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Effect of phenolic acids from black currant, sour cherry and walnut on grain aphid (*Sitobion avenae* F.) development

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ABSTRACT

The influence of naturally-occurring phenolic acid mixtures from selected plants was tested against the grain aphid (*Sitobion avenae* F.). Phenolic acids were extracted from the leaves of black currant (*Ribes nigrum* L.), sour cherry (*Prunus cerasus* L.) and walnut (*Juglans regia* L.), as well as from the green husks of walnut. The highest content of total phenolic acids and individual compounds such as *p*-hydroxybenzoic, *p*-coumaric, chlorogenic and vanillic acids were determined in *J. regia*. Ferulic and tannic acids were found only in *J. regia*. In laboratory bioassays, the phenolic acids extracted from plants prolonged the aphid prereproductive period by 1.5–3.0 days and reduced daily fecundity by 1–1.5 offspring. The strongest effects were observed after application of phenolic acids from the leaves and green husks of *J. regia*. The grain aphid used glutathione *S*-transferase (GST), peroxidase (POD) and polyphenol oxidase (PPO) in response to the application of plant phenolic acids. An increase in aphid GST activity was found in response to treatment with all extracts. Induction of PPO and POD was shown 24 h after the application of phenolic acids mixture from leaves of walnut; inhibition was observed after 48 and 168 h in response to treatment with both extracts of walnut. An inverse relationship between the POD and PPO activity of the aphids was found 24 h after application of the black currant and sour cherry phenolic acids. After 168 h, the activities of these enzymes were higher in treated aphids compared to unsprayed insects. Mixtures of phenolic acids naturally occurring in phenol-rich plants might be used as biopesticides to control the grain aphid as a part of an integrated pest management programme.

1. Introduction

The grain aphid (*Sitobion avenae* F.) is an important pest of cereals in Europe (Larsson, 2005). Aphids ingest phloem sap from sieve elements using their narrow piercing-sucking mouthparts called stylets. While probing, the aphid stylets transiently puncture epidermal, mesophyll and parenchymal cells (Tjallingii and Esch, 1993). Aphids secrete various toxic compounds in their saliva (Karban and Agrawal, 2002) and are capable of inoculating the barley yellow dwarf viruses BYDV-MAV and BYDV-PAV (Brault et al., 2007).

Pest management programmes in cereals rely on balanced fertilization and application of selective insecticides (Korbas et al., 2008). Joshi and Sharma (2008) demonstrated that imidacloprid is effective in controlling the grain aphid. This neonicotinoid insecticide has a mode of action connected with persistent activation of cholinergic receptors which leads to death (Jeschke and Nauen, 2008). However, possible adverse effects of imidacloprid include oncogenicity teratogenicity in rats, DNA damage and neurotoxicity (CDPR, 2011). Moreover, Johnson et al. (2010) described that major honey bee losses were attributable to organochlorine, carbamate, organophosphorous and pyrethroid pesticides exposure. Thus, efforts have investigated plant sources that contain natural biopesticides to control phytophagous insects (Cao et al., 2008; Kim and Kim, 2008; Pan et al., 2009). New biopesticides are also needed to combat the evolution of resistance to common pesticides (Copping and Duke, 2007).

Leszczyński et al. (1985) found that phenylpropenoid acids, pyrocatechol and hydroquinone reduced the feeding of *Rhopalosiphum padi* (L.) on winter wheat (*Triticum aestivum* L.). Ciepiela and Chrzanowski (1999) ascertained a higher content of gallic, chlorogenic and caffeic acids within winter triticale cultivars which possessed a higher level of resistance to *S. avenae*. Moreover, Ciepiela and Chrzanowski (2001) showed the positive association between

Abbreviations: GST, glutathione *S*-transferase; POD, peroxidase; PPO, polyphenol oxidase; TCA, trichloroacetic acid; CDNB, 1-chloro-2,4-dinitrobenzene.

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the concentration of ferulic acid and the resistance of winter triticale to grain aphids. Plant phenolics are considerable to have major roles in plant defence against insect herbivores (Rattan, 2010). The deleterious effects of plant extracts on insect pests can be manifested in several ways, including increased toxicity, mortality, as well as a reduction in fecundity. Multiple mechanisms of action give phytochemicals unique properties that make them useful as part of modern integrated pest management programmes (Copping and Menn, 2000). It has been suggested that extracts from plants, which contain numerous compounds, have more complex effects in comparison to synthetic pesticides and, therefore, may delay the build-up of resistance (Völlinger, 1987; Rice, 1993). However, their specific mode of action is not yet clearly known.

Sour cherry (*Prunus cerasus* L.) and black currant (*Ribes nigrum* L.) have been recommended as anti-inflammatory and antibacterial agents. Rauha et al. (2000) showed the antimicrobial properties of an extract from *R. nigrum*, whereas phenolic substances and antioxidative factors have been found in cherries (Kim et al., 2005). Polyphenols obtained from walnut, *Juglans regia* (L.), have been reported as strong antioxidants (Fukuda et al., 2003). Scavenging of hydroxyl and superoxide radicals was also found in water and methanol extracts of walnut (Ohsugi et al., 1999).

Herbivorous insects can transform plant toxins into less toxic soluble molecules, and this strategy is a major weapon in their co-evolutionary arms race with plants (Despres et al., 2007). Plant phenolic detoxifying systems in insect herbivores have been shown to use enzymes such as glutathione *S*-transferase (Francis et al., 2005), as well as polyphenol oxidase and peroxidase (Giordanengo et al., 2010). Glutathione *S*-transferase (GST, EC 2.5.1.18) is an esterase that metabolises different groups of pesticides and xenobiotics within arthropods. GST belongs to the second phase of detoxifying enzymes which catalyse the conjugation of electrophilic molecules with reduced glutathione (Francis et al., 2005). Polyphenol oxidase (PPO, EC 1.10.3.1) acts as monophenol monooxygenase and/or *o*-diphenol oxidase. The enzyme catalyses hydroxylation of monophenols, and their further oxidation to quinones and non-toxic melanin pigments (Robinson et al., 1991). Peroxidase (POD, EC 1.11.1.7) oxidises phenols and other aromatic derivatives with hydrogen peroxide. Oxidoreductases, e.g. peroxidase and polyphenol oxidase, metabolise plant phenolics and play a fundamental role during food digestion (Miles and Peng, 1989; Urbańska et al., 2002). The presence of polyphenol oxidase and peroxidase within aphids enables them to neutralise the detrimental effects of a wide range of phenolic compounds (Urbańska et al., 1998).

The present study was conducted to elucidate some of the effects of the application of mixtures of phenolic acids obtained from the leaves of *R. nigrum* and *P. cerasus* as well as the leaves and green husks of *J. regia* on grain aphid growth and development. The specific objectives were: (i) to analyse the phenolic acid composition in the tested plant extracts; (ii) to determine the antibiotic parameters on grain aphids after treatment with phenolic acid mixtures; (iii) to determine activities of the detoxification enzyme glutathione *S*-transferase and the oxidoreductases peroxidase and polyphenol oxidase in *S. avenae* in response to exposure to mixtures of phenolic acids extracted from tested plants; and (iv) to examine the relationship between the activities of the analysed enzymes and the development of the *S. avenae* population.

2. Materials and methods

2.1. Aphids

The grain aphid, *S. avenae* (green colour form) was collected from a stock culture raised on winter wheat seedlings cv. Tonacja at the Department of Biochemistry and Molecular Biology at the Siedlce University of Natural Sciences and Humanities. Parthenogenetic morphs of the aphid were reared on wheat seedlings in a climatic chamber (16:8 L:D photoperiod, temperature 24:18 °C and 65% relative humidity) and apterous females were used in all the experiments.

2.2. Phenolic acids

Phenolic acid mixtures were obtained from leaves of black currant cv. Bona, sour cherry cv. Lutówka and leaves and green husks of walnut cv. Albi. Plants were cultivated without chemical control of insects in a small private orchard located in Siedlce. The green and healthy leaves uninfested by aphids and without necrosis as well green husks were harvested in July 2010. The procedure of phenolic acid extraction was partially based on the method described by Kowalski and Wolski (2003). Extraction of the phenolic acids was performed using 25 g of air-dried plant material with 80% methanol. Pigments and fatty substances were removed with petroleum ether and the extracts were then cleared with sodium hydrogen carbonate (5% final concentration). The extracts were acidified to pH 2.0 with 6 M hydrochloric acid and the phenolic acid fraction was immediately extracted with ethyl acetate. The organic phase was evaporated to dryness under a vacuum at 40 °C with a Heidolph Hei-VAP Precision system and the crude extracts were prepared by dissolving the phenolic acid residues in 25 ml of 80% methanol. Spraying mixtures (1% and 5% v/v; containing 4% methanol) were prepared from the crude extracts after further dissolving with distilled water containing 0.075% (v/v) Tween 80.

2.3. HPLC analysis

Phenolic acids for chromatographic analysis were extracted from 1 g of air-dried leaves of black currant, sour cherry and walnut, and green husks of walnut according to Kowalski and Wolski (2003). The final volume of 80% methanol used for dissolving the phenolic residues was 5 ml. The extraction and HPLC separation were repeated three times for each tested plant material. Chromatographic separation of the phenolic acids was carried out based on the method of Kowalski and Wolski (2003) using an HPLC isocratic Varian ProStar system, equipped with a ProStar 210 pump, a ProStar 335 Photodiode Array Detector and a Microsorb MV 100-5C18 (250 × 4.6 mm) column. The mobile phase was a mixture of methanol and water (25:75) with the addition of 1% (v/v) acetic acid. Identification of the phenolic acids was done using Varian software (Star Chromatography Workstation ver. 6.41, Aurora and PolyView 2000). Retention times and the UV/VIS spectra of separated compounds were compared with standards obtained from Sigma Chemical Co., St. Louis, Mo, USA. The quantity of phenolic acids present in each sample was then estimated by calibrating the total peak area against the reference standard of known weight. All experiments were repeated three times and the results are expressed in μg g^{-1} of dry weight (d.w.) as the mean ± standard error.

2.4. Bioassay

Experiments took place in a climatic chamber at 23.5–25.5 °C (light phase) and 18.5–20.5 °C (dark phase) in 65 ± 5% relative humidity and with a photoperiod of 16:8 (L:D). Seven-day old winter wheat seedling, cv. Tonacja at stage of first leaf – G.S. 11 (Tottman and Broad, 1987), was infested with a wingless female (one female was kept on one plant with use of small brash). After 30 min, the aphid was sprayed with phenolic acid mixtures obtained from crude extracts of the tested plants (black currant, sour cherry and walnut; 1% or 5% of the crude extract in 4% methanol, v/v) or 4% methanol (control). Spraying device with atomiser was used (bottle 20 ml;