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Original Research

Cereal Phenolic Compounds as Biopesticides of Cereal Aphids

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Abstract

Triticale (*Triticosecale* Wittmack) is a hybrid crop developed by crossing wheat (*Triticum*) and rye (*Secale*). The associations between the concentrations of total phenols and *o*-dihydroxyphenols within tissues of winter triticale with their resistance to the grain aphid and bird cherry-oat aphid were studied. In the tissues of study plants, the highest amount of phenols was observed in flag leaves of transgenic plants (9.34 $mg \cdot g^{-1}$), and the lowest in ears of wild-type control plants (1.13 $mg \cdot g^{-1}$). Similar clear trends also were obtained for content of *o*-dihydroxyphenols. In general, transgenic triticale plants were more resistant to the cereal aphids than the regular ones. Aphid development time was prolonged while fecundity and intrinsic rate of natural increase (r_m) were reduced. In addition, the triticale phenolics increased the density of the cereal aphid population. The importance of the phenolic compounds in the resistance of plants (cereals) to the aphids (grain aphid) is discussed.

Keywords: phenolic compound, aphids, *Rhopalosiphum padi*, *Sitobion avenae*, transgenic plants

Introduction

Pesticides have traditionally been used to control insect damage on economically important plants. For the future, it is necessary to develop a more environmentally friendly agriculture that will decrease inputs in energy and chemicals, and generate fewer harmful outputs such as pesticide residues.

Plants and insects have coexisted for at least 100 million years, and have evolved a variety of beneficial and deleterious interactions. One of them is a natural plant resistance to pests and pathogens, e.g. aphid-resistant cultivars [1-6].

Plants use many strategies to protect themselves against insects. For example, morphological, anatomical, and chemical plant properties play an important role in the natural plant resistance to aphids. Chemical components are among the most important mediators in the insect-plant interactions. Many of these substances act as biopesticides that naturally occur within the host-plant tissues.

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Most studies on plant-insect interactions have focused on secondary plant compounds [7-12]. Secondary plant substances can be defined as plant compounds that are not universally found in higher plants, but are restricted to certain plant taxa, or occur in certain plant taxa at much higher concentrations than in others. The secondary plant metabolites are an integral part of plant metabolism, and play important ecological and physiological roles in chemical interactions between plants and pathogens [13]. They affect growth, health, and behaviour of other plant or animal species, and are called allelochemicals or xenobiotics [14].

Many of them, including phenolics (especially *o*-dihydroxyphenols, glucosinolates, alkaloids, cyanogenic glycosides, and furanocumarins) are known as the protective agents toward various species of aphids. They seriously affect aphid behaviour, physiology, and metabolism, and as a result they reduce aphid populations on resistant plants [15-19]. Phenols are one of the most active groups of allelochemicals that unfavourably affect aphid growth, development and/or feeding behaviour [20]. In particular, they

are important resistance factors to the grain aphid, and are potentially useful for triticale resistance breeding programs. In some triticale cultivars, high phenol content relates positively to resistance against the grain aphid, *Sitobion avenae*. Phenols also interfere with aphid feeding behavior [21, 22].

Improvement of pest resistance in crop plants by implementing genetic engineering requires more precise knowledge of herbivorous insect-cereal relationships. As part of this effort, the research detailed here reports on the influence of winter triticale phenolics on cereal aphid growth, development, and population density.

Experimental Procedures

Plant Material

Plants of transgenic triticale (MS×325/95, MS×366-3/95) and non-transgenic triticale plants (Tewo, Bogo) were used in the experiments. The studied triticale plants were obtained from the Institute of Plant Breeding and Acclimation at Radzików/Błonie near Warsaw, Poland.

Field Experiments

Entomological Experiment

Field observations were performed on 3.0×3.0 m experimental plots at IHAR. A population study of two species of aphids (bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus, 1758) and grain aphid, *Sitobion avenae* (Fabricius, 1775) was carried out on transgenic (MS×325/95, MS×366-3/95) and non-transgenic plants of winter triticale (Tewo, Bogo). The cereal aphids' density on the studied triticale was estimated according to the method described by Wratten et al. [23] and Lykouressis [24]. The observations were carried out from aphid arrival on the plants until their disappearance (G.S. 47-75; Tottman and Broad scale [25]. Aphids were counted along the diagonal of the plot, five times per season, on 50 randomly chosen plants. The population of the cereal aphids was studied on three replicated plots per genotype of plant. Results of the observations were used to calculate the total number of aphids living on an individual plant and the percentage of infested plants.

Antibiosis Experiment

Plants of transgenic triticale (MS×325/95, MS×366-3/95) and non-transgenic triticale plants (Tewo, Bogo) were examined for their antibiosis to cereal aphids in the field at IHAR Radzików. During the anthesis, plants were covered with 3×3×2 m nylon cages (1×1 mm mesh) to exclude birds and cylindrical, plastic cages (13×4 cm) were placed on the flag leaves of 20 plants inside the large cages. At the time, one apterous adult female was placed in each small cage for one day. The adult and all nymphs but one were then discarded. When the nymph matured and began producing offspring, the offspring were counted and removed daily. The aphid's prereproductive period (time from birth until maturity of female) and daily fecundity were estimated [18]. Population parameters were used to determine the influence of plants on cereal aphid population growth potential. The intrinsic rate of natural increase (r_m) and mean time of generation development (T) were calculated using the following equations after Wyatt and White [26].

$$r_m = 0.738\frac{\ln Md}{d}$$

$$T = \frac{d}{0.738}$$

...where d is the length of the prereproduction period, and Md is the number of larvae born during the reproductive period that equals the d period, 0.738 of the correlation factor.

Chemical Analyses

Phenolic Assay

At the time of peak density of cereal aphids on the triticale (stage of anthesis G.S. 65) noninfested flag leaves and ears (isolated on the field) of studied plants were sampled for analyses. The plant material was placed in solid carbon dioxide (dry ice) and transferred to the laboratory, where it was subjected to freeze-drying.

Phenolic compounds were extracted from the lyophilizate. 5 g samples were extracted continuously in the Soxhlet apparatus using 150 cm^3 of chloroform for 2 h and then 200 cm^3 of 70% ethanol for 6 h. Content of the total phenols, soluble in ethanol within flag leaf and ear tissues of the studied triticale plants was determined using a colorimetric method after Singh et al. [27]. 0.5 cm^3 of ethanol extract was taken for analysis and 14.5 cm^3 of redistilled water and 5 cm^3 of Singh's reagent were added. Absorbance values were measured at the wavelength of 700 nm, against the control sample. Total phenol content was estimated using a standard curve for ferulic acid solutions.

The same samples and extraction method used for total phenol analysis were also used for the analysis of *o*-dihydroxyphenols. The level of the *o*-dihydroxyphenols within the alcohol extracts was measured using Arnov's colorimetric method according to Leszczyński [28]. 5 cm^3 of ethanol extract was mixed with 1 cm^3 of 0.5 M HCl solution, 1 cm^3 of Arnov'a reagent, 2 cm^3 1M NaOH and 1 cm^3 of redistilled water. Absorbance values were measured at the wave length of 520 nm, against the control sample. *O*-dihydroxyphenols content was estimated using a standard curve for catechol solutions.

Total phenol and *o*-dihydroxyphenols concentration was expressed as $mg \cdot g^{-1}$ dry weight. All chemical analyses were performed in three replicates.

Table 1. Occurrence of the cereal aphids on the studied triticale in the field ($\bar{x}$±SD).

Plants	Parameters			
	Number of individuals/blade		Percentage of plants infested	
	S. avenae	*R. padi*	*S. avenae*	*R. padi*
Tewo	0.80^{b}±0.43	0.39^{ab}±1.24	22.89^{a}±1.05	10.67^{b}±1.61
Bogo	1.30^{a}±1.12	0.63^{a}±0.29	27.22^{a}±1.04	17.22^{a}±0.20
MS×325/95	0.27^{c}±0.22	0.19^{b}±1.36	10.67^{b}±1.28	7.78^{b}±2.06
MS×366-3/95	0.40^{c}±1.32	0.20^{b}±2.12	15.55^{b}±2.16	8.89^{b}±2.45

Means in the columns followed by various letters are significantly different at P≤0.05 (Duncan's test).

Statistics

Differences between the means were subjected to one-way Anova, followed by Duncan's test. The linear correlations between the concentrations of the chemical compounds and the population parameters of the aphids on the studied triticale plants were calculated.

Results

Abundance of the Cereal Aphids on the Studied Triticale Plants

On the basis of field observations it was found that the transgenic plants of winter triticale (MS×325/95, MS×366-3/95) were attacked less by the cereal aphids, *S. avenae* and *R. padi,* than the regular ones (Tewo, Bogo). The largest number of grain aphids was on Bogo non-transgenic plants, and the lowest on MS×325/95 transgenic plants. *R. padi* also most often inhabited Bogo and Tewo cultivars, while MS×325/95 and MS×366-3/95 were least accepted. The higher percentage of plants inhabited by cereal aphid was Bogo cultivar (27.22%) the lower MS×325/95 (7.78%) and MS×366-3/95 (8.89%) (Table 1).

Cereal Aphid Performance on Transgenic and Non-Transgenic Plants of the Studied Winter Triticale

Cereal aphid performance on the transgenic plants was clearly reduced in comparison to the non-transgenic plants. Particularly, flag leaves of transgenic triticale plants (MS×325/95, MS×366-3/95 prolonged larval development of the cereal aphids and shortened its reproduction. Moreover, aphid fecundity and the intrinsic rate of natural increase (r_m) were both reduced (Table 2). The transgenic plants of winter triticale (MS×325/95, MS×366-3/95) also caused reduction of average generation development time (T) (Table 2).

Table 2. Values of the cereal aphid population parameters on the studied triticale in the field ($\bar{x}$±SD).

Plants	Parameters			
	Prereproductive period (days)	Daily fecundity per female	Intrinsic rate of natural increase (r_m)	Mean time of generation development (T) (days)
S. avenae				
Tewo	5.6^{d}±0.31	2.6^{ab}±0.92	0.4721^{ab}±0.0070	7.2^{bc}±0.40
Bogo	5.1^{d}±0.28	3.0^{a}±0.17	0.5016^{a}±0.0011	6.9^{c}±0.32
MS×325/95	8.3^{a}±0.09	1.0^{c}±0.33	0.2138^{c}±0.0016	9.7^{ab}±0.14
MS×366-3/95	7.2^{b}±0.23	1.3^{c}±0.54	0.2667^{c}±0.0040	9.3^{ab}±0.28
R. padi				
Tewo	6.4^{c}±0.41	2.3^{b}±0.19	0.4073^{b}±0.0013	8.1^{b}±0.33
Bogo	5.8^{d}±0.13	2.4^{b}±0.27	0.4097^{b}±0.0014	7.9^{b}±0.08
MS×325/95	8.6^{a}±0.65	1.0^{c}±0.39	0.2236^{c}±0.0020	10.2^{a}±0.78
MS×366-3/95	8.5^{a}±0.30	1.2^{c}±0.18	0.2097^{c}±0.0012	10.0^{a}±0.39

Means in the columns followed by various letters are significantly different at P≤0.05 (Duncan's test).

Table 3. Content (mg·g^{-1} dry weight) of the ethanol soluble phenolic compounds within flag leaves and ears tissues of the triticale cultivar ($\bar{x}$±SD).

Plants	Total phenols	
	Flag leaves	Ears
Tewo	4.62^{c}±0.22	2.50^{c}±0.10
Bogo	2.00^{d}±0.30	1.13^{d}±0.14
MS×325/95	9.34^{a}±0.37	8.74^{a}±0.26
MS×366-3/95	8.11^{b}±0.29	7.00^{b}±0.01

Means in the columns followed by various letters are significantly different at P≤0.05 (Duncan's test).

Content of the Phenolic Compound in Tissue Plants of the Studied Winter Triticale

Chemical analysis showed a much higher content of soluble total phenolics extracted from the transgenic triticale plants (Table 3). The mean concentration of total phenols ranged from 2 mg·g^{-1} dry weight in flag leaves of regular cultivar plants Bogo to 9.34 mg·g^{-1} in flag leaves of transgenic triticale plants MS×325/95. The total phenolic concentration in the flag leaves was almost two times higher than concentrations in ears. Wild-type Bogo ears had the lowest amount of phenols (1.13 mg·g^{-1} dry weight), while transgenic triticale MS×325/95 plants had the highest (8.74 mg·g^{-1}) (Table 3).

Similar clear trends also were obtained for content of *o*-dihydroxyphenols (Table 4). The content of *o*-dihydroxyphenols ranged from 3.57 mg·g^{-1} dry weight in transgenic leaves to 0.31 mg·g^{-1} in leaves of wild-type control plants. The highest content of *o*-dihydroxyphenols was observed for MS×325/95, while the lowest was noted for Bogo. In the ears of study plants the highest amount of phenols was also observed on MS×325/95 (3.05 mg·g^{-1}), and the lowest on Bogo (0.22 mg·g^{-1}) (Table 4).

Table 4. Content (mg·g^{-1} dry weight) of the o-dihydroxyphenols within flag leaves and ears of the triticale cultivar ($\bar{x}$±SD).

Plants	o-dihydroxyphenols	
	Flag leaves	Ears
Tewo	1.82^{c}±0.19	1.40^{c}±0.00
Bogo	0.31^{d}±0.00	0.22^{d}±0.03
MS×325/95	3.57^{a}±0.43	3.05^{a}±0.16
MS×366-3/95	2.75^{b}±0.05	2.36^{b}±0.08

Means in the columns followed by various letters are significantly different at P≤0.05 (Duncan's test).

Effect of Phenolics on Cereal Aphid Abundance in the Field

The concentration of total phenols in the flag leaves was negatively significantly correlated with population size of the *S. avenae* (r=-0.9981, P≤0.01) and *R. padi* in field (r=-0.9979, P≤0.01), and the percentage of plants inhabited by *R. padi* (r=-0.9841, P≤0.05). Highly significant negative correlations were also found between the content of total phenols in the ears and the number of grain aphid (r=-0.9939, P≤0.01) and bird cherry–oat aphid (r=-0.9694, P≤0.05), and percentage of plants infested by grain aphid (r=-0.9796, P≤0.05).

Similarly, significant negative correlations were found between the content of *o*-dihydroxyphenols in the flag leaves and number of *S. avenae* (r=-0.9665, P≤0.05) and *R. padi* (r=-0.9717, P≤0.05) and percentage of plants infested by *R. padi* (r=-0.9878, P≤0.05). The concentration of these compounds in the ears was also significantly negatively correlated with the number of *S. avenae* (r=-0.9727, P≤0.05) and *R. padi* (r=-0.9789, P≤0.05), and the percentage of plants inhabited by *R. padi* (r=-0.9998, P≤0.01).

Discussion

Grain aphid *Sitobion avenae* (F.) and bird cherry-oat aphid *Rhopalosiphum padi* (L.) are an important pest of triticale in Poland. Since the cultivation area of this cereal is constantly increasing, there is a strong desire to reduce the application of synthetic pesticides and use alternative plant protection methods. Transgenic cultivars are one of the most promising methods of plant protection. Today, plant biotechnology is being used as a tool to give plants new traits that benefit agricultural production, the environment, human nutrition, and health. However, breeding of such plants requires detailed knowledge of plant structure and chemicals involved in the resistance. The chemical interactions between plants and phytophagous insects include diverse secondary metabolites and also herbivores' adaptations to these substances.

The obtained results also indicate that the level of phenolic compounds might play an important role in the resistance of winter triticale hybrids to cereal aphids. Total phenol and *o*-dihydroxyphenol contents were associated with the values of antibiotic resistance and the population density between the transgenic and non-transgenic plants. According to Levin [29], one of the most important groups of the plant secondary constituents playing a defensive role against pests are phenolics. Phenolics are biologically active secondary metabolites that influence insect growth and feeding [6, 30-33]. The results presented here indicate that the transgenic plants that contain a high level of phenolic compounds were less accepted by the cereal aphid. These compounds are widespread in the plant kingdom. [34]. Eleftherianos et al. [35] revealed an inverse correlation between the total concentration of plant total phenols in

aphid-infested maize and barley plants. Similar relationships have also been observed for other aphid species such as *Metoplophium dirhodum*, *Schizaphis graminum*, and *Diuraphis noxia* [36-38].

There are many examples of negative associations between the secondary compounds present in herbaceous/woody plant species and insects. Cipollini et al. [6] found that leaves of *Lonicera maackii* contain phenolic compounds, including apigenin and chlorogenic acid, that deterred feeding of the generalist herbivore, *Spodoptera exigua*. In the studies carried out by Leiss et al. [39], *Frankliniella occidentalis*-resistant chrysanthemums contained higher amounts of the phenylpropanoids chlorogenic acid and feruloyl quinic acid. Both phenylpropanoids are known for their inhibitory effect on herbivores [40-42]. Moreover, chlorogenic acid has been described as an antifeedant and digestibility reducer in insects such as aphids [43]. Feruloyl quinic acid has been implicated in the resistance of cereal aphids [44, 45] and cereal midges [46, 47].

Leiss et al. [48] showed that resistant hybrids contained higher amounts of the flavonoid kaempferol glucoside. Flavonoids are generally involved in plant resistance to herbivores [19, 49-51]. Kaempferol glucosides also have a negative effect on aphids. Aphid-resistant cow pea lines contained significantly higher amounts of flavanoids, including kaempferol, compared to susceptible lines [30]. Similar results had been obtained earlier by other authors. For example, rose buds contained high concentrations of catechol and catechin that reduced the growth and development of *Macrosiphum rosae* [52]. Cole [53] showed that the high concentration of total phenols within tissues of lettuce was related to their resistance to the *Penphigus bursarius* aphid. Furthermore, 4-hydroxycoumarins and their derivatives and some flavonoids are feeding deterrents to several aphid species [54, 55]. Cichocka et al. [56] found negative relationships between phenolic compound concentrations and the acceptance of broad bean cultivars by the black bean aphid *Aphis fabae*.

Additionally, *in vitro* experiments showed that phenolic compounds exerted negative influence on the feeding, growth, survival, and reproduction of the aphids *Sitobion avenae*, *Schizaphis graminum*, and *Myzus persicae* [57-59, 13, 27, 4]. Similar effects on aphid biology also are exerted by different groups of secondary plant compounds [60-63]. All of the phenolic compounds found to be especially active in the inhibition of the feeding of the insects are dihydroxyphenols [17]. The *o*-dihydroxyphenols occurring in the triticale plants are especially toxic to insects. They exert a negative influence on aphids even at low concentration [57].

Many authors state that plant hybridization may drive the evolution of novel secondary plant metabolites [64, 65], leading to enhanced resistance to herbivores and pathogens [66, 67]. Moreover, Wei et al. [67] found that the density of secretory glandular trichomes was significantly greater in transgenic than in wild-type plants, and mortality of adult whiteflies fed transgenic tobacco plants was significantly higher than those reared on control plants. The results of the present study demonstrated that biochemical composition of plants might be a good indicator of their acceptability by aphids. The transgenic triticale plants containing a higher level of phenolics were less attractive for cereal aphids. These compounds are involved in the defensive strategies of cereal crops against aphids.

Conclusions

The triticale hybrids containing higher phenolic levels were less attractive for cereal aphids than standard, non-transgenic cultivars. The results indicate that the *o*-dihydroxyphenols of the plant tissues may be one of the most important groups of chemicals playing a major role in aphid-cereal interactions. Thus phenolics should be considered within a breeding program of new naturally aphid-resistant cereals.

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