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Control of *Hoplocampa testudinea* using the extract from *Quassia amara* in organic apple growing

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Abstract

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In 2008 and 2009 the effects of quassin and neoquassin (oxygenated triterpenes) on apple sawfly (*Hoplocampa testudinea* Klug, 1814) were studied. In the Czech Republic, monitoring was carried out in small-plot trials and in one laboratory experiment. The extract containing quassin and neoquassin was made by boiling wood chips of a tropical shrub *Quassia amara* L. (Sapindales: Simaroubaceae). The experimental dosages were 3, 4.5, 6, and 9.25 kg of wood chips/ha. Spray treatment with the quassia extract was carried out just before most larvae hatched out. It was statistically proven that the extract from the wood of *Q. amara* reduced the apple sawfly infestation of fruitlets. Extract in the dosage corresponding to 3–4.5 kg of quassia wood chips for 1/ha appeared as optimal. The efficacy of these dosages was approximately 40–50%, and the efficacy above 80% was record.

Keywords: apple orchard; organic farming; *Malus domestica*; pest control; apple sawfly

Apple sawfly (*Hoplocampa testudinea* Klug, 1814) (Hymenoptera: Tenthredinidae) overwinters as a prepupa within the cocoon in the soil (Alford 2007). It has one generation per year. Imagos hatch out during the blossom time of early and mid early apple tree varieties (Graf et al. 2001). This pest causes significant losses and damages on apple fruits in organic orchards in Europe (Kienzle et al. 2006a; Graf et al. 2002).

The possible method acceptable in organic growing is spraying on the basis of natural bitter compounds quassin and neoquassin (Wijnen et al. 1994; Zijp, Blommers 2002). These substances belong among oxygenated triterpenes and are contained in the wood of plants of the Simaroubaceae family (Guo et al. 2005). The source of quassin and neoquassin is a shrub *Quassia amara* L. (Sapindales: Simaroubaceae), its wood contains, depending on the age, 0.14–0.28% of quassinoids (quassin and neoquassin) (Villalobos et al. 1999).

In 2002–2003 a series of experiments with a standard solution containing quassin were performed in Germany. Dosages of pure quassin of 2, 3, 4, 6, and 9 g/ha/m tree height were tested. The efficacy in most cases was over 80%; 6 g of quassin/ha/m tree height being determined as the optimal dosage (Kienzle et al. 2006a). In Germany and Switzerland a commercial preparation with standardized quassin content

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is used; however, its high price is a limiting factor for wider use. A non-standardized extract prepared directly from wood of the shrub *Quassia amara* is a cheaper alternative. This wood is readily available in Europe for a favorable price. The aim of our experiments was to evaluate the efficacy of the simply prepared, non-standardized extract.

MATERIAL AND METHODS

Locations

The research was carried out in a form of a small-plot field trial in two locations in the Czech Republic and one experiment was conducted under the laboratory conditions.

Location 1 was situated in a typical fruit area in South Moravia. The orchard was situated 20 km south from Brno (49°0' N, 16°39' E). In 2008 and 2009 two experiments were performed in an apple orchard of the Idared variety in spacing 3 × 4.5 m.

Location 2 was situated in Eastern Bohemia in the orchards owned by the Research and Breeding Institute of Pomology in the village of Holovousy (50°22'N, 15°34' E). The experiment was carried out in 2009, on apple trees of the Šampion variety in spacing 3 × 4.5 m.

Extract preparation

Extract was prepared from wood chips of the *Quassia amara* shrub. Quassia wood chips were supplied by the firm Keller GmbH & Co. KG (Freiburg, Germany). The supplier did not specify proportions of chips in the product description (our estimation of main fraction: length 0.5–1 cm; width 0.3–0.5 cm; thickness 0.2–0.3 cm). The extract was prepared shortly before its application (2–4 days). Quassia wood chips (100 g) were boiled in 2 l of water for one hour following the procedure described by Dodia et al. (2008) and Zijp, Blommers (2002). During boiling, chips slowly dropped to the bottom and finally they settled there. Subsequently, brown-colored solution was decanted and for a short period (max. 4 days) stored in a fridge.

Small-plot trials

The experiments were established in randomized blocks with four replications of each treatment. One replication included 4 trees in location 1, and 5 trees in location 2. Flight of apple sawfly adults was monitored using white sticky traps based on the method of Lukáš and Kocourek (1998).

The selected dosage of application water volume was 400 l/ha. Volumes of the concentrated extracts applied corresponded to the dosages of 3, 4.5, 6, and 9.25 kg of boiled quassia wood 1/ha. The wetting agent Silwett L-77 (0.1 l/ha) was added to the spray solution.

The spray treatment was applied with a knapsack motor sprayer.

In both seasons, the embryonic development of larvae in egg was monitored. Treatment dates were scheduled according to the embryonic phase of larvae so that spraying could cover a maximal possible number of hatching larvae. The efficacy of the extract was calculated from the acquired data by applying the Abbott's formula (Abbott 1925). Data were statistically evaluated using the analysis of variance (ANOVA) in programs UPAV GEP 1.6 (State Phytosanitary Administration) and Statistica 8 (StatSoft). Statistical difference among the treatments was determined according to the Tukey's test.

Experiment conducted in 2008

In 2008 artificial infestation was performed. On April 17 the first apple sawflies were recorded on white sticky traps. On April 25 trapping of the adults was carried out. Captured flies were closed to monofilament isolators. One shoot at full blossom (April 25) on each tree was covered with the isolator and two apple sawfly females and one male were placed to each isolator. The isolators were removed on April 29.

Subsequently, the embryonic development of larvae was assessed on twenty blossoms with an apple sawfly egg each day. On May 5, it was determined that in 70% of eggs, the sawfly larvae were in the phase before hatching. One day later it was already 90% and 5% of hatched larvae were recorded. Spray treatment was conducted the following day. The tested dosages of quassia wood were 3, 4.5, and 9.25 kg wood/ha in one application (May 7) and the dosage of 3 kg/ha which was applied twice, the second spraying was carried out after five days, it means on May 12. The control variant was only treated with water containing the wetting agent.

On May 16 the infestation on the marked shoots was evaluated. Within each replication, fruitlets with apple sawfly eggs were counted and then compared to a number of fruitlets infested by hatched apple sawfly larvae.

Experiment conducted in 2009

The level of apple sawfly infestation was high. Therefore in 2009 the experiments were carried out with natural level of infestation without any artificial infestation.

Location 1: The first apple sawflies on white sticky traps were recorded on April 16 and on April 20 the infestation nearly finished. From April 20 the embryonic development of larvae in eggs was daily evaluated. The first spray treatment was applied on April 23, it means when most of the larvae (78%) had finished their embryonic development. The second spraying was carried out on April 24 when most of the larvae were just before hatching or had already started hatching. The third spraying was performed on April 25, it means on the day when most of the larvae had already hatched. Variants with one spray treatment (3 or 4.5 kg/ha) were applied on April 23. On April 24 treatment dosage was 6 kg/ha. On April 23 and April 25, variants with both spray treatments (3 and 4.5 kg/ha) were applied. The control was treated only with water containing the wetting agent. The evaluation was carried out on May 11. Within each replication, 150 fruitlets were assessed, i.e. 600 fruitlets/variant. Numbers of non-infested fruitlets and those damaged by sawfly larvae were determined.

Location 2: Flight of the apple sawfly adults was monitored using the white sticky traps from the beginning of April. From April 24 the embryonic development of larvae in eggs was monitored. In this location in addition to the quassia extract, organic insecticide NeemAzal T/S (azadirachtin) and conventional insecticide Mospilan 20 SP (acetamiprid) were applied.

The first spray treatment was applied on April 30 when most of the larvae were just before hatching. The second application was performed on May 4, when most of the larvae had already hatched. Quassia wood extracts 3, 4.5, and 6 kg/ha were applied in two variants; in the variant with one spray treatment (April 30) and the variant with two spray treatments (April 30 and May 4). The insecticide NeemAzal T/S 3 (l/ha) was applied in two variants, i.e. in the variant with one spray treatment (April 30) and in the variant with two spray treatments (April 30 and May 4). The conventional insecticide Mospilan 20 SP was applied only in one dosage of 0.25 kg/ha on May 4. The control was treated only with water containing the wetting agent. The evaluation was performed on May 12 and within each replication 100 fruitlets were evaluated, i.e. 400 fruitlets/variant. Again, the numbers of non-infested fruitlets and those damaged by sawfly larvae were determined.

Laboratory trial

Blossoms with hatched eggs of apple sawfly were collected in location 2 on May 4, 2009. The blossoms were incubated in a climabox under a long-day regime and at the temperature of 20°C in the Crop Research Institute, Prague-Ruzyně. On May 7 most of the larvae were just before hatching according to the embryonic development and the treatment was carried out with an apparatus for manual spraying. The spray treatment was performed in concentrations corresponding to the dosage 3 and 4.5 kg quassia wood chips/ha. The spray solution did not contain any wetting agent to assess its efficiency. The control was left untreated. Each variant had 15 blossoms. On May 13 the experiment was evaluated, the number of fruitlets where sawfly larvae successfully hatched and continued to rear and the number of fruitlets where larvae died after the spray treatment were determined. Statistical evaluation was performed using a simple non-parametric sign test.

RESULTS

Year 2008 (small-plot trial)

In this season, heavy apple sawfly infestation was achieved by use of isolators. Infestation in the control variant was 56.7%. The efficacy of the one spray treatments of 3, 4.5, and 9.25 kg/ha was 55.03%, 58.02%, and 65.43%, respectively. Surprisingly, the variant with two spray treatments of 2 × 3 kg/ha was the least efficient (only 37.92%) (Table 1).

The dosage affected the level of infestation statistically significantly (F = 23.8063, P = 0.05*). All three dosages differed from the control statistically significantly and also the variant with two spray treatments differed statistically significantly from the variant with one treatment. No statistically sig-

Table 1. Infestation, efficacy, and statistical difference of the treatments in location 1 in 2008

Treatment	Infestation (%)	Efficacy (%)	95%
Quassia 3 kg	25.5	55.03	A
Quassia 4.5 kg	23.8	58.02	A
Quassia 9.25 kg	19.6	65.43	A
Quassia 3 kg – 2 ×	35.2	37.92	B
Control	56.7	–	C

Differences between the treatments were determined with the Tukey's test

nificant difference was found between the variants with one treatment (Table 1).

Year 2009 – location 1 (small-plot trial)

In 2009 relatively high natural apple sawfly infestation occurred. In the control, 13.33% of fruitlets were damaged with apple sawfly larvae. The one spray treatments with quassia extracts of 3, 4.5, and 6 kg/ha achieved the efficacy of 50.34%, 28.27%, and 39.86%, respectively. The two spray treatments with 3 and 4.5 kg/ha achieved the efficacy of 39.19% and 41.22%, respectively (Table 2).

Similarly to 2008, the dosage affected the level of infestation statistically significantly (F = 5.033, P = 0.05*). Statistical differences were not detected between the variants with one treatment with quassia extracts of 4.5 or 6 kg/ha and variants with two spray treatments of 3 and 4.5 kg/ha. The one spray treatment of 3 kg/ha was the only one that differed statistically significantly from the control and the other treatments (Table 2).

Table 2. Infestation, efficacy, and statistical difference of the treatments in location 1 in 2009

Treatment	Infestation (%)	Efficacy (%)	95%
Quassia 3 kg	6.13	50.34	B
Quassia 4.5 kg	8.79	28.72	AB
Quassia 6 kg	7.42	39.86	AB
Quassia 3 kg – 2 ×	7.50	39.19	AB
Quassia 4.5 kg – 2 ×	7.25	41.22	AB
Control	13.33	–	A

Differences between the treatments were determined with the Tukey's test

Table 3. Infestation, efficacy, and statistical difference of the treatments in location 2 in 2009

Treatment	Infestation (%)	Efficacy (%)	95%
Quassia 3 kg	2.00	82.61	BC
Quassia 4.5 kg	1.50	86.96	BC
Quassia 6 kg	0.75	93.48	BC
Quassia 3 kg – 2 ×	1.25	89.13	BC
Quassia 4.5 kg – 2 ×	0.25	97.83	C
Quassia 6 kg – 2 ×	0.25	97.83	C
NeemAzal 3 l	2.25	80.43	B
NeemAzal 3 l – 2 ×	1.75	84.78	BC
Mospilan 20 SP	1.00	91.30	BC
Control	11.50	–	A

Differences between the treatments were determined with the Tukey's test

Year 2009 – location 2 (small-plot trial)

Similarly to location 1, heavy natural apple sawfly infestation occurred here. In the control, 11.5% of fruitlets were damaged with apple sawfly larvae. In this experiment surprisingly very high efficacy in all the dosages tested was achieved.

The one spray treatments with quassia extracts of 3, 4.5, and 6 kg/ha achieved the efficacy of 82.61%, 86.96%, and 93.48%. The two spraying treatments with 3, 4.5 or 6 kg/ha achieved the efficacy of 89.13%, 97.83%, and 97.83%, respectively (Table 3).

The dosage had a statistical significant effect on the infestation level (F = 13.081, P = 0.05*). All the treatments tested differed statistically significantly from the control (Table 3). The insecticide NeemAzal T/S achieved the efficacy of 80.43% in the variant with one treatment and the efficacy in the variant with two spray treatments was 84.78%. The efficacy of the insecticide Mospilan 20 SP achieved 91.30%.

Laboratory trial

The variants with one treatment (3 or 4.5 kg/ha) achieved the same efficacy of 36.36% under the controlled conditions of the laboratory trial. Damage of the control treatment was 73.33%.

The tested dosages did not differ statistically from the control (P > 0.05).

DISCUSSION

The present results show that the extract prepared directly from wood chips of *Quassia amara* is statistically significantly efficient against apple sawfly larvae. In 2009, in location 2 this extract was statistically equally effective as the conventional synthetic insecticide Mospilan 20 SC (Table 3).

The achieved efficacy results of the individual experiments show that the optimal dosage is 3–4.5 kg of *Quassia amara* wood chips/ha of an orchard. According to Villalobos et al. (1999) it corresponds to 4.2–8.4 g of quassinoids (3 kg/ha) and 6.3–12.6 g of quassinoids (4.5 kg/ha). Although releasing of quassinoids to water solutions was already confirmed by Roark (1947) we do not suppose that all the quassinoids contained in wood get into the extract. We can assume that the size of chips, i.e. size of the active surface, affects the extraction rate and quantity of the substance extracted into the solution. However, the results indicate that under the conditions of the method used by us, a sufficient amount of the effective substances was extracted into the water solution. The treatment with higher dosages of Quassia extract (6 kg/ha or 9.25 kg/ha) did not achieve statistically significantly higher efficacy. In 2009 in location 1 the most efficient dosage was 3 kg/ha. This result could be affected by considerably uneven infestation of the individual replications.

Kienzle et al. (2006a) recommended the optimal dosage of 6 g of pure quassin/ha. This dosage can be achieved with standardized commercial preparations containing quassin without any problems, however acquisition costs are high. In the case of simply prepared wood extract, not only quassin but also neoquassin get into the extract. Both these substances are effective to newly hatched larvae.

In the laboratory trial, the efficacy of only 36.36% was achieved in the variant with one spray treatment of 3 or 4.5 kg/ha. In the small-plot trials, the efficacy achieved with these dosages was considerably higher. This difference could be caused by the absence of the wetting agent in the laboratory trial. Therefore, we assume that the wetting agent plays an important role in the case of the use of quassia wood extract. It was also confirmed by Kienzle et al. (2006b) who determined higher efficacy of quassin in case a wetting agent was added to the solution. Silwett L-77, the wetting agent used, is not permitted by the Czech law for organic agriculture. The possible wetting agents for organic agriculture are e.g. on the basis of plant oils and natural tensides (State Phytosanitary Administration 2010; Zebitz 2005). In addition, quassia wood extract can be used in integrated production where a wide spectrum of wetting agents (including Silwett L-77) can be applied. Volume of water is also significant. Kienzle et al. (2006b) determined that the efficacy increases with the volume of applied water and the optimal water volume appears to be 500 l/ha.

Based on the results achieved, it is possible to conclude that the use of the aqueous extract prepared from 3 or 4.5 kg quassia wood chips/ha reduced the apple sawfly infestation of apple fruitlets from 50–85%. Extract prepared from higher dosages of quassia wood chips or two consecutive sprays were not statistically significantly more efficient. Further research should focus on the evaluation of quassia extract application in practice; the experiment should be tested in the scale of several hectares.

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Effect of low oxygen storage conditions on volatile emissions and anaerobic metabolite concentrations in two plum fruit cultivars

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Abstract

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By harvest time, small amounts of acetaldehyde were accumulated in the flesh of plums, such as 0.31 mg/l for the cv. Stanley and 1.03 mg/l for the cv. Valjevka. This relative difference in concentrations remained constant throughout the whole period of storage in a regular atmosphere. The long-term effects of higher concentrations of CO_2 are the same as for very low oxygen concentrations; and significant amounts of ethanol accumulate in the tissue. Out of a total number of 42 different odour compounds identified in the juice, there were 11 alcohols, 6 aldehydes, 17 esters, 2 terpenes, 3 organic acids, and 1 lactone. Very low oxygen atmospheres slow down the production of esters and aldehydes, but have little effect on the production of lactones and terpenes. It was shown that a very low oxygen concentration, without much CO_2 (Fluctuating anaerobiosis treatment), does not encourage the production of significant amounts of ethanol and acetaldehyde in the fruit flesh, but does significantly slow the biosynthesis of aromatic volatiles.

Keywords: plum fruit; volatiles; ethanol; acetaldehyde; firmness; headspace gas analysis

The quality of plum fruit following harvest is essentially influenced by the temperature and composition of the ambient atmosphere. The storage period is limited by fruit softening, visible signs of wilting and a physiological disease manifested as an internal browning adjacent to the stone (Taylor et al. 1993; Goliáš 2004; Toivonen, Brummell 2008). Knowing the lower oxygen limit for effective aerobic metabolism is critical for managing the composition of the gaseous atmosphere (Gran, Beaudry 1993; Beaudry 1999; Menniti et al. 2006). A gas atmosphere where the ethanol concentration does not increase over time is considered to be optimal for long-term storage (Smagula, Bramlage 1977; Nichols, Patterson 1987; Pesis 2005). If oxygen levels drop below a certain critical point for the aerobic conversion of storage substrates, then pyruvate is converted into acetaldehyde and ethanol with adverse affects on fruit quality.

Fermentation processes which proceed as a consequence of fruit ripening, even without low oxygen levels in the ambient atmosphere, also result in the accumulation of ethanol and consequently the production of ethyl esters in concentrations which may cause off-flavours. Specifically, an unpleasant odour and taste results from the production of ethyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, ethanol, and acetaldehyde (Lara et al. 2007), whereas there

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