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Effect of triticale surface compounds on growth and development of cereal aphids

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Introduction

The plant cuticle represents the interface between the plant ant its environment (BARGET *et al.*, 2003). The outer surface of the cuticle is covered with epicuticular waxes that present in diverse forms, from amorphous to crystalline deposits, and consists of complex mixtures of aliphatic components and sugars, amino acids, and secondary plant substances such as glucosinolates, furanocumarins, alkaloids and phenolics (TULLOCH, 1976; BAKER, 1982; EIGENBRODE & ESPELIE, 1995; SCHOONHOVEN, 2006).

Although the primary role of epicuticular wax is to prevent uncontrolled water loss, they might also contribute to plant-insect interactions (JUNIPER, 1995; EIGENBRODE *et al.*, 1997; SHEPHERD *et al.*, 1999; WHITE & EIGENBRODE, 2000; EIGENBRODE & JETTER, 2002; DUETHING *et al.*, 2003; HUANG *et al.*, 2003; CHANG *et al.*, 2004; MOHAMMADIAN *et al.*, 2007). The chemical composition and structure of the epicuticular wax mediate the plants attractiveness to pests (EIGENBRODE, 2004). Differences in the chemical composition of the plant surface are associated with resistance to herbivores and/or with their effect on herbivores behavior. They also show a strong influence on aphid behaviour and physiology which results in a reduction of the aphid growth and development (SZAFRANEK & SYNAK, 2006).

Improvement of pest resistance in crop plants by classical breeding methods or genetic engineering requires knowledge of more precise details of herbivore feeding behaviour, plant acceptability and chemical structures involved in the moderate resistance (SCHOONHOVEN, 2006). The present paper reports on the effect of the triticale surface compounds on the growth and development of cereal aphids.

Material and methods

Plant material

The experiments were held on seedlings of wax-covered (RAH 122) and wax-less (RAH 366) triticale hybrids obtained from Institute of Plant Breeding and Acclimation at Radzików/Błonie near Warsaw (Poland). Seeds of the studied hybrids were germinated in a climatic chamber, kept at 20-25°C under a 16h daylight and 8h of darkness, and 70% humidity. The seedlings were grown in a medium nutrient fine structure compost with sand, in 7 cm x 7 cm 9 x cm plastic pots, one plant per pot. The plants were regularly watered and no extra fertilizer was added.

Aphids

The aphids came from a stock culture kept at the University of Podlasie at Siedlce. Parthenogenetic clones of *Sitobion avenae* (F.) and *Rhopalosiphum padi* (L.) were reared on winer wheat seedlings in an environmental chamber (21°C, 70% relative humidity, 16h:8h L:D photoperiod).

Extraction of surface wax

Three various methods of the extraction using three solvents of different polarity (dichloromethane, chloroform, ethanol) were performed to remove the surface chemicals from triticale seedlings. The seedlings were immersed into dichloromethan for 5s, into chloroform for 5s, and into ethanol for 20s at room temperature, and control seedlings were not dipped in solvents.

The effect of triticale surface compounds on cereal aphids

Presence of aphid feeding deterrents/stimuli in surfacing extracts of the waxes on triticale RAH 122 were conducted on the dichloromethane, chloroform and ethanol extracts. Two different acceptance tests were performed. The first was conducted on RAH 122 seedlings with chemically removed surface compounds in comparison to the control (without extraction) seedlings. The second test was made on young seedlings of the wax-less hybrid RAH 366 which were sprayed with the previously obtained extracts of the RAH 122 and related to the control seedlings sprayed only with the used solvents. In both groups of the experimental seedlings cereal growth and development was recorded.

Population tests

The population tests were conducted in an environmental chamber, at a 16 h : 8 h photoperiod, temperature 21±1°C, 70% relative humidity, in Plexiglass cages with a cheese cloth cover. The adult apterous females were caged individually on 5-day-old seedlings and allowed to deposit nymphs. After 24 hours, only one nymph, remained on each single plant, other offspring and the adult were removed. Development time (from birth until maturity) and reproduction of the individual apterous females were observed daily until death (LESZCZYŃSKI, 1996). The experiments were run in 10 independent replicates for each hybride of the studied winter triticale.

Statistics

Differences in the aphid growth and development in the conducted experiments were subjected to one-way ANOVA, followed by Duncan's test.

Result and discussion

Growth and reproduction of the cereal aphid species on control seedlings of the waxy triticale hybrid was clearly reduced in comparison to the wax-less one. Particularly, plants of the waxy triticale cultivar prolonged larval development of the grain aphid and the bird cherry-oat aphid and shortened their reproduction (Tab. 1., 2.). On the waxy triticale reproductive period of the studied aphids began later and finished earlier than on the wax-less hybrid. The waxy triticale seedlings also caused reduction of other studied population parameters such as daily and total fecundity. *S. avenae* proved to be significantly more fecund than *R. padi* on the triticale seedlings (Tab. 1., 2.).

The removal of surface compounds with the used organic solvents clearly shortened prereproductive period of the grain aphid and bird cherry-oat aphid on the waxy hybrid RAH 122 (Tab. 1.). Such treatment with organic solvent, also clearly expended a fecundity of by the grain aphid and bird cherry-oat in comparison to control seedlings of the heavy-waxy hybrid. Among the studied solvents the most effective was dichloromethane (Tab. 1.).

Extracts from RAH 122 hybrid applied on seedlings of wax-less hybrid (RAH 366), significantly prolonged the larval development and decreased daily and total fecundity of both aphid species (Tab. 2.).

Quite often insects appear to be unable to distinguish between plant varieties without tasting them. Therefore, one of the most important factors of preference or non-preference by aphids of the studied hybrids might be chemical attractants and repellents within plant tissues present on their surface. Degree of the preference of the studied hybrids was strongly related to the epicuticular wax layer of the plants.

Table 1. Effect of the removed surface compounds from waxy triticale hybrid RAH 122 on development and fecundity of the grain aphid and bird cherry-oat aphid.

Aphid species and used extractions		**Prereproductive period (days)**	**Daily fecundity (per female)**	**Total fecundity (per female)**
S. avenae	**control (none)**	8.0 a	1.4 b	29 c
	dichlorometan	5.5 c	2.1 a	44 a
	chloroform	5.7 c	1.8 a	39 ab
	ethanol	5.8 c	1.9 a	40 a
R. padi	**control**	9.0 a	1.3 b	27 d
	dichlorometan	6.5 b	2.0 a	42 a
	chloroform	6.4 b	1.7 a	36 b
	ethanol	6.0 c	1.8 a	39 ab

Values in columns followed by various letters are significantly different at p≤0,01 (Duncan's test).

Table 2. Effect of the surface compounds on growth and fecundity of the cereal aphids placed on seedlings of the wax-less hybrid (RAH 366), previously spread with the extracts from RAH 122 hybrid

Aphid species and used extractions		**Prereproductive period (days)**	**Daily fecundity (per female)**	**Total fecundity (per female)**
S. avenae	**control (none)**	5.0 c	2.6 a	55 a
	dichlorometan	8.0 a	1.4 c	29 e
	chloroform	7. ab	1.7 b	35 c
	ethanol	7. ab	1.7 b	36 c
R. padi	**control**	5.4 c	2.3 a	49 b
	dichlorometan	7,5 ab	1.4 c	30 d
	chloroform	7.6 ab	1.6 b	33 cd
	ethanol	7.0 b	1.8 b	37 c

Values in columns followed by various letters are significantly different at p≤0,01 (Duncan's test).

The obtained results also indicated that chemical composition of the epicuticular wax layer may be one of the most important sources of the allelochemicals (repellents) playing a major role in triticale cereal aphid interactions. The layer of the epicuticular waxes may contain aliphatic components, sugars and amino acids (EIGENBRODE & ESPELIE, 1995), as well as secondary metabolites such as glucosinolates, furanocumarins, alkaloids, triterpenoids, flavonoids and phenolics (SCHOONHOVEN *et al.*, 2006). Its composition varies among species, and genotypes of plants during ontogeny (EIGENBRODE & ESPELIE, 1995; JETTER & SCHÄFFER, 2001). Differences in the chemical composition of the plant surface might be also associated with resistance to herbivores including their effect on feeding behavior. For example, removal of the surface waxes with chloroform from seedlings of *Sorghum bicolar* (L.) caused their acceptance by nymphs of *Locusta migratoria* L. (WOODHEAD, 1983). Hexane extracts of surface lipids from resistant rice cultivars deterred feeding of the brown planthopper, *Nilaparvata lungens* (Stäl) (WOODHEAD & PADGHAM, 1988).

Summing up the results presented here suggest that chemicals which occur within epicuticular waxes of the triticale play an important role in its acceptance by the grain aphid and the birch cherry-oat aphid. Thus the surface compounds might be responsible for expression of the triticale resistance to cereal aphids. However, further study is needed to understand the role of waxes in the mechanism of triticale resistance to cereal aphids.

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Wpływ związków powierzchniowych na wzrost i rozwój mszyc zbożowych

Streszczenie

Fizyczne i chemiczne właściwości roślin, oddziałują na receptory wzrokowe, węchowe, smakowe i dotykowe owadów. Szczególnie duży wpływ na zachowanie, wzrost i rozwój roślinożernych owadów wywierają związki chemiczne występujące w epikutikularnych woskach.

W prezentowanej pracy zbadano wpływ substancji występujących na powierzchni wybranych rodów pszenżyta ozimego na mszycę zbożową, *Sitobion avenae* (F.) i czeremchowo-zbożową, *Rhopalosiphum padi* (L.). Usunięcie warstwy wosków epikutikularnych przy użyciu rozpuszczalników o różnej polarności (dichlorometan, chloroform, etanol) znacznie skróciło długość okresu przedreprodukcyjnego i zwiększyło płodność obu badanych gatunków mszyc. Spośród badanych rozpuszczalników największą efektywność w usuwaniu wosków stwierdzono dla dichlorometanu. Ponadto ekstrakty wosków epikutikularnych z silnie pokrytego woskiem rodu RAH 122 znacznie wydłużały długość okresu przedreprodukcyjnego i obniżały płodność bezskrzydłych samic mszycy zbożowej i czeremchowo-zbożowej, po naniesieniu na siewki rodu RAH 366 charakteryzującego się słabym nalotem woskowym.

Na podstawie przeprowadzonych badań stwierdzono, że składniki wosków występujących na powierzchni pszenżyta ozimego wydają się być ważnymi czynnikami odpornościowymi dla badanych gatunków mszyc.