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Original Research

Fecundity and Survival of Grain Aphid at Various Growth Stages of Waxy and Waxless Triticale

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Abstract

Plants use many strategies to protect themselves against insects. Morphological, anatomical, and chemical plant properties play an important role in natural plant resistance to herbivore insects. The effects of triticale genotypes with different wax covers at various growth stages was studied on the fecundity and survival of the grain aphid *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae). The aphids that fed on the organs of the waxless genotypes survived longer and produced significantly more nymphs. Furthermore, mortality of nymphs on the waxy plants was significantly higher than on waxless plants. The chemical analysis also showed that the waxless plants had lower levels of flavonoids in comparison with waxy plants. Waxless seedlings had lower levels of flavonoids at every studied organ. The obtained results indicate that the level of flavonoids might play an important role in the resistance of winter triticale genotypes to grain aphid. The content of flavonoids was associated with the values of resistance between the waxy and waxless plants. It was concluded that the waxy plants that contained high levels of flavonoids were less preferred by the grain aphid.ces, which indicated no significant change in either periphyton or macrozoobenthos following such episodes.

Keywords: flavonoids, *Sitobion avenae*, triticale, resistance

Introduction

Almost all land ecosystems have been strongly shaped by interactions between plants and insects. Plants are attacked by many different herbivores. Some consume whole leaves or roots, while others attack specific types of tissue. For example, piercing-sucking herbivores may feed on sap of xylem, phloem, or other plant cells. Selection of host plants by the aphids involves a sequence of specific behavioral events: attraction in response to visual and olfactory cues, testing of the physiochemical characteristics of the plant surface (including probing by style), penetration to the phloem tissues, and testing of phloem content [1, 2].

The initial line of plant defense against aphids is the epicuticular waxes. After an aphid lands on a plant, various cues on the surface of plants, such as epicuticular wax

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structure and chemical composition, can influence aphid behaviour, growth, and development [3-5]. Volatiles emanating from plant surface waxes can act as attractants or repellents [6, 7]. The layer of the epicuticular waxes may contain aliphatic components, sugars, and amino acids [8-12], as well as secondary metabolites [13-16]. Its composition varies among species and genotypes of plants during ontogeny [17]. Differences in wax chemistry may modulate ecological interactions among plants and insects [7, 11, 18]. Flavonoids are of great interest for their bioactivities, such as their influence on behavior and reduction of fecundity of insects [19]. The feeding and reproduction follow selection of a suitable host. In the absence of the appropriate stimuli, the sequence may be interrupted at any stage, and characteristic behaviour on non-host plants includes increased periods of walking relative to probing, and ultimately the departure of the insect [1, 20, 21].

This aim of this study was to find out the effect of the triticale genotypes with different wax cover at various growth stages on some grain aphid population parameters.

Materials and Methods

Plant Material

Waxy (RAH 122, DED 1137) and waxless (RAH 325, RAH 366) triticale plants were used in the study. The experiment was performed on three organs (fifth leaf, flag leaf, and ear) at three various growth stages (G.S.) of winter triticale determined according to the Tottman and Broad scale [22]. The studied stages were: fifth leaf (G.S. 37), flag leaf (G.S. 46), and heading (G.S. 56). The observations were made on experimental fields in the Institute of Plant Breeding and Acclimatization at Radzików/Błonie near Warsaw, Poland.

Aphids

The grain aphids *Sitobion avenae* Fabricius (Hemiptera: Aphididae) used in the experiments came from a stock culture kept at the University of Natural Sciences and Humanities in Siedlce. The aphid population was reared on winter wheat cv. Tonacja in an environmental chamber (21±1ºC, 70% relative humidity, 16:8 h L:D photoperiod). They were transferred to the studied genotypes for one generation [23]. Then apterous adult females were used in the experiments.

Field Experiments

Field observations were performed on 3.0×3.0 m experimental plots at IHAR. The observations were started with aphid arrival on the plants. Experiments lasted 10 days on 10 randomly chosen plants. During the studied growth stages, plants were covered with nylon cages (3×3×2 m dimension, 1×1 mm mesh) to exclude birds and other insects, and then cylindrical-shaped plastic cages (13×4 cm) were placed on the studied organs of 10 plants inside the large cages. At that time, one apterous adult female was placed in each small cage for 10 days. At 10 days from infestation, surviving apterous adult and nymphs were counted on 10 triticale plants.

Population parameters were used to determine the influence of plant defense on cereal aphid population growth potential. Results of the observations were used to calculate the percentage of adult survival and the number of offspring living on an individual plant. Moreover, mortality of nymphs was calculated. Experiments were conducted on selected organs that corresponded to the grain aphid occurrence in the field at the studied triticale growth stages.

Chemical Analysis

Determinattion of Flavonoids

Noninfested fifth leaves, flag leaves, and ears (isolated in the field) of the 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.

Flavonoid content was determined spectrophotometrically using $AlCl_3$ reagent. The freeze-dried samples were extracted with methanol. The concentration of flavonoids was determined according to the previous published procedure [24]. Briefly, 0.2 ml of $AlCl_3$ 5% in methanol or 0.2 ml of methanol was added to 2 ml of diluted sample. After 30 min the absorbance at 420 nm was measured in both solutions. The concentration was calculated from the differences of both measurements and compared with a routine standard.

Total flavonoids concentration was expressed as $mg \times g^{-1}$ fresh weight. All chemical analyses were performed in three replicates.

Statistical Analysis

Data were subjected to two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test at $P<0.05$. The linear correlations between the concentrations of the chemical compounds and the population parameters of the aphids on the studied triticale genotypes were determined using Pearson correlations.

Results

Population Parameters of the Cereal Aphids on the Studied Triticale Genotypes

The results showed that the survival of *S. avenae* females differed among waxy (RAH 122, DED 1137) and waxless (RAH 325, RAH 366) genotypes on each studied organ (Fig. 1). The mortality of aphid adults was faster on waxy plants than that on waxless ones. The significant higher females survival on waxless genotypes (RAH 325, RAH 366) were observed on all the studied organs (Fig. 2). The highest mortality of females was observed on the fifth-leaf stage /G.S. 37/. Moreover, the highest number of dead females was observed on RAH 122 and DED 1137 genotypes on all the studied organs (Fig. 2).

The results also showed that the *S. avenae* produced significantly more nymphs on waxless than on waxy plants. The significantly higher number of nymphs was produced on waxless plants on all the studied organs. The number of *S. avenae* nymphs ranged from 6.7±2.03 to 36.4±1.38, with the lowest value going to RAH 122 and RAH 366 having the highest number (Table 1). It was also found that the grain aphid was the most abundant on the ears of the tested genotypes of winter triticale. The flag leaves as well as fifth leaves were definitely less suitable for this pest (Table 1).

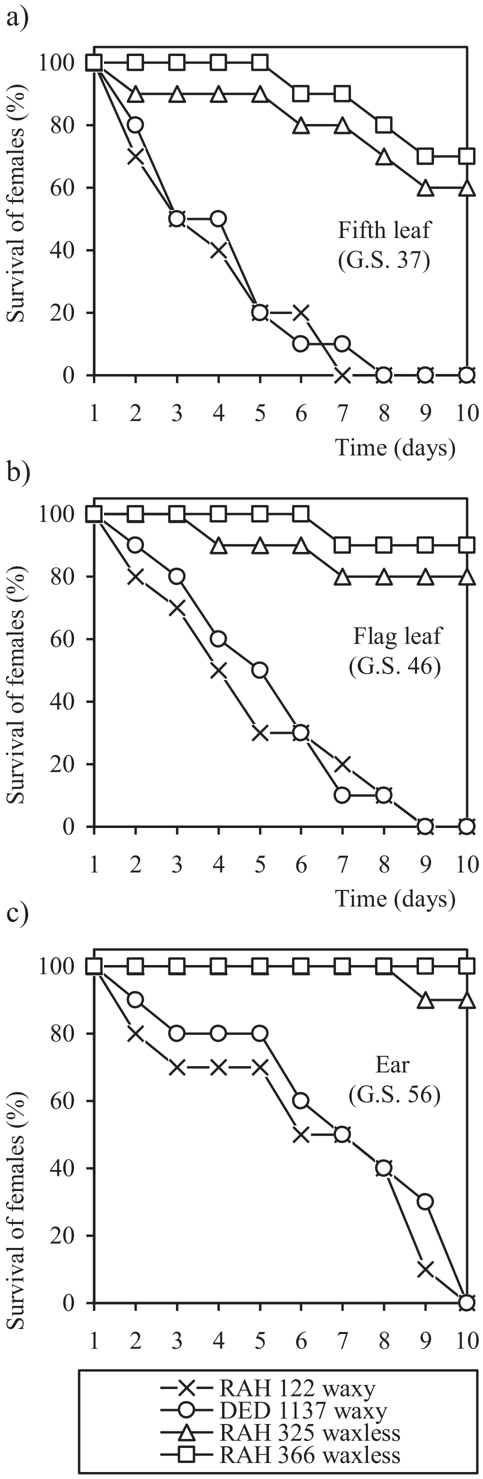

Fig. 1. Survival of *S. avenae* females at various growth stages of waxy and waxless triticale genotypes: a) fifth leaf (G.S. 37), b) flag leaf (G.S. 46), c) ear (G.S. 56).

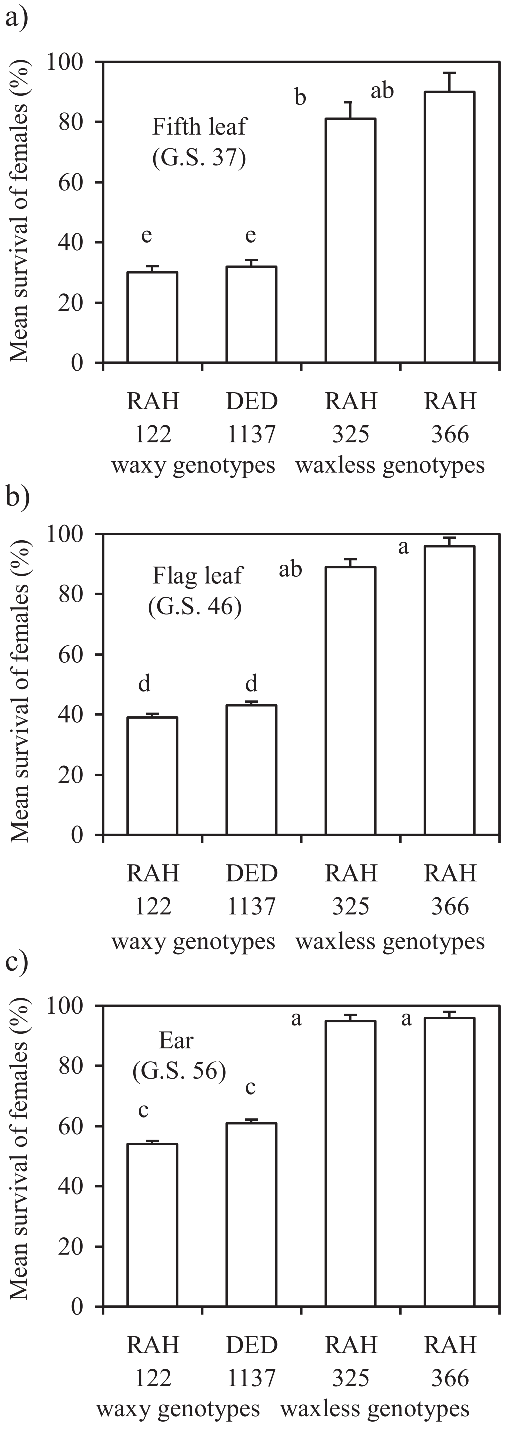

Fig. 2. Mean (±sd) survival of *S. avenae* females at various growth stages of waxy and waxless triticale genotypes. Values signed by various letters are significantly different at P<0.05 (Tukey's multiple comparison test): a) fifth leaf (G.S. 37), b) flag leaf (G.S. 46), c) ear (G.S. 56).

Table 1. The influence of waxy and waxless triticale genotypes and their growth stages on the studied parameters (mean±sd).

Plants			Number of *S. avenae* nymphs	Percentage mortality of *S. avenae* nymphs	Content of the flavonoids (mg×g^{-1} f.w)
Fifth leaf (G.S. 37)	waxy	RAH 122	6.7±2.03 e	35.6±4.71 a	36.5±5.13 a
		DED 1137	7.8±1.01 e	25.4±4.30 b	34.2±4.87 a
	waxless	RAH 325	23.8±1.34 c	10.2±2.10 d	17.0±2.10 b
		RAH 366	25.2±0.78 c	10.0±3.11 d	13.1±2.01 c
Flag leaf (G.S. 46)	waxy	RAH 122	8.±2.10 e	25.5±3.60 b	18.2±2.80 b
		DED 1137	9.2±2.08 e	20.3±3.12 c	13.3±3.92 c
	waxless	RAH 325	26.8±1.00 b	10.1±2.41 d	8.0±2.10 d
		RAH 366	29.2±1.12 b	5.3±0.22 e	7.1±1.24 d
Ear (G.S. 56)	waxy	RAH 122	13.1±1.18 d	20.4±3.52 c	7.3±1.72 d
		DED 1137	15.8±1.05 d	15.5±2.63 cd	5.0±0.06 d
	waxless	RAH 325	33.2±1.21 a	5.2±0.30 e	3.2±0.01 e
		RAH 366	36.4±1.38 a	0.0±0.00 f	3.1±0.01 e

Values in columns not followed by the same letters are significantly different at $P<0.05$ (Tukey's multiple comparison test).

On the basis of field observations it was found that the nymphs mortality on waxless triticale genotypes (RAH 325, RAH 366) was significantly lower than that on the waxy genotypes (RAH 122, DED 1137) (Table 1). Percentage mortalities of *S. avenae* nymphs varied from 0.0±0.00 to 35.6±4.71. The highest number of dead nymphs was observed on RAH 122 for all the studied organs. The lowest number of dead nymphs was observed on RAH 366 (Table 1).

Content of the Flavonoids in Tissue of the Studied Waxy and Waxless Triticale

Chemical analysis showed that the waxless plants had lower levels of flavonoids in comparison with waxy plants. Waxless genotypes had lower levels of flavonoids at every studied organ (Table 1). The concentration of flavonoids in the organs of the four triticale genotypes ranged from 3.1 mg×g^{-1} to 36.5 mg×g^{-1} fresh weight, with RAH 122 and RAH 366 having the highest vs. the lowest concentrations, respectively.

The mean concentration of flavonoids ranged from 36.5 mg×g^{-1} fresh weight in fifth leaves of waxy plants RAH 122 to 13.1 mg×g^{-1} in fifth leaves of waxless plants RAH 366. The flavonoid concentration in the fifth leaves was two times higher than concentrations in flag leaves. Waxless genotype RAH 366 flag leaves had the lowest amount of flavonoids (7.1 mg×g^{-1} fresh weight) while waxy triticale RAH 122 plants had the highest (18.2 mg×g^{-1} fresh weight). Similar clear trends were also obtained for content of flavonoids in ears. The content of flavonoids ranged from 7.3 mg×g^{-1} fresh weight in waxy ears to 3.1 mg×g^{-1} in ears of waxless plants. The highest content of flavonoids was observed for RAH 122, while the lowest was noted for RAH 366 (Table 1).

The concentration of flavonoids in the organs of the four triticale genotypes was significantly and negatively correlated with mean survival of *S. avenae* (G.S. 37 $r = -0.99$, $P \leq 0.01$; G.S. 47 $r = -0.97$, $P \leq 0.05$; G.S. 37 $r = -0.97$, $P \leq 0.05$). Similarly, significant negative correlations were found between the content of flavonnoids in the studied plants of the four triticale genotypes and number of *S. avenae* (G.S. 37 $r = -0.98$, $P \leq 0.02$; G.S. 47 $r = -0.96$, $P \leq 0.05$; G.S. 54 $r = -0.97$, $P \leq 0.05$). The concentrations of flavonoids in the studied plants was significantly and positively correlated with mortality of *S. avenae* nymphs (G.S. 47 $r = 0.99$, $P \leq 0.01$; G.S. 54 $r = 0.99$, $P \leq 0.01$).

Discussion

Favourable plant surface characteristics for successful colonization by aphids include good surface adhesion and minimization of physical impediments to movement, probing, and stylet penetration. These traits are often associated with glossy phenotypes that usually have reduced coverage of wax, reduced complexity of cuticular wax microstructure, and altered chemical composition when compared to the normal waxy phenotypes [7, 10, 18, 25-27]. On the basis of field observations it was found that the survival of the grain aphid *S. avenae* on plants of the waxy triticale genotypes (RAH 122, DED 1137) was clearly reduced in comparison to the waxless genotypes (RAH 325, RAH 366). Furthermore, the cereal aphid was the most numerous on plants of the waxless genotypes than that on the waxy triticale genotypes. It is reported that cuticular waxes can protect plants against invasion of herbivore insects [28, 29]. Most studies on plant-insect interactions have focused on the chemical composition of surface wax layers. There are many examples of negative associations between surface waxes and insect infestation. For example, epicuticular waxes were studied in *Brassica* crops, and evidence shows that wax blooms on glaucous surfaces reduced adult and larval feeding of some herbivores [8, 30]. Increased surface wax levels were correlated with resistance of cabbage (*Brassica oleracea* L.) to the aphid, *Bravicoryne brassicae* L., of sorghum (*Sorghum bicolour* L.) to the green bug *Schizaphis graminum* (Rondani), of winter wheat (*Triticum aestivum* L.) to the English grain aphid *S. avenae* (F.) [1]. Similarly, Ulrich et al. [31] showed the insecticidal and antifeedant properties of *Porteresia coarctata* Takeoka leaf extract against the generalist pest *Spodoptera litura* (F.), leading to biochemical changes in the insect body. The chemicals have also been attributed with beneficial properties as they necessarily represent the first line of contact with organisms [3, 11]. Among these compounds are those that exhibit a wide range of biological activities, including neurotoxicity, cytotoxicity, and antimitotic [32, 33].

The chemical interactions between plants and phytophagous insects include diverse secondary metabolites. The secondary plant metabolites are an integral part of plant metabolism and play important ecological and physiological roles in chemical interactions between plants and pests [34, 35]. Many of them, including flavonoids, glucosininolates, alkaloids, cyanogenic glycosides, and furanocumarins are known as the protective agents toward various species of herbivores. They seriously affect aphid behaviour, physiology, and metabolism, meaning that they can reduce aphid populations on resistant plants [4, 36-40]. According to Simmonds [41] one of the most important groups of the plant secondary constituents playing a defensive role against pests are flavonoids. These compounds are widespread in the plant kingdom. The concentration of flavonoids in plants depends on cultivar, plant age, and abiotic and biotic factors [42-45]. Flavonoids are biologically active secondary metabolites that influence insect growth and development [46-47]. The obtained results also indicate that the level of flavonoids might play an important role in the resistance of winter triticale genotypes to grain aphid. The content of flavonoids was associated with the values of resistance between the waxy and waxless plants. The result presented here indicates that the waxy plants that contained high level of flavonoids were less accepted by the grain aphid. There are many examples of negative associations between the flavonoids present in herbaceous/woody plant species and insects. 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 [49-52]. Kaempferol glucosides also have a negative effect on aphids. For instance, aphid-resistant cow pea lines contained significantly higher amounts of flavanoids, including kaempferol, compared to susceptible lines [53]. Lahtinen et al. [54] showed that aglycones flavonoids reduce the growth rate and prolong the duration of the first instar *Epirrita autumnata* larvae. Birch leaf surface flavonoid aglycones waere shown to correlate negatively with the growth rate of the fifth instar and the pupal mass of the Lepidoptera *E. autumnata* [55]. Ateyyat et al. [56] showed that the three tested flavonoids (quercetin dehydrate, naringin, and rutin hydrate) were active as aphicides against the woolly apple aphid *Eriosoma lanigerum*, and the mortality to nymphs was higher than that obtained against apterous adults.

The physical and chemical characteristics of plants play important roles in controlling pests in an agroecosystem. Some aphid-resistant cultivars have been introduced into the modern agroecosystem, therefore understanding the interaction of host plants and aphids is important for determining ecological safety and optimizing pest management techniques.

The results of the present study indicate that epicuticular waxes of the triticale play an important role in its acceptance by the grain aphid. The aphids that fed on the organs of the waxless genotype survived longer and produced significantly more larvae. The obtained results also indicate that the level of flavonoids might play an important role in the resistance of winter triticale genotypes to grain aphid. The content of flavonoids was associated with the values of resistance between the waxy and waxless plants. The result presented here indicate that the waxy plants that contained high levels of flavonoids were less accepted by the grain aphid.

Conclusions

The current studies suggest that surface waxes may have potential as triticale resistance factors toward the grain aphid. However, cereal resistance to the aphids is a complex phenomenon and other plant metabolites such as allelochemicals (flavonoids) also play a significant role in this process.

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