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ROLE OF AN INDOLE ALKALOID IN THE RESISTANCE OF BARLEY SEEDLINGS TO APHIDS

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Key Word Index—*Hordeum*, Gramineae, *Schizaphis graminum*, greenbug, gramine, benzyl alcohol

Abstract—The content of the simple indole alkaloid gramine in barley leaves decreased with age Conversely, susceptibility to aphids increased in older plants Population growth rate of the greenbug *Schizaphis graminum* correlated with gramine content of leaves of several barley cultivars Gramine decreased rate of feeding, survival and reproductive index of aphids feeding on artificial diets at concentrations similar to those found in plant leaves Thus, it is suggested that gramine plays a role in the resistance of barley seedlings to *S graminum* Benzyl alcohol, a previously reported insect resistance factor from barley, was absent from all barley cultivars analysed

INTRODUCTION

Plant chemicals may affect resistance of several Gramineae to aphids Resistance of *Sorghum* to the aphid *Schizaphis graminum* has been associated to the degree of methylation of the intercellular pectin [1] Hydroxamic acid content in leaves of corn, rye and wheat correlates with plant resistance to aphids [2–4] It has been proposed that resistance of some barley varieties may be attributable to the presence of several phenolics or flavonoids [5] Benzyl alcohol also has been suggested as a possible resistance factor of small grains to the greenbug [6], but these results have not been confirmed

Gramine (1), a simple indole alkaloid, is present in several barley cultivars reaching concentrations of up to 8 mg/g dry wt [7–9] Indole alkaloids are responsible for toxicity of *Phalaris* pastures to sheep and cattle [10–12] These compounds cause various effects on ruminants [13], decrease food palatability to lambs [14], and cause kidney lesions, glycosuria and weight losses in meadow voles fed on gramine [15] Indole alkaloids also decrease survival of aphids feeding on artificial diets and are feeding deterrents [16] In this paper, the possible roles of benzyl alcohol and gramine in the resistance of barley to *S graminum* are discussed

RESULTS

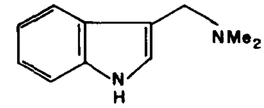
Benzyl alcohol and gramine in barley

Benzyl alcohol a previously reported plant resistance factor to *S graminum* [6] was not detected in any of the barley cultivars analysed (cv Rogers, Omugi, F Union, Cruzat and Australiana) Plants analysed were 10-day-old, grown in the dark, or 20–30-day-old, grown under light at 25° The limit of detection of the method was 0 01 mmol/kg fr wt Since a much higher concentration of benzyl alcohol is required to cause deleterious effects on *S graminum* feeding on artificial diets (LD_{50} 0 3 mM) it may be suggested that this compound is not a resistance factor of barley to aphids

Seeds of 34 barley cultivars were germinated in the dark, at 25° for 6 days, and the gramine content of the shoots (mesocotyl and coleoptile) was determined by using Ehmann's reagent for indoles (see Experimental) Each extract was also analysed by TLC, gramine being the only indole alkaloid present Cultivars differ in their gramine content, ranging from 0 to 4 8 mmol/kg fr.wt Gramine was not detected in seeds Cultivars Rogers and Omugi had a gramine content of 0 25 and 0 7 mmol/kg fr wt, respectively

Gramine content and resistance to aphids

Varieties that lack benzyl alcohol and differ in gramine content were selected for aphid infestation experiments Ten-day-old plants were infested with aphids and the insect population and gramine content of leaves were determined at 10 and 20 days (Fig 1) Gramine content decreased with plant age The most susceptible variety was F Union, a gramine-lacking plant, while varieties with a higher gramine content than average were more resistant to the aphids Older plants were more susceptible to aphids (not shown) To explore further this apparent effect of gramine on aphid population growth rate, several barley varieties were infested with *S graminum* and the population growth rate was determined over a 6-day interval A correlation ($r = -0.98$) was observed between gramine content and aphid population growth rate (Fig 2)



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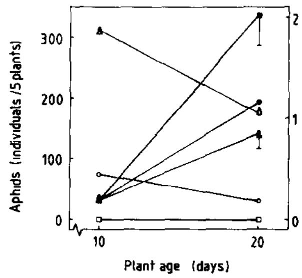

Fig 1 Gramine content and susceptibility of barley leaves to *Schizaphis graminum* Filled and open symbols indicate aphid population and gramine content, respectively (▲△, barley cv Cruzat, ● ○, cv Australiana, ■ □ cv F Union) Two nonalate adults were placed on each 10-day-old plant Values are the average of three samples of five plants each Vertical bars are 1 s e

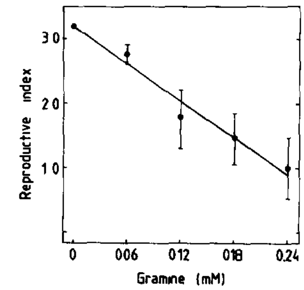

Fig 4 Effect of gramine on reproduction of *Schizaphis graminum* reared with artificial diet The reproductive index (number of nymphs/average number of adults) was measured after feeding aphid adults for 72 hr Each point is the average of three samples consisting of five aphids each Vertical bars are 1 s e

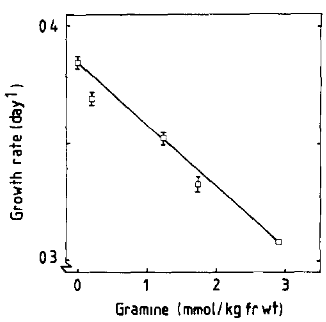

Fig 2 Gramine content and population growth rate of *Schizaphis graminum* on barley cultivars Nine-day-old plants were infested with two nonalate adults Aphids were counted and leaves analysed for gramine content after 6 days Values represent the average of three samples of five plants each Vertical bars are 1 s e Aphid population growth rate = $\ln(Nf/Ni)/\Delta t$

Effects of gramine on aphids

Gramine decreased survival of aphids reared on artificial diets with an LD_{50} of 0 8 mM (Fig 3) after 24 hr of feeding In addition, gramine also decreased diet intake by aphids with an ED_{50} of 2 1 mM after 5 hr of feeding Lower gramine concentrations decreased reproduction (Fig 4) Thus, gramine had deleterious effects on aphids at concentrations similar to those found in barley leaves

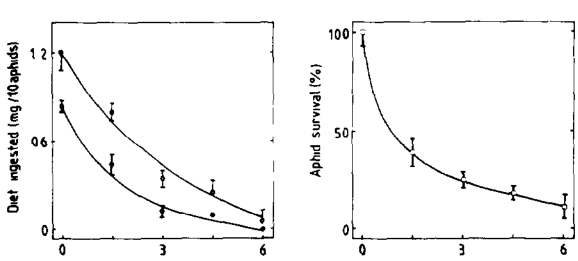

Fig 3 Effect of gramine on diet ingestion and survival of nymphs of *Schizaphis graminum* Diet ingestion was the difference in wt of the diet between the beginning of the experiment and at 5 (●) and 7 (○) hr Survival was measured after 24 hr of feeding Each point is the mean of three samples consisting of 10 aphids each Vertical bars are 1 s e

DISCUSSION

Since indole alkaloids cause acute toxicity and decrease palatibility of several grasses to mammals, it has been proposed that alkaloid content of various Gramineae be reduced by plant breeding [9, 17] Also, it has been suggested that gramine accumulation in barley plants grown in hot environments may have potentially deleterious consequences, both for the productivity of livestock and for the heat tolerance of the crop itself [9]

This and previous work [16] have shown that gramine causes toxicity, feeding deterrence and decrease in reproduction rate of aphids reared in artificial diets Also, gramine content correlated with resistance to the greenbug *S graminum* Moreover, gramine concentration in barley is sufficiently high to cause toxicity or feeding deterrence to aphids Thus, it is likely that gramine plays a role in protecting barley from aphid attack

Eliminating or decreasing gramine content of plants by plant breeding may be convenient for feeding livestock, but it may also increase susceptibility of the crop to insects and other herbivores It would be advisable to study alternative resistance mechanisms of this plant to insects before recommending the selection of cultivars with a low gramine content

EXPERIMENTAL

Aphids Individuals of the greenbug *Schizaphis graminum* (Rondani) were reared on *Hordeum distichum* cv F Union Plants and aphids were kept at 28° under permanent light

The diet was a pH 6 soln of 35% sucrose, amino acids and mineral salts [16], placed between two layers of Parafilm M [18] All feeding experiments were performed at 28° under permanent fluorescent light

Infestation experiments Seed were planted in pots filled with soil and grown under continuous light in a greenhouse (23–28°) Plants were infested with nonalate adults and covered with a nylon net

Preparation of plant extracts for benzyl alcohol analysis Barley leaves (30–50 g) of various ages grown in the dark or under light were macerated with a mortar and pestle The extract was filtered through cheesecloth, pH adjusted to 3 with 1 M HCl and centrifuged to 5000 *g* for 5 min The aq extract was divided into 4-ml aliquots to which benzyl alcohol was added to reach various concns (0, 0 2, 0 4, 0 6, 0 8 and 1 0 mM) These solns were extracted into EtOAc Benzyl alcohol recovery was 88 % These samples were used for analyses in a Perkin–Elmer 900 gas chromatograph equipped with FID and a 3 % Carbowax 20 M glass column (2 m × 3 mm) Nitrogen (40 ml/min) was used as a carrier Chromatograms were performed under isothermal conditions (135°) Benzyl alcohol and int standard (3-phenyl-1-propanol) had retention times of 3 3 and 6 5 min, respectively

Preparation of extracts for gramine analysis Plant leaves (2–3 g) were frozen and then macerated using a mortar and pestle with 20 ml MeOH–NH_4OH (100 1) The extract was filtered through glass-wool The filtrate was evapd and the residue was dissolved in 5 ml 0 1 M HCl This soln was filtered in Whatman No 1 paper, adjusted to pH 9 with conc NH_4OH and extracted into $CHCl_3$ (× 2) Gramine recovery under these conditions was 97 % The organic phase was evapd to dryness These extracts were used for gramine analyses Indoles form colored complexes with Ehmann's reagent [19] Gramine forms a pink complex (λ_{max} 550 nm, ε 4207) Each extract was also analysed by TLC (silica gel GF 254 plate, MeOH–NH_4OH (7 1), gramine R_f 0 55) Gramine was the only indole alkaloid in barley detected by this procedure

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