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The performance of *Melolontha* grubs on the roots of various plant species

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Abstract: *Melolontha* grubs are polyphagous and are adapted to feeding on plants of varying nutritional value. Our research sought to investigate whether host plant quality affects first-instar grub development, weight gain, or mortality. Ten plant taxa of the families Polygonaceae, Brassicaceae, Asteraceae, Fabaceae, and Pinaceae were tested. The quality of each plant species was estimated based on the root content of phenols, condensed tannins, soluble sugars, starch, nitrogen, and carbon. Retarded development, high mortality, and low weight gain were observed in grubs feeding on roots of *Fagopyrum esculentum*, *Brassica rapa* subsp. *rapifera*, and *B. napus*, whereas *Tanacetum vulgare*, *Trifolium repens*, and *Lupinus polyphyllus* roots proved to be the most beneficial for larval performance. Weight gain was positively correlated with the concentration of soluble sugars and starch in the plant roots. Starch was also positively correlated with the percentage of molted grubs. The revealed positive correlations may be explained by the fact that nonstructural sugars constitute an energy source that is essential for grub movement in the soil. Plant species that negatively affect cockchafer grubs may be used in integrated plant protection against these pests in agriculture, horticulture, and, to some extent, in forestry, e.g., in nurseries and postagricultural lands.

Key words: Cockchafer, legumes, mustards, plant quality, Scots pine, tansy

1. Introduction

Two species of *Melolontha* (Coleoptera, Scarabaeidae) are widely distributed throughout Poland: *M. melolontha* (L.) (the common or May cockchafer) and *M. hippocastani* F. (the forest cockchafer). Their biology is very similar. The beetles usually fly in late April and in May. After emerging from the soil, they begin maturation feeding in the crowns of the host trees. They then mate, and the females lay eggs in the soil. The larvae (grubs) feed initially on humus and later on the fine roots of grasses and other plants. Their development usually lasts 4 years, although it may be shortened or extended by environmental conditions (Sierpiński, 1975; Śliwa, 1993).

The adult cockchafers feed on the leaves of various tree species, and substantial losses in agriculture, horticulture, and other production systems in different European countries are caused by root-feeding grubs (Keller and Zimmermann, 2005), similar to other below-ground herbivores across the world (Hunter, 2001). Since the 1990s, cockchafers have become increasingly harmful pests in forestry, affecting nurseries and young plantations (Tóth, 1998; Delb and Mattes, 2001; Švestka, 2006; Malinowski, 2007). The high population density of grubs in certain regions makes reforestation or afforestation impossible. In 2006, the total loss of Polish state forests due to grub activity was approximately 7 million euro (Malinowski, 2007).

The insecticides that were previously used to control grubs, such as Marshall SuSCon 10 CG, Pyrinex 480 SC, Diazinon 10 GR, and Dursban 480 EC, have been banned as a consequence of regulations concerning plant protection, including Directive 91/414/EWG (currently Regulation 1107/2009), introduced by the European Commission to protect the environment. Similar restrictions apply not only to chemical pesticides but also to available or potential biological products based on entomopathogenic nematodes, bacteria, and fungi, which were tested in different countries (Keller et al., 1999; Koppenhöfer et al., 2000; Enkerli et al., 2004; Grewal et al., 2004; Benker and Leuprecht, 2005; Keller and Zimmermann, 2005; Dolci et al., 2006; Erbaş et al., 2014). Mechanical methods, such as deep plowing and other techniques involving intensive soil cultivation, are frequently recommended for cockchafer control (Horber and Wüst, 1958; Fröschle, 1996; Stocki and Malinowski, 2000). However, these methods are highly invasive to the soil environment, particularly sandy soils, and are not always feasible. Therefore, it is necessary to develop novel approaches and/or improve the

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current methods of grub control. The use of plants with potentially detrimental effects on grubs appears to be an environmentally friendly alternative method. We focused on representatives of the families Asteraceae, Fabaceae, Brassicaceae, and Polygonaceae, which had already been observed to affect grub behavior or performance to some extent, but in many cases, the observations were not well documented, as presented below.

The repellent effects of the common tansy, *Tanacetum vulgare* L. (Asteraceae), on *Melolontha* grubs were observed in a 2-choice bioassay (Tol et al., 2011), although they have not been confirmed in another experiment (L Sukovata, personal observations). However, no studies have examined the effects of this plant on the white grubs that feed on its roots.

Some species of legumes (Fabaceae) were selected to test the effect of elevated nitrogen in plant tissues on the grubs (Hirsch, 1992; Kumar and Goh, 2002; Askegaard and Eriksen, 2007; Wagner, 2011). It has been shown for both *M. melolontha* and *M. hippocastani* that first- and second-instar grubs reached the highest weight and body fat tissue percentages when feeding on the roots of Scots pine *Pinus sylvestris* L. and silver birch *Betula pendula* saplings, whereas lower values were observed in grubs feeding on the roots of *Sambucus nigra* L., *Caragana arborescens* Lam., and the common oak *Quercus robur* L. (Sierpiński, 1975, after Gur'ânova 1954; Voroncov, 1982, after Berezina 1957). Chemical analyses of roots allowed these authors to conclude that a low carbohydrates-to-nitrogen ratio had a negative effect on the performance of grubs; however, the suggested relationship was not supported by statistical analyses. We selected some species of legumes with a potentially high N content to test the effect of elevated N on the grubs.

According to Rożyński (1926), the buckwheat *Fagopyrum esculentum* Moench (Polygonaceae) is particularly useful for cockchafer grub control. In the years after sowing this plant the author observed a high survival rate of tree seedlings in portions of the nursery that were heavily infested with grubs. Similar experiments in nurseries and postagricultural lands showed a decrease in the population density of grubs (Malinowski et al., 2001). Malinowski (2009) suggested that tannins in the buckwheat roots have a poisoning effect on grubs and could thus be responsible for the observed changes. Furthermore, gardeners' guides included guidelines on the use of plants within the family Brassicaceae as pest control, by simply sowing, for example, the oilseed rape *Brassica napus* L. or by covering the soil with *Brassica oleracea* L. leaves and plowing after the first autumn frosts. It has been speculated that compounds released by the decaying plant material exert toxic effects on the grubs (Rewieński, 1887).

Despite the availability of the above information, sufficiently convincing evidence that demonstrates the deleterious effects of these plants on the grubs is lacking. Thus, the aim of our study was to test selected plants for their potential detrimental effects on the development, mortality, and weight gain of grubs in a no-choice experiment. We also attempted to explain the mechanism underlying the effects of these plants on grubs by assessing the relationship between the content of the selected chemical compounds in plant roots and the vital parameters of the grubs. We focused on constitutive chemical resistance based on primary and secondary metabolites, rather than on induced defense mechanisms.

2. Materials and methods

2.1. Study species

In our studies, we used the first-instar *Melolontha* grubs from a young pine plantation in the Sobibór Forest Division (51°28′N, 23°37′E). The grubs were collected at the end of May 2011 after overwintering in soil. In contrast to the adults, which can be easily distinguished by the shape of the last abdominal segment (pygidium), the larvae of both *Melolontha* species are almost identical and there is no reliable diagnostic key that can be used for their discrimination (Krell, 2004). Thus, the grubs used in our experiment were only identified to genus level (*Melolontha* sp.) using the key presented by Sierpiński (1975). The instar was determined by measuring the width of the head capsule (L1: 2.6–2.7 mm, L2: 4.2–4.5 mm, L3: 6.5–6.9 mm) (Śliwa, 1993).

2.2. Experimental design

The research was conducted in 2011 in seminatural conditions using a tent with a wooden frame, which was covered with a net providing 30% shade.

In mid-April, the seeds of the following 9 plant species were sown in plastic boxes of 40 × 30 × 24 cm in size and filled with garden soil (peat deacidified to pH (H_2O) = 5.5–6.5 with chalk and with macro- and microelements added; Agrohum, Łomianki, Poland): buckwheat *F. esculentum* (Polygonaceae); white mustard *Sinapis alba* L., turnip rape *Brassica rapa* L. var. *silvestris* (Lam.) Briggs, oilseed rape *B. napus*, and turnip greens *B. rapa* L. subsp. *rapifera* Metzger (Brassicaceae); tansy *T. vulgare* (Asteraceae); and alfalfa *Medicago sativa* L., large-leaved lupine *Lupinus polyphyllus* L., and white clover *Trifolium repens* L. (Fabaceae).

The numbers of seeds to be sown in the boxes were calculated on the basis of the maximum values of the seeding standards recommended in agriculture, namely 80 kg/ha for *F. esculentum*, 25 kg/ha for *S. alba*, 20 kg/ha for *B. rapa* var. *silvestris*, 15 kg/ha for *B. napus*, 12 kg/ha for *B. rapa* subsp. *rapifera*, 15 kg/ha for *T. vulgare*, 25 kg/ha for *M. sativa*, 180 kg/ha for *L. polyphyllus*, and 10 kg/ha for *T. repens*.

One-year-old *Pinus sylvestris* seedlings (12 seedlings/box) were used as the control. *Pinus sylvestris* is the principal forest tree species in Poland and its seedlings are produced in all forest nurseries. This plant species has also been most often damaged by white grubs in young forest plantations. The seedlings were obtained from a forest nursery in the Bałtów Forest District, Ostrowiec Świętokrzyski Forest Division (51°00′N, 21°32′E).

Five replicates (boxes) of each plant species were used. The grubs were weighed individually with an accuracy of 0.001 g on an AD 300 scale (Axis sp. z o.o., Gdańsk, Poland) and placed in boxes with growing plants (2 specimens/replicate) on 19 June, i.e. 68 days after sowing or planting. The experiment was completed 51 days later on 10 August. The soil from each box was sieved and the grubs were counted again and weighed. The final instar was also determined.

To obtain undamaged plant material for chemical analyses, the seeds of each plant species mentioned above were sown, and pine seedlings were planted in additional boxes (3 boxes/plant species) at the same time and in the same conditions as described above.

2.3. Chemical analyses

The soluble sugar, starch, nitrogen and carbon, condensed tannin, and soluble phenolic compound contents were determined in the fine roots of each plant species as indicators of plant quality. The chemical analyses described below had already been used successfully in earlier studies (Karolewski et al., 2010).

Four months after sowing, the additional sown plants were removed from the soil and cleaned. The plants from different boxes were kept separately and then used as 3 replicates of the roots per plant species for chemical analyses. The roots were cut, placed in paper envelopes, and dried for 3 days at 38 ± 2 °C using a ULE 400 Memmert dryer (Memmert GmbH & Co. KG, Germany) with forced air circulation. Fine roots (up to 2 mm in diameter) were then sampled from the dried material and ground into powder with a Mikro-Feinmühle-Culatti MFC mill (IKA-Labortechnik Staufen, Janke & Kunkel GmbH & Co. KG, Germany). The condensed tannin content was determined at this stage, whereas the remaining material was used for other analyses after drying at 65 °C.

After extraction (0.025 g dry mass (d.m.)) with absolute methanol (2.5 cm^{-3}) at room temperature for 20 min, the condensed (catechol) tannins were detected by colorimetry using a color reaction with vanillin in an acid medium (Price et al., 1978). Readings of absorption at λ = 500 nm were taken. The results were converted into μM catechin g^{-1} d.m. and (+)-catechin hydrate (Sigma–Aldrich C1251) was used for the standard curve.

The concentration of total soluble phenols was measured in 0.1-g samples of root dry mass after a boiling extraction for 15 min in 95% ethanol and 10 min in boiling 80% ethanol, according to Johnson and Schaal (1957) and as modified by Singleton and Rossi (1965). Accordingly, the determination was performed using Folin–Ciocalteu phenol reagent (Sigma F – 9252) at λ = 660 nm, and the results were expressed as μM chlorogenic acid g^{-1} d.m. Chlorogenic concentrations were calculated from standard curve linear regression equations.

The concentrations of total nonstructural carbohydrates, namely soluble carbohydrates and starch, were determined according to Haissig and Dickson (1979) and Hansen and Møller (1975). Soluble carbohydrates were assayed in methanol-chloroform-water (12:5:3 by volume) extracts. The precipitate after extraction was evaluated to determine starch content. The starch analysis consisted of transformation into glucose with amyloglucosidase and oxidation using the peroxidase-glucose oxidase complex. The concentrations of soluble carbohydrates were measured at λ = 625 nm after a colorimetric reaction with anthrone for 30 min, while the starch concentrations were measured at λ = 450 nm following a reaction with dianisidine dihydrochloride after 30 min of incubation at 25 °C. The total concentration of soluble carbohydrates and starch was expressed as a percentage of the dry mass. Soluble carbohydrate concentrations were calculated using standard regression equations based on the glucose (β-glucose, Sigma, catalog no. 635-100) standard solution.

In all cases, absorbance was determined with a spectrophotometer (UV-1700 PharmaSpec, UV-Visible Spectrophotometer, Shimadzu Corporation, Japan).

The nitrogen concentration (% d.m.) was determined with an Elemental Combustion System CHNS-O 4010 analyzer (Costech Instruments, Italy/USA), an automatic analytical unit using the combustion/gas chromatography technique.

2.4. Statistical analyses

The percentage of molted grubs and the mortality rate were estimated by dividing the number of molted or dead grubs in a treatment (plant species) by the surviving or total number of grubs (N = 10 grubs/treatment), respectively, and then multiplying by 100. It was not possible to determine the weight gain of each grub kept in a box because they were not marked; therefore, we calculated the average weight for 2 grubs at the beginning of the experiment and repeated the calculation for those that survived to the end of the experiment.

The differences in molting and mortality percentage of the grubs between different treatments were tested using the Fisher exact test for 2 × 2 tables. The weight gain of the grubs and the content of chemical compounds in the roots were analyzed with one-way ANOVA, followed by

a Tukey–Kramer test for the comparison of means with unequal sample sizes in the former case, or by a Tukey honestly significant difference test in the latter case. If the assumptions of a normal distribution and homogeneity of variance were violated, the data were transformed using for total phenols, for tannins, and for starch (Sokal and Rohlf, 1995). The data were back-transformed so as to graphically represent the results of the analyses.

Linear and nonlinear correlation analyses were performed to determine the relationship between the chemical compound contents in the plant roots (average of 3 replicates for each plant species) and the molting rate, mortality, and weight gain (N = 10 plant species).

The analyses were performed using Statistica 8 software (StatSoft Inc., 2007) with the significance level set at $\alpha = 0.05$.

3. Results

3.1. Grub mortality

The mortality rate of the grubs ranged from 10% and 20% for those feeding on *T. vulgare* and *S. alba*, respectively, to 80% for those feeding on *B. napus* (Table), with significant differences between the latter and 2 former species (Fisher's exact test: P = 0.0055 and P = 0.0230, respectively). The mortality of the grubs feeding on the *P. sylvestris* control seedling roots was 40%. The study showed that only grubs feeding on *F. esculentum*, *B. napus*, and *B. rapa* subsp. *rapifera* exhibited higher mortality.

3.2. Grub development

All surviving grubs that fed on the roots of *T. vulgare, B. rapa* var. *silvestris,* or plants of the family Fabaceae molted to the second instar. In the other 5 plant species, the percentage of grubs that molted varied from 0% in *B. napus* to 83% in *P. sylvestris* (Table). We found significant differences between *T. vulgare* and 2 representatives of the family Brassicaceae, specifically *B. napus* and *S. alba* (Fisher's exact test: P = 0.0182 and P = 0.0294, respectively). The percentage of molted grubs on *B. napus* was also significantly lower than on *L. polyphyllus*, *T. repens*, *M. sativa*, and *B. rapa* var. *silvestris* (P = 0.0278 in the first case and P = 0.0357 in the other 3 cases).

3.3. Grub weight gain

The greatest weight gain was found in the grubs feeding on *T. repens* (0.285 ± 0.003 g (mean ± standard error)), *T. vulgare* (0.276 ± 0.028 g), and *L. polyphyllus* (0.264 ± 0.043 g). Their weight gain was significantly higher than that of the grubs feeding on the other plants (Figure 1). The weakest performance was observed for the grubs feeding on the roots of *F. esculentum* (0.033 ± 0.015 g), *B. napus* (0.051 ± 0.025 g), and *B. rapa* subsp. *rapifera* (0.054 ± 0.027 g).

The performance of the grubs feeding on the roots of *P. sylvestris* seedlings was slightly better than on the 3 plant species mentioned above and differences were not significant (Figure 1).

Table. The mortality of the first-instar *Melolontha* grubs feeding on the roots of various plant species (N = 10 grubs/plant species) and the percentage of specimens that molted to the second instar.

Plant family	Plant species	Number of surviving grubs	% mortality	Number of molted grubs	% molted grubs (of all that survived)
Asteraceae	*T. vulgare*	9	10 a*	9	100 a*
Fabaceae	*L. polyphyllus*	7	30 ab	7	100 ab
	T. repens	6	40 ab	6	100 ab
	M. sativa	6	40 ab	6	100 ab
Brassicaceae	*S. alba*	8	20 a	4	50 bc
	B. rapa var. *silvestris*	6	40 ab	6	100 ab
	B. rapa subsp. *rapifera*	4	60 ab	2	50 abc
	B. napus	2	80 b	0	0 c
Polygonaceae	*F. esculentum*	4	60 ab	3	75 abc
Pinaceae	*P. sylvestris*	6	40 ab	5	83 abc

*Different letters indicate significant differences between plant species at $\alpha = 0.05$.

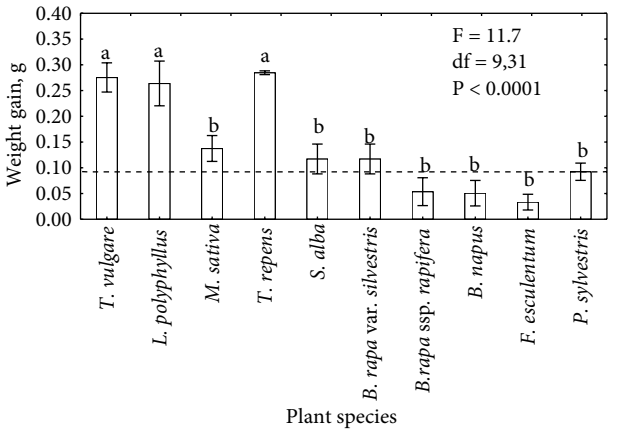

Figure 1. The weight gain (mean ± SE) of the first-instar *Melolontha* spp. grubs feeding on the roots of various plant species (dotted line indicates the level of weight gain in grubs feeding on *P. sylvestris* roots as a control; different letters indicate significant differences at α = 0.05).

3.4. The effects of root chemical composition on grub performance

The tested plants exhibited significant differences in the contents of total phenols, condensed tannins, soluble sugars, starch, nitrogen, and carbon.

The roots of *T. vulgare* contained the highest phenol concentrations (274.9 ± 8.9 μM/g d.m.), which were significantly higher than in all other plant species. High levels of phenols were also detected in the roots of *P. sylvestris* (131.6 ± 14.9 μM/g d.m.), *L. polyphyllus* (111.1 ± 4.1 μM/g d.m.), and *F. esculentum* (106.5 ± 1.3 μM/g d.m.). These plants contained significantly more phenols than all other plants except *T. vulgare* (Figure 2a).

The highest condensed tannin content (333.8 ± 47.9 μM/g d.m.) was found in the roots of *P. sylvestris* seedlings (Figure 2b). The level of tannins in the roots of *F. esculentum* (54.7 ± 16.5 μM/g d.m.) was significantly lower than in *P. sylvestris*, yet higher than in all other plants. The tannin content in the roots of *T. vulgare* and the Fabaceae and Brassicaceae plants was relatively low and ranged from 22.9 ± 0.2 to 24.6 ± 0.5 μM/g d.m.

The analyzed plant species can be divided into 3 groups in terms of the soluble sugar contents of their roots. The high-sugar group comprised *L. polyphyllus* (8.3 ± 0.6% d.m.), *M. sativa* (6.9 ± 0.3% d.m.), and *T. vulgare* (6.6 ± 1.3% d.m.). This group differed significantly from the group with the lowest root soluble sugar contents (1.5%–2.2% d.m.), including *S. alba*, *B. rapa* var. *silvestris*, and *F. esculentum* (Figure 2c). Only *L. polyphyllus* was found to contain significantly more soluble sugars than *P. sylvestris*. The concentration of soluble sugars in *T. repens* did not differ significantly from that in the other species.

Medicago sativa was characterized not only by high soluble sugar content but also by a significantly greater amount of starch (22.3 ± 3.7% d.m.) compared with the other species, including *P. sylvestris*. Generally, all plants belonging to the family Fabaceae showed significantly higher concentrations of starch (2.5%–22.3% d.m.) than the other species, whose starch concentrations ranged from 0.42% to 0.48% d.m. (Figure 2d).

Considerable variations in nitrogen concentration (Figure 2e) but only slight differences in carbon content (Figure 2f) were detected in the roots of the tested plants. The highest nitrogen concentration was observed in the *B. rapa* subsp. *rapifera* roots (3.1 ± 0.2% d.m.), and this concentration differed significantly from that in the roots of the other plants, except *T. repens* (2.6 ± 0.3% d.m.). The concentration of nitrogen in the roots of *P. sylvestris* (1.1 ± 0.1% d.m.) was significantly lower than that in the roots of *T. repens*, *B. rapa* var. *silvestris*, and *B. rapa* subsp. *rapifera* (Figure 2e). In contrast, *P. sylvestris* and *T. repens* roots contained the highest carbon levels (50.7 ± 1.1% d.m. and 49.7 ± 0.8% d.m., respectively), which differed significantly from those in the roots of *B. rapa* var. *silvestris* (42.6 ± 1.8% d.m.) and *B. rapa* subsp. *rapifera* (42.8 ± 0.5% d.m.) (Figure 2f).

None of the analyzed substances were found to be significantly correlated with the mortality of the grubs. However, the concentration of soluble sugars and starch showed a strong positive correlation with weight gain (Figures 3a and 3b, respectively). Starch was also significantly and positively correlated with the percentage of molted grubs (Figure 4). Neither C:N ratio nor carbohydrates-to-N ratio was significantly correlated with any studied parameters of grub performance.

4. Discussion

4.1. Plant effects on grubs

Cockchafer white grubs are polyphagous, and this characteristic may indicate extensive adaptations to feeding on plants with varying nutritional values. Our research aimed to examine whether selected plant species exert any deleterious effect on L1 grubs. These plants could be used in the future to target *Melolontha* populations and reduce their damage to plants in agriculture, horticulture, and forestry.

Our results generally indicated that lower mortality rates and higher weight gain were observed in grubs feeding on the roots of *T. vulgare* and plants belonging to the family Fabaceae (except for *M. sativa*) compared with *F. esculentum* and the species belonging to Brassicaceae. *Pinus sylvestris* usually occupied an intermediate position between these 2 groups.

We were unable to show that *T. vulgare*, *L. polyphyllus*, *T. repens*, or *M. sativa* negatively affected the performance

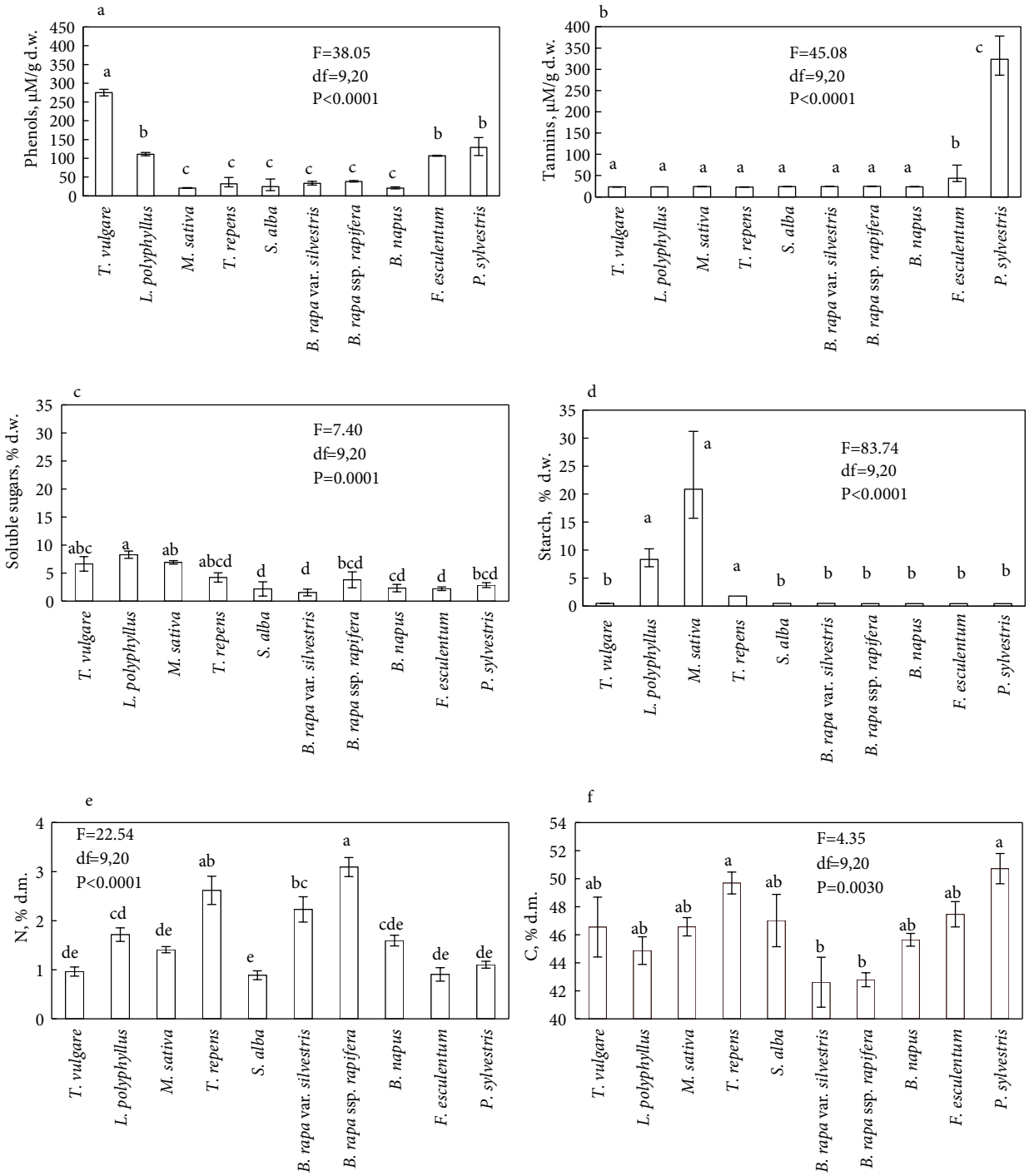

Figure 2. The contents of total phenols (a), condensed tannins (b), soluble sugars (c), starch (d), nitrogen (e), and carbon (f) in the roots of various plant species (columns and bars indicate means ± SE; ANOVA was performed on the transformed data for phenols, tannins, and starch; different letters indicate significant differences at α = 0.05).

of grubs. The results of our study are consistent with previous observations by other authors who demonstrated the beneficial effects of *T. repens* on grub development in another species belonging to the family Scarabaeidae, namely *Costelytra zealandica* (White) in New Zealand (Farrell and Sweney, 1974; Wilson, 1978). However, in contrast to the positive effect of *L. polyphyllus* on *Melolontha* grubs in our study, *L. angustifolius* L. and *L.*

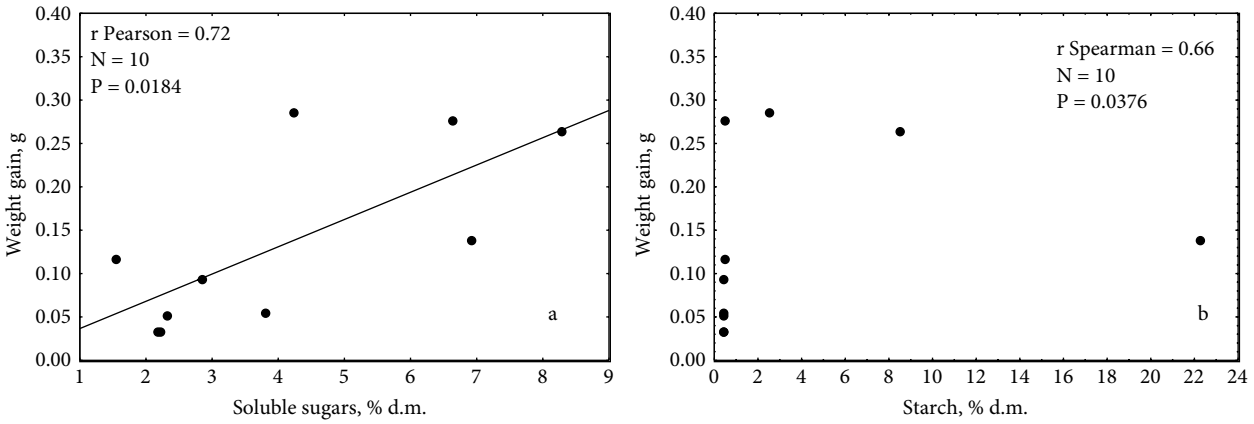

Figure 3. Correlation between the weight gain of the first-instar *Melolontha* spp. grubs and the concentration of soluble sugars (a) and starch (b) in the roots of the tested plant species.

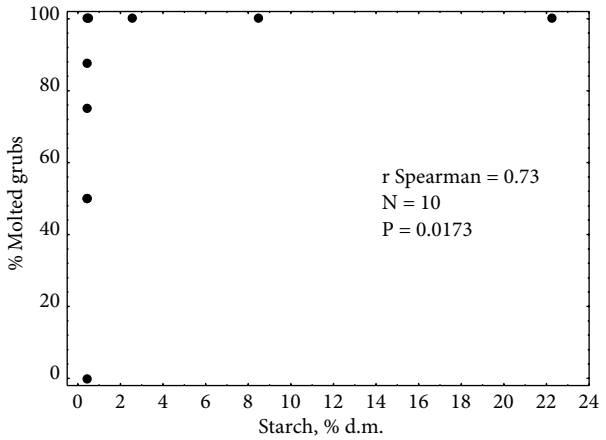

Figure 4. Correlation between the percentage of the first-instar *Melolontha* spp. grubs that molted to the second instar and the concentration of starch in the roots of the tested plant species.

luteus L. had a detrimental effect on *C. zealandica* larvae, resulting in reduced weight gain (Farrell and Sweney, 1974; Farrell and Stufkens, 1977). Although *M. sativa* belongs to the same family as *L. polyphyllus* and *T. repens*, the grubs feeding on its roots gained relatively little weight and the resulting mortality was similar. The difference in weight gain, although not significant, could have been caused by saponins in the roots of *M. sativa*, which are known to have negative effects on insects (De Geyeter et al., 2007). Kain and Atkinson (1970) and Farrell and Sweney (1972) suggested that the high concentrations of saponins in the roots of *M. sativa* were most likely responsible for the negative effect of this plant species on *C. zealandica* larvae. As confirmed in later studies, saponins may exert both deterrent (Sutherland et al., 1975) and toxic (Sutherland et al., 1982) effects on *C. zealandica* larvae.

The survival rate and weight gain of *Melolontha* grubs was the lowest, and the development of the grubs was most retarded by *F. esculentum* and plant species of the family Brassicaceae, particularly *B. rapa* subsp. *rapifera* and *B. napus*. Thus, we confirmed the deleterious effects of these plants on the performance of the first-instar grubs.

4.2. Plant quality

It has been proposed that the negative effects of *F. esculentum* on white grubs may be due to the high tannin concentrations in its roots (Malinowski, 2009). The results of our correlation analysis allowed us to reject the hypothesis that both tannins and phenols have negative effects on the plant roots on grubs, although these substances are often regarded as antagonistic towards insects (Feeny, 1970; Rossiter et al., 1988; Lattanzio et al., 2006). Likewise, Sutherland et al. (1982) did not observe any effects of condensed tannins on the survival rates of *C. zealandica* larvae.

Among the compounds that could potentially promote the development of cockchafer grubs, such as nitrogen, carbon, starch, and soluble sugars, only the nonstructural sugars (soluble sugars and starch) were significantly positively correlated with weight gain. Starch also showed a strong positive correlation with the percentage of molted grubs. Sutherland (1971) and Sutherland and Hillier (1974) showed similar positive effects of sucrose and ascorbic acid on *C. zealandica* white grubs. However, our results did not support the statement made by Gur'ânova (Sierpiński, 1975, after Gur'ânova 1954) and Berezina (Voroncov, 1982, after Berezina 1957) that a low carbohydrates-to-N ratio has a negative effect on the performance of *Melolontha* grubs.

Carbohydrates are an important source of energy necessary for the grubs to move through the soil in search of food. Bauchop and Clarke (1975) did not find cellulose-

degrading bacteria in the larval hindgut of *C. zealandica*. Similar conclusions may be drawn from studies of *M. melolontha* grubs in which glucose concentration in the hindgut was found to be much lower than in the midgut (Egert et al., 2005). Xylanolytic bacteria were found in both the mid- and hindgut of *M. hippocastani* grubs, although their ability to degrade cellulose was not tested (Arias-Cordero et al., 2012). Generally, these results may indicate that structural carbohydrates, which are mostly concentrated in roots, are not as essential for the *Melolontha* grubs as nonstructural carbohydrates, which are present in significantly lower quantities. This fact may explain the voracity of these insects, which must ingest large quantities of plant roots to obtain enough sugars to satisfy their energy requirements (Bauchop and Clarke, 1975). Sugars are known to act as phagostimulants in a wide range of insect species, including 3 scarab species (Johnson and Gregory, 2006); however, the positive effects of soluble sugars and starch on *Melolontha* grub performance were presented in our study for the first time.

The poorest vital parameters were observed in the grubs feeding on *F. esculentum* and Brassicaceae plants, which have low root sugar and starch concentrations. However, glucosinolates, compounds that are characteristic of the family Brassicaceae but were not analyzed in our study, might also be of great importance. These compounds have been shown to have toxic effects on a variety of organisms (Kirkegaard and Sarwar, 1998; Ahuja et al., 2011), including *Cyclocephala* spp. white grubs (http://www.plantmanagementnetwork.org/pub/php/research/chafer/).

In summary, we failed to show any relationship between *Melolontha* L1 grub performance and the root contents of tannins and phenols as secondary metabolites playing an important role in plant defense. However, we presented evidence that the high levels of nonstructural sugars were favorable for the grubs. The results of our study suggest that the negative effects of plants on cockchafer grub populations may be either immediate, i.e. direct toxic effects resulting in high mortality, or long-term, i.e. effects that decrease weight gain. Retarded development and low weight gain potentially affecting the mobility of grubs may result in their increased mortality due to higher risk of exposure to natural enemies and/or unfavorable microclimatic conditions, including drought or extended excessive soil humidity. The use of plant species and varieties that negatively affect cockchafer grubs may prove to be one of the important aspects of integrated plant protection against these pests in agriculture, horticulture, and forestry (mainly nurseries and postagricultural lands). Among the tested plants, *B. napus*, *B. rapa* subsp. *rapifera*, and *F. esculentum* may be of greatest efficacy against first-instar grubs.

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