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CHANGES IN FERULIC ACID AND LIPID CONTENT IN APHID-INFESTED BARLEY

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Key Words Index—*Hordeum vulgare*; Gramineae; *Schizaphis graminum*; aphid infestation; ferulic acid; lipids.

Abstract—Aphid infestation reduced the susceptibility of barley cultivars to new infestations. The intrinsic growth rate of aphids was lower in the populations reared on previously infested plants, than in populations reared on barley without previous infestation. Changes in the content of ferulic acid, and other aromatic compounds and lipids, were detected in barley seedlings infested by the aphid, *Schizaphis graminum*. Ferulic acid concentration increased from 4 to 14 μmol kg^{-1} fresh wt, as a function of infestation level by aphids. Survival of aphids reared with diets containing this compound was lower than the controls. Aphid survival at 24 hours was under 58% and 40% in diets with 10 and 20 μM ferulic acid, respectively. In diets without the compound, survival was 84%. Analysis of alicyclic and aliphatic compounds in extracts of infested and non-infested barley plants showed differences in the relative concentrations of compounds. Our results suggest that the increase of ferulic acid induced by initial infestation may protect the plant against new infestations. Changes in lipid concentrations could be an acclimatization response of the plant to water-stress caused by the aphids.

INTRODUCTION

Aphid infestation removes resources from plants and causes metabolic changes in barley [1]. Some of these changes could be related to the reparative process of the damaged tissue, others to defence against the invader.

Phenolic compounds are widely distributed in higher plants and several have been proposed as defensive factors against insects [2] and fungal diseases [3]. Some of these phenolic compounds appear around infection points [4]. Caffeic, ferulic, *p*-coumaric acids and others are common constituents of plants [2]. Several reports have shown that they are released from cell walls of Gramineae by treatment with NaOH, because these acids are linked to lignins with high polymerization [5]. Ferulic acid, a methoxy hydroxylated derivative of cinnamic acid, has been found to be linked to xylose and arabinose in cell walls of barley straw [6]. Dimers of ferulic acid are also found in carbohydrates of the cell wall in several grass species [5]. Free ferulic acid has been found in sugar beet and cereal weeds and it has been reported as an inhibitor of seed germination [7]. Phenolic compounds are oxidized to toxic derivatives (quinones). Compounds having *ortho*-hydroxyl groups, including catechol, tannic acid, quercetin, chlorogenic acid and protocatechuic acid, are detrimental to greenbug growth and progeny survival [8]. However, the role of these compounds in plants is not clear. Most of them are thought to affect plant growth regulation [8]. These compounds also inhibit enzyme activities and reduce the nutritive value of plants to herbivores [9].

Barley lipid composition is known to be affected by water-stress [10]. The physical properties of lipids are markedly dependent upon their fatty acid composition. Changes in lipid composition have been reported mainly during the ripening of seeds [11]. Also, the composition of these compounds is affected by genotype and physiological conditions [12]. Since aphids cause water-stress symptoms [13], it is likely that the lipid composition of the leaves is also affected.

We report here in on an increase of ferulic acid, and the effect of this compound on the survival of the greenbug, *Schizaphis graminum*, and the variations in the composition of aliphatic and alicyclic compounds in barley infested by this aphid.

RESULTS AND DISCUSSION

Development rates and mean relative growth rate of aphids on barley previously infested

Previously infested and non-infested barley were infested with nymphs of *S. graminum* (see Experimental). After three days, growth and development rates of aphids were measured (Fig. 1). Nymphs on previously infested barley

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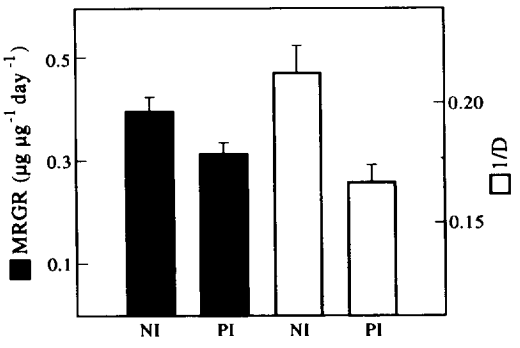

Fig. 1. Mean relative growth rates (MRGR) and development rates ($1/D$) for *Schizaphis graminum* reared on pre-infested and on non-infested barley seedlings. Nymphs of aphids weighed at the 1st or 2nd instar stage were placed on barley leaves (20 plants each with a single aphid per treatment). MRGR was calculated with aphid weights over a 72 hr period and $1/D$, where D is the time taken by an aphid to reach the adult stage and onset of reproduction. Each value is the mean ($n = 8$) ± standard error. NI = non-infested, PI = previously infested.

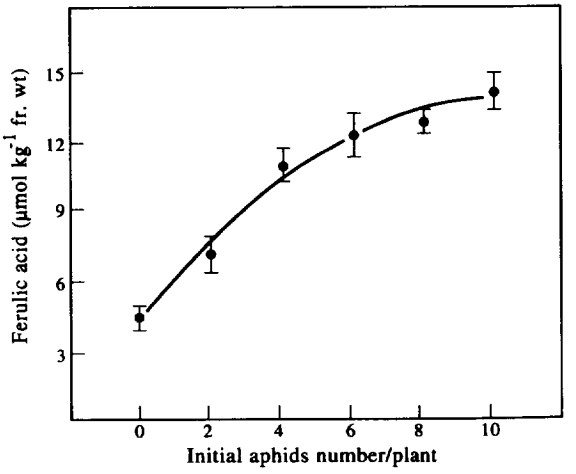

Fig. 2. Effect of aphid infestation on ferulic acid content in leaves of barley seedlings. Infestations carried out on 6-day-old, barley. Each value for ferulic acid represents the mean of 4 samples ± standard errors.

had lower development and mean relative growth rates than those on previously uninfested plants. These experiments show that pre-infested plants have a lower nutritional value for aphids than non-infested plants. This deterioration in the nutritive quality of the plant caused by aphids could be partially due to the effect of new toxic or repellent compounds present in plants.

Effect of aphid infestation on ferulic acid content of barley

Ferulic acid increased as a function of aphid infestation (Fig. 2). In extracts of non-infested plants, the basal content was 4 μmol kg^{-1} fr. wt and in samples initially infested with 10 individuals it reached 14 μmol kg^{-1} fr. wt. Events that occur during the introduction of aphid stylets into the tissue may provoke an increase in the concentration of free ferulic acid. It has been reported that ferulic and *p*-coumaric acids are bound to the cell walls of barley straw. The linkages are the same as those shown earlier for ferulic acid in the cell walls of sugar cane and maize [14].

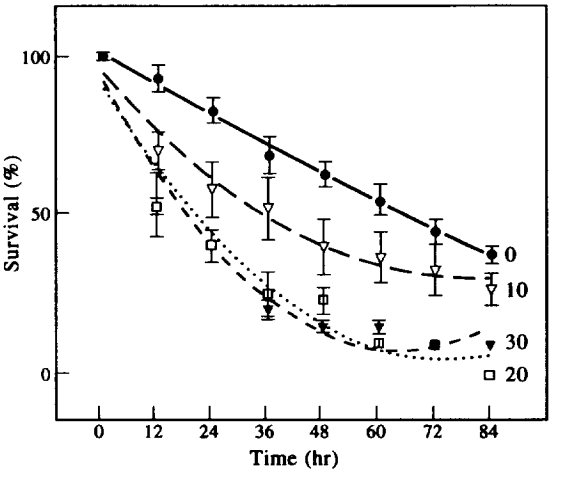

Fig. 3. Effect of ferulic acid on survival of aphids fed with artificial diets. Survival was measured every 12 hr during feeding with diets containing 0, 10, 20 and 30 μM of ferulic acid. Each point is the mean of 4 samples ± standard errors.

Effects of ferulic acid on aphids

This effect was studied by rearing aphids on artificial diets containing ferulic acid in concentrations similar to those found in plant extracts (Fig. 3). Ferulic acid decreased aphid survival in all treatments, the lowest being with 30 and 20 μmol ferulic acid kg^{-1} fr. wt. Effects of the compound were visible 12 hr after the beginning of the assay.

Previous reports have shown that ferulic acid incorporated into artificial diets reduces the reproductive index of aphids [2]. Moreover, it has been established that several monocotyledonous families, including the Gramineae, have phenolic acids linked to their cell walls, and that 1–3% of their dry matter is in the form of these phenolic compounds, mainly *trans*-ferulic and *trans-p*-coumaric acids [14].

To conclude that ferulic acid is effectively protecting barley against aphids, it would be necessary to study its concentrations in specific tissues, such as phloem, the main feeding site of the aphid and to determine whether it is present in the honeydew of the insects.

Aliphatic and alicyclic compounds in extracts of infested and non-infested barley

Several sterols and aliphatic derivatives of fatty acids with a high degree of unsaturation were detected in barley. Analysis of hydroxylated compounds by GC-

Table 1. Aliphatic and alicyclic compounds in extracts from barley infested and non-infested with *S. graminum*

Compound	Relative amount	
	Non-infested	Infested
2-Hydroxybutanedioic acid	3.8	nd
Methyl 8-hydroxyoctanoate	nd	1.4
Ethyl hexadecanoate	10.2	12.7
7-Hexadecenioc acid	4.8	nd
6-Hexadecenioc acid	nd	4.0
Hexadecanoic acid	53.3	100
cis-9,12,15-Octadecetrienoic acid (i)	27.0	51.5
11,14,17-Icosatrienoic acid	93.5	nd
Octadecanoic acid	2.3	23.2
8-Octadecenoic acid nd	nd	60.1
1-Hexacosanol (i)	1.2	29.5
Campes-5-en-3-β-ol	0.8	9.8
Ergost-5-en-3-β-ol	nd	36.5
Stigmast-5-en-3-β-ol	1.3	9.8

Values represent amounts relative to the major component (hexadecanoic acid). nd = not detected. (i) = isomer.

mass spectrometry in infested and non-infested barley showed more than 14 aliphatic and steroidal compounds. Some differences between infested and non-infested plants were noted (Table 1). 2-Hydroxybutanedioic, 7-hexadecenoic and 11,14,17-icosatrienoic acids were detected only in non-infested plants. Methyl 8-hydroxyoctanoate, 6-hexadecenoic acid, 8-octadecenoic acid and ergost-5-en-3-β-ol, were detected only in infested plants. 11,14,17-Icosatrienoic acid was the main compound in extracts of non-infested plants and hexadecanoic acid was the main compound in extracts of infested plants. Such compounds, however, are typical of plant tissues where they are the main components of membranes [15]. With few exceptions, steryl esters and lipids of membranes have the same fatty acid moieties [15]. They typically range from C_{12} to C_{22}. Physicochemical properties of membranes depend mainly on the composition of these mixtures of fatty acids [16]. An increase in the free sterols has been found in some cultivars of *Hordeum vulgare* when progressing from the dried seed up to day 8 after germination, whereas the steryl fatty acid esters undergo a depletion during the first five days and then remain stable [15]. It has been reported that an increase in the amount of both free sterols and steryl fatty acyl esters is induced in *Avena sativa* as a result of water-stress [15]. The relative free sterol and steryl fatty acyl ester composition of membranes will determine membrane fluidity and permeability. Hormonal changes induced by water-stress could partially explain these observations [15]. Since aphids cause water-stress-like symptoms, it is likely that infestation by aphids induces similar changes in barley membranes. The significance of changes in aliphatic and alicyclic compounds caused by aphids is not clear at present, but it could be an adaptation process to the water-stress caused by aphid infestation [13].

Some of the changes in steryl ester concentration observed during different physiological processes in plants have been explained by speculating that steryl esters are the form in which sterols are stored when present in amounts greater than those immediately required by the plant. An example is senescence, when free sterols may be released from the degenerating membranes [15]. Moreover, during germination of *Phaseolus vulgaris*, senescence of cotyledons was accompanied by a decrease in free sterol content and a concomitant increase in steryl esters. It was postulated that as the cotyledon membranes became disorganized, the free sterols released from the membranes became esterified and were transported to growing tissues [15]. It is possible that similar events may occur when barley tissues are damaged by the aphids.

EXPERIMENTAL

Plant growth and aphid infestation of seedlings. Six-day-old *H. vulgare* seedlings, cv. Aramir 57 were infested with 0, 2, 4, 6, 8 and 10 individuals of *S. graminum*. Six days later, the ferulic acid content of leaf extracts was analysed by HPLC as described below.

Growth and development rates of S. graminum *on pre-infested and non-infested barley.* Nymphs of aphids weighed at the 1st and 2nd instar stage were placed on pre-infested plants with 20 aphids per plant during 4 days and non-infested leaves. The mean relative growth rate (MRGR) $\mu g\ \mu g^{-1}\ day^{-1}$ was calculated from aphid wts over a 72 hr period as described in ref. [17], viz., MRGR = [$\log_e$ (final wt, μg) $-\log_e$ (initial wt, μg)]/3. Aphid development rate was calculated as $1/D$, where D = time in days from birth to adulthood.

Identification and quantification of compounds. Ferulic acid. Leaves were cut into small pieces and submerged in 80% EtOH for 24 hr. The EtOH extract from each treatment was filtered through a 0.45 μm Millipore filter and analysed by HPLC using a RP18 column. Operating conditions were, flow rate 1.3 ml min^{-1}, mobile phase, linear gradient of solvent A (H_2O, pH 3) and B (MeCN), 0–20 min 0–55%B, 20–22 min 100% B, 22–24 100% B and 25 min 0% B. Detection was at 236 nm. R_ts for ferulic and *p*-coumaric acid standards were 7.5 and 8.6 min, respectively. Both acids were isolated by prep. HPLC and identities confirmed by ^{1}H NMR at 300 MHz, using $CDCl_3$ as ref. and by comparison with an authentic sample (mp, IR, MS). *Aliphatic and alicyclic compounds.* EtOH extracts of samples were evapd under a stream of N_2 and residual material briefly freeze-dried (5 min) to remove H_2O. TMSi derivatives for GC were prepd by addition of 50 μl pyridine and 100 μl BSTFA. Derivatized samples were sepd and analysed by a GC-MS system. The GC system was fitted with a 50 m, 0.3 mm i.d., and 0.5 μm given thickness bonded OV1 column and a splitless injector with a flush 30 s after sample injection to remove residual gases. The end of the column was introduced directly into the mass spectrometer analyser chamber. The system was operated under the following conditions: He pressure 3 kg cm^{-2}, injector temp. 310°, GC temp. 85–310° at 3° min^{-1}. The mass spectrometer was set to scan 40–650 mu per nominal s with an ionizing voltage of 70 eV. Peaks were identified by computer search of user-generated reference libraries incorporating GC R_ts times and MS. Peaks were examined by single-ion chromatographic reconstructions to confirm their homogeneity; mixed peaks were resolved by a computer program aimed at resolving the mass spectral data of one compound from overlapping mass spectra of another [16].

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