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Effect of 6-Methoxybenzoxazolin-2-one (MBOA) on the Reproduction Rate of the Grain Aphid (*Sitobion avenae* F.)

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Partial host-plant resistance could make a substantial contribution to reducing the damage caused by economically important grain aphids and, therefore, to reduced insecticide use. Naturally occurring hydroxamic acids, in particular 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), have been shown to be involved in the resistance of cereals to insects. DIMBOA is unstable in aqueous solutions and has been reported to decompose to 6-methoxybenzoxazolin-2-one (MBOA). MBOA was tested on grain aphids living on artificial diet incorporated with MBOA. From 0 to 0.1 mM, the intrinsic rate of increase (r_m) increased. From 0.1 to 0.3 it decreased by 73%. The r_m was calculated to be 0 at 1.0 mM. Consequently, even low concentrations of MBOA may reduce the aphid multiplication to a level below the economic damage threshold. Therefore, it is possible to breed wheat varieties with a sufficiently high content of DIMBOA to decrease grain aphid populations.

KEYWORDS: Aphids; *Sitobion avenae*; artificial food; antibiosis; DIMBOA; MBOA

INTRODUCTION

Antibiosis is one of the most important resistance mechanisms of modern wheat (*Triticum aestivum* L.) cultivars to grain aphids (*Sitobion avenae* F.) (*1*). Grain aphids are major pests of winter wheat in temperate climates (*2*), damaging plants by consuming nutrients, injecting toxins, providing—through their honeydew—a rich medium for fungal establishment, and transmitting viral diseases such as barley yellow dwarf virus (BYDV) (*3*).

Explosive increases in aphid populations can occur, but a number of factors operate to control them (*4*). Under favorable conditions, biological control of aphids by insect predators, or parasites, can have a decisive effect on aphid populations. However, under normal conditions predator population growth lags somewhat behind host population growth, and control is seldom complete. Therefore, it is necessary to use pesticides in some cases to avoid large yield reductions.

The rising costs of pesticides, plus increased insect resistance and their undesirable effects on the environment, have led to a renewed effort to exploit host-plant resistance to pests and diseases. Cereals, although constituting the main food crops in the world, have imperfectly described chemical defenses.

Partial host-plant resistance could make a substantial contribution to reducing the damage effects of grain aphids and, therefore, to reducing the use of insecticides.

The secondary plant substances are important in determining the host range of many insects. Cyclic hydroxamic acids are allelochemicals present in the common agricultural crops such as wheat in concentrations up to 8 mmol/kg of fresh weight, but in most cases in lesser concentrations (*5*). The hydroxamic acids are the main group of secondary substances involved in cereal resistance (*6*−*8*). The most abundant of these acids in wheat extracts is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), produced by hydrolyses of the naturally occurring glucoside by β-glucosidases released upon tissue injury (*9*).

The defense mechanisms of wheat against aphids are poorly understood, but resistance to the grain aphid in relation to levels of DIMBOA has been described in many papers. Antibiosis, measured as intrinsic rate of increase (r_m), shows a strong negative correlation between r_m and DIMBOA levels (*7*, *9*−*14*).

DIMBOA is found mainly as glucoside (DIMBOA-Glc) in intact wheat plants (*10*). DIMBOA-Glc and the hydrolyzing enzyme β-glucosidase are kept apart by compartmentalization. When the tissue is injured, DIMBOA-Glc comes into contact with the enzyme and the glucose moiety is cleaved off. DIMBOA decomposes spontaneously to 6-methoxy-2-benzoxazolin-2-one (MBOA) (*15*−*19*). Therefore, MBOA is formed when the plant tissue is injured.

The dose−response effects of MBOA to grain aphid reproduction have not previously been reported. For that reason, we have tested MBOA and not DIMBOA in the present research, as a contribution to better understanding the defense mechanisms of wheat against aphids.

MATERIALS AND METHODS

Aphid Stock Culture. The grain aphids (*S. avenae* F.) (Insecta, Hemiptera, Aphididae) came from a stock culture kept at the Danish Institute of Agricultural Sciences, Research Centre Flakkebjerg. These were reared in a glasshouse on winter wheat (*T. aestivum* L. cv. Herzog) at 20 °C, relative humidity of 70%, and light/dark 16/8 h.

Artificial Diet. Aphids are specialized plant-sucking insects that probe through the plant epidermis and mesophyll to access their final feeding site, the sieve elements. To construct experimental bioassays

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Table 1. Composition on the Pure Artificial Diet Fed to *S. avenae* (Milligrams per 100 mg of Diet)[a]

alanine	100	leucine	80
arginine (HCl)	270	lysine (HCl)	120
ascorbic acid	100	methionine	80
asparagine monohydrate	550	$MgSO_4 \cdot 7H_2O$	123
aspartic acid	140	$MnCl_2 \cdot 4H_2O$	0.4
biotin	0.1	*myo*-inositol·$2H_2O$	50
choline chloride	50	nicotinic acid	10
citric acid	10	phenylalanine	40
$CuCl_2 \cdot 4H_2O$	0.2	proline	80
cysteine (HCl) monohydrate	40	pyridoxine–HCl	2.5
D-calcium pantothenate	5	serine	80
$FeCl_3 \cdot 6H_2O$	1.1	sucrose	15000
folic acid	2	threonine (allo free)	140
glutamic acid	140	thiamin–HCl	2.5
glutamine	150	tryptophan	80
glycine	80	tyrosine	40
histidine	80	valine	80
isoleucine	80	$ZnSO_4$	1.7
$K_2HPO_4 \cdot 3H_2O$	1500	demineralized water	100 mL

[a] Amino acids are all L-.

testing MBOA, it was necessary to produce an artificial diet from which the grain aphids could suck through a membrane. The diet was produced following the methods of Kunkel (*20*) and Dadd and Mittler (*21*). The composition of the diet used in all experiments is shown in **Table 1**.

Solutions containing 0, 0.05, 0.1, 0.2, 0.3, 0.5, and 8 mM concentrations of MBOA (purity = 98+%, Lancaster Synthesis Inc.) were made and sterilized though a Millipore (0.20 μm) filter. Subsequently, they were stored at −18 °C for a period not exceeding 1 month. Then, new solutions were made.

When MBOA was incorporated into the diet, the diet was stirred until all of the MBOA appeared to be incorporated.

Aphid Bioassay. In offering the diet to the grain aphids, a small droplet was placed in an envelope composed of opposing pieces of Parafilm M as first described by Mittler and Dadd (*22*).

To get the Parafilm M sachets antiseptic, they were irradiated by UV light for an hour. They were stretched into thinner membranes in two directions at right angles to each other. Then, the sachets were draped over plastic cylinders (diameter = 3.3 cm; height = 7.5 cm). After this, one aphid was placed on the lower membrane of the sachet and the cylinder was sealed with a cork.

Procedure for Testing Experiments. The aphids were 1−3-day-old apterous virginoparous nymphs that were born of aphids raised on wheat plants from the stock culture. The experiments were carried out in a controlled chamber at 20 °C and light/dark 16/8 h. Twice a week for 4 weeks, the newborn aphid nymphs were counted and removed. At the same time the sachets with artificial food were replaced to prevent bacterial and fungal contamination. Only aphids surviving all 4 weeks are included in the statistics. The number of aphids in the tests was between 20 and 30. However, ∼13% of the aphids developed wings and are not included in the statistics.

The effect of MBOA on the reproduction rate of aphids was calculated as the intrinsic rate of increase (r_m) from the following equation: $N_t = N_0\, e^{r_m t}$ (*23*), where N_0 is the number of aphids at the start of the experiment (here 1), N_t is the number of aphids at time t (here N_0 + number of offspring after 28 days), and t is the time calculated as day degrees (DD) with a developmental temperature at 3 °C [here DD = (20 − 3) × 28 = 476].

The intrinsic rate of increase could subsequently be calculated as $r_m = \log(N_t)/476$. The dimension of r_m is the number of aphids per aphid per DD.

The intrinsic rate of increase was determined by offspring produced in 28 days, the period of time nearly equivalent to twice the prereproductive period (*24*).

Statistical Analyses. Results from the separate concentrations of MBOA were tested by a Duncan test in the SAS GLM Procedure, and for the calculation of the relationship between the concentration of MBOA and the intrinsic rate of increase a NLIN procedure was used (*25*). The relationship between the intrinsic rate of increase (r_m) and the concentration of MBOA is calculated from the equation $y = a(\log)^2$, where $y = r_m$ and x = concentration of MBOA in mM.

Table 2. Mortality of *S. avenae* Raised on Pure Artificial Diet

date	% mortality	no. of aphids tested	significance, $p < 0.05$
Sept 9, 2002	50.0	14	ns
March 11, 2003	60.0	20	ns
March 14, 2003	63.2	19	ns
Feb 15, 2004	68.4	19	ns
April 20, 2004	73.3	15	ns
Oct 2, 2004	67.9	28	ns
Oct 7, 2004	62.5	16	ns

RESULTS AND DISCUSSION

Pure Artificial Diet. Results from aphids raised on a purely artificial diet are shown in **Table 2**. For the included aphids the mortality, as an average, was 64.1 ± 4.2%, which was high. There was no significance between the separate experiments.

One reason for high mortality could be starvation due to broken stylets done when aphids were moved and sachets replaced. For that reason, all stylets were examined after handling to be sure that there was no damage. In these, no stylets were broken, so this was not the explanation.

In addition to the high mortality, it was a long time before the first nymph was born: in these experiments 16.8 ± 3.3 days, compared with 8−9 days if the aphids were reared on wheat plants.

Bioassay conditions represent, in general, a stress to the test individuals. It is obvious that the quality of the present artificial diet is not 100% optimal to the grain aphids. Sutter and Kieckhefer (*26*) report a mortality of 90% after 6 days for grain aphids feeding on artificial diet if the sachets were changed every 72 h. If they were changed every 24 h, the mortality was only 60%.

Other authors report mortalities of 90% (*27*) and 20−45% for *Metopolophium dirhodum* (Walker) (*28*), also for a 6-day period when the sachets were changed daily. Dadd and Mittler (*21*) have reared 30 generations of *Myzus persicae* (Sulzer) on an artificial diet. On the same diet Dadd and Krieger (*29*) reared 25 generations of *Aphis fabae* (Scopoli), but they do not report mortality frequencies.

Ehrhardt (*30*) was able to rear bean aphids though only two generations on a synthetic diet developed for green peach aphids and pea aphids. Growth was slow in the first generation, and mortality was high. He stated that the limitation on growth must have been due, at least in part, to the absence from the diet of appropriate levels of trace metals. He specifically mentioned iron, zinc, manganese, and copper. We incorporated all metals into the artificial diet we used.

When aphids are moved from one food source to another, in this case from wheat plants to artificial diet, it can take some time, maybe generations, before the aphids accustom themselves to the new food source and obtain a high level of reproduction again. This could be part of the explanation. Another part could be that the artificial diet is not appropriate. The separate aphid species need different food compositions, and despite extensive information retrieval the optimal composition was obviously not found.

However, the mortality rate is significantly constant through the whole experimental period, and only aphids surviving the whole testing period at 28 days are included in the statistics. Therefore, it was concluded that the experimental design could

Table 3. Effect of MBOA on Reproduction of *S. avenae* Raised on Artificial Diet

concentration (mM)	intrinsic rate of increase[a] (r_m)	no. of aphids testet
0.00	0.00162 B	131
0.05	0.00150 B	167
0.10	0.00296 A	62
0.20	0.00139 BC	99
0.30	0.00075 CD	83
0.50	0.00048 D	58
8.00	0.00000 D	60

[a] Numbers with the same letter are not significantly different, $p < 0.05$.

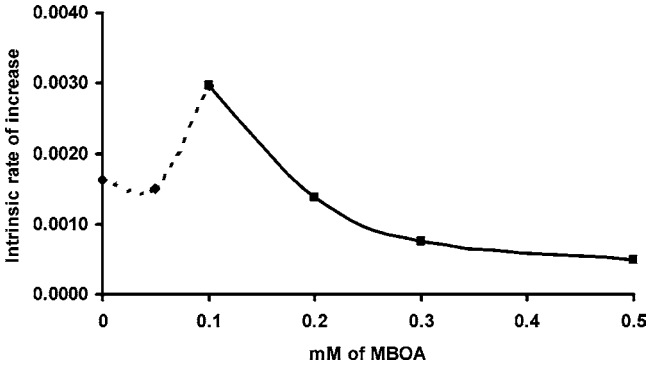

Figure 1. Relationship between intrinsic rate of increase and concentration of MBOA.

be used for testing the effects of MBOA on the reproduction rate of the grain aphids.

Artificial Diet with MBOA Incorporated. Results from grain aphids raised on artificial diet with MBOA incorporated are shown in **Table 3**. As it appears from **Figure 1**, incorporation of up to 0.1 mM of MBOA will increase aphid reproduction. Concentrations incorporated higher than 0.1 mM cause a continuous decrease in the intrinsic rate of increase.

On the face of it, the correlation seems to be curious. However, Liu and Lovett (*31*) reported a similar correlation between responses in radicle length of white mustard to Gramineae. Lovett et al. (*32*) stresses that similar curves can be produced for responses by many organisms to a range of biologically active chemicals, both organic and inorganic.

The relationship between the intrinsic rate of increase (r_m) and the concentration of MBOA $\geq$ 0.1 mM (**Figure 1**) is $r_m = 0.00056 \log(\text{mM MBOA})$, $r^2 = 0.99$; $F = 214.6$ (*25*).

When the concentration of MBOA increases from 0.1 mM to 0.2 or 0.3 mM, a reduction of r_m is obtained at 51 and 73%, respectively. For that reason, even minor increases in concentrations of MBOA in the plants will strongly reduce the population buildup of the grain aphids. When the concentration of MBOA is 1.0 mM, the value of r_m is 0, and no more population buildup will take place.

DIMBOA is unstable in aqueous solutions (pH 5−7) at room temperature and decomposes spontaneously to MBOA with a half-life of $\leq$1 day (*33*). Bredenberg et al. (*34*) concludes that the degradation is quantitative, whereas Wodward et al. (*16*) states that it is not. They found that the yield of MBOA from degradation of DIMBOA varied with temperature, pH, and the presence of uncharacterized solutes. However, under no condition that was tried was >75% MBOA obtained. Thus, it is desirable to study the activity of MBOA.

It was previously thought (*35*) that concentrations of hydroxamic acids (Hx) declined rapidly during seedling growth, reaching very low levels in maturing plants. Measurements of mean concentrations of benzoxazinone derivatives (the hydroxamic acids DIMBOA and 2,4-dihydroxy-2(*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA), the benzoxazolinones MBOA and 2-hydroxy-2(*H*)-1,4-benzoxazin-3(4*H*)-one (BOA), and the lactams 2-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HMBOA) and 2-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HBOA) at various growth stages supported this observation (*36*, *37*). However, Thackray et al. (*7*) have shown that the concentration may be relatively high in newly emerging leaves, including emerging flag leaves. For example, concentrations of Hx in newly emerged flag leaves of *T. aestivum* cv. Mission were 10.8 mmol/kg of dry weight, compared with values of 12.9 and 21.7 mmol/kg in the new leaves of 9-day-old seedlings of *T. aestivum* cv. Likay and *Triticum durum* cv. SNA3, respectively. The relationship between volumes of dry and fresh weight is about 1:10, which means that concentration of Hx in flag leaves could be ~1 mM/kg of fresh weight.

Åhman and Johansson (*38*) found concentrations up to 7.5 mmol/kg of fresh weight in 4-day-old wheat seedlings, and Givovich et al. (*39*) found concentrations in the phloem sap of wheat seedling up to 4 mM.

In mature plants, the youngest tissue retains a high concentration of Hx (*9*−*11*, *17*−*20*). Within the aerial parts of wheat seedlings they are present in the mesophyll as well as in the vascular bundles (*32*, *39*). Recently, it has been shown that the phloem sap of wheat seedlings, collected though excised aphid stylets, contains Hx glucosides (*40*). Furthermore, aphid feeding provoked an increase in Hx levels in some wheat cultivars (*41*, *42*).

In a tillering plant, a relatively high proportion of leaf material might therefore have a high concentration of Hx, thus giving a higher degree of protection to the young tissues of the plant than was previously supposed.

Niemeyer et al. (*43*) reported that DIMBOA concentrations used in artificial diets measured in millimolar levels were comparable with the range in plant leaves measured as millimoles per kilogram of fresh weight. Hence, it follows that concentrations of DIMBOA at ~1 mmol/kg of fresh weight in plants will decrease population development of grain aphids heavily and prevent severe yield losses from the aphids.

In light of the presented data, it can be concluded that even lower concentrations of DIMBOA-glu and consequently DIMBOA and MBOA concentrations will be able to reduce the multiplication of aphids to a level below the economic damage threshold.

Allelopathy is one of the many stresses with which plants must cope in their environment. Strictly, in the context of plants, the allelopathy may represent a chemical contribution to defense adaptations.

Reports of allelopathic phenomena most frequently identify effects that are readily observed in the field or under controlled conditions. The visible effects of allelopathy are merely secondary expressions of primary effects upon metabolic processes (*39*).

There is vast knowledge of insect−plant relationships on which future breakthroughs in the breeding of cultivars resistant to insect pests are dependent. Therefore, it is possible to breed varieties with a sufficiently high content of DIMBOA. In the future this could be one of the tools for handling aphids in winter wheat and other cereal products without increasing the application of insecticides.

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