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The Relationship between DIMBOA Concentration in Corn Lines and Resistance to Aphids

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2, 4-Dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA), a hydroxamic acid is considered to be one of the components responsible for the resistance to pest insects in cereal plants. The relationship between concentration of DIMBOA and aphid infestation on 21 corn lines was investigated in 1990 and 1991.

DIMBOA was detected in leaves of all corn lines tested, contained larger amounts in young plants and gradually decreased with growth. This property was the same as observed in wheat. A more than ten times difference in DIMBOA concentration was observed in corn lines.

However, there was no positive correlation between DIMBOA concentration and aphid density. Most of the lines used had resistance to aphids. Resistance may be built together with other components such as (*E*)-aconitic acid.

Key words : Aphid, Resistant substance, Hydroxamic acid, DIMBOA, Corn

INTRODUCTION

The corn leaf aphid, *Rhopalosiphum maidis* (Fitch) and bird-cherry aphid, *R. padi* (L.) infest corn, reduce corn yield by sucking on leaves, silk and kernels, interfere with tassel development as well as pollen shedding, and sometimes transmit several virus diseases such as maize dwarf mosaic, sugarcane mosaic and barley dwarf of barley (Painter 1951, Long *et al.* 1977).

We have been examining the factors involved in the resistance and susceptibility of some cereal plants to aphids, and found many conferring

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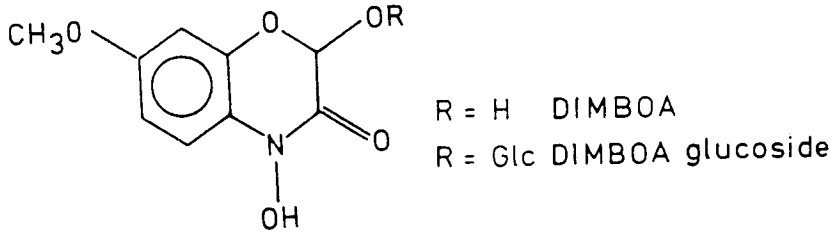
resistance factors. Gramine (Kanehisa *et al.* 1990), (*E*)-aconitic acid (Rustamani *et al.* 1992) and waxes (Tsumuki *et al.* 1989) may be resistance factors.

2, 4-Dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) has been reported to be important for confering resistance to insects and fungi in some species of Graminae. A correlation between concentration of hydroxamic acids in the maize leaves and strength of resistance to European corn borer *Ostrinia nubilalis* (Hubner) was studied (Klun *et al.* 1966, Gutierrez *et al.* 1988, Richardson and Bacon 1993). Long *et al.* (1977) have assayed DIMBOA toxicity to *R. maidis* by the artificial rearing method, and observed that DIMBOA was a resistance factor.

Previously, we reported that DIMBOA was responsible for resistance along with (*E*)-aconitic acid in wheat (Kanehisa *et al.* 1995). Herein, we describe the relationship between DIMBOA concentration and resistance to aphids in 21 corn lines obtained from Hokkaido Agricultural Experiment Station.



MATERIALS AND METHODS

1. *Corn*

Thirty lines were obtained from the Hokkaido Agricultural Experiment Station. They were cultured from 1989 to 1991, sown in middle May in a greenhouse, and transplanted in an experimental field after about 20 to 30 cm height in middle June. For obtaining seed, self-breeding was conducted by covering whirls. Five plants in each line were usually used for the aphid infestation experiment. Some lines were difficult to get seeds; so finally 21 lines were examined for assay of DIMBOA amounts and aphid populations.

2. *Aphid infestation*

The number of aphids per stem was counted at adequate time intervals in resistant and susceptible lines. There had been no natural aphid infestation at the experimental fields for the past three years. *R. maidis* was introduced from heavily infested corn fields. Thirty to 50 aphids were attached to each stem, and the change in aphid density was examined.

3. *Extraction of DIMBOA*

DIMBOA was extracted by the method of Woodward *et al.* (1978), the second and third leaves from the top leaf were homogenized in distilled water, adjusted to pH 3.0 with 1 M HCl, and centrifuged at 10,000 *g*. The supernatant solution was partitioned to diethylether, concentrated and assayed by HPLC.

4. *Thin layer chromatography*

Silica gel (Merck 60G) was used for the adsorbent, and a mixture of methanol, chloroform and 99.5% acetic acid (50 : 50 : 1) for the developing solvent. Ferric chloride solution was used to detect the compound. In 50 m*l* EtOH 0.5 m*l* conc. HCl and 5 g $FeCl_3 \cdot 6H_2O$ was dissolved. DIMBOA was stained blackish indigo blue.

5. *Liquid chromatography*

DIMBOA was determined by means of a Shimadzu HPLC 5A with a SPD-2A detector, a Hibar R column with precolumn (Cica-Merck), and a Shimadzu C-6RA recorder. For elution we used a mixture of 0.05 M phosphate buffer (pH 3.5) and acetonitrile (80 : 20 v/v). The flow rate was adjusted to 1 m*l*/min. Components were detected at 280 nm.

DIMBOA was obtained from Dr. Osamu Saito of the Hokkaido Agricultural Experiment Station. DIMBOA was detected at about 10 min after injection.

6. *Artificial rearing and toxicity assays of DIMBOA to R. padi*

Artificial diet for *R. padi* (Kieckhefer and Derr 1967) was prepared and assayed by the parafilm method (Kanehisa *et al.* 1990). *R. padi* was reared on barley seedlings (Kikaihadaka). Usually 3 aphids per bottle (mostly second instar nymphs) were used for the experiment. The effects of DIMBOA, gramine and (*E*)-aconitic acid on survival rates were determined.

These rates were compared with those obtained by rearing on distilled water only.

RESULTS

1. *Aphid infestation*

The natural infestation was rarely observed on the 21 lines used in the experimental years, from 1989 to 1991, at the institute fields. During the same spring (until early June) and the same area, *R. maidis* and *R. padi* were abundant on barley and wheat and *Melanaphis sachari* (Zehntner) was abundant on sorghum. All corn lines used showed resistance. Therefore, *R. maidis* was introduced from the heavily infested corn fields at farmer's fields.

Population changes were recorded intermittently (Tables 1 and 2). Although the population of transplanted aphids decreased at the early growth stage during which the DIMBOA concentration was high, the survival rate increased at mature and senescent stages in which DIMBOA concentration was low. The oriental corn borer, *Ostrinia furnacalis* (Guenee), was found in several plants, but we could not discriminate whether they were resistant or susceptible.

2. *Thin layer chromatography*

Acid extracts from corn leaves were examined and stained with $FeCl_3$ solution. As shown in Fig. 1, DIMBOA was stained blackish indigo blue. All corn leaves contained DIMBOA, but mature sorghum was not stained.

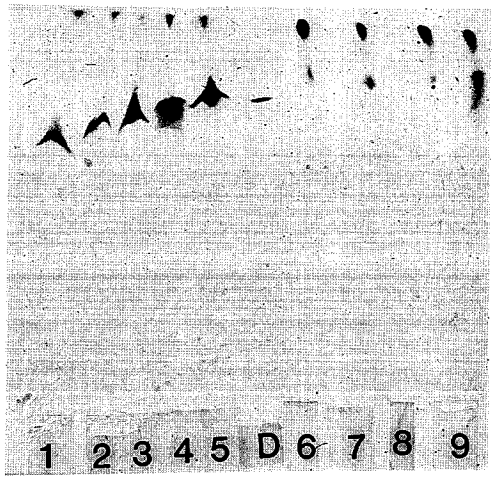


Fig. 1. Thin layer chromatography of DIMBOA in corn cultivars. Developing solvent was MeOH : $CHCl_3$: 50% acetic acid (50 : 50 : 2), and color developer was a $FeCl_3$ reagent. D: DIMBOA, 1: Ho-8, 2: A-654Ht, 3: A-619Ht, 4: A-554, 5: N-132, 6 A-619Ht(scenescent), 7, 8, 9: Sorghum, 7: PE-954177 (resistance to aphids), 8: E-59+ (resistance to greenbug), 9: Redlan B (susceptible to aphids).

3. *Changes of DIMBOA amounts with growing*

Acid extracts from corn leaves contained many substances in HPLC, but the peaks of DIMBOA (about 10 min after injection) and aconitic acid (about 3.7~4.0 min) were the major ones. DIMBOA accompanied with a small peak which was shown at 7~8 min after injection. This was unidentified, but might be a DIMBOA-related compound. DIMBOA-glucoside was a precursor of DIMBOA in a healthy plant, and DIMBOA was liberated by glucosidase activity, enhanced by insects or fungi attack (Klun *et al.* 1967,

Corcuera *et al.* 1982). This glucoside was observed at the peak of 4.0～4.5 minutes on HPLC, younger plants contained more than mature plants.

Table 1 (1990) and Table 2 (1991) show the DIMBOA contents during the growth stages of corn. DIMBOA was found in larger amounts in young plants than in mature plants. The DIMBOA amounts gradually decreased and were very little in senescent plants. There was more than ten times difference among lines tested at the same growth stage. However, there was no relationship between aphid population and DIMBOA amount. All corn lines tested have resistance to aphids more or less. In natural conditions, all lines showed almost no aphid infestation and only decreasing aphid population after introduction from other heavily infested corn fields. Line, A-554, A-632Ht and A-661 in 1990, and A-632Ht and SD-10 in 1991 appeared to be a susceptible in the first year, but it did not show the same characteristic in the second year.

Table 3 shows the DIMBOA concentration in aphid-infested tassels and leaves. The tassels and leaves contained less DIMBOA than those in Tables 1 and 2 in the same growth stages, less than about ten times, especially tassels had no DIMBOA with highly populated aphids.

Table 1. DIMBOA concentration (μg/g fresh weight) and aphid populations on corn leaves in 1990

Line (corn stage)	June 12 (80 cm)	June 21	July 3 (e.e.)	July 11	July 17 (l.e.)	July 18	July 21 (mature)	July 27 (mature)
A-34	63.5 (0)	*	28.5 (0)	*	[53]	*	[37]	1.56 (2)
A-171	99.8 (0)	*	25.5 (0)	*	[68]	*	[7]	1.28 (0)
A-334	12.6 (0)	*	5.6 (0)	*	[22]	*	[69]	0.66 (8)
A-554	7.6 (0)	*	3.3 (0)	*	[93]	*	[396]	0.19 (4)
A-619Ht	42.1 (0)	*	15.5 (0)	*	[830]	*	[40]	1.05 (7)
A-632Ht	25.5 (0)	*	9.2 (0)	*	[370]	*	[385]	1.15 (15)
A-654Ht	14.3 (0)	*	6.5 (0)	*	[20]	*	[48]	0.85 (12)
A-661	88.0 (0)	*	17.4 (0)	*	[201]	*	[237]	1.05 (0)
CM-51	16.4 (0)	*	3.6 (0)	*	[25]	*	[21]	0.40 (2)
Co-108	101.4 (0)	*	41.5 (0)	*	[6]	*	[0]	1.03 (0)
D-321	15.8 (0)	*	6.6 (0)	*	[134]	*	[10]	0.46 (0)
Ho-6	34.5 (0)	*	12.5 (0)	*	[117]	*	[70]	1.00 (3)
Ho-11	43.0 (0)	*	8.0 (0)	*	[721]	*	[241]	1.22 (2)
N-132	20.0 (0)	*	9.5 (0)	*	[1]	*	[4]	2.00 (0)
N-150	57.6 (0)	*	16.5 (0)	*	[8]	*	[25]	1.44 (7)
ND-100	40.5 (0)	*	15.6 (0)	*	[2]	*	[6]	2.00 (0)
SD-10	15.5 (0)	*	4.8 (0)	*	[5]	*	[10]	0.36 (0)
W-15	70.8 (0)	*	20.0 (0)	*	[3]	*	[4]	1.06 (0)
W-540	22.5 (0)	*	9.8 (0)	*	[11]	*	[0]	1.45 (0)

e.e.: early earring stage, l.e.: late earring stage
R. maidis was introduced from other corn fields three times, June 21, July 11 and July 18, as a colony on a leaf (30～50 aphids, marked as *). The number in bracket is the number of aphids on a stem; the number is the number of aphids on leaves from which 10 grams of homogenized sample was prepared.

Table 2. DIMBOA concentration (μg/g fresh weight) and aphid population on corn leaves in 1991

Line (corn stage)	June 12 (80 cm)	June 21 (90 cm)	June 24	June 28	July 2 (e.e.)	July 8 (e.e., l.e)	July 12	July 22 (mature)
A-34	*	25.8 (0)	[2]	*	[0]	10.0 (0)	*	1.32 (0)
A-334	*	14.2 (0)	[0]	*	[0]	6.3 (0)	*	0.43 (0)
A-619Ht	*	44.8 (0)	[45]	*	[0]	12.0 (10)	*	1.02 (0)
A-632Ht	*	13.8 (3)	[490]	*	[103]	5.2 (5)	*	0.55 (14)
A-654Ht	*	39.6 (0)	[0]	*	[0]	12.6 (0)	*	1.59 (0)
A-661	*	69.1 (0)	[40]	*	[26]	20.0 (11)	*	1.86 (20)
CM-51	*	11.4 (2)	[270]	*	[39]	5.6 (38)	*	0.85 (11)
CO-108	*	20.7 (8)	[276]	*	[40]	9.5 (23)	*	1.05 (11)
D-321	*	21.8 (0)	[10]	*	[0]	9.5 (0)	*	0.95 (0)
Ho-6	*	68.8 (0)	[0]	*	[0]	11.0 (0)	*	2.00 (0)
N-132	*	15.6 (0)	[2]	*	[0]	5.5 (0)	*	0.36 (0)
N-150	*	47.6 (0)	[2]	*	[2]	14.0 (0)	*	1.88 (0)
ND-100	*	17.2 (6)	[832]	*	[113]	5.5 (15)	*	0.30 (13)
SD-10	*	12.5 (15)	[2115]	*	[1004]	5.8 (23)	*	0.45 (21)
W-540	*	29.4 (7)	[47]	*	[60]	6.2 (20)	*	0.56 (20)
W729D	*	3.7 (15)	[2307]	*	[22]	2.5 (38)	*	0.40 (21)

e.e. : early earring stage, l.e. : late earring stage
R. maidis was introduced from other corn fields three times, June 12, June 28 and July 12, as a colony on a leaf (about 30~50 aphids, marked as *). The number in bracket is the number of aphids on a stem ; The number is the number of aphids on leaves from which 10 grams of homogenized sample was prepared.

Table 3. DIMBOA concentration (μg/g fresh weight) in corn plants collected from farmer's fields

Sample	Date	DIMBOA
Tassel (many *R. maidis*)	18 July 1990	no peak
Leaf (early earring 650 〃)	〃	0.50
〃 (〃 1000 〃)	〃	0.05
〃 (〃 1000 〃)	〃	0.31
Tassel (many *R. maidis*)	11 July 1991	no peak
〃	12 〃	no peak
Leaf (early earring 500 〃)	11 〃	0.17
〃 (〃 750 〃)	〃	0.10
〃 (〃 1400 〃)	〃	0.19
〃 (〃 400 〃)	12 July 1991	0.26
〃 (〃 900 〃)	〃	0.31
〃 (〃 2500 〃)	〃	0.10

4. *The toxicity of DIMBOA to R. padi*

Table 4 shows the effects of DIMBOA in comparison with gramine and (*E*)-aconitic acid on *R. padi* survivals. DIMBOA showed a toxicity at a concentration of 0.01%, about a tenth of the toxicity of gramine and was slightly more toxic than (*E*)-aconitic acid.

Table 4. Survival percentage of *Rhopalosiphum padi* on diet with different concentrations of DIMBOA, gramine and (*E*)-aconitic acid

Treatment		Total number of aphids (aphids × trials)	Days									
			1	2	3	4	5	6	7	8	9	10
Control		36 (3×12)	100	95	85	81	75	60	46	29	17	8
H_2O		24 (3× 8)	100	83	58	25	8	0				
DIMBOA	0.03%	24 (3× 8)	100	87	62	50	37	13	4	0		
	0.01%	24 (3× 8)	100	96	83	71	50	25	17	8	4	0
	0.003%	24 (3× 8)	100	96	83	71	62	42	25	17	8	0
	0.001%	24 (3× 8)	100	96	83	79	62	50	29	17	8	4
Gramine	0.03%	24 (3× 8)	100	78	30	13	0					
	0.01%	24 (3× 8)	100	93	71	42	13	8	4	0		
	0.003%	24 (3× 8)	100	96	71	71	50	25	17	4	0	
	0.001%	24 (3× 8)	100	96	83	67	58	50	42	17	4	0
(*E*)-	0.3%	24 (3× 8)	100	80	58	38	33	25	17	0		
Aconitic	0.1%	24 (3× 8)	100	80	69	50	42	29	17	4	0	
acid	0.03%	24 (3× 8)	100	96	83	71	42	29	17	13	4	0
	0.01%	24 (3× 8)	100	96	92	75	50	29	17	13	4	4

DISCUSSION

All corn lines tested contained DIMBOA, and there were no high aphid population-increasing lines, suggesting that they are resistant to aphids. Typical susceptible lines were found in barley (Kanehisa *et al.* 1990) and less resistant lines in wheat varieties (Kanehisa *et al.* 1995). The DIMBOA concentration in corn leaves decreased with plant growth, 10 to 100 μg/g fresh weight of leaves elongation stage, 2 to 20 μg/g at the earing stage, less than 1 μg/g at the mature stage and almost none at the senescent stage. The aphid population seemed to increase on mature and senescent corn plants, accompanied by a decrease of DIMBOA. Aphid-infested leaves and especially the tassels of plants in heavily infested field contained very little DIMBOA.

Aphids are phloem sap feeders and hydroxamic acid was detected only in central and lateral veins, and not detected in guttation drops and xylem exudate (Argandona and Corcuera 1985). Therefore, DIMBOA may be directly injested to digestive organs and may act as antibiotic substance.

(*E*)-aconitic acid, another water-soluble phloem-localizing antibiotic component, was detected in corn (200-500 μg/g), wheat (10-30 μg/g), and barley (1-10 μg/g) (Rustamani *et al.* 1992). DIMBOA may play a role in the resistance to aphids with (*E*)-aconitic acid, as observed in wheat (Kanehisa *et al.* 1995).

The three most important detoxication mechanisms in insects are dependent on the activity of the enzymes: glutathion S-transferases,

polysubstrate monooxygenase, and /or carboxyesterases (Terriere 1984, Ahmad *et al.* 1986). In general, DIMBOA decreases the activity of glutathion S-transferases (Leszczynski and Dixon 1992). This may be the action mechanism of DIMBOA. Aconitic acid may be a competitive inhibitor of the aconitase in tricarboxylic acid cycle in the cellular respiration (Saffran and Prado 1949).

Gramine, indole alkaloid, is responsible for the resistance to aphids in barley lines and this water insoluble compound is in the mesophyll parenchyma cells and in epidermis. There was no gramine in corn leaves (Kanehisa *et al.* 1993). The toxicity of DIMBOA to *R. padi* was about a tenth of that of gramine and several times stronger than (*E*)-aconitic acid.

DIMBOA and (*E*)-aconitic acid may act as antibiotics in corn leaves against aphids. The relationship between the feeding behavior and phloem localization of these substances is important.

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REFERENCES

Ahmad, S., Brattsten, L.B, Mulin, C.A. and Yu, S.J. 1986. Enzymes involved in the metabolism of plant allelochemicals. 73-152. *In* "Molecular aspect of insect-plant associations" (Brattsten, L.B. and Ahmad, S. eds.) London, New York.

Argandona, V.H. and Corcuera, L.J. 1985. Distribution of hydroxamic acids in *Zea mays* tissues. Phytochemistry 24 : 1778-178.

Corcuera, L.J., Argandona, G.F., Pena, G.F., Perez, F.J. and Niemeyer, H.M. 1982. Effect of a benzoxazinone from wheat on aphid. 33-39. *In* "Proc. 5th Symp. Insect-Plant Relationships" (Visser, J.H. and Minks, A.K. eds.) Centre Agric. Publ. Doc., Wageningen.

Gutierrez, C., Castanera, P. and Torres, V. 1988. Wound-induced changes in DIMBOA (2, 4-dihydoxy-7-methoxy-2H-1, 4 benzo-xazin-3(4H)-one) concentration in maize plants caused by *Sesamia nonagrioides* (Lepidoptera : Noctuidae). Ann. Appl. Biol. 113 : 447 -454.

Kanehisa, K., Tsumuki, H., Kawada, K. and Rustamani, M.R. 1990. Relations of gramine and aphid populations on barley lines. Appl. Entomol. Zool. 25 : 251-259.

Kanehisa, K., Rustamani, M.A., Tsumuki, H., Kasahara, T. and Shiraga, T. 1993. Changes of gramine contents as an aphid resistant substance with barley growth. (in Japanese) Bull. Res. Inst. Biores. Okayama Univ. 1 : 105-112.

Kanehisa, K., Rustamani, M.A., Cheng, W-I. Tsumuki, H. and Shiraga, T. 1995. DIM-

BOA, resistance substance to aphid, contents in wheat varieties. Bull. Res. Inst. Bioresour. Okayama Univ. 3 : 17-26.

Kieckhefer, R.W. and Derr, R.F. 1967. Rearing three species of cereal aphids on artificial diets. Jour. Econ. Entomol. 60 : 663-665.

Klun, J.A., Tipton, C.L. and Brindley, T.A. 1967. 2, 4-Dihydroxy-7-methoxy/1, 4-benzoxazin-3 (4H)-one (DIMBOA), an active agent in the resistance of maize to the European corn borer. Jour. Econom. Entomol. 60 : 1529-1533.

Leszczynski, B. and Dixon, A.F.C. 1992. Resistance of cereals to aphids : Interaction between hydroxamic acids and glutathion S-transferases in the aphid *Sitobion avenae* (F.) (Homoptera : Aphididae). Jour. Appl. Entomol. 113 : 61-67.

Long, B.J., Dunn, G.M., Bowman, J.S. and Routley, D.G. 1977. Relationship of hydroxamic acid content in corn and resistance to the corn leaf aphid. Crop Sci. 17 : 55-58.

Painter, R.H. 1951. Insect resistance in crop plants. 1-520. The Macmillan Co., New York.

Richardson, M.D. and Bacon, C.W. 1993. Cyclic hydroxamic acid accumulation in corn seedlings exposed to reduced water potentials before, during, and after germination. Jour. Chem. Ecol. 19 : 1613-1624.

Rustamani, M.A., Kanehisa, K. and Tsumuki, H. 1992. Aconitic acid content of some cereals and its effect on aphids. Appl. Entomol. Zool. 27 : 79-87.

Saffran, M. and Prado, J.M. 1949. Inhibition of aconitase by *trans* aconitate. Jour. Biol. Chem. 180 : 1301-1309.

Terriere, L.C. 1984. Induction of detoxification enzymes in insects. Ann. Rev. Entomol. 29 : 71-88.

Tsumuki, H., Kanehisa, K. and Kawada, K. 1989. Leaf surface wax as a possible resistance factor of barley to cereal aphids. Appl. Entomol. Zool. 24 : 275-281.

Woodward, M.D., Corcuera, L.J., Helgeson, J.P., Kelman, A. and Upper, C.D. 1978. Quantitation of 1, 4-benzoxazin-3-ones in maize by gas-liquid chromatography. Plant Physiol. 63 : 14-19.

トウモロコシに含まれるDIMBOAとアブラムシ抵抗性の関係

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ハイドロキサム酸のDIMBOAは特定のイネ科植物にあり，寄生する昆虫や病原菌に対して抵抗性物質となっている．一方，トウモロコシにはトウモロコシアブラムシとムギクビレアブラムシが寄生し，養分奪取，排泄する甘露によるスス病や萎縮病ウイルスの伝播者としての害をもたらしている．

トウモロコシの21系統を1990年と1991年に栽培し，生育に伴うDIMBOA含量の消長とア

ブラムシの寄生の変化を調べた．DIMBOA は単位体重当り若齢期に多量を含有し，生育に伴って少量になり，老齢期になると検出不可能の量となった．

使用した系統は全て多少ともアブラムシに対して抵抗性を示し，オオムギやコムギなどで見られた増殖は見られなかった．大繁殖している農家の圃場から移植したが，DIMBOA 含量の多い若齢期においては急速に死滅した．DIMBOA 含量の少ない成熟期から老齢期にかけては若干生存した．

ムギミドリアブラムシを用いて，人工飼育液に DIMBOA 等を添加して生存率を調べて，他の作用物質との関連を考察した．トウモロコシには同じ篩管に含有されるアコニット酸も多量にあり，両物質が共に抵抗性物質であると考えられた．

キーワード：アブラムシ，抵抗性物質，ハイドロキサム酸，DIMBOA，トウモロコシ