PHYTOCHEMICAL NATURE OF WHEAT (TRITICUM AESTIVUM L.) AND BARLEY (HORDEUM VULGARE L.) RESISTANCE TO THE CEREAL LEAF BEETLE (OULEMA MELANOPUS (L.))

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
JOHN IRVING WILLARD
1975

This is to certify that the

thesis entitled

Phytochemical Nature of Wheat (Triticum Aestivum L.)
and Barley (Hordeum Vulgare L.) Resistance to
the Cereal Leaf Beetle (Oulema Melanopus L.)

presented by

John Irving Willard

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Crop Science

Major professor

Date October 9, 1975

O-7639

ABSTRACT

PHYTOCHEMICAL NATURE OF WHEAT (TRITICUM AESTIVUM L.) AND BARLEY (HORDEUM VULGARE L.) RESISTANCE TO THE CEREAL LEAF BEETLE (OULEMA MELANOPUS (L.))

By

John Irving Willard

Cultivars of wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.) with varying degrees of resistance to the cereal leaf beetle (Oulema melanopus (L.)) were studied for possible physiological or phytochemical differences related to their resistance. Plants were grown either in the growth chamber or the greenhouse in sand, soil or nutrient solution.

Tolerance to preemergence application of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) was inversely related to observed resistance to the cereal leaf beetle in selected cultivars of barley seedlings grown in the greenhouse in sand and soil. In wheat this relation did not hold among all cultivars. This relationship was not as evident when evaluated among 219 backcross lines. A summer adult cereal leaf beetle bioassay was used for measuring feeding preference. Quantitative and qualitative differences were observed among the cultivars in the benzoxazinone glucosides extracted from seedling leaves.

Reducing sugar content of the seedling leaves could not be related to cereal leaf beetle resistance.

The cultivars resistant to the cereal leaf beetle with leaf pubescence were found to contain greater deposits of silica than the non-pubescent cultivars. The silica was associated with the pubescence. Cultivars with high calcium concentrations in the seedling leaves were rated most susceptible to the cereal leaf beetle. The high calcium content of the leaves was correlated to high pectin levels. The higher concentration of pectic substances in susceptible cultivars may result in softer more palatable cell walls. Of the cultivars studied, those most resistant to the cereal leaf beetle were the least succulent. The results reported indicate that although pubescence was important, other factors are related to the resistance of wheat and barley to the cereal leaf beetle.

PHYTOCHEMICAL NATURE OF WHEAT (*TRITICUM AESTIVUM* L.) AND BARLEY (*HORDEUM VULGARE* L.) RESISTANCE TO THE CEREAL LEAF BEETLE (*OULEMA MELANOPUS* (L.))

By

John Irving Willard

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

1975

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to his major professor, Dr. Donald Penner, for his support and guidance throughout this study. I am most grateful for the opportunity of working with him.

The assistance of Dr. D. H. Smith, Dr. S. G. Wellso, Dr. W. F. Meggitt, and Dr. J. W. Hanover as Guidance Committee members is gratefully acknowledged. The technical assistance of Ricky Rodden and Barbra Reinhardt is especially appreciated.

Special thanks is given to my loving wife, Jean, for her patience and encouragement that has helped make this dissertation possible. To my daughter, Jill, my thanks for her unsuspecting sacrifices.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

INTRODUCTION

The importance of establishing the phytochemical characteristics involved in plant resistance to pests, i.e., insects, disease, weeds, or nematodes, cannot be overlooked in a program for their control. Not only does this contribute to our knowledge and understanding but presents the possibility of using this knowledge in developing screening programs for finding resistant lines. If the physiological or phytochemical character can be quickly and economically determined in the laboratory or greenhouse in large numbers of lines the tremendous investment in time required to screen using only traditional plant nurseries can be reduced considerably. By defining as many factors as possible which contribute to resistance the plant breeder can incorporate these into new lines giving greater resistance. This also reduces the chances of shifts developing in the pest population allowing it to overcome the resistance affected by the so-called "resistant" cultivars.

The cereal leaf beetle (Oulema melanopus (L.)), a Eurasian introduction into Michigan in 1962, has now spread into 15 states and Canada. Losses in yield of small grains as a result of cereal leaf beetle infestation have been reported as high as 48%. Considerable research has been done with the cereal leaf beetle and in the development of resistant cereal lines. However, only the presence of leaf pubescence on resistant cultivars has been correlated with resistance.

The objectives of this study were to (1) determine whether characteristics other than pubescence in the area of plant anatomy, morphology, physiology or phytochemistry were involved in the resistance of cereals to the cereal leaf beetle, (2) evaluate these characteristics among several cultivars varying in resistance to the cereal leaf beetle, and (3) develop a bioassay to test response of the cereal leaf beetle to the different characteristics.

CHAPTER 1

BENZOXAZINONES - CYCLIC HYDROXAMIC ACIDS FOUND IN PLANTS

Introduction

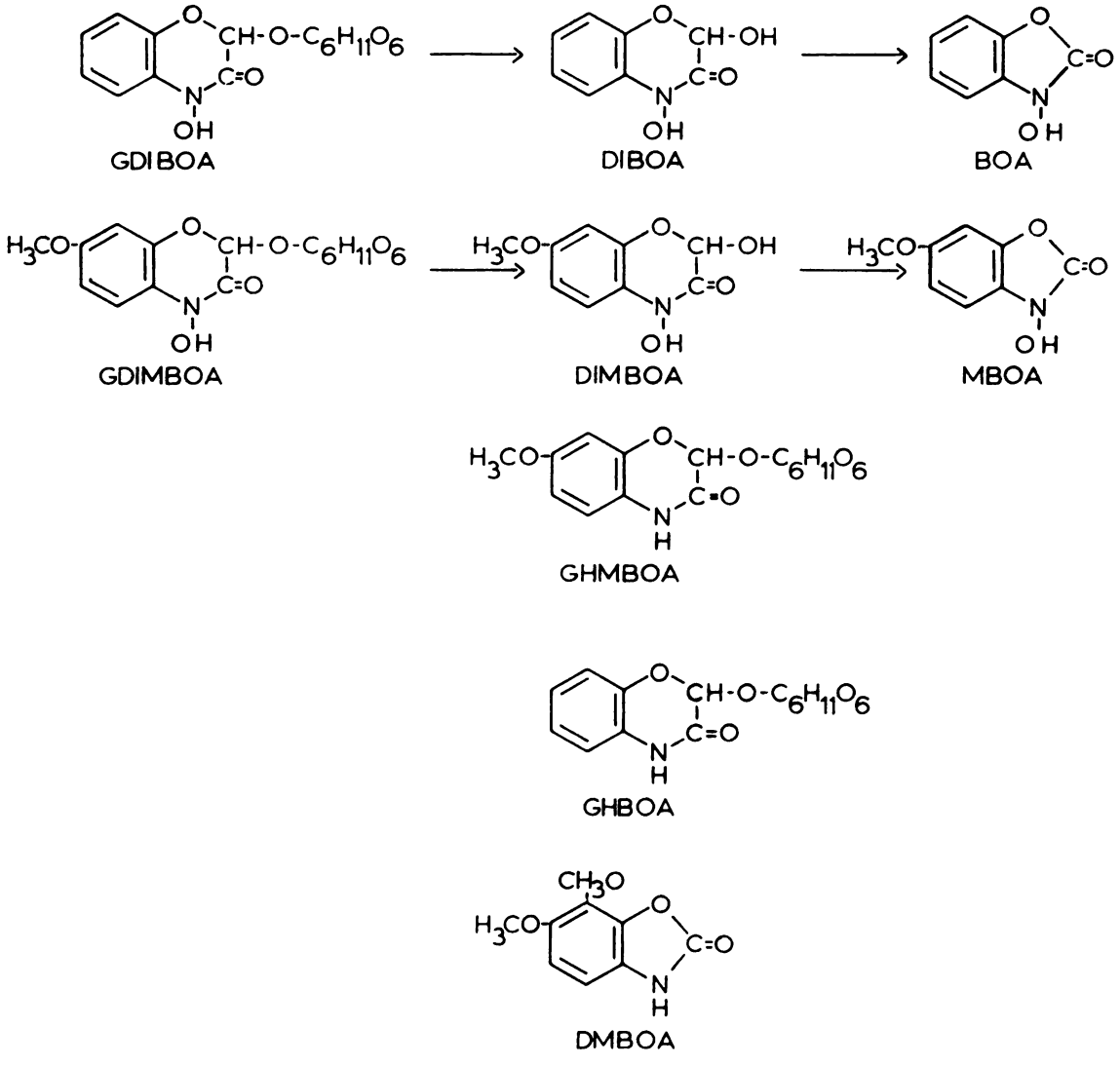
The benzoxazinones are naturally occurring cyclic hydroxamic acids found in numerous plant species. Their possible role in insect and disease resistance, and detoxication of the triazine herbicides has aroused scientific interest in several disciplines.

Identification and subsequent manipulation of a naturally occurring chemical or group of chemicals responsible for resistance to several pests and for partial selectivity to a prominent group of herbicides offers tremendous potential for the plant breeder to develop new cultivars allowing more effective pest control.

Chemistry

The first published record of the occurrence of 2(3)-benzoxazolinone (BOA) was reported by Virtanen and Hietala (1955). It was extracted from rye (Secale cereale L.) seedlings. The structures of BOA and other related compounds are shown in Figure 1. The methoxy derivative 6-methoxy-2(3)-benzoxazolinone (MBOA) was subsequently isolated from wheat (Triticum aestivum L.) and corn (Zea mays L.) by Virtanen et al. (1956) and from corn by Loomis (1957) nearly simultaneously. Following further research it was proposed that the naturally occurring precursor to BOA in rye was a glucoside (Virtanen and Hietala 1959), and that the precursor in wheat and corn was a glucoside of MBOA (Wahlroos and Virtanen 1959). In subsequent publications, the structures of the true natural precursors of BOA found in rye were identified as the glucoside 2-(2,4-dihydroxy-1,4(2H)-benzoxazin-3(4H)-one)-B-D-glucopyranoside (GDIBOA) Hietala and Virtanen (1960) and the aglucone 2,4-dihydroxy-1,4(2H)-benzoxazin-3-one (DIBOA) (Virtanen and Hietala 1960). The structures of the benzoxazinone precursors of MBOA in wheat and corn were also determined to be

Figure 1. Benzoxazinones, their glucosides and benzoxazolinone derivatives.


GDIBOA
DIBOA
BOA
GDIMBOA
DIMBOA
MBOA
GHMBOA
GHBOA
DMBOA

2-(2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazin-3(4H)-one)-B-D-glucopyranoside (GDIMBOA) and 2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one (DIMBOA) Wahlroos and Virtanen 1959. The structure of DIMBOA from corn was confirmed by Hamilton et al. (1962). Much of the initial research and some of the recent studies on the relationship of benzoxazinones to pest resistance have been done with BOA and MBOA. However, these compounds are not naturally occurring plant products but are artifacts of extraction. It has been shown that DIMBOA has a higher degree of biological activity than does MBOA (Klun et al. 1967). The conversion of the glucoside to the aglucone in rye (Virtanen and Hietala 1959), wheat and corn (Wahlroos and Virtanen 1959) takes place enzymatically upon crushing the plant tissue. The heat and other extraction reagents cause the formation of the benzoxazolinone from the aglucone. This accounts for the initial observation that BOA and MBOA were the naturally occurring compounds in plants as enzymatic activity was not stopped upon initial extraction of the plant material (Virtanen and Wahlroos 1963). Hofman and Hofmanova (1971) showed that when extreme care is taken to stop the enzymatic action immediately, only the glucoside (GDIMBOA) is present in uninjured corn. The mechanism for the conversion of the aglucones DIBOA and DIMBOA to BOA and MBOA respectively was shown to occur by Honkanen and Virtanen (1961) with the liberation of formic acid which was derived from carbon atom 2 of the aglucone structures. More recently Smissman (1972) has proposed an alternate mechanism to the formation of BOA and MBOA. Undoubtedly the conversion of the benzoxazinones to the benzoxazolinones during extraction in many of the studies accompanied with loss or changes in biological activity has greatly hindered the elucidation of the true biological role of the benzoxazinones.

Studies on the localization of B-glycosidases responsible for the formation of DIMBOA in corn have shown that they are associated with the phloem of the small vascular bundles in the leaves (Mace 1973) and in the lateral root meristems (Ashford and McCully 1973). Other cyclic hydroxamic acids have also been described in corn in lesser quantities, i.e. 2-(2-hydroxy-7-methoxy-1,4-benzoxazin-3-one)-B-D-glucopyranoside (GHMBOA) (Gahagan and Mumma 1967), 2-(2-hydroxy-1,4-(2H)-benzoxazin-3(4H)-one)-B-D-glucopyranoside (GHBOA) (Hofman and Hofmanova 1969), and 6,7 -dimethoxy-2-benzoxazolinone (DMBOA) (Klun et al. 1970). A review of the isolation and characterization of most of the aforementioned cyclic hydroxamic acids was made by Tipton et al. (1967). The biosynthesis and interconversion of these compounds in corn has also been discussed by Tipton et al. (1973). It appears doubtful that the identity of all of the naturally occurring benzoxazinones has been determined.

Antimicrobial Properties

Reviews on plant diseases (Beck et al. 1957, Maehr 1971 and Ingham 1972) have pointed out the importance of the cyclic hydroxamic acids to plant resistance. The initial report of their presence in plants was related to their anti-fusarium properties in rye varieties which were resistant to these fungi (Virtanen and Hietala 1955). Later both BOA and MBOA were shown to have inhibitory effects on the growth of Fusarium nivale, Sclerotinia trifoliorum, Pennicillium roquefortii, Mucor K. species, Staphylococcus aureus, and Pseudomonas fluorescens at a concentration of 0.5 mg per ml of culture medium (Virtanen et al. 1957). Although Virtanen and his co-workers have shown the inhibitory effect of both BOA and MBOA, neither of which are naturally occurring, this evidence, and that of others, is still pertinent to disease resistance as both the glucosides and aglucones present in the plant may have antimicrobial

properties. Whitney and Mortimore (1959, 1959a) showed that an antifungal substance in field corn, which they thought to be MBOA, prevented the growth of Gibberella zeae and Fusarium moniliforme fungi responsible for root and stalk rot. It was also shown that MBOA at a concentration of 0.15 mg/ml of media inhibited the growth of bacterial wilt (Xanthomonas stewartii) in sweet corn (Whitney and Mortimore 1959). The resistance of corn varieties to the stalk rot fungi Diplodia zeae was shown by BeMillar and Pappelis (1965) to not only be related to the GDIMBOA content of pith cores but also to the density of those cores. Dabler et al. (1969) showed that as little as 100 ppm of GDIMBOA completely inhibited the germination of Diplodia zeae spores. Research with inbred corn lines which showed resistance to northern corn leaf blight (Helminthosporium turcicum) has shown a correlation coefficient of -0.95 between the injury ratings of H. turcicum and $\log_{10}$ of the MBOA concentration (Molot and Anglade 1968, Molot 1969). Later Couture et al. (1971) showed that less than 10 ppm DIMBOA was required to inhibit the germination of H. turcicum spores. The relationship between the genetic inheritance of resistance of H. turcicum and the DIMBOA content of inbred lines has been shown to be related to the Ht gene and the Bx gene respectively (Couture et al. 1971). The homozygous dominant HtHtBxBx line showed the greatest resistance to H. turcicum and the homozygous recessive hthtbxbx line the least, with the intermediate heterozygous lines showing intermediate resistance (Calub et al. 1974). Long et al. (1975) suggested that screening corn lines for DIMBOA content at 30 to 40 cm in height could be of value for selecting lines resistant to H. turcicum. The B-glucosidase content of isolines of corn resistant and susceptible to H. turcicum were shown to be the same, indicating the GDIMBOA content was of greater importance

to the resistance to northern corn leaf blight (Mace 1973). Although the majority of the research has been done with corn, perhaps due to the nearly ten fold greater concentration of cyclic hydroxamic acids than in wheat (Hamilton 1964), investigations into the resistance of wheat to stem rust (Puccinia graminis var. tritici) have shown some interesting results. Elnaghy and Linko (1962) reported that the concentration of GDIMBOA in stem rust-resistant wheat lines was higher than in lines susceptible to P. graminis var. tritici Erikss. and Henn. They also reported that the GDIMBOA content in necrotic areas of a resistant line was lower than that in healthy tissue, suggesting the breakdown of GDIMBOA to DIMBOA. More recent studies however, have indicated that the above relationship only holds for the wheat lines at the extreme ends of the resistance scale and that lines with intermediate concentrations of GDIMBOA did not show the expected degree of resistance to P. graminis var. tritici Erikss. and Henn. (Elnaghy and Shaw 1966, Knott and Kumar 1972).

Resistance to Insects

Several reviews have discussed the resistance of plants to insects (Beck 1965, Maxwell et al. 1972, Gallun et al. 1975). One of the classic examples of the relationship of the genetics and phytochemistry to resistance is the resistance in corn to the European corn borer (Ostrinia nubilalis, (Hubner)). After a satisfactory artificial diet was developed for the O. nubilalis, Loomis et al. (1957) found that extracts from young corn leaves had inhibitory effects on the borer. They were able to isolate this factor and labeled it Resistance Factor A (RFA). The quantity of RFA in any particular corn line followed the mortality observed for borer larvae. After additional research, RFA was identified as MBOA. Virtanen (1961) suggested however, that MBOA was probably not the active compound

but that DIMBOA, its precursor, was more likely the active product. The role of MBOA was further studied by Klun and Brindley (1966). They showed that the highly resistant corn lines had 10 times more MBOA than the highly susceptible lines and that 0.5 mg of MBOA was capable of inhibiting borer pupation on artificial diet. They also felt that MBOA could not be the primary factor in borer resistance as it was an artifact of extraction but that its precursors may have biological activity. In another study, Klun et al. (1967) showed that DIMBOA was actually the chemical factor associated with the resistance of corn to first-brood European corn borer. It was also reported (Klun and Robinson 1969) that those corn inbreds that maintained high concentrations of DIMBOA in the whorl at later stages of maturity showed greater resistance to the borer. In a study of the genetic nature of borer resistance, significant correlations between the concentration of DIMBOA and the resistance to first-brood borers in 11 inbred lines ($r=-0.89$) and the single crosses ($r=-0.74$) were shown (Klun et al. 1970). They also reported significant effects due to general and specific combining ability for DIMBOA concentration and borer resistance.

A recent study into the physiological resistance of cereals to the cereal leaf beetle (Oulema melanopus (L.)) has shown both quantitative and qualitative differences in glucosides present in resistant and susceptible lines of both wheat and barley (Hordeum vulgare L.) (Willard et al. 1974).

Detoxication of Triazine Herbicides

Hamilton and Moreland (1961) reported both in vivo and in vitro conversion of simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) to hydroxy-simazine and that this conversion could be effected in vitro by both DIMBOA and GDIMBOA from corn. It was later reported that the ability

of excised roots of corn, wheat, rye, Coix lacryma-jobi L., sorghum (Sorghum vulgare Pers.), oats (Avena sativa L.), and barley to convert atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) to hydroxy-atrazine was correlated to their content of benzoxazinone derivatives (Hamilton 1964). Many other reports have indicated that in a wide variety of species, the hydroxy-derivative of triazine herbicides catalytically formed by DIMBOA or GDIMBOA is important in their detoxication, i.e. Coix lacryma (Hurter 1966), cotton (Gossypium hirsutum L.) and soybeans (Glycine max Merr.) (Sikka and Davis 1968), bananas (Musa acuminata L.) (Barba and Romanowski 1969), Norway Spruce (Picea abies L.) (Lund-Hoie 1969). Tipton et al. (1970) reported on studies on the kinetics of the catalysis of simazine hydrolysis by DIMBOA. They were able to show that hydrolysis increased with the concentration of DIMBOA and that the reaction was greater than first order, indicative of its catalytic properties. In the aforementioned report by Hamilton (1964) he suggested that the DIMBOA content did not totally explain the differential susceptibility of the various species observed and that other selective mechanisms were probably present. Since that time other mechanisms for the metabolism of the triazines have been reported; the N-dealkylation (Shimabukuro et al. 1966, Shimabukuro 1967, 1967a, 1968, Shimabukuro and Swanson 1970, and Roeth and Lavy 1971) and the enzymatic detoxication in the leaves by glutathione s-transferase to form glutathione and glutamylcysteine conjugates (Shimabukuro et al. 1971 and Lamoureux et al. 1972). The possibility for another mechanism for resistance to simazine in common groundsel has been reported by Radosevich and Appleby (1973) as they were unable to find any of the above mentioned metabolites in one biotype of resistant groundsel. Although N-dealkylation and conjugation have

been shown to be the most important metabolites of triazines in the leaves of various species, the catalytic hydrolysis by DIMBOA in the roots also plays an important role in those species which have DIMBOA present in sufficient quantities.

Summary

Benzoxazinones, cyclic hydroxamic acids found in plants as glucoside s or the aglucone, have been related to plant-pest resistance. Their benzoxazolinone derivatives were originally thought to be naturally occurring, however, they have been shown to be artifacts of the extraction procedures used. In the uninjured plant the benzoxazinones are found as glucosides, upon crushing or injuring the plant tissue the benoxazinone aglucone is released as the result of cleavage of the O-glycosyl bond by B-glycosidases. The benzoxazinone in rye (Secale cereale L.) has been identified as 2,4-dihydroxy-1,4(2H)-benzoxazine-3-one and in corn (Zea mays L.) and wheat (Triticum aestivum L.) as 2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazine-3-one. These compounds have been shown to have antimicrobial properties. Resistance of corn to Fusarium nivale and wheat to Puccinia graminis var. tritici Erikss. and Henn. have been related to benzyoxazinone content. It has been well documented that the resistance in corn to the European corn borer is related to the concentration of the benzoxazinone present in the resistant lines. Detoxication of s-triazine herbicides in the roots of corn and other species has been to the conversion of the herbicides to their hydroxy derivative by the catalytic action of benzoxazinone. The total contribution of the benzoxazinones to herbicide selectivity has not been determined.

Although a rapid and inexpensive assay for the quantitative determination of benzoxazinones content in plant tissue has not been developed. Such a method presents great potential for the screening of many crop lines for resistance to several pest problems.

REFERENCES

Ashford, A.E. and M.E. McCully: Histochemical localization of B-glycosidases in roots of Zea mays. Protoplasma 77, 411 (1973).

Barba, R.C. and R.R. Romanowski, Jr.: Differential phytotoxicity of atrazine and ametryne to bananas. Weed Res. 9, 114 (1969).

Beck, S.D.: Resistance of plants to insects. Ann. Rev. Entomol. 10, 207 (1965).

Beck, S.D., E.T. Kaske and E.E. Smissman: Quantitative estimation of the resistance factor, 6-methoxybenzoxazolinone, in corn plant tissue. J. Agr. Food Chem. 5, 933 (1957).

BeMiller, J.N. and A.J. Pappelis: 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside in corn. I. Relation of water-soluble, I-butanol-soluble glycoside fraction content of pith cores and stalk rot resistance. Phytopathology 55, 1237 (1965).

Calub, A.G., G.M. Dunn, D.G. Routley, and R.M. Couture: Genetic and environmental effects on production of inhibitory compounds in corn resistant to Helminthosporium turcicum. Crop Sci. 14, 359 (1974).

Couture, R.M., D.G. Routley and G.M. Dunn: Role of cyclic hydroxamic acids in monogenic resistance of maize to Helminthosporium turcicum. Physiol. Plant Pathol. 1, 515 (1971).

Dabler, J.M., A.J. Pappelis, and J.N. BeMiller: Effect of phenolic acids and corn extracts upon spore germination of Diplodia zeae. Phytopathology 59, 1098 (1969).

Elnaghy, M.A. and P. Linko: The role of 4-O-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in resistance of wheat to stem rust. Physiol. Plant. 15, 764 (1962).

_____ and M. Shaw: Correlation between resistance to stem rust and the concentration of glucoside in wheat. Nature 210, 417 (1966).

Gahagan, H.E. and R.O. Mumma: The isolation of 2-(2-hydroxy-7-methyoxy-1,4-benzoxazin-3-one)B-D-glucopyranoside from Zea mays. Phytochemistry 6, 1441 (1967).

Gallun, R.L., K.J. Starks, and W.D. Guthrie: Plant resistance to insects attacking cereals. Ann. Rev. Entomol. 20, 337 (1975).

Hamilton, R.H.: Tolerance of several grass species to 2-chloro-s-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. J. Agr. Food Chem. 12, 14 (1964).

_____, and D.E. Moreland: Simazine: Degradation by corn seedlings. Science 135, 373 (1961).

_____, R.S. Bandurski, and W.H. Reusch: Isolation and characterization of cylic hyroxamate from Zea mays. Cereal Chem. 39, 107 (1962).

Hieta, P.K. and A.I. Virtanen: Precursors of benzoxazolinone in rye plants II. Precursor I., the glucoside. Acta Chem. Scand. 14, 502 (1960).

Hofman, J. and O. Hofmanova: 1,4-Benzoxanine derivatives in plants: Sephadex fractionation and identification of a new glucoside. European J. Biochem. 8, 109 (1969).

_____, _____ 1,4-Benzoxazine derivatives in plants: Absence of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one from uninjured Zea mays plants. Phytochemistry 10, 1441 (1971).

Honkanen, E. and A.I. Virtanen: On the reaction of 2,4-dihydroxy-1,4-benzoxazin-3-one to 2(3)-benzoxazolinone. Acta Chem. Scand. 15, 221 (1961).

Hurter, J.: Abbauprodukte von Simazine in Gramineen. Experientia 22, 741 (1966).

Ingham, J.L.: Phytoalexins and other natural products as factors in plant disease resistance. The Bot. Rev. 38, 343 (1972).

Klun, J.A. and T.A. Brindley. Role of 6-methoxy benoxazolinone in inbred resistance of host plant (Maize) to first-brood larvae of european corn borer. J. of Econ. Entomol. 59, 711 (1966).

_____, W.D. Guthrie, A.R. Hallauer, and W.A. Russell: Genetic nature of the concentration of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one and resistance to the european corn borer in a diallele set of eleven maize inbreds. Crop Sci. 10, 87 (1970).

_____, and J.F. Robinson: Concentration of two 1,4-benzoxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the european corn borer J. Econ. Entomol. 62, 214 (1969).

_____, C.L. Tipton, J.F. Robinson, D.L. Ostrem and M. Beroza: Isolation and identification of 6,7-dimethoxy-2-benzoxazolinone from dried tissues of Zea mays (L.) and evidence of its cyclic hydroxamic acid precursor. J. Agr. Food Chem. 18, 663 (1970).

_____, _____, and T.A. Brindley: 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one(DIMBOA), an active agent in the resistance of maize to the european corn borer. J. Econ. Entomol. 60, 1529 (1967).

Knott, D.R. and J. Kumar: Tests of the relationship between a specif phenolic glucoside and stem rust resistance in wheat. Physiol. Plant Pathol. 2, 393 (1972).

Lamoureux, G.L., L.E. Stafford and R.H. Shimabukuro: Conjugation of 2-chloro-4,6-bis(aklylamino)-s-triazines in higher plants. J. Agr. Food Chem. 20, 1004 (1972).

Long, B.J., G.M. Dunn, and D.G. Routley: Relationship of hydroxamic acid content (DIMBOA) in maize to resistance to Helminthosporium turcicum. Crop Sci. 15, 333 (1975).

Loomis, R.S., S.D. Beck and J.F. Stauffer: The European corn borer, Pyrausta nibilalis (Hubn). and its principal host plant. V. A chemical study of host plant resistance. Plant Physiol. 32, 379 (1957).

Lund-Hoie, K.: Uptake, translocation and metabolism of simazine in Norway Spruce (Picea abies). Weed Res. 9, 142 (1969).

Mace, M.E.: Histochemistry of beta-glucosidase in isolines of Zea mays susceptible or resistant to Northern Corn Leaf Blight. Phytopathology 63, 243 (1973).

Maehr, H.: Antibiotics and other naturally occurring hydroxamic acids and hydroxamates. Pure Appl. Chem. 28, 603 (1971).

Maxwell, F.G., J.N. Jenkins, and W.L. Parrot: Influence of constituents of the cotton plant of feeding, oviposition, and development of the boll weevil. J. Econ. Entomol. 60, 1294 (1967).

_____, _____, _____ Review of european corn borer resistance. Adv. in Agron. 24, 187 (1972).

Molot, P.M.: Recherches sur la resistance du mais a l'Helminthosporiose et aux Fusarioses II. Ann. Phytopathol. 1, 353 (1969).

_____, and P. Anglade: Resistance commune des lignees de mais a L'Helminthosporiose (Helminthosporium turcicum Pass.) et al la Pyrale (Ostrinia nubilalis Hbn.) en relation avec la presence d'une substance identifiable a la 6-methoxy-2-(3)-benzoxazolinone. Ann. Epiphyties. 19, 75 (1968).

Radosevich, S.R. and A.P. Appleby: Studies on the mechanism of resistance to simazine in common groundsel. Weed Sci. 21, 497 (1973).

Roeth, F.W. and T.L. Lavy: Atrazine translocation and metabolism in Sudangrass, sorghum and corn. Weed Sci. 19, 98 (1971).

Shimabukuro, R.H.: Significance of atrazine dealkylation in root and shoot of pea plants. J. Agr. Food Chem. 15, 557 (1967).

_____ Atrazine metabolism and herbicidal selectivity. Plant Physiol. 42, 1269 (1967).

_____ Atrazine metabolism in resistant corn and sorghum. Plant Physiol 43, 1925 (1968).

_____, D.S. Frear, H.R. Swanson, and W.C. Walsh: Glutathione conjugation of atrazine. Plant Physiol. 47, 10 (1971).

_____, R.E. Kadunce, and D.S. Frear: Dealkylation of atrazine in mature pea plants. J. Agr. Food Chem. 14, 392 (1966).

_____, and H.R. Swanson. Atrazine metabolism in cotton as a basis for intermediate tolerance. Weed Sci. 18, 231 (1970).

Sikka, H.C. and D.E. Davis: Absorption, translocation and metabolism of prometryne in cotton and soybean. Weed Sci. 16, 474 (1968).

Smissman, E.E., M.D. Corbett, N.A. Jenny, and O. Kristiansen: Mechanism of the transformation of 2,4-dihydroxy-1,4-benzoxazin-3-ones to 2-hydroxy-2-methyl-4-methoxy-1,4-benzoxazin-3-one to 2-benzoxazolinone. J. Organ. Chem. 37, 1700 (1972).

Tipton, C.L., R.R. Husted, and F.H.C. Tsao: Catalysis of simazine hydrolysis by 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one. J. Agr. Food Chem. 19, 484 (1971).

_____, J.A. Klun, R.R. Husted, and M.D. Pierson: Cyclic hydroxamic acids and related compounds from maize. Isolation and characterization. Biochem. 9, 2866 (1967).

_____, M.C. Wang, F.H.C. Tsao, C.C.L. Tu, and R.R. Husted: Biosynthesis of 1,4-benzoxazin-3-ones in Zea mays. Phytochemistry. 12, 347 (1973).

Virtanen, A.I.: Some aspects of factors in the maize plant with toxic effects on insect larvae. Suomen Kemistilehti B. 34, 29 (1961).

_____ and P.K. Hietala: 2(3)-Benzoxazolinone an anti-fusarium factor in rye seedlings. Acta Chem. Scand. 9, 1543 (1955).

_____, _____ and O. Wahlroos: An antifungal factor in maize and wheat plants. Suomen Kemistilehti B. 29, 143 (1956).

_____, _____, _____ Antimicrobial substances in cereals and fodder plants. Arch. of Biochem. and Biophys. 69, 286 (1957).

_____, _____ On the structure of the precursors of benzoxazolinone in rye plants. II. Suomen Kemistilehti 32, 252 (1959).

_____, _____ Precursors of benzoxazolinone in rye plants I. Precursors II, the aglucone. Acta Chem. Scand. 14, 499 (1960).

_____ and O. Wahlroos: Absence of 6-methoxybenzoxazolinone in uninjured maize tissue. J. Pharm. Sci. 52, 713 (1963).

Wahlroos, O. and A.I. Virtanen: The precursors of 6-methoxy-benzoxazolinone in maize and wheat plants, their isolation and some of their properties. Acta Chem. Scand. 13, 1906 (1959).

_____, _____ On the formation of 6-methoxybenzoxazolinone in maize and wheat plants. Suomen Kemistilehti B. 32, 139 (1959).

Walker, J.C. and M.A. Stahmann: Chemical nature of disease resistance in plants. Ann. Rev. Plant Physiol. 6, 351 (1955).

Whitney, N.J. and G.G. Mortimore: An antifungal substance in the corn plant and its effect on growth of two stalk-rotting fungi. Nature 183, 341 (1959).

_____, _____ Isolation of the antifungal substance, 6-methoxy benzoxazolinone from field corn (Zea mays L.) in Canada. Nature 184, 1320 (1959).

_____, _____ Effect of 6-methoxy benzoxazolinone on the growth of Xanthomonas stewartii (Erw. Smith) Dowson and its presence in sweet corn (Zea mays var. saccharata Bailey). Nature 189, 596 (1959).

Willard, J.I., D. Penner and D.H. Smith: Physiological tolerance in cereals to the cereal leaf beetle. Amer. Soc. of Agron. Abstr. p. 78 (1974).

CHAPTER 2

PHYTOCHEMICAL ASPECTS IN WHEAT AND BARLEY RESISTANCE TO THE CEREAL LEAF BEETLE

Abstract

Tolerance to preemergence application of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) was inversely related to observed resistance to the cereal leaf beetle in selected cultivars of barley seedlings grown in the greenhouse in sand and soil. In wheat this relation did not hold among all cultivars. This relationship was not as evident when evaluated among 219 backcross lines. A summer adult cereal leaf beetle bioassay was used for measuring feeding preference. Quantitative and qualitative differences were observed among the cultivars in the benzoxazinone glucosides extracted from seedling leaves. Reducing sugar content of the seedling leaves could not be related to cereal leaf beetle resistance.

INTRODUCTION

Resistance in wheat (Triticum aestivum L.) to the cereal leaf beetle (Oulema melanopus (L.)) has been associated with leaf pubescence by numerous investigators (1, 8, 9, 10, 13). Less pubescence has been found in barley (Hordeum vulgare L.) and oats (Avena sativa L.) and neither have the degree of resistance found in wheat (13). However, some non-pubescent barley lines show a degree of resistance to the cereal leaf beetle (personal communication[4]). In searching for phytochemical factors involved in insect-host resistance, the evidence relating cyclic hydroxamic acids, i.e., 2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one (DIMBOA), present in corn (Zea mays L.) to the resistance of the European corn borer (Pyrausta nubilalis, Hubn.) (5) appeared of interest in cereal leaf beetle resistance. These compounds have also been related to the detoxication of triazine herbicides in several resistant species (2). The objective of this study was to investigate phytochemical cereal leaf beetle resistance relationships in cereals.

MATERIALS AND METHODS

The following cereal lines were examined for their physiological and phytochemical characteristics with respect to resistance to the cereal leaf beetle. CI refers to Cereal Investigation accession number of the USDA.

---

[4]Smith, D. H., Jr.

'Selkirk' CI 13100 - a hard red spring wheat which possesses some moderate resistance to the cereal leaf beetle . Developed in Canada.

'Fletcher' CI 13985 - a hard red spring wheat developed at the Minn. Agr. Exp. Sta. which is susceptible to the cereal leaf beetle.

'Vel' CI 15890 - a soft red winter wheat developed at Purdue Univ. ARS, USDA which is resistant to the cereal leaf beetle.

'Chris' CI 13751 - a hard red spring wheat developed at the Minn. Agr. Exp. Sta. which is susceptible to the cereal leaf beetle.

'Era' CI 13986 - a hard red spring wheat developed at the Minn. Agr. Exp. Sta. which is susceptible to the cereal leaf beetle.

CI 8519 - a soft red winter wheat of Russian origin which is resistant to the cereal leaf beetle.

CI 11490 - a soft red spring wheat which is resistant to the cereal leaf beetle which was developed in Russia.

CI 9321 - a soft white spring wheat of Russian origin which is resistant to the cereal leaf beetle.

CI 9294 - a soft white spring wheat of Russian origin which is resistant to the cereal leaf beetle.

'Avon' CI 13477 - a soft white winter wheat developed by the Cornell Univ. Agr. Exp. Sta. which is susceptible to the cereal leaf beetle.

'Waldron' CI 13958 - a hard red spring wheat susceptible to the cereal leaf beetle which was developed at N. Dak. Agr. Exp. Sta.

'Lakeland' CI 13734 - a winter barley susceptible to the cereal leaf beetle which was developed by the Mich. Agr. Exp. Sta.

'Larker' CI 0649 - a spring barley susceptible to the cereal leaf beetle which was developed by the N. Dak. Agr. Exp. Sta.

CI 6469 - a spring barley of Polish origin which is moderately resistant to the cereal leaf beetle.

CI 6671 - a spring barley developed in Iran which is moderately resistant to the cereal leaf beetle.

Relationship of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) tolerance to cereal leaf beetle resistance.

The tolerance of cereals to atrazine was studied with selected cultivars grown in a randomized block design in 0.3 L. paper cups, 10 seeds per cup, in soil or sand and given a preemergence application of 2.2 kg/ha (active ingredient) of atrazine. The plants were grown in the greenhouse at 25 ± 2C with supplemental fluorescent lighting to assure a 16-hr day. Injury ratings on a scale of 1 to 5 (1 = no injury, 5 = death) were taken at the end of 17 days. Data reported are the means of two experiments with four replications each.

Seed from three backcrosses of wheat (CI 9321/Era//Era, CI 9321/Fletcher//Fletcher, and CI 9321/Waldron//Waldron) were planted in 0.3 L paper cups, 10 seeds per cup, in silica sand and grown in a randomized block design in the greenhouse at 25 ± 2 C with supplemental fluorescent lighting to assure a 16-hr day. Five replications of 219 lines were treated with 10^{-5} M atrazine and compared to non-treated controls. All plants were supplied with a modified Hoagland's No. 1 solution (3) with or without the atrazine. The plants were rated every 2 days for atrazine injury on a 1 to 10 scale (1 - no injury, 10 = death). The results of the final rating, 14 days after planting, were analyzed and correlated with field ratings made on the same lines for cereal leaf beetle resistance.

Cereal leaf beetle feeding bioassay

Selected cereals were grown in 0.3 L paper cups, 10 seeds per cup, in a sandy clay loam in the greenhouse at 25 ± 2 C with supplemental fluorescent lighting to assure a 16-hr day to develop an adult feeding bioassay. The plants were allowed to grow for 7 to 10 days at which time the soil was covered with a thin layer of plaster and placed in cages 0.91 by 2.13 m in a randomized block design with three replications. The adult beetles, which had been starved for 24 hours, were released from the far end of the cage from the plants, one insect per four plants, and were allowed to feed until damage was observed in the third replication. The plants were then removed from the cage, the insects removed, and the feeding damage rated on an existing scale of 0 to 6 (0 = no feeding, 6 = maximum feeding). Means for each replication were obtained from the mean rating of all leaves in each pot. The data reported are the means of two experiments with three replications each.

To assay the effects of 2 (3)-benzoxazolinone (BOA) on larval weight gain, seedlings of CI 9321 were grown in silica sand in 0.3 L paper cups, 10 seeds per cup, with 10 replications in a randomized block design in a growth chamber at 21 C with 16-hr day and a light intensity of 19 klux. The plants were daily supplied with modified Hoagland's No. 1 nutrient solution containing 0%, 0.25%, 0.5% and 1.0% BOA. After 1 week plants were thinned to six plants per pot and one larva was put on each plant. The larvae were allowed to feed for 3 days and then were removed and their weight determined.

'In vivo' detoxication of atrazine by 2(3)-benzoxazolinone

Ten seeds of CI 9321 were planted in 0.3 L paper cups, 10 seeds per cup, in silica sand supplied with modified Hoagland's No. 1 solution and grown in a growth chamber at 21 C with 16 hr day and a light intensity of 19 klux. When the plants were all 2 to 4 cm high, they were treated with Hoagland's No. 1 nutrient solution containing 0.0%, 0.25%, or 0.50% 2(3)-benzoxazolinone. After 3 days the 2(3)-benzoxazolinone treatments were terminated, and a portion of the plants treated with 2(3)-benzoxazolinone received nutrient solution containing 10^{-5} M atrazine for 24 hours. All treatments subsequently received regular nutrient solution. After 14 days, the plants were harvested and the fresh weight per plant determined. The results were analyzed as a randomized block with three replications.

Benzoxazinone content and activity

A root tip $FeCl_3$ analysis for benzoxazinone content was made with cereal seeds germinated in Petri-dishes on Whatman No. 1 filter paper for 3 to 4 days (coleoptiles were 2 to 3 cm long) at 28 C. Root tips were assayed in a randomized block design by crushing the root tips of the seedlings on Whatman No. 1 filter paper saturated with 0.1 N $FeCl_3$. The roots were rated by the blue colored chelate which is formed when either the glucoside or aglucone of the cyclic hydroxamic acids react with the $FeCl_3$. The roots were rated from 0 to 2 (0 = no color, 2 = full blue color). The rating for each replicate was calculated as a mean of five to ten seedling roots. The data reported are the means of two experiments with four replications each.

Benzoxazinone glucoside content was determined by harvesting twenty-five grams of 7-day old plants and immediately placing them into boiling water. The mixture was cooled to room temperature, filtered, and the plant material was ground three times in 100 ml of water for 5 min. each. After each grinding the mixture was filtered and all water fractions combined, centrifuged to remove remaining plant material and the volume was reduced 'in vacuo' to approximately 60 ml. This water fraction was partitioned four times against ethyl ether followed by four extractions against n-butanol. The n-butanol fraction was reduced to dryness 'in vacuo' and brought to 1 ml with n-butanol. Avicel microcrystaline cellulose TLC plates (500 microns thick) were spotted with 20 ul of the above extract and developed in a two dimensional system of n-butanol:methanol:benzene:water (3:1:1:1) followed by 2% acetic acid. After development in the two solvents, the glucoside spots were examined under U V light, scraped, eluted into n-butanol, and the U V absorption determined at 260nm.

To bioassay the glucoside, the glucosides from 1.2 kg fresh weight of corn seedlings approximately 35 cm high were extracted as described by Wahlroos and Virtanen (12) and the fractions were combined into 10 fractions as they came off the cellulose 300 column. The fractions were dried under nitrogen and the samples weighed and divided to be used in the nutrient solution cereals were grown in. Seeds of selected cultivars were germinated in petri dishes, the seedlings placed between layers of filter paper wrapped around a screen cylinder and covered

with plastic film, two seedlings per cylinder. Four cylinders were placed in plastic boxes containing nutrient solution (modified Hoagland's No. 1) with 0, 1X, and 2X glucoside dissolved in it. Plants were grown for 1 week in the greenhouse at 25 ± C with supplemental fluorescent lighting to assure a 16-hr day. After 1 week the nutrient solutions were changed for fresh solutions containing the designated glucoside treatment. The plants were then placed in cages and an adult feeding bioassay with summer adult beetles was conducted as described above.

Role of glucose in cereal leaf beetle resistance

Free reducing sugar was determined in selected cultivars grown in a growth chamber in soil at 21 C with 16 hr day and a light intensity of 19 klux. When seedlings were 1 week old they were harvested and freeze-dried. The free reducing sugars were determined by extracting the dried ground samples in 80% ethanol for 1 hour at 70 C. The plant material was removed by centrifugation and the ethanol was reduced nearly to dryness and the chlorophyll and other interfering material removed by passing the samples through a 10.0 by 1.3 cm G 10 Sephadex column. The reducing sugar content was then determined by Nelson's Test (5). The results, reported as mg glucose equivalent per g dry weight of plant material, are the means of two experiments with four replications each.

Free reducing sugar content of field plants was determined in selected cultivars of wheat and barley grown in the field on Miami Loam, soil management group 2.5a, in a randomized block design with

four replications in East Lansing, Michigan. Leaf samples were collected in the field, immediately frozen and subsequently freeze-dried, ground and the free reducing sugar content determined as described above. Three physiological stages of leaf growth were collected; 1) first leaf below the flag leaf, 2) just expanding flag leaf, and 3) fully expanded flag leaf. These samples were collected from different plants when the uppermost leaf was in the stage described.

The response of the cereal leaf beetle to various glucose dosages was bioassayed with four selected cultivars of wheat and barley grown in silica sand in 0.3 L paper cups, 10 seeds per cup, in the greenhouse at 25 ± 2 C with supplemental fluorescent lighting to assure a 16-hr day. Modified Hoagland's No. 1 nutrient solution with 0.0 M, 0.0005 M and 0.001 M glucose was supplied the plants daily for one week at which time they were placed in a cage and an adult feeding bioassay with summer adult beetles was conducted, as described above. The feeding damage ratings reported are the mean of two experiments with six replications each.

RESULTS AND DISCUSSION

Relationship of atrazine tolerance to cereal leaf beetle resistance

The preemergence application of atrazine to selected cultivars of barley resulted in an inverse relationship between atrazine resistance and observed field resistance to the cereal leaf beetle (Table 1). In wheat, the cultivar with the most consistent field resistance, CI 9321, was damaged the most, while the susceptible cultivars, Chris and Vel, showed less injury. However, CI 8519 and CI 11490 did not

Table 1. Atrazine tolerance of cereals grown for 17 days with a 2.2 kg/ha preemergence application of atrazine.

Cultivar	Injury rating*
Wheat	
CI 8519	2.7 a†
CI 11490	3.0 abc
Chris	3.5 cde
Vel	3.7 def
CI 9294	4.0 ef
CI 9321	4.5 g
Barley	
Larkland	2.8 ab
Larker	3.3 bcd
CI 6671	3.7 def
CI 6469	4.2 fg

* Ratings: 0 = no injury, 5 = death

† Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

follow the same trend indicative of a multifactor basis for the observed field resistance to the cereal leaf beetle. Because of the involvement of benzoxazinone and its glucosides in detoxication of atrazine in roots, higher concentrations of these cyclic hydroxamic acids may be related to cereal leaf beetle susceptibility.

To test whether atrazine tolerance could be used in screening large numbers of lines, 219 lines resulting from three backcrosses of susceptible lines to the highly resistant line CI 9321 were treated with atrazine. When the injury results were correlated with field ratings for cereal leaf beetle resistance on the same backcross lines, weak negative correlations were obtained (Table 2). The correlation of -0.335 obtained from the CI 9321/Waldron//Waldron backcross was significant at the 5% level but was weak, possibly due to poor germination in the screening study, no replication of the field ratings because of limited seed supply, and multifactor basis for resistance. These results present a possible method for screening large numbers of lines for beetle resistance.

Cereal leaf beetle feeding bioassay

The development of a meaningful bioassay to differentiate between variable resistance in cultivars proved to be possible when summer adult beetles were used in the test (Table 3). Significant differences between resistant (CI 9294) and susceptible (Chris) wheat cultivars were observed after the beetles were allowed free choice between cultivars in the bioassay. No meaningful differences were observed between

Table 2. Correlation between atrazine tolerance ratings and field ratings of cereal leaf beetle resistance of 219 backcross lines.

Backcross lines	Correlation
All	-0.147
CI 9321/Era//Era	-0.094
CI 9321/Fletcher//Fletcher	-0.002
CI 9321/Waldron//Waldron	-0.335*

* Significant at 5% level

Table 3. Summer adult feeding damage on 13 selections of 7-day-old cereal seedlings.

Cultivar	Feeding damage rating*
Wheat	
Vel	0.4 a†
CI 9294	0.4 a
CI 11490	0.7 ab
CI 8519	0.7 ab
CI 9321	0.8 ab
Selkirk	1.2 abc
Era	1.4 bc
Chris	1.5 bc
Fletcher	1.7 c
Barley	
Larker	0.8 ab
CI 6469	2.0 c
Lakeland	2.0 c
CI 6671	2.2 c

* Ratings: 0 = no damage, 6 = maximum damage

† Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

barley cultivars. However, when the same bioassay was conducted with pre-ovipositing spring adult beetles (Table 4) the significant difference between cultivars was not observed in either wheat or barley cultivars.

Although no activity of 2(3)-benzoxazolinone toward atrazine detoxication was observed, a study was initiated to determine if, by supplying wheat seedlings with 2(3)-benzoxazolinone at several concentrations, a difference could be observed in the weight gain of cereal leaf beetle larvae allowed to feed on those seedlings. The data, not presented, showed that 2(3)-benzoxazolinone had no biological activity towards weight gain of cereal leaf beetle larvae.

2(3)-benzoxazolinone detoxication of atrazine

It appears from data presented in Table 5 that 2(3)-benzoxazolinone, the only commercially available cyclic hydroxamic acid, which is an artifact of extraction from rye, has no activity with respect to the detoxication of atrazine when fed to wheat plants. There was no significant difference between those plants pretreated with 2(3)-benzoxazolinone and those treated with atrazine alone.

Benzoxazinone content and activity

Determination of benzoxazinone content through $FeCl_3$ complex formation in root tips was used to determine possible difference between selected cultivars of wheat and barley. Although significant differences did exist between cultivars, there was no possible explanation with respect to known differences in cereal leaf beetle resistance (Table 6) or atrazine tolerance (Table 1). Whether any correlation between root tip determination and benzoxazinone content

Table 4. Spring adult feeding damage on nine selections of 7-day-old cereal seedlings.

Cultivar	Feeding damage rating*
Wheat	
CI 9321	0.3 a†
CI 9294	0.5 ab
Vel	0.6 ab
Fletcher	0.9 abcd
Era	1.0 abcde
Chris	1.4 bcdef
Barley	
CI 6671	1.8 def
CI 6469	1.9 ef
Larker	2.2

* Ratings: 0 = no damage, 6 = maximum damage

† Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 5. 'In vivo' detoxication of atrazine by BOA in wheat (CI 9321).

Treatment	Fresh wt/plant (gm)
Control	1.58 b*
BOA, 0.25%	1.32 b
BOA, 0.50%	1.44 b
Atrazine, 10^{-5}M	0.39 a
Atrazine, 10^{-5}M + BOA, 0.25%	0.25 a
Atrazine, 10^{-5}M + BOA, 0.50%	0.31 a

* Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 6. Analysis for colored complex formed between benzoxazinone and $FeCl_3$ in crushed root tips of 13 cereal selections.

Cultivar	Color rating*
Wheat	
Era	0.4 b†
Fletcher	0.6 b
CI 9294	0.9 c
CI 9321	1.0 cd
Vel	1.1 cd
Selkirk	1.2 cd
CI 11490	1.2 cd
CI 8519	1.2 d
Chris	1.5 e
Barley	
CI 6469	0.0 a
CI 6671	0.0 a
Larker	0.0 a
Lakeland	0.0 a

* Color ratings: 0 = no color, 2 = full blue color
† Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

of leaf tissue, which the cereal leaf beetle use as a food source, is not known.

Upon extraction and separation of tentatively designated benzoxazinone glucosides on TLC plates, qualitative differences between cultivars were observed (Figure 1). CI 9321 contained one glucoside not present in Avon (spot e) and Avon had two glucosides not present in CI 9321 (spots f and g). Upon elution of these compounds from the TLC plates, UV spectra determinations were made and the spectra compared favorably to those reported by Hietala and Virtanen (4). When the relative concentrations of the different glucosides were determined by UV absorption at 260nm, quantitative differences were shown to exist between cultivars (Table 7). These glucosides may be involved in cereal leaf beetle resistance.

With differences between glucoside content of CI 9321 and Avon and successful development of the bioassay described above, a study was conducted to determine if differences in glucoside content and concentration in the nutrient solution used to grow wheat and barley would result in different feeding damage. The results shown in Table 8, however, indicate that the insect feeding damage observed did not show any significant differences toward either different glucoside fractions or relative concentrations.

Role of glucose in cereal leaf beetle resistance

Considering the results of the atrazine detoxication and beetle resistance and the report by Panella et al. (7) that the cereal leaf beetle was attracted by sugars, we speculated that a relation might

Figure 1. Tracing of thin-layer chromatogram of benzoxazinone glucoside extracts of 7-day-old seedlings of CI 9321 and Avon wheat developed in (1) n-butanol: methanol: benzene: water (3:1:1:1) and (2) 2% acetic acid on microcrystalline cellulose plates.

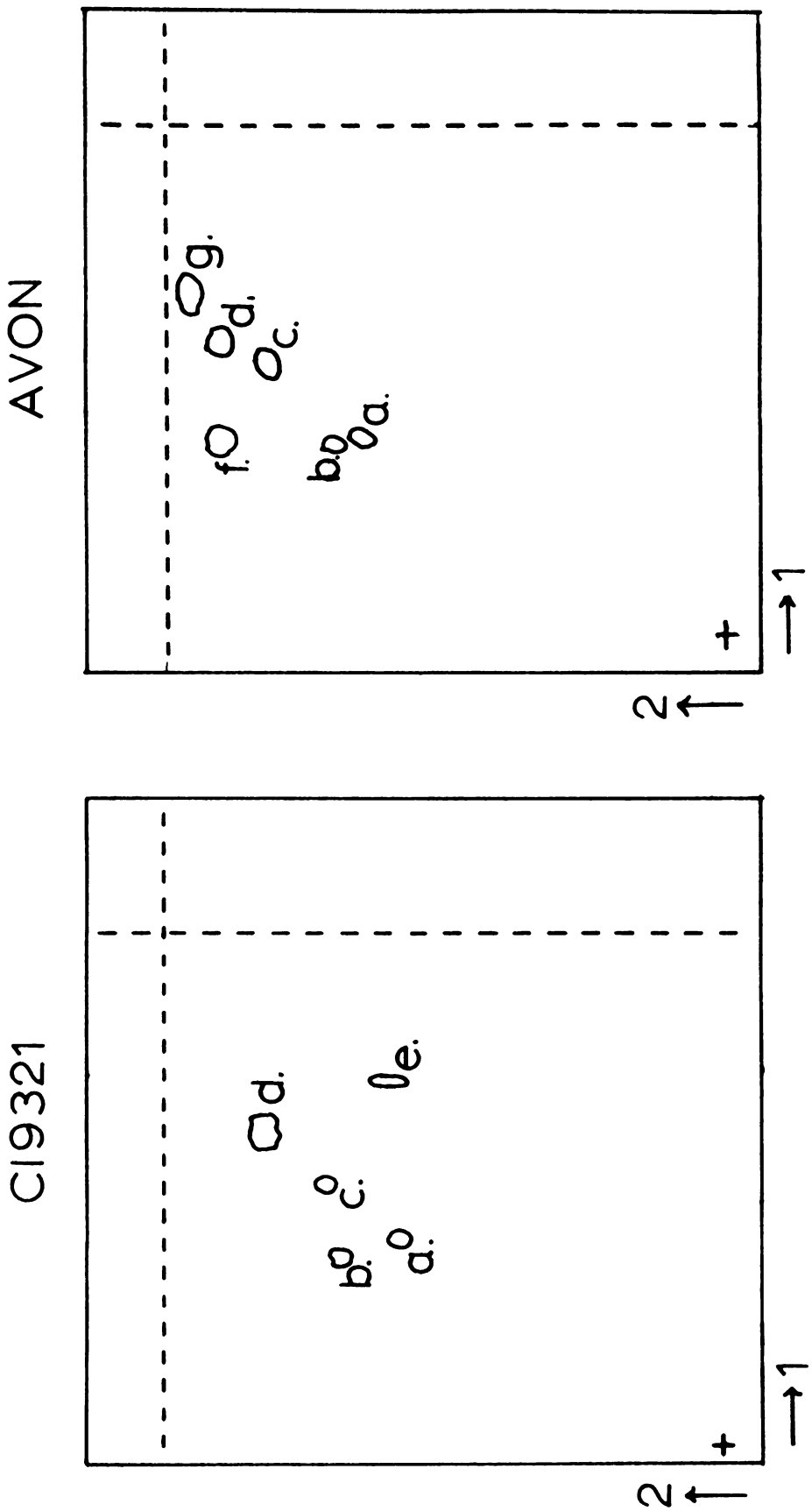

AVON
g.
d.
c.
f.
b.
a.
+
1
2
CI9321
d.
e.
c.
b.
a.
+
1
2

Table 7. Quantitative distribution of glucosides from TLC system.

Cultivar	Spot	R_f	O.D. 260 nm
CI 9321	a	0.35/0.57	0.02
	b	0.39/0.67	0.07
	c	0.48/0.70	0.08
	d	0.59/0.80	0.66
	e	0.67/0.57	>0.01
Avon	a	0.39/0.58	0.12
	b	0.36/0.63	0.12
	c	0.53/0.73	0.14
	d	0.58/0.81	0.15
	f	0.39/0.80	0.07
	g	0.68/0.85	0.21

Table 8. Glucoside dosage effect on summer adult feeding damage.

Glucoside fraction No.	Glucoside treatment (mg)	Feeding damage ratings*
1	0.0	2.3 a†
	4.8	1.4 a
	9.6	1.7 a
2	0.0	2.3 a
	4.9	1.8 a
	9.8	2.3 a
3	0.0	0.5 a
	4.4	1.8 a
	8.8	2.4 a
4	0.0	2.5 a
	4.2	1.4 a
	8.4	1.9 a
5	0.0	2.1 a
	2.3	1.4 a
	4.6	2.3 a
6	0.0	0.5 a
	0.1	0.8 a
	0.2	0.3 a
7	0.0	0.9 a
	0.9	1.6 b
	1.8	0.4 a
8	0.0	0.7 a
	2.0	0.5 a
	4.0	0.6 a
9	0.0	0.6 a
	0.7	0.4 a
	1.4	0.7 a
10	0.0	0.3 a
	0.1	0.3 a

* Ratings: 0 = no damage, 6 = maximum damage.
† Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

exist between the benzoxazinone glucosides and the sugar content of the various cultivars. However, results of free reducing sugar determinations of 10-day-old seedlings and three more mature physiological stages of development show significant differences between the different ages of selected cereal leaves but no difference between cultivars of the same age (Tables 9 and 10). From these results it would seem that free reducing sugars are not involved in differential beetle resistance.

The adult feeding bioassay described above was used to determine if the beetle could detect differences in the glucose content of four selected cultivars (Table 11). Although there were significant differences in feeding damage between wheat (Era, CI 9321) and barley (Larker, CI 6469) cultivars, no differences were detected between those plants with different glucose content.

Development of a negative relationship between atrazine tolerance in barley and cereal leaf beetle resistance, possible involvement of benzoxazinone glucosides, and an adult feeding bioassay have led to a better understanding of the phytochemical properties of cereals with respect to their resistance to the cereal leaf beetle. Although all of the phytochemical differentiations discussed above do not agree as to the relative resistance of each cultivar, these differences point out that the observed resistance under field conditions is made up of several factors. Any combination of these factors may lead

Table 9. Levels of free reducing sugars in 10-day-old seedling leaves.

Cultivar	Reducing sugar mg glucose equivalent / g dry wt.
Wheat	
CI	6.10 a*
Selkirk	7.04 a
CI 11490	7.45 ab
Fletcher	7.51 ab
Vel	7.68 ab
Chris	10.25 abc
Era	10.85 abc
CI 8519	10.96 abc
CI 9294	11.31 abc
Barley	
Larker	9.60 abc
CI 6469	10.02 abc
Lakeland	12.30 abc
CI 6671	19.04 c

* Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 10. The free sugar content of field grown wheat and barley harvested at three physiological stages of growth.

Cultivar	Physiological stage*	Leaf free sugar content (mg sugar/g dry wt)
Wheat		
Chris	1	7.27 a†
	2	57.25 bcd
	3	68.25 bcde
Selkirk	1	9.00 a
	2	58.50 bcd
	3	59.00 bcd
CI 9321	1	12.50 a
	2	83.35 def
	3	55.00 bc
Era	1	7.75 a
	2	50.75 b
	3	60.25 bcd
Barley		
CI 6469	1	7.75 a
	2	69.00 bcde
	3	95.50 ef
CI 6671	1	11.75 a
	2	91.00 ef
	3	95.50 ef
Larker	1	6.50 a
	2	82.75 cdef
	3	98.85 f

* 1 = first leaf below the flag leaf, 2 = just expanding flag leaf, 3 = fully expanded leaf.

† Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 11. Effect of glucose dosage on summer adult feeding damage on four selections of cereal seedlings.

Cultivar	Glucose treatment*	Feeding† damage ratings
Era	0	0.8 ab‡
	1X	0.7 ab
	2X	0.6 a
CI 9321	0	0.4 a
	1X	0.3 a
	2X	0.3 a
Larker	0	1.4 c
	1X	1.6 c
	2X	1.6 c
CI 6469	0	1.2 bc
	1X	1.3 c
	2X	1.2 bc

* 0 = 0.0M Glucose, 1X = 0.005 M Glucose, 2X = 0.001 M Glucose

† Ratings: 0 = no damage, 6 = maximum damage

‡ Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

to a high degree of resistance to the cereal leaf beetle and reduce the chance of shifts developing in the beetle population allowing it to overcome the resistance in the so-called "resistant" cultivars. Therefore, none should be ignored when screening cultivars for resistance.

REFERENCES

1. Gallun, R. L., R. Ruppel, and E. H. Everson. 1966. Resistance of small grains to the cereal leaf beetle. J. Econ. Entomol. 59:827-829.

2. Hamilton, R. H. 1964. Tolerance of several grass species to 2-chloro-s-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. J. Ag. Food Chem. 12:14-17.

3. Hoagland, D. R. and D. I. Arnon. 1950. The water culture method for growing plants without soil. California Agr. Exp. Sta. Circ. 347. 32 pp.

4. Hietala, P. K., and A. I. Virtanen. 1960. Precursors of benzoxazolinone in rye plants II. Precursor I. the glucoside. Acta Chem. Scand. 14:502-504.

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

6. Nelson, N. 1944. A photometric adaptation of the Somogyl method for the determination of glucose. J. Biol. Chem., 153:375-377.

7. Panella, J. A., J. A. Webster, and M. J. Zabik. 1974. Cereal leaf beetle host selection and plant resistance: olfactometer and feeding attractant tests. J. Kansas Entomol. Soc. 47:348-357.

8. Ringlund, K., and E. H. Everson. 1968. Leaf pubescence in common wheat, Triticum aestivum L., and resistance to the cereal leaf beetle, Oulema melanopus (L.). Crop Sci. 8:705-710.

9. Schillinger, J. A., Jr., and R. L. Gallun. 1968. Leaf pubescence of wheat as a deterrent to the cereal leaf beetle, Oulema melanopus. Ann. Entomol. Soc. Am. 61:900-903.

10. Smith, D. H., Jr., T. Ninan, E. Rathke, and C. E. Cress. 1971. Weight gain of cereal leaf beetle larvae on normal and induced leaf pubescence. Crop Sci. 11:639-641.

11. Smith, D. H., Jr., and J. A. Webster. 1974. Leaf pubescence and cereal leaf beetle resistance in Triticum and Avena species. Crop Sci. 14:241-243.

12. Wahlroos, O. and A. I. Virtanen. 1959. The precursors of 6-methoxy-benzoxazolinone in maize and wheat plants, their isolation and some of their properties. Acta Chem. Scand. 13:1906-1908.

13. Webster, J. A., D. H. Smith, Jr., E. Rathke, and C. E. Cress. 1975. Resistance to cereal leaf beetle in wheat: density and length of leaf-surface pubescence in four wheat lines. Crop Sci. 15:199-202.

CHAPTER 3

RESISTANCE TO CEREAL LEAF BEETLE IN WHEAT AND BARLEY: SILICA CONTENT, CALCIUM CONTENT AND LEAF SUCCULENCE

Abstract

Cultivars of wheat (Triticum aestivum L.) and barley (Hordeum vulgare L.) with varying degrees of resistance to the cereal leaf beetle (Oulema melanopus (L.)) were studied for possible physiological or phytochemical differences. Plants were grown either in the growth chamber or the greenhouse in sand, soil or nutrient solution. The cultivars with leaf pubescence were found to contain greater deposits of silica than the non-pubescent cultivars. The silica was associated with the pubescence. Cultivars with high calcium concentrations in the seedling leaves were most susceptible to the cereal leaf beetle. The high calcium content of the leaves was correlated to high pectin levels. The higher concentration of pectic substances in susceptible cultivars may result in softer, more palatable cell walls. Of the cultivars studied, those most resistant to the cereal leaf beetle were the least succulent. The results reported indicate that although pubescence was important, other factors are involved in the resistance of wheat and barley to the cereal leaf beetle.

INTRODUCTION

Resistance in wheat (Triticum aestivum L.) to the cereal leaf beetle (Oulema melanopus (L.)) has been associated with leaf pubescence by numerous investigators (4, 6, 10, 11, 12, 14, 15). Less pubescence was found in barley (Hordeum vulgare L.) and oats (Avena sativa L.) and neither have the degree of resistance found in wheat (14). Earlier studies have shown that several grass species, including the cereals, have the ability to accumulate silica in their epidermal cells including the tricomes (1, 7, 13), and accumulation of silica has been related to insect resistance (3, 9). In rice (Oryza sativa L.) this accumulation of silica in specific varieties has been shown to be related to resistance of those varieties to Asiatic rice borer (Chilo suppressalis, Walker) (3).

The objective of this study was to determine whether the silica content, calcium content or the succulence of cereal cultivars were important in their resistance to the cereal leaf beetle.

MATERIALS AND METHODS

Thirteen cereal cultivars as previously described by Willard et al. (16) were examined in this study with respect to their resistance to the cereal leaf beetle.

Silica determination

To determine the presence of silica in selected cultivars of wheat, plants were grown in the greenhouse in sandy loam soil in 12.0 cm pots at 25 ± 2 C with supplemental fluorescent lighting to assure a 16-hr day until they reached the flag leaf stage of development. Flag leaves were then removed, cut with a razor blade, and

immediately mounted and frozen in Optimum Cutting-Temperature Compound. Cross sections of the flag leaf were cut 16 μ thick at -16 C in a cryostat and were mounted on polished carbon discs (2.5 cm in diameter) at room temperature. The mounted samples were allowed to air dry before analysis on an electron microprobe (Applied Research Laboratories, Model EMX-SM). Microprobe conditions used were 15 kV acceleration voltage and 0.02 μA sample current. No conductive coating was used for thin sections of this type. Scanning electron micrographs and oscillograms of silica X-ray distribution were made of each sample.

To control the silica deposition in wheat, plants were grown on screen cylinders in plastic containers as described by Willard et al. (16), in growth chambers at 20 C with 16-hr day and a light intensity of 19 klux. Water for the nutrient solution was produced by a metal still and passed through a mixed deionization column to remove any soluble ions. High grade reagents were used in the preparation of the nutrient solution to avoid contamination with silica. All plants were supplied with a modified Hoagland's No. 1 solution (5) with no silicic acid added (0 Si), 50 ppm silicic acid added (50 ppm Si) or 100 ppm silicic acid added (100 ppm Si). Seedling leaves were harvested, immediately frozen and subsequently freeze-dried. Small sections of these leaves were then mounted on polished carbon discs and were coated several times with conductive coatings of carbon. Four random areas of 200 x 160 μm were scanned with the microprobe for silica with the value for each replication composed of the mean of five scans of the same area. Data reported are the means of two experiments with four replications each. Microprobe

conditions were the same as above.

An experiment was designed to resolve if the cereal leaf beetle could detect differences in silica deposition in cereals. Six replications of selected cultivars were grown on screen cylinders in plastic containers in the greenhouse at 25 ± 2 C with supplemental fluorescent lighting to assure a 16-hr day. Precaution was taken to keep the amount of silica in the water to a minimum as described above. All plants were grown in Hoagland's No. 1 nutrient solution with 25 and 50 ppm silicic acid added to the silica treatments and no silicic acid added to the control. When the plants were 7 days old they were put into the bioassay cages and an adult bioassay was conducted as described by Willard et al. (16).

Calcium determination

Quantitative calcium determinations were made on selected cereal cultivars grown in the greenhouse at 25 ± 2 C with supplemental fluorescent lighting to assure a 16-hr day in flats in sandy loam soil. The seedlings were later harvested, immediately frozen and freeze-dried. The freeze-dried samples were ground, combusted at 499 C and the calcium content determined with the atomic absorption spectrophotometer. The data reported are the means of two experiments with three replications each.

To control calcium deposition in selected cereal cultivars, plants were grown in 0.3L paper cups, 10 seeds per cup, in silica sand in a growth chamber at 20 C with 16 hr day and a light intensity of 19 klux. The pots were supplied with nutrient solution containing the following treatments; no calcium (0 Ca), 5 ml of 1M $Ca(NO_3)_2$ per liter (1X Ca) and 10 ml of 1M $Ca(NO_3)_2$ per liter (2X Ca) along with the normal amount of all other nutrients used in modified Hoagland's No.1

solution. Seedlings were harvested, immediately frozen and subsequently freeze-dried. The samples were ground and the calcium content determined as described above. The data reported are the means of two experiments with three replications each.

To evaluate whether the cereal leaf beetle could detect different calcium dosages in cereals, selected cereal cultivars were grown in 0.3 L paper cups, 10 seeds per cup, in silica sand in the greenhouse at 25 ± 2 C with supplemental fluorescent lighting to assure a 16-hr day. The pots were supplied with three different calcium treatments as described above. When the plants were 7 days old they were placed into cages for bioassay with summer adult cereal leaf beetles as previously described. The feeding damage ratings reported are the means of two experiments with six replications each.

Pectin determinations

To determine if pectic substances were related to calcium content in the leaves of selected cultivars, pectin content determinations were made on plant material not used in the calcium determinations. Ten mg of plant material were extracted four times at 58 C in 0.05 M phosphate buffer (pH 6.5) containing 1% ammonium oxalate as a chelate. The supernatant fluid was collected after centrifugation between each extraction and all four were combined and brought to 4 ml. The uronic acid content of the samples were determined as described by Blumenkrantz and Asboe-Hansen (2) and were compared to standards of glucuronic acid. The data reported are the means of two experiments with three replications each.

Succulence

The succulence or percent moisture of 13 selected cultivars was

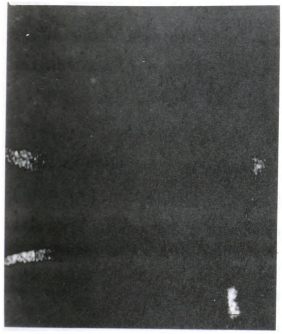
determined on plants grown in a growth chamber in 0.3 L paper cups, 10 seeds per cup, in sandy loam soil at 21 C with 16-hr day and a light intensity of 19 klux. When seedlings were 1 week old they were harvested, fresh weight determined, freeze-dried, and dry weight determined. The data reported for the succulence of 13 cereal cultivars are the means of two experiments with four replications each. The succulence of field collected leaves of seven selected cultivars was determined as above from plants grown in the field on Miami loam soil, soil management group 2.5a, in a randomized block design with four replications in East Lansing, Michigan. Three physiological stages of leaf growth were collected: 1) first leaf below the flag leaf (June 9), 2) just expanding flag leaf (June 20), and 3) fully expanded flag leaf (June 27). These samples were collected from different plants when the uppermost leaf was in the stage described.

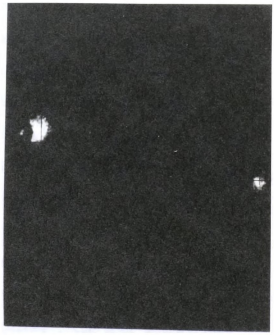
RESULTS AND DISCUSSION

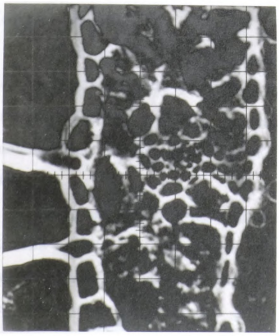
Upon determination of the relative content of silica in selected cultivars on the microprobe, the highest concentrations were found associated with tricomes of those cultivars which were pubescent. Figure 1 shows the cross section of the resistant, pubescent wheat cultivar CI 9321 and the susceptible wheat cultivar 'Era.' In the oscillogram of the X-ray distribution of silica in CI 9321 the silica was deposited largely in the tricomes while considerably less silica was found in Era. These results posed the question whether the resistance observed in the pubescent cultivars was due to the presence of the pubescence or their silica content.

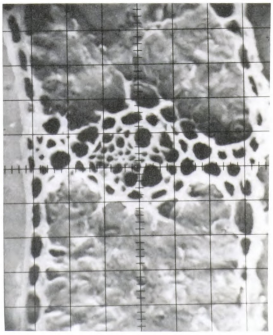
An experiment was designed to determine whether the silica deposition in seedling cereals could be controlled by altering the amount

Figure 1. Electron photomicrographs (left) and x-ray oscillograms (right) of cross sections of CI 9321 (upper) and Era (lower) wheat leaves.









of silica available to the seedlings. The results shown in Table 1 indicate that silica deposition in leaves can be controlled in culture. However, the adult feeding bioassay previously described showed no significant decrease in feeding damage with an increase in silica concentration in the leaves of any of the cultivars (Table 2).

Concurrent with the silica determination on the microprobe, differences in the calcium content of these cultivars was also observed. Furthermore, they appeared to be related to resistance to the cereal leaf beetle. When the calcium concentration of selected cultivars was determined, it was found that wheat cultivars resistant to the cereal leaf beetle had significantly lower calcium concentration than the susceptible cultivars (Table 3). The results were not as consistent in the barley; however, 'Larker', the most susceptible of all wheat and barley cultivars studied, had the highest calcium content observed.

It was subsequently found that the concentration of calcium in seedling plants could be altered by use of different nutrient solutions (Table 4). However, the increased calcium concentration did not increase feeding damage by the adult cereal leaf beetle (Table 4).

Since an important phytochemical function of calcium in the plant is the formation of salts with pectic substances in the middle lamela and cell walls of the leaf, perhaps pectin content could be related to the calcium concentration as well as cereal leaf beetle resistance. The data presented in Table 5 show significant differences between resistant and susceptible cultivars with respect to pectin concentration.

Table 1. Relative silica concentration in the leaves of Era wheat seedlings grown in nutrient solutions without silicic acid (-Si) and with 50 ppm and 100 ppm silicic acid.

Treatment	Average counts Scan
-Si	32.41 a*
50 ppm Si	240.69 b
100 ppm Si	345.03 c

* Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. Silica dosage effect on the feeding damage of summer adult cereal leaf beetles.

Cultivar	Silica treatment	Feeding damage rating†
Wheat		
Era	-Si	0.5 ab*
	25 ppm Si	0.1 a
	50 ppm Si	0.3 ab
CI 9321	-Si	0.3 ab
	25 ppm Si	0.1 a
	50 ppm Si	1.2 abc
Barley		
Larker	-Si	1.8 bc
	25 ppm Si	2.2 c
	50 ppm Si	1.8 bc
Ci 6469	-Si	2.7 c
	25 ppm Si	2.5 c
	50 ppm Si	1.7 bc

* Means followed by the same letter do not differ significantly at the 5% level by Duncan's multiple range test.
† Ratings: 0 = no damage, 6 = maximum damage.

Table 3. Calcium concentration of selected cereal seedling leaves.

Cultivar	Calcium concentration (ppm Ca/g dry wt.)
Wheat	
CI 9321	106.7 a*
Selkirk	107.2 a
CI 11490	114.5 ab
CI 8519	115.2 ab
CI 9294	116.7 abc
Vel	120.9 bcd
Fletcher	128.7 cde
Chris	138.7 ef
Era	146.3 fg
Barley	
CI 6469	115.2 ab
Lakeland	116.5 abc
CI 6671	152.9 gh
Larker	162.6 h

* Means followed by the same letter do not differ significantly at the 5% level by Duncan's multiple range test.

Table 4. Calcium concentration in the leaves of seedling cereals and calcium dosage effect on feeding damage of summer adult cereal leaf beetles grown without Ca (0), with normal Ca (1X), and with two times Ca (2X) concentration in nutrient solution.

Cultivar	Treatment	Calcium Concentration ppm Ca / gram dry wt.	Feeding damage rating†
Wheat			
CI 9321	0 Ca	42.7 a*	1.2 bcde
	1X Ca	97.0 bc	1.0 abcd
	2X Ca	144.7 d	0.8 abc
Era	0 Ca	57.5 a	0.4 a
	1X Ca	102.7 c	0.6 ab
	2X Ca	132.6 cd	0.4 a
Barley			
Larker	0 Ca	62.8 ab	1.6 def
	1X Ca	147.1 d	2.0 f
	2X Ca	195.1 e	1.7 ef
CI 6469	0 Ca	46.5 a	1.7 ef
	1X Ca	144.6 d	1.4 cdef
	2X Ca	148.0 d	1.6 def

* Means within columns followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

† Ratings: 0 = no damage, 6 = maximum damage.

Table 5. Pectin concentration of selected cereal seedling leaves.

Cultivar	Pectin concentration µg glucuronate equivalent mg dry weight
Wheat	
CI 9294	3.10 a*
CI 9321	3.20 ab
Vel	3.67 abc
CI 11490	3.97 abcd
CI 8519	4.22 abcd
Fletcher	4.25 abcd
Selkirk	4.53 bcd
Chris	4.62 cd
Era	5.03 d
Barley	
Lakeland	3.10 a
CI 6469	4.33 abcd
CI 6671	4.55 bcd
Larker	4.87 d

* Means followed by the same letter do not differ significantly at the 5% level by Duncan's multiple range test.

A significant correlation coefficient of $r = 0.66$ was obtained between the calcium and pectin concentration of seedling leaves for those cultivars studied.

These results indicate that susceptible cultivars are high in pectic substances which may make the cell walls softer and therefore more palatable to the cereal leaf beetle.

Casual observation of the seedlings grown for the bioassays indicated that the resistant cultivars appeared less succulent than the susceptible ones. This observation was substantiated upon determination of succulence as shown in Table 6. In a study conducted to determine if the same trend persisted as the cereal plants matured, the trend persisted in the wheat cultivars until they were 44 days old, but not beyond (Table 7), but no significant differences were observed either in seedling plants or more mature plants of any of the barley cultivars studied.

The succulence of cereal cultivars in the field may contribute more to susceptibility in the spring than later in the season as the most significant differences are observed in young plant material.

Higher silica deposition, low calcium and pectin concentrations and lower succulence in cultivars resistant to the cereal leaf beetle indicate that the presence or absence of pubescence is not the only factor involved in the resistance in wheat and barley. In developing a screening program to find cultivars resistant to the cereal leaf beetle, the factors discussed above could easily be used to speed up the process.

Table 6. Succulence of 13 cereal cultivar seedlings expressed as percent moisture.

Cultivar	Moisture (%)
Wheat	
CI 9321	87.55 a*
CI 9294	87.80 ab
Fletcher	88.26 abc
Vel	88.59 abcd
CI 11490	88.80 bcde
CI 8519	88.85 bcde
Chris	88.90 bcde
Era	89.21 cde
Selkirk	89.51 cde
Barley	
Lakeland	89.57 de
Larker	89.86 de
CI 6671	89.95 e
CI 6469	90.00 e

* Means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 7. Succulence of field collected leaves of seven cultivars.

Cultivar	Plant age 44 days	55 days	62 days
	(% moisture)	(% moisture)	(% moisture)
Wheat			
CI 9321	74.1 a*	97.0 ab	96.6 a
Era	76.0 b	97.1 ab	96.8 a
Selkirk	78.1 c	97.2 bc	96.8 a
Chris	78.3 c	96.9 a	96.7 a
Barley			
CI 6469	79.3 cd	97.4 c	96.8 a
CI 6671	80.7 d	97.4 c	97.0 b
Larker	80.0 d	97.1 ab	96.8 a

* Means within column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

REFERENCES

1. Blackman, E. 1969. Observations on the development of the silica cells of the leaf sheath of wheat (Triticum aestivum). Can. J. Botany 47:827-838.

2. Blumenkrantz, N. and G. Asboe-Hansen. 1973. New method for quantitative determination of uronic acids. Anal. Biochem. 54:484-489.

3. Djamin, A. and M. D. Pathak. 1967. Role of silica in resistance to Asiatic Rice Borer, Chilo suppressalis (Walker), in rice varieties. J. of Econ. Entomol. 60:347-351.

4. Gallun, R. L., R. Ruppel, and E. H. Everson. 1966. Resistance of small grains to the cereal leaf beetle. J. Econ. Entomol. 59:827-829.

5. Hoagland, D. R. and D. I. Aron. 1950. The water culture method for growing plants without soil. California Agr. Exp. Sta. Circ. 347, 32 pp.

6. Hoxie, R. P., S. G. Wellso, and J. A. Webster. 1975. Cereal leaf beetle response to wheat trichome length and density. Envir. Entomol. 4:365-370.

7. Kaufman, P. B., W. C. Bigelow, L. B. Petering, and F. B. Drogosz. 1969. Silica in developing epidermal cells of Avena internodes: electron microprobe analysis. Science 166:1015-1017.

8. Miller, B. S., R. J. Robinson, J. A. Johnson, E. T. Jones, and B. W. X. Ponnciya. 1960. Studies on the relation between silica in wheat plants and resistance to Hessian fly attack. J. Econ. Entomol. 53: 995-999.

9. Okuda, A. and E. Takahashi. 1964. The role of silica. The Mineral Nutrition of the Rice Plant. The John Hopkins Press, Baltimore pp. 123-146.

10. Ringlund, K., and E. H. Everson. 1968. Leaf pubescence in common wheat, Triticum aestivum (L.) and resistance to the cereal leaf beetle, Oulema melanopus (L.). Crop Sci. 8:705-710.

11. Schillinger, J. A., Jr., and R. L. Gallun. 1968. Leaf pubescence of wheat as a deterrent to the cereal leaf beetle, Oulema melanopus. Ann. Entomol. Soc. Am. 61:900-903.

12. Smith, D. H., Jr., T. Ninan, E. Rathke. and C. E. Cress. 1971. Weight gain of cereal leaf beetle larvae on normal and induced leaf pubescence. Crop Sci. 11:639-641.

13. Soni, S. L., P. B. Kaufman, and R. A. Jones. 1972. Electron microprobe analysis of the distribution of silicon and other elements in rice leaf epidermis. Bot. Gaz. 133:66-72.

14. Webster, J. A., D. H. Smith, Jr., E. Rathke, and C. E. Cress. 1975. Resistance to cereal leaf beetle in wheat: density and length of leaf-surface pubescence in four wheat lines. Crop Sci. 15:199-202.

15. Wellso, S. G. 1973. Cereal leaf beetle: Larval feeding, orientation, development, and survival on four small-grain cultivars in the laboratory. Ann. Entomol. Soc. Am. 66:1201-1208.

16. Willard, J. I., D. Penner, and D. H. Smith, Jr. Phytochemical aspects in the resistance to the cereal leaf beetle in wheat and barley. Crop Sci. (in preparation).

CHAPTER 4

SUMMARY AND CONCLUSIONS

Barley cultivars resistant to the cereal leaf beetle were least tolerant to preemergent application of atrazine. However, this relation did not hold among all wheat cultivars. When atrazine was used to screen 219 backcross lines, this relationship was not as evident. This may be due to the complex nature of resistance in the cereal lines which caused a masking of the negative relationship to atrazine in some lines. The development of a summer adult cereal leaf beetle bioassay which gave significant differences between cultivars of variable resistance however, was not sensitive enough to detect imposed increases in dosage of benzoxazinone glucosides or glucose. The differences observed in benzoxazinone-glucoside content in seedling leaves between resistant and susceptible cultivars may be involved in the variability observed in atrazine tolerance and also as a source of glucose. The reducing sugar content of the cultivars studied, however, could not be related to cereal leaf beetle resistance.

The resistant cereal cultivars with pubescent leaves had high silica content associated with the pubescence, while the susceptible lines had less silica deposition. However, increased silica content, imposed through addition of salicic acid in the nutrient solution, did not decrease the feeding damage observed in a summer adult bioassay.

The calcium and pectin concentration of cereal leaf beetle susceptible cultivars was shown to be significantly higher than in the resistant cultivars. The succulence of the susceptible cultivars was higher than the resistant cultivars studied. The correlation between calcium and pectin content as well as the higher succulence of susceptible cultivars indicates that the cereal leaf beetle prefers softer leaf tissue for feeding.

In conclusion, resistance in wheat and barley is not totally dependent on the presence of leaf pubescence. Many factors contribute to the resistance observed in the cereal cultivars studied and the relative contribution of each of these factors varies from one resistant cultivar to the next. It is imparative therefore, for the plant breeder to incorporate as many anatomical, morphological, physiological and phytochemical factors into new cultivars giving greater resistance. The incorporation of many factors also reduces the chances of a shift in the beetle population allowing it to overcome the resistance affected by the so-called "resistant" cultivars.