheless, the varying results of the dose-effect-trials in the field, which are confirmed by similarly varying experiences in farmers practice cannot yet be totally explained. In most trials and in most of the commercial orchards with uncertain results with low rates it can be excluded that the results are due to an application date that was too late (larvae already hatched). Further research has to be done to allow a reduction of the application rate and, therefore, of the cost. The results obtained until now allow to hope that this will be possible.

Another important result of these experiments is the effect of Quassia on secondary infestation. If the application date is too late and the primary infestation cannot be stopped, the secondary infestation is reduced considerably. Thus, the addition of NeemAzal-T/S to the Quassia treatment, which reduces mainly the secondary infestation, does not seem necessary – provided the Quassin rate is high enough.

Concerning the criteria for the definition of the quality of “Quassia wood” by the active ingredients Quassin and Neoquassin it can be confirmed, that Quassin is the most important active ingredient. For the difference in efficacy of both substances on elder larvae, it is not possible to summarize both substances together as “Quassinoids” since an extract with high content of Neoquassin and low content of Quassin may have a lower efficacy in control of secondary infestation than an extract with high content of Quassin.

The side effects of both Neoquassin and Quassin and the Quassia extract on the beneficials tested (*Aphelinus mali, Chrysopa carnea, Coccinella septempunctata, Forficula auricularia*) can be neglected.

## Acknowledgements

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