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Effects of botanical antifeedants on *Melolontha melolontha* grub feeding on Scots pine roots

Iwona Skrzecz ✉, *Alicja Sowińska*, *Wojciech Janiszewski*

[1] Forest Research Institute, Department of Forest Protection, Sękocin Stary, Braci Leśnej 3, 05-090 Raszyn, Poland, phone: 48 22 7150541, fax: 48 22 7150557, e-mail: i.skrzecz@ibles.waw.pl

Abstract

The aim of the study was to evaluate the possibility of using botanic antifeedants to reduce the damage caused by Melolontha spp. grubs. To achieve the objective, the experiments were established in semi-field conditions to estimate the antifeedant activity of rutin, quercetin (flavonoids from buckwheat *Fagopyrum esculentum*) and an extract from black alder *Alnus glutinosa* leaves against *Melolontha melolontha* grubs. The grubs were placed individually in the pots with a soil in which 2 year old *Pinus sylvestris* trees were planted. The pots were put in garden pavilions placed in the open area. Then the soil in the pots were watered with the emulsions of rutin, quercetin, an extract from *A. glutinosa* leaves, and with pure water-comparative variant. After 4 months, the weight and mortality of grubs were compared, as well as the weight of tree roots in all pots.

There was no effect of the antifeedants on the development and extent of damage caused by *M. melolontha* grubs. The results do not indicate the use of botanic antifeedants in the protection of forests against the cockchafer grubs.

Key words

botanical antifeedants, *Melolontha melolontha*, protection of forest

Introduction

Control of grubs and imagines of common and forest cockchafers (*Melolontha melolontha* L. and *Melolontha hippocastani* Fabr.) has recently become one of the most important challenges in plant protection. Not long ago, the most frequently used method of forest protection against *Melolontha* spp. grubs was the application of organophosphate and carbamate insecticides into soil (Malinowski 2009). Nonetheless, regular pesticide use reduction in plant protection practice due to law regulations within the European Union resulted in eliminating from the market the majority of chemical insecticides so far used for cockchafer control.

At the present time, one of the orientations of scientific research on non-chemical protection of agricultural and forest crops against insects are studies on utilization of naturally occurring substances in certain plants, which negatively affect insect feeding (antifeedants). In forestry, such studies have been mainly focused on using antifeedants in the protection of afforested areas against pine weevils *Hylobius abietis* L. (Korczyński and Owczarek 2001; Kuźmiński 2002; Schlyter 2004; Månsson et al. 2005), and not so much attention has

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been drawn to investigations on the use of these substances for diminishing cockchafer grub feeding in forests (Malinowski 1997; Woreta 1997).

A possibility of application of antifeedants to decrease damages due to cockchafer grub feeding was pointed out by Malinowski et al. (1999) who observed reduced grub numbers on buckwheat *Fagopyrum esculentum* Moench fields, and therefore they advocated that buckwheat plants had cockchafer antifeedant properties. Yet, even though the results obtained showed potential, further studies on reducing cockchafer numbers in afforested areas were focused on the use of soil chemical insecticides (Malinowski 2009, 2011).

The lack of future prospects for further development of the methods connected with chemical insecticide treatments or else effective alternate means motivated the present study, which was carried out in 2011–2013 and aimed to assess the possibility of utilization of botanical antifeedant substances for decreasing damages due to *Melolontha* grubs in forests. To achieve the objectives laid down, the experiments were conducted on antifeedant activity of buckwheat *F. esculentum* flavonoids (rutin and quercetin) and that of black alder *Alnus glutinosa* Gaertn. extract against common cockchafer *M. melolontha* grubs.

Material and methods

Research material

Biological material consisted of second instar (L2) *M. melolontha* grubs collected annually in the middle of May on afforested areas within the Forests Districts Zwierzyniec (east-southern Poland) and Smardzewice and Opoczno (central Poland).

The grubs observed were fed on the roots of 2-year-old pine *Pinus silvestris* L. seedlings obtained from forest nursery in the Forest District Skierniewice (central Poland).

Antiffedant formulations for testing were prepared by the Chemipan R&D Laboratories (Institute of Physical Chemistry in Warsaw, Polish Academy of Sciences). The following formulations were tested:

– black alder extract: an extract from 50 g of black alder leaves mixed with 100 ml of rapeseed oil,
– rutin: 2g rutin/100 ml canola oil,
– quercetin: 2g quercetin/100 ml canola oil.

Cockchafer grub breeding in soil treated with water-based antifeedant formulations

Cockchafer grubs were bred in 20 cm wide and 30 cm high pots filled with peat soil mixed with sand at 1 : 1 ratio. One pine seedling was grown in each pot.

The grubs were weighed just before placing single specimens into soil in each pot. Then pot soil was treated with 10% water emulsion of rutin, quercetin and black alder extract formulations at a rate 0.5 l/pot. The pots with individual grubs feeding on pine seedlings treated with pure water (0.5 l/pot) represented the control treatment. At the same time, the pots with pine seedlings but no grubs were put aside for observations of tree growth in the pots irrigated at the same timing and with the same volume of water as it was done for other experimental variants (comparative treatment).

Altogether 400 grubs were observed under the conditions of 4 treatments (rutin, quercetin, black alder extract, control – 100 grubs in 100 pots for each treatment). The pots with grubs as well as those without them (comparative treatment – 100 pots) were placed in 2.5 m high net tents (gardening pavilions) with 9 m^2 basal area. The net had 2x2 mm holes which allowed appropriate light conditions and free air exchange. At the same time, the net constituted a barrier against other insects and also – birds (photograph 5.2). The tents were situated within an open area (around the Research Forest Institute, Warsaw).

The observations were carried out starting from the fourth decade of May until the first decade of September. During this period, every 2–3 weeks, pot soil in all experimental variants was treated with the formulations tested mixed with water or only with water. At the end of June and at the end of observations in a given year, the health and weight of the grubs were assessed. At the end of the observations, there was also assessed mortality of pine trees. The roots of all trees observed were cut off, dried out and then weighed.

During the observations carried out in 2013, there was used AgroHydroŻel (AGROIDEA, Krakow, Poland), the gel which is commonly applied in agricultural production, among others to improve soil structure as well as its water capacity through absorption and then slow release of water. Soil application of the gel helps to prevent soil drying out, and as a result plant growth conditions are improved. In the present

study, the gel was used so as to prolong the period of antifeedants' activity in soil and also to enhance their adsorption on pine seedling roots as well as to reduce antifeedant treatment frequency (in other words: to decrease of the number of antifeedant and water treatments).

In this part of the study, 10% water emulsion of rutin, quercetine and black alder extract formulations were supplemented by AgroHydroŻel at a rate recommended by the producer – 50g/10l of the liquid. Next, pine seedling roots were dipped (5 s) in the antifeedant liquids and placed individually into soil in 60 pots per each treatment (with one cockchafer grub per each pot). The control treatment embraced the pots with individual specimens of grubs and pine seedlings, the roots of which were dipped in no more than AgroHydroGel mixed with water before placing in the pots. At the same, the comparative treatment consisting of 60 pots with pine seedlings with the roots dipped in AgroHydroGel mixed only with water and no grubs put in, were set aside for observations of pine seedling growth.

In 2013, on the whole 260 grubs were observed under the conditions of 4 treatments (rutin, quercetine, black alder extract, control – 60 grubs in 60 pots for each treatment). The observations were carried out from the third decade of May until the end of August. The pots were irrigated only with water every 2–3 weeks. At the end of August grub health and weight were assessed. At the same time, pine seedling mortality was evaluated. Next, the roots of all trees observed were cut off, dried out and weighed.

Statistical analyses

The differences between the weights of the cockchafer grubs bred in differently treated soils as well as those between the weights of the roots of pine seedlings grown in these soils were statistically tested by means of one-way ANOVA. Homogenous groups were determined with the use of Tukey's test. All the tests were performed using Statistica® 8.

Results

Effect of antifeedant treatments on cockchafer grub development and damages in pine seedlings

The weight of grubs

The weights of cockchafer grubs at the start of observations (May) were comparable – at a range from 0.56 to 0.68 g, with the mean value 0.62 ± 0.06 g (±SD, $F = 2.649712$, $p = 0.069785$). With time, grub weights were gradually increasing in all the treatments. In July, grub weights were at a range 0.88–1.12 g (on average 0.89 ± 1.12 g), and in September – from 1.06–1.47 g (on average 1.34 ± 0.06 g) (fig. 1). In September, at the end of the observations, no statistical differences were found between body weights of the grubs bred in soil treated either with specific antifeedants or only water ($F = 0.487632$, $p = 0.693849$).

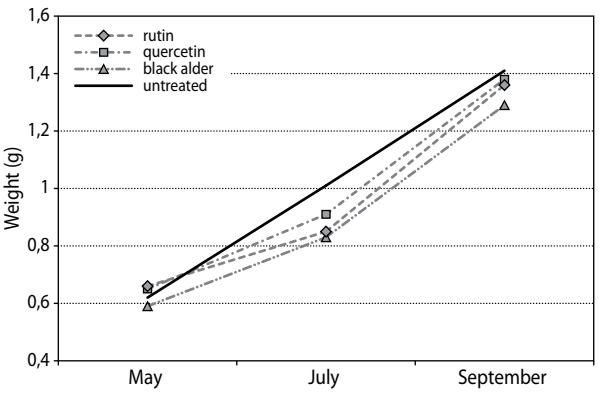

Figure 1. Changes of grub weight depending on applied treatment

Cockchafer grub mortality

Mortality of the grubs was comparable for all the treatments and amounted to 25% in case of specimens feeding on pine roots treated with rutin, 29% – with quercetin, 24% – with black alder extract and 26% in the control treatment.

Mortality of pine seedlings

The lowest mortality (39%) was observed in the group of pine seedlings treated with quercetin. In the rest of antifeedant treatments, there died 43–48% of trees. The highest mortality of pine trees was observed in the con-

trol treatment – 52%, whereas only 5% of the trees died in the comparative treatment group.

Comparison of seedling root dry weights

One–way ANOVA showed no statistically significant differences between mean root weights in the pine seedlings treated with the antifeedants when compared with the control trees, which all grew in the pots with soil infested by grubs. Regardless of the treatment, all the roots were damaged and their weights were significantly lower than in the trees grown in the pots with no grubs in soil (comparative treatment) ($F = 9.76$; $p < 0.0001$; tab. 1).

Table 1. Mean weights of roots of pine seedlings depending on treatment variants

Treatment	Root weight (g) ± SD
Rutin	0.579 ± 0.121a*
Quercetin	0.594 ± 0.367a
Black alder extract	0.627 ± 0.425a
Control**	0.631 ± 0.346a
Comparative***	2.571 ± 0.651b

* different letters indicate statistically significant differences between means at $\alpha = 0.05$; ** trees dipped with water without antifeedant and grown in soil with 1 grub; *** trees dipped with water without antifeedant and grown in soil with no grub.

Effects of antifeedants supplemented with AgroHydroĐel on cockchafer grub development and damages in pine seedlings

Body weight of the grubs

The weights of the cockchafer grubs at the start of observations (May) were comparable – at a range from 0.52 to 0.81 g (on average 0.68 ± 0.09 g). At the end of August, grub body weights ranged from 0.99 g in rutin treatment to 1.14 g in quercetin treatment. No statistical differences between the treatments tested were found (tab. 2).

Mortality of the grubs

Comparable mortality of the grubs was observed in all the treatments tested. In case of the specimens feeding on pine roots dipped in rutin it was 29%, quercetin – 32%, black alder extract– 28% and when the grubs fed on not treated roots it was 27%.

Table 2. Weight of *M. melolontha* grubs feeding on *P. sylvestris* seedling roots dipped in antifeedant emulsions supplemented with AgroHydroŻel

Treatment	Mean grub weight (g) ± SD
Rutin	0.99 ± 0.15
Quercetin	1.14 ± 0.23
Black alder extract	1.02 ± 0.17
Control*	1.04 ± 0.25

* grub breeding on pine roots dipped in AgroHydroŻel without antifeedant.

Mortality of pine seedlings

Mortality of pine seedlings grown in the pots infested by cockchafer grubs was similar in all the treatments. When treated with antifeedant and AgroHydroŻel, tree mortality was 23%, 25% and 29% for rutin, quercetine and black alder extract, respectively, and 28% for the control treatment. There survived all trees in the comparative treatment group.

Comparison of root dry weights

One-way ANOVA showed no statistical differences between mean dry weights of seedling roots dipped either in antifeedant formulations mixed with water or only in water, which all grew in the pots infested with cockchafer grubs. All the roots were damaged and their weights were significantly lower than those in the control group ($F = 11.97$; $p < 0.0001$) (tab. 3).

Table 3. Weight of *P. sylvestris* seedling roots dipped in antifeedant emulsions supplemented with AgroHydroŻel

Treatment	Mean root weight (g) ± SD
Rutin	0.629 ± 0.268a*
Quercetin	0.775 ± 0.146a
Black alder extract	0.872 ± 0.173a
Control**	0.942 ± 0.512a
Comparative***	2.851 ± 0.355b

* different letters indicate statistically significant difference between means at $\alpha = 0.05$; ** trees grown with AgroHydroŻel without antifeedant in soil with 1 grub; *** trees grown with AgroHydroŻel without antifeedant in soil with no grub.

Discussion

In Poland, initial studies pointing to prospects of the use of specific plants for reducing damages due to cockchafer feeding were carried out already at the end of the 19th century. Satkowski (1899) observed and described that afforested areas were free of grubs if there was grown tartary buckwheat *Fagopyrum tataricum* Gaertn. At the same time, forest areas around – where this plant was not cultivated, were highly infested by cockchafer grubs. In 1926, there were published the results of Różyński who observed that cockchafer grubs did not feed on black alder roots. The same author confirmed that the grubs occurred considerably deeper in soils where buckwheat or lupine *Lupinus* L. were grown when compared with the areas with no cultivation of these plant species. Similarly, Ulatowski (1932) reported considerably lower grub numbers in afforested areas on which buckwheat was grown before planting forest trees. More than six decades later, Malinowski et al. (1999) also showed comparatively lower cockchafer grub numbers in the areas where buckwheat was cultivated.

In search for the reasons behind repellent activity of buckwheat plants against insects, there were carried out chemical analyses, the results of which pointed out the occurrence of large amounts of flavonoid compounds in this plant species: rutin (quercetin 3-rutinoside), quercetin (quercetin 3-rhamnoside) and hyperoside (quercetin 3-galactoside) (Campbell 1997). In the following studies, there were confirmed antifeedant properties of the above substances against aphids (Dreyer and Jones 1981) and butterflies of the genus *Heliothis* (Harbourne 1997). Concurrently, Parmar and Walia (2001) listed rutin in the group of compounds bringing about pupa deformations as well as larva mortality in tobacco hornworm *Manduca sexta* L. and Colorado potato beetle *Leptinotarsa decemlineata* Say.

In the present study, there were found no effects of rutin, quercetine and black alder leaf extract on cockchafer grub development and the extent of damage caused by grub feeding. Regardless of the treatment applied to the pine seedlings (watering with antifeedant formulations or irrigation with water) no differences in weights and mortality of the grubs developing in soil around the trees were found. At the same time, no differences were observed in root weights of antifeedant treated seedlings which grew in the presence of cockchafer grubs when compared to the control trees (treated with water, growing in soil with one grub). Similar results were obtained when seedling roots were dipped in water emulsions of the antifeedants tested which were mixed with AgroHydroŻel added to increase stability of the compounds applied in soil. The results indicate that application of rutin, quercetin or black alder leaf extract formulations does not protect pine seedling roots against *Melolontha* spp. grubs.

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