

Effect of phenolic acid content on acceptance of hazel cultivars by filbert aphid

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Abstract: The allelopathic effect of phenolic acids contents in the leaves of six cultivars of hazel (*Corylus* L.) on the choice of plants by *Myzocallis coryli* Goetze (filbert aphid), one of the most important pest of hazel in Poland and throughout the world, was identified. The cvs White Filbert, Mogulnus, and Luizen Zellernus were more resistant to the feeding of aphids in all the years than cvs Minnas, Barra, and Halls Giant. The highest content of total phenolic acids was reported in the leaves of cvs White Filbert and Luizen Zellernus, with a low level of acceptance by aphids. These cultivars demonstrated a high concentration of gallic acid and caffeic acid. In the leaves of cvs Minnas and Halls Giant, much infested by aphids, the total content of phenolic acids was significantly lower. Moreover, gallic and caffeic acids occurred at significantly lower concentrations. The chromatographic analysis of hazel leaf extracts revealed the presence of eight phenolic acids: gallic, protocatechuic, *p*-hydroxybenzoic, salicylic, chlorogenic, ferrulic, caffeic, and α -resorcinolic. The leaves of the tested cultivars, irrespective of the level of resistance to filbert aphid, showed a definitely higher concentration of acids, derivatives of *trans*-cinnamic acid, if compared to the amount of acids – derivatives of benzoic acid.

Keywords: *Corylus* L.; *Myzocallis coryli*; chromatographic analyse

Secondary plant metabolites are considered the key source of plant resistance to pests. They act both during the plant selection and during the physiological pest–plant relations, conditioning the phenomenon of natural resistance (GATEHOUSE 2002; BERNARDS & BASTRUP-SPOHR 2008). Their mechanisms vary since they involve both the substances which occur in peripheral tissues (epidermis, mesophyll) and in the vascular bundles (LESZCZYŃSKI 2001a, b; GOGGIN & ZHU-SALZMAN 2015). They affect olfactory, tactile, and taste receptors in the herbivores. In that group of secondary metabolites the derivatives of shikimic acid and acetate prevail, however, in woody plants the derivatives of shikimic acid are more important (LESZCZYŃSKI 2001b).

Their effect on pests is usually toxic and they are considered as food attractants (HARBORNE 1997; PÉLLISIÉ *et al.* 2018). In the leaves of the common hazel (*Corylus avellana* L.) flavonoids (myricitrin and quercetin as well as tannins) have been identified and determined (FRAISSE *et al.* 1999; AMARAL *et al.* 2005). In the research performed in Portugal the content and the composition of phenolic acids in the leaves of three cvs of cultivable hazel were determined (OLIVEIRA *et al.* 2006).

It is known that plant defense response to pathogen infection involves many physiological and biochemical processes. As a result of an infestation by pathogens some specific elicitor molecules may be generated by chemical or physical damage to plant cell walls. Plant

response may also involve such reactions as synthesis of pathogenesis related (PR) proteins, phenolic compounds (e.g. phytoalexins), lignin deposition, the production of signalling compounds, and the generation of reactive oxygen species (ROS), e.g. hydrogen peroxide. H_2O_2 increases the synthesis of another phenolic compound – salicylic acid, a known elicitor of plant defense response to stresses and is also a substrate for peroxidases oxidising phenolic alcohols, which subsequently polymerise to lignins (BOLWELL *et al.* 2002).

Phenolic compounds: phenols, alcohols, phenolic acids, phenylpropanoids, coumarins, flavonoids, and tannins are recognised as the most active groups of plant allelocompounds. Phenols are plant proteolytic inhibitors of enzymes of insects (LESZCZYŃSKI 2001b). Their higher concentration, often observed in the plants of resistant cvs, may inhibit the digestive processes of phytophages, decrease the availability of the feed uptaken by irritating the walls of the alimentary canal, and decrease permeability of the intestine for digested nutrients (CZERNIEWICZ *et al.* 2017). It was demonstrated that the higher level of phenols positively correlated with the resistance of hazel cultivars to *Curculio nucum* (PISKORNIK 1994), or wheat to *Sitobion avenae* and *Oulema melanopus* (CZERNIEWICZ *et al.* 2017). A negative effect of phenolic compounds can be observed in a form of prolonged pre-reproduction period, a decrease in the fertility and the population growth rate. A gradual decrease in the content of phenols in the seedlings of maize and barley occurred together with the development of the population of aphids *Rhopalosiphum padi* and *Sitobion avenae* (ELEFThERIANOS *et al.* 2006), which confirms the hypothesis that young plant leaves are more abundant in secondary metabolites than older leaves (WALCZYŃSKA 2009).

The aim of the study was to identify the allelopathic effect of phenolic acids contents in the leaves of six cultivars of hazel (*Corylus* L.) on the choice of plants by *Myzocallis coryli* Goetze (filbert aphid), one of the most important pest of hazel in Poland and throughout the world.

MATERIAL AND METHODS

Plant and insect material. The level of leaves colonisation by *M. coryli* was determined over 2008–2009 under field conditions on an experimental plantation at Motycz, in the vicinity of Lublin (eastern

Poland, 51°14'N; 22°34'E), with no chemicals applied. The studies were performed in five shrubs of six hazel cvs: Minnas, Barra, Halls Giant Luizen Zellernuss, Mogulus, and White Filbert, demonstrating a different resistance to the pest.

The population of filbert aphid was monitored from early spring till the occurrence of the first larvae on hazel leaves. The method used involved counting the aphids on 100 leaves of each plant. The aphid population was controlled with 10-day intervals until complete disappearance. The number of larvae and winged females as well as the number of colonized leaves were noted. The level of the pest population on the cultivars tested was expressed as a cumulative aphid index (the sum of aphids on 100 leaves) and the mean percentage of the colonised leaves was established.

Besides, the abundance of aphids under insectarium conditions was determined, too. To do so, in late May, on the shoots of the same trees as above (of all six cultivars), 10 bags per each were placed. They were made of the flour mesh and tied with a ribbon. In each isolator 20 leaves non-infected by aphids together with one larva of *M. coryli*, feeding on a given hazel cultivar, were closed. Population growth observations were made every 5 days, each time adult individuals and new born larvae were counted. The population growth of *M. coryli* on the cultivars tested was expressed with the so-called aphid index (the total number of larvae and adult individuals) as well as the larval index (the number of the larvae born). The acceptance of the host by feeding aphids was evaluated with the significant differences between the values of both indicators analysed.

Phenolic acid analyses. The study material were the leaves of the six above-mentioned hazel cultivars. The leaves (undamaged by aphids) were collected in June upon the maximum occurrence of aphids. The leaves were covered due to the abundant aphid honeydew. The plant material was obtained before noon, on sunny and dry days. Directly after the harvest, the material was dried at 35°C in natural shade and in the air flow.

Phenolic extraction and determination. The extraction was performed according to the Arnov method (Polish Pharmacopoeia VI, 2002). Amount of 1 ml of the sample was mixed with 5 ml of distilled water, 1 ml 0.5 M HCl, 1 ml of Arnov reagent, and 1 ml 1 M NaOH, and, subsequently, adjusted to 10 ml of distilled water. The absorbance was measured at 490 nm. The total phenolic acid content was expressed as the caffeic acid equivalent (CAE).

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The content of phenolic acids in healthy plant material was compared. For this purpose, the leaves were left uncontrolled (previously covered with insulators) and colonised by the aphids, which were then subjected to laboratory analysis. About 50 mg of the powdered leaf samples were weighed in triplicates and extracted with 1.5 ml 50% ethanol (purity 99.98%) containing 0.05 mol/l H_3PO_4 in water. The extracts were vortexed and sonicated in an ultrasonic bath (Bandelin Sonorex, Berlin, Germany) at room temperature for 15 min and centrifuged at 15 300 g for 10 minutes. The supernatant was transferred to a vial prior to HPLC analyses (NAJDA *et al.* 2014).

The isolation and purification of phenolic acids in the tested raw materials was carried out using the HPLC method. The chromatographic analysis was performed in a reversed phase system using a liquid chromatograph with a DAD diode detector (L-7450) and a LiChrospher 100 RP-C18 steel column. The mobile phase was the solvent gradient of the solutions: acetonitrile + water + 1% acetic acid (NAJDA 2004). Identification of phenolic acids was carried out by comparing their retention times (tR) with standards and spectroscopically determining their spectra in the UV range (220–400 nm) (NOLLET 2000). The content of individual phenolic acids in the tested raw materials was calculated on the basis of a calibration curve determined for each identified phenolic acid. The HPLC analyses were performed using three technical replicates for each cultivar and thereafter means of the three replicates.

Statistical analysis. For all the statistical laboratory experiments and analysis of the results, the software package STATISTICA 9.1 (StatSoft, Tulsa, USA) was used. ANOVA with the Tukey simultaneous test was applied, with $P < 0.05$. The average results on the figures were determined with standard deviation ($\pm$ SD). The correlation coefficients between the total aphid population and the number of the leaves colonized were calculated. All the determinations were performed in pentaplicates (5 reps, field experiments) and in triplicate (laboratory experiments).

RESULTS

The field observations showed that the hazel cultivar plants demonstrate a varied resistance to colonization by filbert aphid. More individuals were recorded on cvs Minnas, Barra, and Halls Giant. A significantly lower number of aphids were recorded on cvs Luizen

Table 1. Total mean number ($\bar{x} \pm \text{SD}$) of *M. coryli* individuals on 100 leaves and of settled leaves of hazelnut cultivars in field conditions in years 2007–2008

Cultivar	Aphids on 100 leaves	Leaves settled by aphids
Minnas	856.8 $\pm$ 95.0 ^c	257.1 $\pm$ 58.9 ^f
Barra	759.1 $\pm$ 42.6 ^{bc}	244.1 $\pm$ 56.3 ^{ef}
Hall's Giant	567.0 $\pm$ 96.2 ^{abc}	187.5 $\pm$ 46.7 ^{cde}
Luizen Zellernuss	485.7 $\pm$ 85.8 ^{abc}	144.5 $\pm$ 26.9 ^{abcd}
Mogulus	347.1 $\pm$ 151.1 ^{ab}	119.6 $\pm$ 36.2 ^{ab}
White Filbert	251.0 $\pm$ 15.5 ^a	128.6 $\pm$ 39.9 ^{abc}
LSD	508.4	58.83

Cultivars marked with different letters differ significantly at $P = 0.05$

Zellernuss, Mogulus, and White Filbert (Table 1). The cultivars differed also significantly with the mean number of leaves infested by aphids. The largest number of leaves infested by *M. coryli* was reported on cvs Minnas and Barra, and the lowest number on cvs Mogulus, White Filbert, and Luizen Zellernuss (Table 1).

The comparison of the aphid index (the total number of adults and larvae individuals on the leaves of the plant throughout the observations) and the larval index (the number of larvae born) across the cultivars shows that the larval development and feeding of filbert aphid appears with a varied intensity on the cultivars. The largest values of both indicators were recorded on leaves of cvs Minnas, Barra, and Halls Giant. Different and rather worse conditions for the colony development and feeding of the aphids were

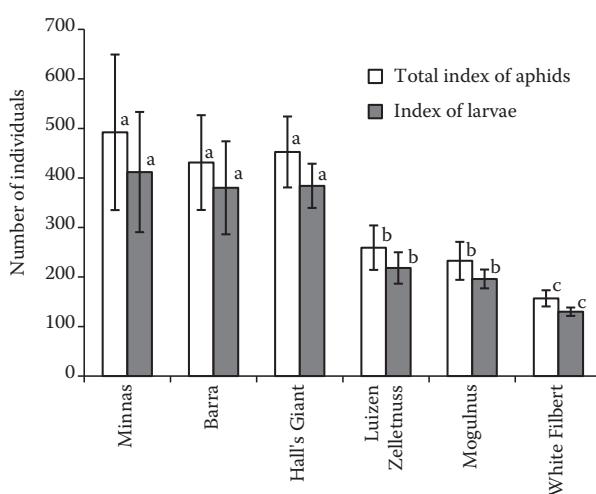


Figure 1. Increase in *M. coryli* population in chiffon insulators in hazel leaves (cultivars marked with different letters differ significantly at $P = 0.05$)

Table 2. Content of phenolic acids ($\mu\text{g/g}$ dry weight) in non-infested leaves of hazel cultivars in 2007 and 2008

Phenolic acid	Hazel cultivar						LSD ($P = 0.01$)
	Minnas	Barra	Hall's Giant	Luizen Zellernuss	Mogulnus	White Filbert	
Gallic	20.71 ± 2.24^a	22.19 ± 0.78^{ab}	18.89 ± 6.39^a	32.58 ± 9.02^c	29.98 ± 7.08^{bc}	32.12 ± 7.09^c	8.23
Protocatechuic	0.61 ± 0.01^{ab}	0.78 ± 0.04^c	0.51 ± 0.21^a	0.73 ± 0.09^{bc}	0.58 ± 0.03^a	0.69 ± 0.03^b	0.11
<i>p</i> -Hydroxy-benzoic	0.15 ± 0.13^a	0.16 ± 0.17^a	0.16 ± 0.15^a	0.13 ± 0.11^a	0.12 ± 0.08^a	0.14 ± 0.12^a	0.08
Salicylic	1.40 ± 0.55^a	1.54 ± 0.70^{ac}	0.00 ± 0.00^a	0.00 ± 0.00^a	2.23 ± 1.19^{bc}	2.28 ± 1.48^c	0.82
Chlorogenic	0.33 ± 0.00^b	0.21 ± 0.00^b	0.19 ± 0.00^b	0.03 ± 0.00^a	0.04 ± 0.00^a	0.67 ± 0.00^c	0.15
Feluric	7.60 ± 2.28^b	5.96 ± 2.30^b	1.94 ± 0.67^a	2.34 ± 1.73^a	2.04 ± 0.87^a	2.69 ± 1.63^a	2.56
Caffeic	139.61 ± 54.87^{ab}	152.97 ± 69.84^{bc}	120.35 ± 22.28^a	228.38 ± 156.03^d	171.62 ± 68.38^c	226.71 ± 147.72^d	25.44
α -Resorcylic	2.02 ± 0.54^b	2.33 ± 0.66^{bc}	0.52 ± 0.20^a	0.75 ± 0.09^a	1.09 ± 0.71^a	2.99 ± 1.45^c	0.77
Total	172.42 ± 56.12	186.11 ± 72.85	142.54 ± 15.77	264.92 ± 167.08	207.68 ± 78.34	268.27 ± 159.45	

Cultivars marked with different letters differ significantly at $P = 0.05$

noted on cvs Luizen Zellernuss and Mogulus, but the lowest value of both indicators were reported on the cv. White Filbert (Figure 1).

Based on the chromatographic analysis of the extracts made from hazel leaves, the presence of eight phenolic acids (four representing the derivatives of benzoic acid: gallic, protocatechuic, *p*-hydroxybenzoic, and salicylic acid; four representing the derivatives of *trans*-cinnamic acid: chlorogenic, feluric, caffeic, and α -resorcylic acid) was documented. The presence of salicylic acid in the leaves of cvs Halls Giant and Luizen Zellernuss was not identified (Table 2). Total phenolic acids content ranged from $142.54 \mu\text{g/g}$ in the leaves of Halls Giant to 264.92 and $268.27 \mu\text{g/g}$ in the leaves of cvs Luizen Zellernuss and White Filbert, respectively (Table 2).

The leaves of the tested cultivars, irrespective of the level of resistance to filbert aphid, showed definitely higher concentration of acids, derivatives of

trans-cinnamic acid, if compared to the amount of acids – derivatives of benzoic acid (Figure 2).

Of all the phenolic acids identified, caffeic acid was dominant and its mean content varied from $120.35 \mu\text{g/g}$ to $226.71 \mu\text{g/g}$ in the leaves of cvs Halls Giant and White Filbert, respectively. It was also found that *p*-hydroxybenzoic acid occurred at the lowest concentration in the extracts, regardless of tested cultivar (Table 2).

The leaves of the hazel cultivars differed significantly in concentration of all phenolic compounds. Only the content of *p*-hydroxybenzoic acid was the same in the tested cultivars (Table 2). The highest content of total phenolic acids was reported in the leaves of cvs White Filbert and Luizen Zellernuss, with a low level of acceptance by aphids. These cultivars demonstrated a high concentration of gallic acid and caffeic acid. In the leaves of cvs Minnas and Halls Giant the total content of phenolic acids was significantly

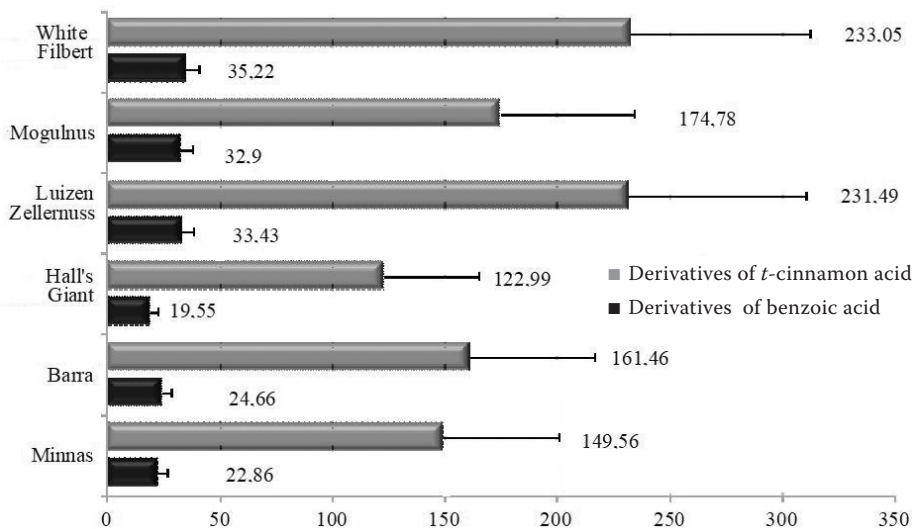


Figure 2. Profile of phenolic acids of crop hazel leaves (collected in spring) depending on the cultivar

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lower. Moreover, gallic and caffeic acids occurred at significantly lower concentrations (Table 2).

DISCUSSION

Host plant resistance is an efficient and environmentally friendly means of controlling insects, including aphids (LOMBARDO *et al.* 2016). However, in Poland, the selection of hazel cultivars does not consider the resistance to diseases and pests, except for the nut weevil (*Curculio nucum*) (PISKORNIK 1994). The global studies are mostly focused on the cultivar-specific hazel resistance to diseases caused by endemic fungus *Anisogramma anomala* or bacteria *Pseudomonas syringae* pv. *avellanae*. Growing hazel cultivars with an increased resistance could decrease the abundance of pests and decrease losses caused by pesticides (GANTNER 2000, 2008),

The observations showed that the cultivars demonstrated a different level of resistance to aphid *Myzocallis coryli*. A partial resistance to filbert aphid was found in cvs White Filbert, Mogulus, and Luizen Zellernuss. Significantly more larvae and adult individuals were noted on the leaves of cvs Barra and Halls Giant, what is not in agreement with WOJCIECHOWICZ-ŻITKO (2003), but is in accord with other reports (GANTNER 2000, 2008).

The research shows that the level of phenolic acids plays an important role in developing the resistance of hazel cultivars on the occurrence of filbert aphid. According to QAWASMEH *et al.* (2012a), phenolic acids are the key group of compounds responsible for protecting the plants from pest attack. Induction of phenol production and antioxidative potential are crucial mechanisms that enhance the plant resistance to oxidative stress (QAWASMEH *et al.* 2012b). PAŃKA *et al.* (2013) found that *Neotyphodium lolii* was involved in the production of phenolic compounds by colonised plants before contact with the pathogen. The level of these chemicals was significantly higher in plants colonised by the endophyte. This suggests the strong influence of the plant and fungal genotype on phenolics production. QAWASMEH *et al.* (2012a) observed a significant influence of infection of perennial ryegrass with *Neotyphodium lolii* on the phenolics content and antioxidant activity.

Phenolic acids represent secondary metabolites affecting the development and feeding of insects (MALLIKARJUNA *et al.* 2004; CIPOLLINI *et al.* 2008). In the leaves of the tested hazel cvs eight phenolic acids

(gallic, protocatechuic, *p*-hydroxybenzoic, salicylic, chlorogenic, feluric acid, caffeic, and α -resorcylic acid) were identified. To compare our findings, OLIVEIRA *et al.* (2006) detected five phenolic acids in hazelnut leaves: 3-, 4-, and 5-caffeoylquinic acids, caffeoyltartaric acid, and *p*-coumaroyltartaric acid. Moreover, their presence, except for 4-caffeoylquinic acid, was reported in earlier research by AMARAL *et al.* (2005). OLIVEIRA *et al.* (2006) demonstrated a quantitative variation in the phenolic acids identified in tested cultivars, what suggests that the cultivar is an important factor affecting both the quantity and the quality of phenolic acids. The available data confirms the important role of phenolic compounds in the defense reaction of plants to pathogen attacks (LEI *et al.* 2014).

Plants defend themselves against insect attacks using three possible mechanisms: antibiosis, when host plant constituents cause adverse effects in the pest life cycle; antixenosis, in which plant acts as bad host so the insect shifts to another plant; and tolerance or ability to withstand or recover from insect damage is possible (TEFERA *et al.* 2016). Thus, both constitutive and induced defenses may contribute to the protection of the plant against herbivorous enemies (SMITH 2007). GANTNER (2008) reported the partial and complex resistance to *M. coryli* in some cultivars of hazel, qualified both by the fully-developed mechanism of lack of acceptance and mostly by mechanism of antibiosis. The leaves of cv. Luizen Zellernuss were generally not attractive for aphids, because they had a thick leaf blade, small quantity of stomata, and very long mechanical and secretory trichomes. The mechanism of antibiosis was affected by the unfavourable level of protein in relation to sugars, high proportion of saccharose in total sugars, as well as high content of tannins. In our research, the leaves of cv. Minnas, abundantly infested by aphids, showed the lowest content of all the phenolic acids. While the leaves of cvs White Filbert and Luizen Zellernuss demonstrated partial resistance to hazel aphid. A similar outcome concerning the resistance of plants to aphids was reported by others (MALLIKARJUNA *et al.* 2004; ELEPHTERIANOS *et al.* 2006). According to our own results, the cultivars with a higher resistance to filbert aphid contained significantly higher amounts of gallic and caffeic acids. In the available literature a negative effect of phenolic compounds on insects feeding can be found. A deterrent effect of chlorogenic acid on feeding of *Spodoptera exigua* was noted by CAPOLLINI *et al.*

(2008). A similar chlorogenic acid relationship was reported by MAO *et al.* (2007) who demonstrated clear anti-feedant properties of chlorogenic acid for *Ostrinia nubilalis* feeding on *Zea mays*. In the present research the largest content of chlorogenic acid was found in the leaves of cv. White Filbert, with the lowest susceptibility to filbert aphid feeding. As reported by CHRZANOWSKI and LESZCZYŃSKI (2008), a cultivar with a larger resistance to aphid feeding contained higher concentration of ferulic and *o*-coumaric acid compared to more susceptible wheat cultivars. The present research demonstrated that cvs Minnas and Barra, more susceptible to aphids, showed a high concentration of ferulic acid (7.60 and 5.96 µg/g, respectively) compared to more resistant cultivars such as Mogulnus or Luizen Zellernus (2.04 and 2.34 µg/g, respectively). As reported by CABRERA *et al.* (1995), ferulic acid decreases the survival rate of aphids and limits their reproduction potential, however, it is one of the components of induced plant resistance to pest feeding. Under the field conditions, we could not affect the total number of aphids occurring on the tree analysed, despite covering the parts of leaves with an insulator and this may be the reason of its high concentration in the leaves of cultivars heavily infested by individuals of *M. coryli* aphids. CZERNIEWICZ *et al.* (2017) identified five phenylpropanoid acids (caffeic, chlorogenic, *o*-coumaric, *p*-coumaric, and ferulic) in seedlings of winter triticale cultivars. The grain aphid feeding on resistant cv. Lamberto caused an increase in the content of four phenolic acids, and only the level of ferulic acid was unaltered. The research performed by the authors show that a low acceptability of hazel cultivars by hazel aphid was determined by the total phenolic acids, which affect a lack of acceptability of a given hazel cultivar by hazel aphid females. The aphid index and the larval index recorded the lowest values in leaves of cv. White Filbert, with the largest content of the total phenolic acids identified. CZERNIEWICZ *et al.* (2017) reported new insight into the role of phenolic compounds during antioxidative responses of the winter triticale plants colonised with pierce-sucking or chewing insects. The earliest response of plants to insects attack is the generation of ROS, which can elicit oxidative burst within plant tissues, and plants employ antioxidant systems against these radicals. Due to their chemical structures, polyphenols are able to diminish the level of ROS and enhance antibiosis against insects in resistant plants.

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