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ROLE OF HYDROXAMIC ACIDS IN THE RESISTANCE OF CEREALS TO APHIDS

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Key Word Index—*Triticum aestivum*; *T. durum*; *Secale cereale*; *Hordeum distichum*; Gramineae; *Metopolophium dirhodum*; aphid: 1,4-benzoxazin-3-ones; 1,3-benzoxazolinones; resistance factor in Gramineae.

Abstract—Hydroxamic acid concentration in Gramineae, both natural and incorporated, correlates with resistance to the aphid *Metopolophium dirhodum*. 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one, a hydroxamic acid isolated from corn extracts, is deleterious to aphids fed on artificial diets. It is proposed that hydroxamic acids act as naturally-occurring protective factors against *M. dirhodum*.

INTRODUCTION

Secondary compounds can act as protective agents in plants against insects by causing repellency, or by direct toxicity [1]. The variation in susceptibility of several Gramineae to the aphid *Metopolophium dirhodum* (Walker) may be due to the presence, in various concentrations, of secondary compounds such as cyclic hydroxamic acids. Some of these acids, in particular 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA, **1**), have been reported to be inhibitory to insects [2, 3], fungi [4–6] and bacteria [7, 8].

We report here on the relationship between hydroxamic acid concentration in Gramineae and susceptibility of those plants to infestation by *M. dirhodum*, and on the effect of DIMBOA on survival of aphids fed on an artificial diet.

RESULTS

Hydroxamic acid concentration and susceptibility of Gramineae to aphids

Twenty-day-old plants of barley, wheat and rye were infested with aphids. Six days later the aphids present on the leaves were counted and the concentration of hydroxamic acids in extracts of those leaves measured. Table 1 shows that as the concentration of hydroxamic acids increases, the aphid population growth rate and hence the degree of infestation decrease.

Effect of plant age on hydroxamic acid concentration and susceptibility to aphids

Plants of wheat and rye of 10, 16, 22 and 28 days of age were infested with aphids. Six days after infestation the aphids on the plants were counted and the hydroxamic acid concentration measured. Figs. 1 and 2 show that as plants grow older, the concentration of hydroxamic acids decreases and the growth rate of the aphid population increases.

Effect of incorporation of DIMBOA on susceptibility of barley to aphids

Freshly cut leaves of barley (a plant lacking hydroxamic acids) were partially immersed in solutions at pH 5 containing various concentrations of DIMBOA, and were

Table 1. Hydroxamic acid concentration and susceptibility of several Gramineae to *M. dirhodum*

Plant	Hydroxamic acids* in leaf extracts (mmol/kg fr. wt)	Aphids/sample Initial	Aphids/sample Final*	Population‡ growth rate (per day)
H. distichum cv Fola Union	nd†	60	518 ± 20	0.35
T. aestivum cv Huenufen	0.09 ± 0.01	60	296 ± 20	0.27
T. durum cv Quilafen	0.76 ± 0.07	60	246 ± 25	0.24
T. durum cv SNA-3	1.00 ± 0.06	60	210 ± 20	0.21
S. cereale cv Emerald	2.09 ± 0.10	60	130 ± 8	0.13

* Mean ± s.e. of three samples of 30 plants each. The infestation was carried out on 20-day-old plants and the experiment lasted for 6 days.

† Not detected. The detection limit under the conditions of the experiment is 8×10^{-4} mmol/kg fr. wt.

‡ Growth rate = $(\ln N_f / N_i)/\Delta t$.

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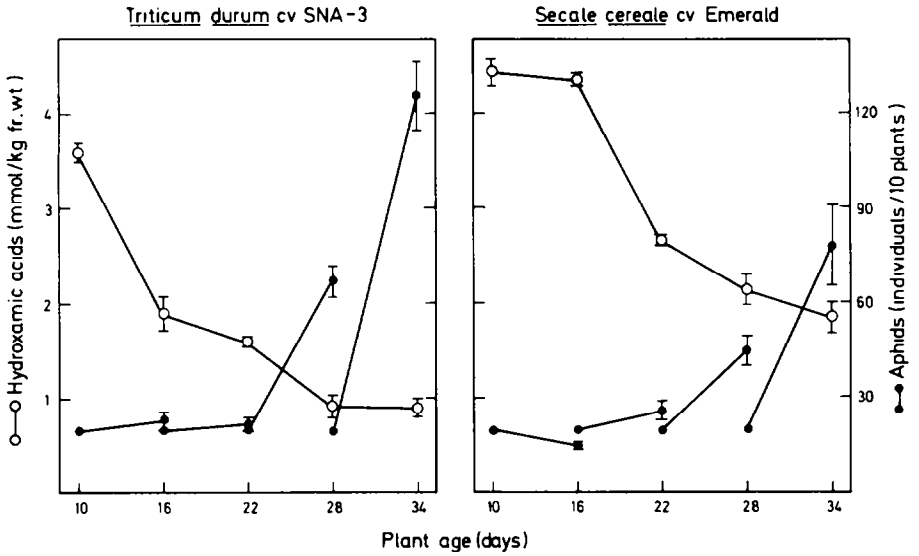


Fig. 1. Effect of plant age on hydroxamic acid concentration in leaf extracts and on susceptibility to *M. dirhodum* in plants of *Triticum durum* cv SNA-3 and *Secale cereale* cv Emerald. Each point is the mean of nine samples. A sample consisted of ten plants infested with two aphids each. Vertical bars are standard errors of the mean. Hydroxamic acid concentrations: ○——○; aphids: ●——●.

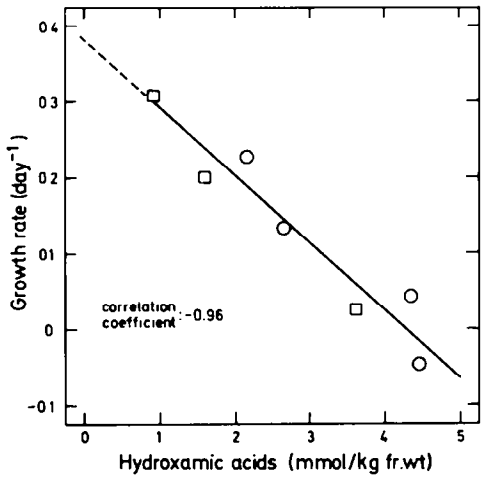


Fig. 2. Effect of hydroxamic acid concentration in leaf extracts on *M. dirhodum* population growth rate in plants of *Triticum durum* cv SNA-3 (□) and *Secale cereale* cv Emerald (○). This figure is based on data presented in Fig. 1. Growth rates were determined as $(\ln N_f/N_i)/\Delta t$.

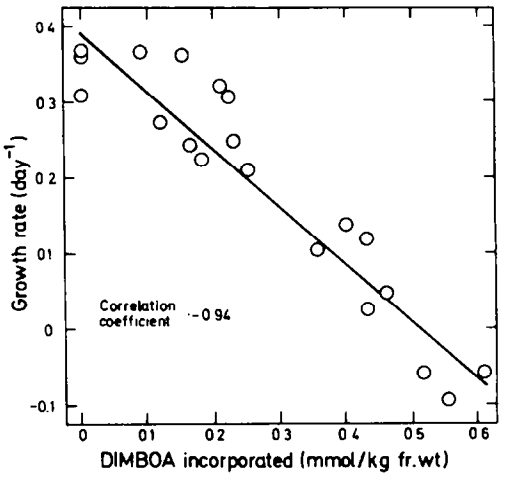
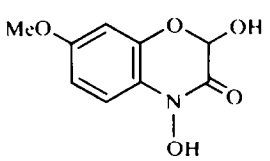


Fig. 3. Effect of incorporation of DIMBOA (1) on growth rate of *M. dirhodum* in excised leaves of *Hordeum distichum* cv Fola Union. Ten-day-old leaves were invested with aphid adults. DIMBOA and aphid populations were measured after 5 days. Each sample is composed of eight leaves initially infested with two aphids each. Growth rates were determined as $(\ln N_f/N_i)/\Delta t$.

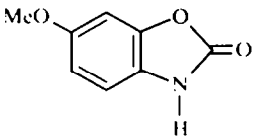
infested with aphids 24 hr later. Hydroxamic acids were determined and aphids counted 5 days later. Fig. 3 shows the inverse relationship found again between aphid-population growth rate and hydroxamic acid concentration. The concentrations in the leaves found to be active were lower than those normally found in Gramineae.

Effect of DIMBOA and of 6-methoxy-1,3-benzoxazolinone (MBOA, 2) upon aphids fed with artificial diets

DIMBOA decomposes in aqueous solutions to MBOA and other products that have not been identified [9]. Thus, it is desirable to study not only the activity of DIMBOA on aphids but also that of MBOA. Groups of aphid nymphs were fed for 5 days with an artificial diet containing various



1 DIMBOA



2 MBOA

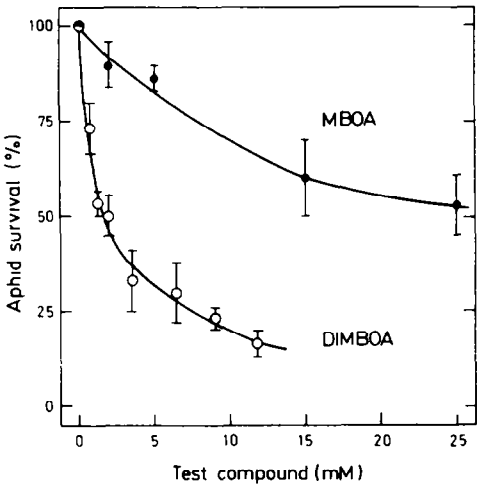


Fig. 4. Effect of DIMBOA (1) and of MBOA (2) upon *M. dirhodum* fed with artificial diets. Survival was measured after feeding the aphids for 24 hr. Each point is the mean of three samples consisting of ten aphid nymphs each. Vertical bars are standard errors of the mean.

concentrations of DIMBOA or MBOA. Every 24 hr dead individuals and progeny were withdrawn and the remaining insects were counted. The diet was renewed every 48 hr. In control experiments with diets lacking DIMBOA or MBOA, aphid survival was 100% at the fifth day. In experiments with inhibitors, mortality was related to the concentration of test compound. Fig. 4 shows the results obtained after 24 hr. The deleterious effect of DIMBOA appears at concentrations lower than those found in grasses which are resistant to the aphid. The effect of DIMBOA on aphids is greater than that of MBOA.

DISCUSSION

The correlations between hydroxamic acid concentration and resistance to *M. dirhodum* in barley, wheat and rye (Table 1) at different plant ages (Figs. 1 and 2) and when DIMBOA is incorporated into leaves of susceptible plants lacking it (Fig. 3), support the general hypothesis that susceptibility of Gramineae to aphid infestation is affected by the concentration of certain secondary metabolites in the plants.

The aphid population growth rates extrapolated to zero concentration of hydroxamic acids are similar for intact plants of wheat and rye, and for excised leaves of barley (0.38 and 0.39 per day respectively, Figs. 2 and 3). A similar value is obtained for the growth rate in an intact hydroxamic acid-lacking plant (see barley, Table 1). Moreover, the points determined by growth rate and hydroxamic acid concentration for both wheat and rye fall on a common line (Fig. 2). These data are internally consistent and confirm the idea that hydroxamic acids are a major resistance factor to *M. dirhodum* in the Gramineae tested. Additionally, the growth rate is more affected by incorporated DIMBOA in excised leaves than by the hydroxamic acid concentration in intact plants, as shown by the slopes of Figs. 3 and 2 (-0.76 and -0.089 respectively).

The interpretation given to the correlations between susceptibility and hydroxamic acid concentration is strongly supported by the experiments with artificial diets. These indicate that DIMBOA is in fact deleterious to aphids in concentrations similar to those found in resistant plants (Fig. 4). The experiments with diets also show that the deleterious effect of MBOA is smaller than that of DIMBOA. The LD_{50} for MBOA is over 15 times greater than that of DIMBOA, indicating that in bioassays carried out with DIMBOA the contribution of MBOA to the deleterious effect is insignificant. Although we have not tested the activity of the other decomposition products of DIMBOA, it has been reported that they do not significantly inhibit other plant pathogens [9].

Hydroxamic acids are present in plants as glycosides which are hydrolysed upon crushing the tissue [10]. The effect of the glycosides on the aphids is not known at present. However, the insect could cause their hydrolysis by disrupting the cellular structure during the feeding process, thus freeing the deleterious aglycone. It would be of interest to determine whether these compounds act as feeding deterrents or are directly toxic.

It has been suggested that hydroxamic acids present in maize are also detrimental to the European corn borer *Ostrinia nubilalis* (Hübner), to the corn leaf aphid *Ropalosiphum maidis* (Fitch) [2, 3] and to several plant pathogens [4–8]. The high concentration of hydroxamic acids in young seedlings (Fig. 1) might be of particular importance since this is the stage of development of many plants when they are most susceptible to attack by pathogens. The use of varieties with high hydroxamic acid concentrations would perhaps allow a natural control of aphid populations during the cultivation of cereals. However, field observations would be desirable.

EXPERIMENTAL

Plant materials. Seeds of barley (*Hordeum distichum* L. cv Fola Union), rye (*Secale cereale* L. cv Emerald) and maize (*Zea mays* L. cv LH Rinconada) were obtained from Departamento de Sanidad Vegetal, Facultad de Agronomia, Universidad de Chile. Wheat seed (*Triticum aestivum* L. cv Huenufen; *T. durum* L. cv Quilafen and SNA-3) were donated by Instituto Nacional de Investigaciones Agropecuarias and Sociedad Nacional de Agricultura, Santiago.

Aphids. Individuals of *Metopolophium dirhodum* (Walker) were collected from a wheat field near Santiago, and allowed to reproduce on barley plants kept inside a nylon net under continuous light in the laboratory.

Growth of plants and infestation assays. Seeds were planted in pots filled with soil and grown under continuous light in a greenhouse (23–27°). Plants were infested with non-alate adults and covered with a nylon net. After 6 days the aphids present on the leaves were counted.

Preparation of extracts and quantification of hydroxamic acids. Leaf tissue was crushed in H_2O, filtered through cheesecloth and left 15 min at room temp. The extract was adjusted to pH 3 with N HCl and centrifuged at 8500 *g* for 25 min. The pellet was discarded and the supernatant was extracted into Et_2O (2 vol. × 3). The organic phases were evapd to dryness. Hydroxamic acids were determined with ferric chloride reagent which forms a blue-coloured complex with an absorption max at 590 nm. The validity of this test for determining hydroxamic acids in plants has been discussed previously [11].

Isolation of DIMBOA. DIMBOA was isolated from maize seedlings by the procedure described previously [9].

Diet composition. The aphids were fed with a chemically defined diet [12] placed between two layers of Parafilm 20 M. The diet was a pH 5.5 aq. soln of 30% sucrose, amino acids, vitamins and mineral salts.

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