Phytochemistry, Vol 27, No 11, pp 3349–3358, 1988
Printed in Great Britain

0031-9422/88 $3 00+0 00

REVIEW ARTICLE NUMBER 39

HYDROXAMIC ACIDS (4-HYDROXY-1,4-BENZOXAZIN-3-ONES), DEFENCE CHEMICALS IN THE GRAMINEAE

HERMANN M NIEMEYER

Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

(*Received* 2 *June* 1988)

Key Word Index—Gramineae, cereals, pest resistance, disease resistance, *Ostrinia nubilalis*, European corn borer, aphids, hydroxamic acids, benzoxazinones, DIMBOA

Abstract—Hydroxamic acids of the type 4-hydroxy-1,4-benzoxazin-3-ones constitute one of the most extensively studied secondary metabolites in relation to host plant resistance to pests and diseases They play a major role in the defence of cereals against insects, fungi and bacteria, in the detoxification of herbicides and in allelopathic effects of the crop Although other mechanisms have also a bearing on these effects, more intensive exploitation of hydroxamic acids in cereal crops is indicated.

INTRODUCTION

The rising costs of pesticides, the increasing resistance of insects to them and their undesirable effects on the environment, has lead to renewed efforts to describe and to exploit host plant resistance to pests and diseases Cereals, while constituting the main food crops in the world, have imperfectly described chemical defences Three decades ago, a series of 1,4-benzoxazin-3-ones were discovered in rye plants in relation to resistance of the plants to fungal infection. The compounds were later found in maize and wheat, and relationships were established between their levels in plants and degree of resistance of the plant to insects, fungi and bacteria, detoxification of herbicides and allelopathic effects. These properties have had a considerable economic impact in agricultural systems.

More than a decade has elapsed since a review on these compounds was published [1]. Since then, the range of known activities of these compounds has substantially widened and the understanding of these activities increased It thus seemed worthwhile to attempt to summarize the currently available information.

Structural diversity

1,4-Benzoxazin-3-ones are naturally present in the plants as 2-β-*O*-D-glucosides [2, 3], which may be isolated if care is taken to inactivate the enzymes in the tissues before extraction [4–6] Aqueous extracts made at room temperature provide the aglucones. Heating of these extracts causes the decomposition of the aglucones to give benzoxazolin-2-ones as main products The reaction is discussed in a later section.

The structures of 1,4-benzoxazin-3-ones and related compounds are collected in Fig 1 Some entries deserve additional comments Although DIM_2BOA has not been isolated in a pure state, its presence in corn extracts was inferred from the mass spectrum of a mixture of aglucones [7] and from the mass spectrum of its trimethylsilyl derivative [8] This is consistent with the increase in decomposition rate expected from methoxylation of hydroxamic acids [9].

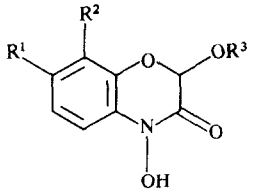
The aglucone of HDMBOA-Glc has also eluded efforts to obtain it in pure form, due to its ease of decomposition [10]. The evidence for the presence of TRIBOA in maize extracts comes only from the mass spectrum of its *tris*-trimethylsilyl derivative [8]. Recently, 5-chloro-HMBOA-Glc was isolated as a peracetate from young corn roots, and claimed to be a natural product. Detailed experimental procedures were unfortunately not reported [11]. The crystalline structures of DIBOA [12] and HMBOA-Glc [13] have been determined. In the latter case, the data was used to ascertain that the absolute configuration of the epimeric carbon atom was *R*.

The term hydroxamic acids (Hx), as used in this review, refers to 4-hydroxy-1,4-benzoxazin-3-ones (Fig. 1).

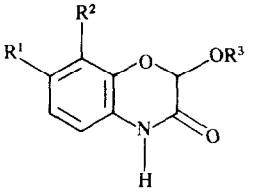
Distribution

Hydroxamic acids have been found in members of the following genera in the Gramineae: *Aegilops* [14], *Arundo* [15], *Chusquea* [15], *Coix* [13, 16], *Elymus* [15], *Secale* [17], *Sorghum* [18; see however refs 19 and 20], *Tripsacum* [21], *Triticale* [22], *Triticum*, including ancient wheats [14] and *Zea*, including *Z. mexicana* (teosinte) [21]. Hydroxamic acids have not been found in *Avena* [19, 20], *Hordeum* [19, 20, 23] or *Oriza* [20].

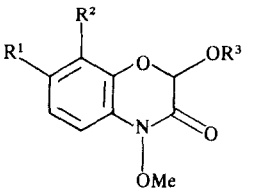
There has been a single report of benzoxazinones outside the Gramineae: DIBOA-Glc was found in the seeds of *Acanthus mollis* (Acanthaceae) [24]. Additionally, MBOA was found in the dried roots of *Scoparia dulcis* (Scrophulariaceae) [25] However, the treatments suffered by the extracts in this case makes it highly probable that the compound originally in the plant was the corresponding hydroxamic acid.



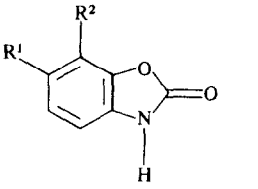
R^1	R^2	R^3	Abbreviation	References
Hydroxamic acids (4 - hydroxy - 1,4 - benzoxazin - 3 - ones				
H	H	H	DIBOA	2 17
H	H	Glc	DIBOA-Glc	17, 37
MeO	H	H	DIMBOA	35
MeO	H	Glc	DIMBOA-Glc	35
MeO	MeO	H	DIM_2BOA	7
MeO	MeO	Glc	DIM_2BOA-Glc	179
OH	H	H	TRIBOA	8



Lactams (1,4 - benzoxazin - 3 - ones)				
H	H	H	HBOA	3
H	H	Glc	HBOA-Glc	3
MeO	H	H	HMBOA	180, 181
MeO	H	Glc	HMBOA-Glc	16, 180, 182
MeO	MeO	H	HM_2BOA	
MeO	MeO	Glc	HM_2BOA-Glc	179
OH	H	Glc	DHBOA-Glc	13, 183



Methyl derivative (4 - methoxy - 1,4 - benzoxazin - 3 - one)				
MeO	H	Glc	HDMBOA-Glc	13, 184



Benzoxazolinones				
H	H		BOA	185
MeO	H		MBOA	25, 50, 81, 186
MeO	MeO		M_2BOA	7

Fig 1 1,4-Benzoxazin-3-ones and benzoxazolin-2-ones from Gramineae

Within the cereals, DIBOA is the main hydroxamic acid occurring in rye, whereas DIMBOA is the main one in wheat and maize DIM_2BOA has only been found in maize

Analysis of hydroxamic acids

Several methods have been utilized to quantitate hydroxamic acids in cereal extracts. In a first group of methods, hydroxamic acids are decomposed to benzoxazolinones by heating Quantitation of these derivatives is carried out by chromatographic separation followed by UV spectroscopy [26, 27], spectrofluorimetry [28] or infrared spectrometry [29], by isotopic dilution [30], by gas chromatography [20, 31], by high performance liquid chromatography [32, 33], or by visual rating after thin layer chromatographic separation [34] The basic assumption of these methods is that stoichiometric quantities of benzoxazolinones are obtained from decomposition of hydroxamic acids. This assumption is based on the comparison of the final UV spectrum of decomposed DIMBOA with that of MBOA [35] and the appearance of isosbestic points in the UV spectrum of a decomposing solution of DIBOA [36, but see however ref 37] Both assumptions may not be valid if products with spectral characteristics similar to benzoxazolinones or starting hydroxamic acids are formed, as has been found to be the case in some instances [38, 39] In fact, it is clear that the decomposition of DIMBOA to MBOA is not quantitative, the yield of MBOA being a function of pH, temperature, and composition of the reaction medium [40, 41].

Another group of methods is based on the quantitation of Fe (III) complexes of hydroxamic acids [19, 42, 43] These methods produce reliable values for total hydroxamic acids within a series of related cereals [44]

Lastly, methods have been developed which allow the quantitation of individual hydroxamic acids, following separation by gas chromatography [8, 44], thin layer chromatography [15], or high performance liquid chromatography [45–47]. The latter method [47] utilizes crude aqueous extracts and its speed, sensitivity, reliability and use of small quantities of tissue makes it very

attractive for extensive screening in plant breeding programs.

Hydroxamic acid levels in a plant

Hydroxamic acids, while not present in the seeds of cereals [23, 31], appear upon germination in maize [48], wheat [23] and rye [23]. Hx levels increase with age reaching a maximum a few days after germination in maize [27, 48, 49] and in wheat [23] The maximum level attained and the subsequent rate of decrease are dependent upon the cultivar studied [48].

Hx are found in all plant parts. The relative levels in aerial parts and in roots varied within species and cultivar analysed [18, 48, 50, 51]. Hx levels are higher in stem than in leaf tissue [52] Hx were not found in xylem exudates or in guttation drops of maize [53] or wheat [54] plants

Younger leaves contain higher Hx levels than older ones [27, 50, 51, 55]. Within a leaf, the distal section contains higher Hx levels than the basal one [47] Hx levels are higher in the vascular bundles than in the complete leaf of maize [53] and wheat [54] plants, and higher in the lateral veins than in the central vein of maize leaves [53]. Hx were not detected in the lower epidermis of wheat leaves [54] In maize seedlings, Hx levels are higher in the stele than in the cortex [53].

Hx levels depend also on extrinsic factors. Iron deficiency in the growth medium provokes an increase in Hx levels in maize [56]. Lower growth temperature decreases Hx levels in maize roots [57], but increases Hx levels in aerial parts of wheat seedlings [31] In this latter case, the effect is obscured by the fact that plants grown under low temperature regime were shorter and the sample analysed contained a higher proportion of younger (higher Hx) tissue This effect may also be operating in the finding that a low intensity light regime produced maize plants with higher Hx levels [58], and that rye seedlings grown in the dark showed lower Hx levels than green seedlings [50]. Longer photophase, between 8 and 16 hr, lead to lower Hx levels [31], without systematic variation in the height of a wheat seedling. In maize, the effect was different: longer photoperiod, although increasing plant fresh weight, did not influence the concentration of either DIMBOA or its glucoside [59].

Finally, while in some maize cultivars the effect of nitrogen application on Hx levels was not significant [56, 60], in others Hx levels increased upon addition of nitrogen [56, 61, 62]

Biosynthesis of benzoxazin-3-ones

Benzoxazinones share with tryptophan a substantial part of their biosynthetic pathway. Thus, label from the shikimate precursors quinic acid [63, 64] and anthranilic acid [65] were incorporated into the aromatic ring of benzoxazinones The carbon atoms of the heterocyclic ring stem from ribose, C-2 from C-2 of ribose and C-3 from C-1. The methoxyl group in DIMBOA-related compounds is derived from various C1 sources such as methionine, glycine and glycerate [64]. An intermediate of the type 1-(α-carboxy-phenylamino)-1′-deoxyribose-5′-phosphate may participate in the biosynthesis of benzoxazinones [63, 64]

The interconversion of lactams and hydroxamic acids could not be detected in cell-free extracts. However, in experiments with seedlings, label from HBOA was incorporated rapidly into DIBOA, less rapidly into HMBOA and least rapidly into DIMBOA. These results suggest that the substrates for interconversion are the glucosides, with the lactams being alternatively oxidized at nitrogen or oxidized at the aromatic carbon atom and further methylated [65]. The initial and final steps in the biosynthesis of benzoxazinones seem to be clear and are shown in Fig. 2.

Inheritance of hydroxamic acids

The level of hydroxamic acids in maize line BxBx is conditioned primarily by a major, partially dominant gene [66, 67]. Monosomic and B-A translocational analysis, coupled with conventional linkage analysis, showed that the benzoxazinless (bx) locus is in the short arm of chromosome 4, near Rp4 (map position 27) [68]. Inheritance of DIMBOA was also quantitative in inbred lines of maize [69]. Among these, inbreds B49 and B37 did not possess any gene or genes with dominance effects comparable to BxBx [67]. Five loci were estimated to condition hydroxamic acid levels in B49, and two loci in B37 [67].

Chemistry of hydroxamic acids

Hydroxamic acids decompose to benzoxazolinones with liberation of formic acid. The mechanism of the reaction appears to involve the fast formation of aldol **1**, and the rate limiting formation of isocyanate **2**, as shown in Fig. 3 [36] This mechanism is supported by studies in aprotic solvents, where the aldol has been quantified [70] and the participation of the hydroxamic oxygen atom as a nucleophile has been confirmed [71], and in alcoholic solvents, where the participation of the hydroxamic carbonyl group in the rate limiting step has been demonstrated [41]. Additionally, higher pH values of the decomposing media [36, 39] as well as solvents of higher electron donating properties [39] increase the reaction rates and yields of benzoxazolinone, as expected from the mechanism proposed.

Alternative mechanisms have been proposed on the basis of the higher reactivity of the analogues **3** [72] and **4** [10] methylated at the hydroxamic oxygen, with respect to their corresponding parent compounds, and the lack of reactivity of compound **5** [72] with an alkylated phenolic hydroxyl group, with respect to DIBOA. These facts may however be rationalized by the proposed mechanism by taking into account the higher nucleophilicity of a methoxyl oxygen atom as compared to a hydroxyl oxygen atom, and the possibility of aldol **1** adopting a conformation with an intramolecular hydrogen bond between the phenolic hydroxyl and the hydroxamic carbonyl groups, which exposes the aldehyde carbonyl group to attack by the hydroxamic hydroxyl group (structure **6**), thus leading to a lower energy transition state.

The reaction of benzoxazinones with nucleophiles resembling fragments of biological macromolecules has been studied, in an attempt to provide a chemical rationale to their biological activities. In one group of studies, *N*-acetylated derivatives of benzoxazinones were studied under the assumption that hydroxamic acids would first be derivatized in the recipient organism. Compound **7** underwent nucleophilic substitution to give products of the type **8** and **9** with phenols, indoles and thiols [73], as well as with aminoacid derivatives [74] as models for

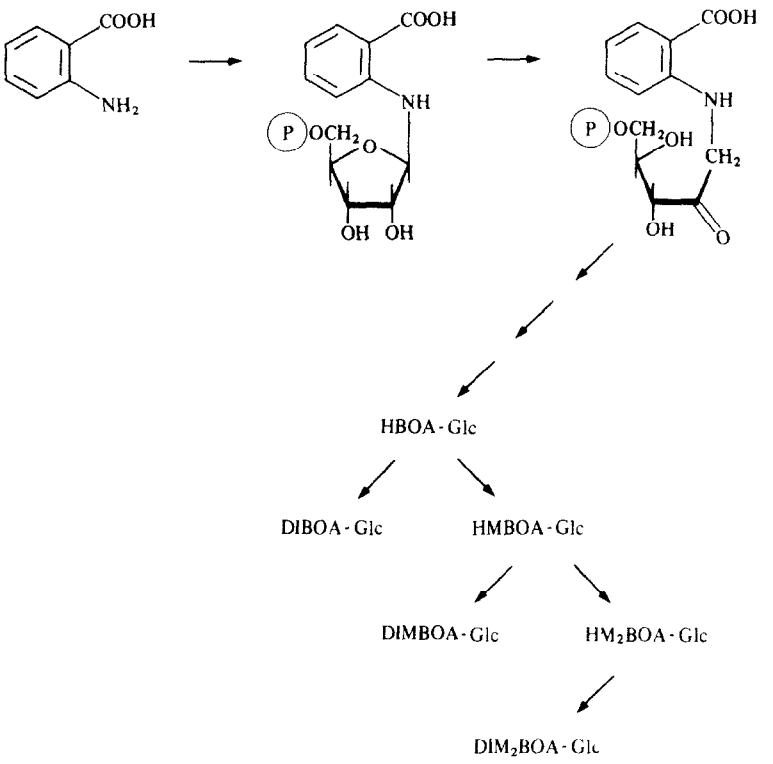


Fig 2 Biosynthesis of 1,4-benzoxazin-3-ones

proteins, and with nucleotides [75] as models for nucleic acids

It was later shown that derivatization of hydroxamic acids was not necessary to account for their biological activity [76–78] and in fact, that *N*-derivatization was neither necessary for their reaction with nucleophiles [9, 79] The reactive sites of DIMBOA were shown to be the nitrogen atom [9] as well as the aldehyde group of the aldol form **1** [79]

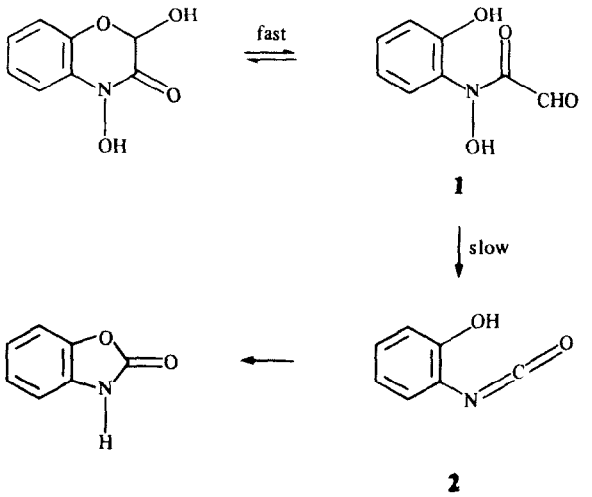


Fig 3 Mechanism of decomposition of hydroxamic acids (2,4-dihydroxy-1,4-benzoxazin-3-ones)

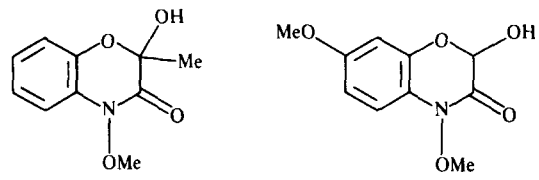
Biological activity of hydroxamic acids

Hydroxamic acids have been associated with resistance of cereals to insects, fungi and bacteria, with triggering the reproduction of grass-feeding mammals, and with allelopathic effects of cereals The presence of hydroxamic acids has also been related to the detoxification of herbicides and pesticides, and to the mineral nutrition of the plant Additionally, they have been shown to be mutagenic agents and to bind to auxin binding sites

Resistance of maize to the European corn borer, Ostrinia nubilalis Much research has been carried out on this devastating maize pest Initial reports on plant chemical resistance factors [80] focused attention on benzoxazolinones [27, 30, 81–85] It was then suggested that the hydroxamic acid precursors may be the actual resistance factors [30, 86] This hypothesis was later confirmed The correlations between resistance and benzoxazolinone levels were complemented by bioassays in artificial diets which showed hydroxamic acids to be the most active plant components [87]

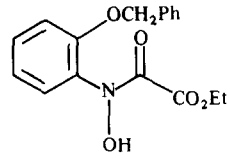
Maize tissue containing different Hx levels, when added to meridic diets where borer larvae feed, showed antibiotic effects, as reflected in larval mortality, slower development, smaller individuals, poorer matings, and fewer offspring [88] Additionally, diets to which DIMBOA was added showed feeding deterrent effects [89, 90] This non-preference component to borer resistance was also present in field [91] and laboratory [91, 92] tests.

In a study with a set of maize inbred varieties, correlations were found between hydroxamic acid levels and leaf feeding resistance by first generation (brood) larvae,

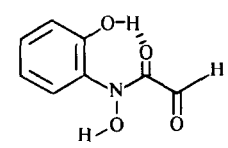


3

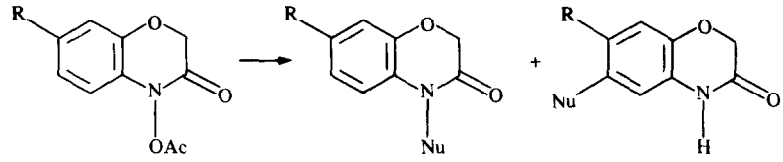
4



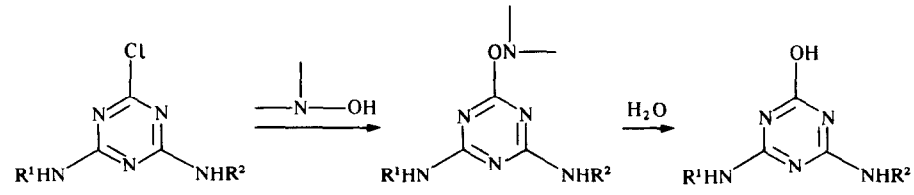
5



6

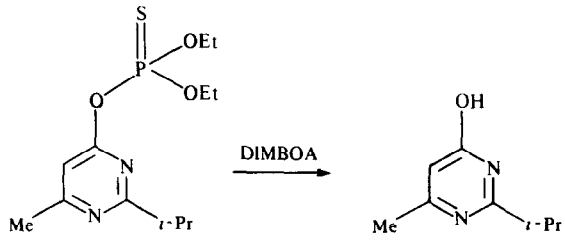


7 R = H, MeO 8 9



Simazine $R^1 = R^2 = Et$

Atrazine $R^1 = Et$, $R^2 = i\text{-Pr}$



Diazinon

which fed on young maize plants. As the plants aged and the hydroxamic acid levels decreased, resistance levels decreased, except in varieties in which the Hx levels remained high [48] Resistance levels to leaf feeding decreased under greenhouse conditions, with respect to field conditions [93]. When a diallel set of 11 maize inbreds was examined for Hx levels and resistance level to first brood larvae, highly significant correlations were found between these variables both in the inbreds and in the single crosses [69]. It was suggested that breeding for first brood resistant maize be carried out by selecting for high hydroxamic acid levels in the plant [69, 89, 94–98]. This leads in fact to inbred lines highly resistant to leaf feeding by first brood borers [99]. Additionally, recurrent selection breeding leads to improved first brood resistance and DIMBOA levels, each method of selection

accumulating about the same level of resistance [100]

In studies using a wider range of maize lines, resistance both to first and second brood borers could not be solely attributed to hydroxamic acid levels [43, 92, 101, 102] Silica content was found to make a significant contribution to resistance to second brood borers [103]. Resistance of maize to a related borer, *Sesamia nonagrioides*, was also attributed to hydroxamic acids [104]

Resistance to aphids Aphids are among the most important pests of cereals on account of direct feeding damage and transmission of viral diseases Inverse relationships were obtained between Hx levels in a maize plant and infestation numbers of the corn leaf aphid *Rhopalosiphum maidis* [105], under both field and greenhouse conditions [106] Similar relationships were obtained for Hx levels in a wheat plant and infestation numbers of the rose-grain aphid *Metopolophium dirhodum* [23], the greenbug *Schizaphis graminum* [51] and the English grain aphid *Sitobion avenae* [107] under greenhouse conditions

When severed barley leaves lacking Hx were immersed in DIMBOA containing solutions, the levels of DIMBOA incorporated also correlated with infestation numbers of *M dirhodum* [23] Aphid infestation numbers were lower in those tissues with lower Hx levels resulting from older plant [23] or older plant parts [51] being studied

DIMBOA exhibited both antibiotic [15, 22, 23, 51, 105, 108–110] and antifeedant [109, 110] effects on cereal aphids when incorporated into holidic diets The aphid most sensitive to DIMBOA levels was *S. graminum*, the least sensitive *R maidis* [111] The glucoside of DIMBOA was less active than DIMBOA itself against *S graminum* [110]

Inverse relationships were obtained between DIMBOA levels in wheat plants and DIMBOA levels in aphids feeding on them [112]. This result, coupled with a tendency towards decreased honeydew production and weight gain, indicated feeding deterrency by DIMBOA also under natural conditions [112]

Resistance to fungi. Infection ratings of a series of maize inbreds to Northern corn leaf blight-producing fungus *Helminthosporium turcicum*, inversily correlated with Hx levels in the plants [27, 49, 52, 113, 114]. The presence of the Bx allele conferred additional resistance with respect to lines containing the Hx-deficiency allele bx, both in monogenic resistant Ht and susceptible ht maize lines [115]

Germination of spores of *H turcicum* was inhibited in DIMBOA solutions [115]. Additionally, diffusates from young maize tissue were more inhibitory to spore germination and to germ tube lengthening than diffusates from older tissue [116]

Linear relationships were described between resistance levels to *H turcicum* and to leaf feeding by the European corn borer, using a limited number of maize lines in the field [85] It was suggested that breeding for high Hx levels may increase resistance to European corn borer, Northern corn leaf blight and maize stalk rot (see below) [99]

When a broader array of maize genotypes was screened, a correlation between resistance ratings to borer leaf feeding and to *H turcicum* was not apparent [117] Additionally, recurrent selection for resistance to leaf feeding by the European corn borer affected negatively resistance to *H turcicum* and had no effect on *Diplodia maydis* stalk rot ratings [118] This points again to the complex and diverse nature of resistance phenomena (see section on resistance to the borer).

Histological observations of the infection of multigenic [119] and monogenic [120] resistant and susceptible maize lines by *H turcicum* showed that differences in mycelial growth between resistant and susceptible lines occurred when the mycelium reached the vascular bundles This is consistent with higher levels of hydroxamic acids being found in the vascular bundles [53, 54].

Correlations have also been reported between Hx levels in the plant and resistance of maize to stalk rot *D maydis* [121, 122], of wheat to stem rust *Puccinia graminis* [123–125], of maize to stalk rot caused by *Fusarium moniliforme* [126], *Gibberella zeae* [126] and *Cephalosporium maydis* [98], of rye to snow mold *Fusarium nivale* [127, 128], of maize to stem and ear infections caused by *Fusarium graminearum* [129]

Weed competition resulted in higher DIMBOA content of maize plants, presumably through lower water availability, and a concomitant decrease in infestation by common smut *Ustilago maydis* [130] Application of simazine (see section on detoxification of herbicides) increased the levels of DIMBOA and of its glucoside in wheat plants and concomittantly reduced attack by *Erysiphe graminis* and *Cercosporella herpotrichoides* [59]

In the case of the obligate parasite *P graminis*, it was noted that infection provoked a breakdown of the glucoside of DIMBOA. The phytotoxic properties of DIMBOA [76] were suggested to cause the death of the host cells and hence that of the parasites Conversely no correlations were observed between Hx levels in wheat and resistance to various strains of the root rot agent *Helminthosporium sativum* [131] Additionally, although Hx levels in wheat seedlings were capable of totally inhibiting *Septoria nodorum*, in the adult plants they were suggested to be unimportant in the resistance to the fungus [132]. Finally, the correlation between Hx levels and resistance to *P graminis* was criticized on the basis that the correlation coefficient substantially decreased when cultivars of extreme resistance ratings were withdrawn [125, 133]

Resistance to bacteria. The inability of certain species of soft rotting bacteria *Erwinia* spp to attack maize was attributed to the presence of DIMBOA in the plant [134] Thus, DIMBOA accounted for most of the capacity of maize extracts to inhibit soft rot bacteria non-pathogenic to maize It did so by prolonging the lag phase of bacterial growth, while the log phase was not altered [134] It was noted however, that after DIMBOA had decomposed in the bacterial medium to non-inhibitory levels, inhibition persisted [135]. The effect could be due to products from the decomposition of DIMBOA, other than MBOA, in the bacterial medium

When the range of strains studied was broadened, it was found that strains pathogenic to maize were more resistant to Hx levels than strains pathogenic to other hosts [136] However, it was also found that several DIMBOA-susceptible strains pathogenic to other hosts produced stalk rot symptoms in DIMBOA-containing maize plants. It was suggested that DIMBOA was not the primary means of resistance of maize to *Erwinia* spp

Allelopathic effects. Both germination and seedling growth of velvetleaf *Abutilon theophrasti* were affected by DIBOA-Glc, DIBOA and BOA, the most active com-

pound being DIBOA [24]. DIBOA and BOA were found to be involved in the well described allelopathic effects of rye [137, 138] Compounds were tested in germination and root and stem elongation of monocots and dicots. DIBOA was the most active compound against monocots [138, 139] while BOA was most active against dicots [138].

The symptoms of DIBOA injury on cress *Lepidium sativum* resembled that of the photosynthetic inhibitor herbicides [138]. This result agrees with the known inhibitory effects of hydroxamic acids on energy metabolism of chloroplasts [76, 77]. Similar symptoms by BOA may be related to the presence of a –N–C=O group [140]. Hydroxamic acids may also be responsible for the allelopathic effects of wheat [141].

Triggering of reproduction of the montane vole. The onset of reproductive activity of the montane vole *Microtus montanus* is correlated with beginning of vegetative growth of the grasses and sedges that constitute their main food source [142]. Plant extracts containing the DIMBOA derived compound MBOA stimulated reproductive activity of *M montanus* [143, 144]. Stimulatory effects by MBOA on the breeding performance of *M montanus* [143–145] and on reproductive responses of rats [146] and other vertebrates [143, 147] were demonstrated

Hx levels in perennial wild Gramineae increased at the onset of the vegetative growth season [15], in agreement with the finding of higher Hx levels in younger tissue. Hence, Hx levels may be the naturally occurring environmental cue triggering the reproductive efforts of *M. montanus* The occurrence of the compounds in the principal food source of the vole, saltgrass *Distichlis stricta*, was demonstrated [147].

Mineral nutrition. The involvement of Hx in mineral nutrition has been considered on account of their generally high complexation constants The formation constants of complexes of DIMBOA and DIMBOA-Glc with ferric ion [148], and those of DIMBOA with several bivalent cations [149, 150] have been measured. The constants, while several orders of magnitude smaller than those of hydroxamic acids which function in microbial iron metabolism, are higher than those of other anions involved in mineral nutrition, such as citric and malic acids. Calculations based on concentrations of chelating agents in the whole plant indicated that a substantial proportion of metal ions would be bound to Hx [148, 149]. Knowledge of concentrations of Hx at relevant plant compartments is necessary before a role in mineral nutrition is adscribed to them.

Modification of auxin-receptor interaction. Maize coleoptiles contain substances capable of inhibiting the binding of 1-naphthylacetic acid (NAA) to auxin receptors [151]. The compounds were initially identified as MBOA and BOA [152]. It was later shown that the parent hydroxamic acid DIBOA was more active than the corresponding decomposition product BOA [153, 154] The compounds inhibited not only saturable binding of NAA to auxin receptors, but also auxin-induced coleptile growth [153], providing evidence that the binding sites represent physiological receptor sites for auxin action. Additionally, BOA inhibited radicle elongation of several plant species [155].

The binding affinity of the auxin analogue 2,4-dichlorophenoxyacetic acid (2,4-D) was increased by Hx-containing extracts [156], a result that has been associated with greater capacity for detoxification of 2,4-D of plants with higher Hx levels [157].

Detoxification of herbicides and pesticides The 2-chloro-*s*-triazine derived herbicides atrazine and simazine are of paramount importance in maize production due to the tolerance exhibited by this crop. Detoxification of these herbicides occur by hydroxylation [158], dealkylation [159–161] or glutathione conjugation [162] The mechanism prevailing in maize is hydroxylation [20, 163–165]. This reaction has been associated with the presence of Hx in the plant. Thus, the Hx-rich Bx line of maize is tolerant while the Hx-lacking bx line is susceptible [66], cereals with lower Hx levels are more susceptible than those with higher levels [158, 166, 167] or less able to detoxify the herbicides by hydroxylation [19]; higher Hx levels in a plant attained through added fertilizer are associated with higher hydroxylation capacity [61]; lower Hx levels attributed to cold growing conditions increased susceptibility to the herbicides [57], and higher Hx levels were associated with lower activity of the herbicides as plant hormones [168]. Finally, a relationship was described between resistance to stalk rot and corn borer, and tolerance to atrazine and simazine in maize plants [169].

Hydroxylation of these herbicides is catalysed by hydroxamic acids [170–172]. It was suggested that a molecular aggregate of DIMBOA was the reactive species [172]. It was later shown, with the use of stable analogues, that the reaction occurred by direct nucleophilic attack of the hydroxamic oxygen atom on the carbonyl bearing the chlorine atom in the herbicide with formation of an intermediate, from which the hydroxamic acid moiety was later displaced by water [173], confirming an earlier mechanistic hypothesis [158]. In agreement with this mechanism, only *s*-triazine derivatives containing a good leaving group are selective for weed control in maize [174]. A similar mechanism may prevail in the detoxification of the organophosphorous insecticide diazinon, by hydroxylation catalysed by DIMBOA [175, 176].

Mutagenicity. Both DIBOA and DIMBOA were shown to be mutagenic in a test with *Salmonella typhimurium* TA 100 and TA 98 [177, 178]. Studies with analogues of these compounds showed that the 2-hydroxyl and 7-methoxyl groups greatly increased mutagenic activity These groups are known to enhance the electrophilic reactivity of the parent molecules [9, 70, 73, 79] Mutagenicity was also associated with the ability of the 4-acetoxy compounds to react with DNA and with model nucleotides [75]. It is worthwhile reiterating that hydroxamic acids have not been found in the grains of cereals

Conclusions

Host plant resistance to pests and diseases is a complex phenomenon in which multiple factors are involved. Factors which may be of major importance in relation to one pest or disease, may only be secondary or not relevant in relation to another. Hydroxamic acids, while of paramount importance in numerous cases of pest and disease resistance of cereals, have been shown to be of lesser importance in others, in which there is a breakdown in the resistance–Hx level correlations, the inheritance of resistance and of Hx are different, or recurrent selection for resistance to one pest does not lead to the desired effects in the resistance to another pest The present knowledge of the biological activity of hydroxamic acids

suggests that their further exploitation as a source of resistance is desirable However, the search for additional sources of resistance should be continued

Acknowledgements--I wish to thank the International Program in the Chemical Sciences (IPICS) at Uppsala University for continued support, and in particular for a fellowship permitting the writing of this work The kind hospitality of Prof Jan Pettersson at Swedish University of Agricultural Sciences (SLU) is gratefully acknowledged So is the help obtained from the library staff at SLU

REFERENCES

1 Willard, J I and Penner, D (1976) *Residue Rev* **64**, 67
2 Virtanen, A I and Hietala, P K (1960) *Acta Chem Scand* **14**, 499
3. Hofman, J and Hofmanova, O (1969) *Eur J Biochem* **8**, 109
4. Wahlroos, O and Virtanen, A I (1959) *Suomen Kemistilehti* **B32**, 139
5 Virtanen, A I and Wahlroos, O (1963) *J Pharm Sci* **52**, 713
6 Hofman, J and Hofmanova, O (1971) *Phytochemistry* **10**, 1441
7 Klun, J A, Tipton, C L, Robinson, J F, Ostrem, D L and Beroza, M (1970) *J Agric Food Chem* **18**, 663
8 Woodward, M D, Corcuera, L J, Schnoes, H K, Helgeson, J P and Upper, C D (1979) *Plant Physiol* **63**, 9
9 Pérez, F J and Niemeyer, H M (1985) *Phytochemistry* **24**, 2963
10 Grambow, H J, Luckge, J, Klausener, A and Muller, E (1986) *Z Naturforsch* **41c**, 684
11 Le-Van, N and Wratten, S J (1984) *Tetrahedron Letters* **25**, 145
12 Kutschabsky, L, Dathe, W and Schneider, G (1982) *Crys Struct Comm* **11**, 841
13. Nagao, T, Otsuka, H, Kohda, H, Sato, T and Yamasaki, K (1985) *Phytochemistry* **24**, 2959
14 Niemeyer, H M (1988) *Euphytica* **37**, 289
15 Zúñiga, G E, Argandoña, V H, Niemeyer, H M and Corcuera, L J (1983) *Phytochemistry* **22**, 2665
16 Shigematsu, N, Kuono, T and Kawano, N (1981) *J Pharm Soc Jpn* **101**, 1165
17 Virtanen, A I and Hietala, P K (1959) *Suomen Kemistilehti* **B32**, 252
18 Malan, C, Visser, J H and van de Venter, H A (1984) *S Afr J Plant Soil* **1**, 99
19 Hamilton, R H (1964) *J Agric Food Chem* **12**, 14
20 Tang, C-S, Chang, S H, Hoo, D and Yanagihara, K H (1975) *Phytochemistry* **14**, 2077
21 Chang, S, Brewbaker, J L and Tang, C (1976) *Maize Gen Coop Newslett* **50**, 32
22 Corcuera, L J, Argandoña, V H and Niemeyer, H M (1982) in *Chemistry and Biology of Hydroxamic Acids*, p 111
23 Argandoña, V H, Luza, J G, Niemeyer, H M and Corcuera, L J (1980) *Phytochemistry* **19**, 1665
24 Wolf, R B, Spencer, G F and Plattner, R D (1985) *J Nat Prod* **48**, 59.
25 Chen, C and Chen, M (1976) *Phytochemistry* **15**, 1997 227
26 Beck, S D, Kaske, E T and Smissman, E E (1957) *J Agric Food Chem* **5**, 933
27 Molot, P-M and Anglade, P (1968) *Ann Epiphyt* **19**, 75
28 Bowman, M C, Beroza, M and Klun, J A (1968) *J Econ Entomol* **61**, 120
29 Scism, A J, BeMiller, J N and Caskey, A L (1974) *Anal Biochem* **58**, 1
30 Klun, J A and Brindley, T A (1966) *J Econ Entomol* **59**, 711
31 Epstein, W W, Rowsemitt, C N, Berger, P J and Negus, N C (1986) *J Chem Ecol* **12**, 2011
32 Pessi, A and Scalorbi, D (1979) *J Chromat* **177**, 162
33 Malan, C, Visser, J H and van de Venter, H A (1986) *S Afr J Plan Soil* 3, 82
34 Robinson, J F, Klun, J A, Guthrie, W D and Brindley, T A (1982) *J Kansas Entomol Soc* **55**, 297
35 Wahlroos, O and Virtanen, A I (1959) *Acta Chem Scand* **13**, 1906
36 Brendenberg, J-B, Honkanen, E and Virtanen, A I (1962) *Acta Chem Scand.* **16**, 35
37 Hietala, P K and Virtanen, A I (1960) *Acta Chem Scand* **14**, 502
38 Virtanen, A I and Hietala, P K (1960) *Acta Chem Scand* **14**, 499
39 Bravo, H R and Niemeyer, H M (1986) *Heterocycles* **24**, 335
40 Woodward, M D, Corcuera, L J, Helgeson, J P and Upper, C D (1978) *Plant Physiol* **61**, 796
41 Bravo, H R and Niemeyer, H M (1986) *Heterocycles* **24**, 2809
42 Long, B J, Dunn, G M and Routley, D G (1974) *Crop Sci* **14**, 601
43 Sullivan, S L, Gracen, V E and Ortega, A (1974) *Environ Entomol* **3**, 718
44 Woodward, M D, Corcuera, L J, Helgeson, J P, Kelman, A and Upper, C D (1979) *Plant Physiol* **63**, 14
45 Gutierrez, C, Guerrero, A, Castañera, P and Torres, J V (1982) *J Agric Food Chem* **30**, 1258
46 Lyons, P C, Hipskind, J D, Wood, K V and Nicholson, R L (1988) *J Agric Food Chem* **36**, 57
47 Niemeyer, H M, Pesel, E, Copaja, S V, Bravo, H R, Franke, S and Francke, W (1989) *Phytochemistry* **28** (in press)
48 Klun, J A and Robinson, J F (1969) *J Econ Entomol* **62**, 214
49 Toth Toldi, E (1984) *Novenytermeles* **33**, 213
50 Virtanen, A I, Hietala, P K and Wahlroos, O (1957) *Arch Biochem Biophys* **69**, 486
51 Argandoña, V H, Niemeyer, H M and Corcuera, L J (1981) *Phytochemistry* **20**, 673
52 Long, B J, Dunn, G M and Routley, D G (1978) *Crop Sci* **18**, 573
53 Argandoña, V H and Corcuera, L J (1985) *Phytochemistry* **24**, 177
54 Argandoña, V H, Zúñiga, G F and Corcuera, L J (1987) *Phytochemistry* **26**, 1917
55 Guthrie, W D, Tseng, C T, Russell, W A, Coats, J R, Robbins, J C and Tollefson, J J (1986) *J Kansas Entomol Soc* **59**, 356
56 Manuwoto, S. and Scriber, J M (1985) *J Chem Ecol* **11**, 1469
57 Thompson Jr, L, Slife, F W and Butler, H S (1970) *Weed Sci* **18**, 509
58 Manuwoto, S and Scriber, J M (1985) *Agric Ecosys Environ* **14**, 221
59 Brandes, W and Heitefuss, R (1971) *Phytopath Z* **72**, 34
60 Manuwoto, S and Scriber, J M (1985) *Agric Ecosys Environ* **14**, 25
61 Sosnavaya, O N and Merezhinskii, Yu G (1979) *Soviet*

Agric Sci 14.
62. Tang, D Z and Ge, Y H (1981) *Plant Protection* (*Zhiwu Baohu*) **7**, 2
63 Reimann, J E and Byerrum, R. U. (1964) *Tetrahedron Letters* 211
64. Reimann, J E. and Byerrum, R. U. (1964) *Biochemistry* **3**, 847.
65 Tipton, C. L., Wang, M.-C, Tsao, F. H.-C., Lin Tu, C.-C. and Husted, R. R (1973) *Phytochemistry* **12**, 347
66. Hamilton, R. H. (1964) *Weeds* **12**, 27.
67. Dunn, G. M, Long, B. J. and Routley, D. G (1981) *Can J. Plant. Sci.* **61**, 583
68 Simcox, K D. and Weber, D F (1985) *Crop Sci.* **25**, 827
69 Klun, J. A., Guthrie, W. D., Hallauer, A. R. and Russell, W A. (1970) *Crop Sci* **10**, 87.
70. Copaja, S V., Bravo, H R and Niemeyer, H. M. (1986) *J. Org Chem* **51**, 3542.
71. Bravo, H. R. and Niemeyer, H. M. (1985) *Tetrahedron* **41**, 4983
72 Smissman, E E, Corbett, M. D., Jenny, N. A. and Kristiansen, O (1972) *J Org. Chem.* **37**, 1700
73 Hashimoto, Y. Ohta, T., Shudo, K. and Okamoto, T (1979) *Tetrahedron Letters* 1611
74. Ishizaki, T, Hashimoto, Y., Shudo, K and Okamoto, T. (1983) *Heterocycles* **20**, 1481
75. Ishizaki, T, Hashimoto, Y., Shudo, K. and Okamoto, T. (1982) *Tetrahedron Letters* 4055
76. Queirolo, C. B., Andreo, C. S, Vallejos, R. H., Niemeyer, H M. and Corcuera, L J. (1981) *Plant Physiol* **68**, 941.
77. Queirolo, C. B, Andreo, C S., Niemeyer, H. M and Corcuera, L J (1983) *Phytochemistry* **22**, 2455.
78 Niemeyer, H. M, Calcaterra, N. B and Roveri, O. A (1986) *Biochem Pharmacol* **35**, 3909.
79 Niemeyer, H. M, Corcuera, L. J. and Pérez, F J (1982) *Phytochemistry* **21**, 2287.
80 Beck, S. D. and Stauffer, J. F (1957) *Ann. Entomol. Soc Am.* **50**, 166.
81. Smissman, E E, LaPidus, J. B. and Beck, S D. (1957) *J. Org. Chem.* **22**, 220
82 Loomis, R S, Beck, S. D and Stauffer, J F. (1957) *Plant Physiol.* **32**, 379
83. Beck, S. D (1957) *J Insect Physiol* **1**, 158
84. Beck, S. D and Smissman, E E. (1961) *Ann. Entomol. Soc. Am* **54**, 53.
85 Anglade, P and Molot, P-M (1967) *Ann. Epiphyt.* **18**, 279
86 Virtanen, A I (1961) *Suomen Kemistilehti* **B34**, 29.
87 Klun, J A., Tipton, C L and Brindley, T A. (1967) *J Econ Entomol* **60**, 1529
88 Reed, G L., Brindley, T. A. and Showers, W B. (1972) *Ann. Entomol. Soc Am.* **65**, 658.
89. Gahukar, R T (1979) *Ann Soc. Entomol France* (*N.S.*) **15**, 649
90 Robinson, J. F, Klun, J. A, Guthrie, W D. and Brindley, T A. (1982) *J Kansas Entomol. Soc* **55**, 357
91. Robinson, J F, Klun, J A and Brindley, T. A (1978) *J Econ Entomol* **71**, 461.
92. Scriber, J M, Tingey, W M, Gracen, V E and Sullivan, S L (1975) *J. Econ Entomol.* **68**, 823
93 Guthrie, W. D, Wilson, R L, Coats, J. R, Robbins, J C., Tseng, C T, Jarvis, J L. and Russell, W. A (1986) *J Econ Entomol.* **79**, 1492
94. Klun, J. A. (1970) *Proc 24th Ann Corn Sorghum. Res. Conf*, p 55
95. Hudon, M and Chaing, M S (1977) *Can. Entomol* **109**, 931
96. Cabulea, I, Mustea, D, Ardelean, P, Agbary, A W. and Terbea, M (1977) *Prob Gen. Teor. Apl* **9**, 155
97. Hudon, M, Chiang, M. S., Shapiro, I D. and Pereverzev, D S (1980) *Ann Soc Entomol Québec* **25**, 36
98. Kostandi, S F., Koraiem, Y. S., Kamara, A and Omar, M A. (1981) *Agrochimica* **25**, 367
99 Russell, W. A, Guthrie, W D., Klun, J A. and Grindeland, R (1975) *J. Econ. Entomol.* **68**, 31
100. Tseng, C. T., Guthrie, W. D., Russell, W. A., Robbins, J C, Coats, J. R. and Tollefson, J J. (1984) *Crop Sci* **24**, 1129
101 Mustea, D. (1973) *Prob Prot. Plant.* **1**, 218
102. Chiang, M. S and Hudon, M (1976) *Phytoprot* **57**, 36.
103. Rojanaridpiched, C, Gracen, V. E, Everett, H L, Coors, J. G., Pugh, B. F. and Bouthyette, P. (1984) *Maydica* **24**, 305
104 Gutiérrez, C and Castañera, P (1986) *Inv Agrar Prod Prot. Veg.* **1**, 109
105. Long, B J, Dunn, G M., Bowman, J S. and Routley, D. G (1977) *Crop Sci.* **17**, 55.
106. Beck, S D., Dunn, G. M., Routley, D. G and Bowman, J S. (1983) *Crop Sci.* **23**, 995.
107 Bohidar, K, Wratten, S D. and Niemeyer, H M (1986) *Ann. Appl Biol.* **109**, 193
108 Argandoña, V H., Peña, G. F., Niemeyer, H M. and Corcuera, L. J (1982) *Phytochemistry* **21**, 1573.
109 Argandoña, V H., Corcuera, L. J., Niemeyer, H M and Campbell, B C (1983) *Entomol. Exp. Appl* **34**, 134
110. Corcuera, L J., Queirolo, C. B, and Argandoña, V H (1985) *Experientia* **41**, 514.
111 Corcuera, L. J, Argandoña, V H., Peña, G. F., Pérez, F J and Niemeyer, H. M. (1982) *Proc. Fifth Int. Symp. Insect-Plant Relationships* (Visser, J H. and Minks, A K, eds), p 33 Pudoc, Wageningen.
112. Niemeyer, H M., Pesel, E., Franke, S. and Francke, W (1988) *Entomol Exp Appl* (in press)
113 Molot, P-M (1969) *Ann. Phytopath.* **1**, 353
114 Long, B. J., Dunn, G. M. and Routley, D. G. (1975) *Crop Sci* **15**, 333.
115. Couture, R M, Routley, D G and Dunn, G M (1971) *Physiol Plant Path* **1**, 515.
116 Calub, A. G, Dunn, G M, Routley, D. G and Couture, R M (1974) *Crop Sci* **14**, 359.
117. Guthrie, W. D, Barry, B D., Rossman, E C and Jarvis, J L (1985) *J Econ. Entomol.* **78**, 811
118. Klenke, J R., Russell, W A., Guthrie, W. D., Martinson, C. A. and Pedersen, W L. (1987) *Phytopathology* **77**, 735
119 Hilu, H M and Hooker, A L. (1964) *Phytopathology* **54**, 570
120 Mace, M E. (1973) *Phytopathology* **63**, 243
121. BeMiller, J M and Pappelis, A J. (1965) *Phytopathology* **55**, 1237
122 Dabler, J M., Pappelis, A. J. and BeMiller, J. N. (1969) *Phytopathology* **69**, 1098.
123 Elnaghy, M. A and Linko, P (1962) *Physiol Plant* **15**, 764.
124. ElNaghy, M A and Shaw, M (1966) *Nature* **210**, 417
125. Chkanikov, D I, Tarabrin, G. A., Shabanova, A M, Konstantinov, P F and Solomatin, D. A (1968) *Proc First Int Cong Plant Path*, London, p. 29.
126 Whitney, N J. and Mortimore, C G (1959) *Nature* **183**, 341
127. Virtanen, A. I. and Hietala, P K (1955) *Suomen Kemistilehti* **B28**, 165
128 Honkanen, E and Virtanen, A I (1960) *Acta Chem Scand.* **14**, 1214
129. Cabulea, I, Ardelean, P, Monteanu, I and Agbary, A W (1976) *Prob Gen. Teor Apl* **8**, 33

130 Pfister, J-A and Grossmann, F (1979) *Phytopath Mediterr* **18**, 102
131 Fadeev, Yu N, Tarabrin, G A and Tishenkov, A D (1982) *Soviet Agric Sci* **4**, 15.
132 Baker E A. and Smith, I M (1977) *Ann Appl Biol* **87**, 67
133 Knott, D. R and Kumar, J (1972) *Physiol Plant Path* **2**, 393
134 Corcuera, L J, Woodward, M D, Helgeson, J P., Kelman, A and Upper, C D (1978) *Plant Physiol* **61**, 791
135 Woodward, M D, Corcuera, L J, Helgeson, J P, Kelman, A and Upper, C D (1978) *Plant Physiol* **61**, 803
136 Lacy, G H, Hirano, S S, Victoria, J I, Kelman, A and Upper, C D (1979) *Phytopathology* **69**, 757
137 Fuerst, E P and Putman, A R (1983) *J Chem Ecol* **9**, 937.
138 Barnes, J P and Putman, A R (1987) *J Chem Ecol* **13**, 889
139 Barnes, J P, Putman, A R, Burke, B A and Aasen, A J (1987) *Phytochemistry* **26**, 1385
140 Moreland, D E and Hill, K L (1963) *Weeds* **11**, 284
141 Lodhi, M A K, Bilal, R. and Malik, K A (1987) *J Chem Ecol* **13**, 1881.
142 Negus, N C, Berger, P J and Forslund, L G (1977) *J Mammal* **58**, 347
143 Sanders, E H, Gardner, P D, Berger, P J and Negus, N C (1981) *Science* **214**, 67
144 Berger, P J, Negus, N C., Sanders, E H and Gardner, P D (1981) *Science* **214**, 69.
145 Berger, P J, Negus, N C and Rowsemitt, C N (1987) *Biol Reprod* **36**, 255
146 Butterstein, G M, Schadler, M H, Lysogorski, E, Robin, L and Sipperly, S (1985) *Biol Reprod* **32**, 1018
147 Negus, N C, Berger, P J and Brown, B W (1986) *Can J Zool* **64**, 785
148 Tipton, C L and Buell, E L (1970) *Phytochemistry* **9**, 1215
149 Dabed, R G, Toral, M. I, Corcuera, L J and Niemeyer, H M (1983) *Polyhedron* **2**, 106
150 Hiriart, M V, Corcuera, L J, Andrade, C and Crivelli, I (1985) *Phytochemistry* **24**, 1919
151 Ray, P M, Dohrmann, U and Hertel, R (1977) *Plant Physiol* **59**, 357
152 Venis, M A and Watson, P J (1978) *Planta* **142**, 103
153 Venis, M A (1979) in *Plant Growth Substances* 1979 (Skoog, F, ed), p 61 Springer, Berlin
154 Venis, M. A (1980) in *Aspects and Prospects of Plant Growth Regulators*, British Growth Regulator Group, Monograph No 6, p 187
155 Alupova, A T, Molchanov, L V, Kadryov, C S, Aliev, N A, Giasov, K, Loi, N P, Tsoi, Z and Umarov, A A (1979) *Agrokhimiia* **10**, 107
156 Ray, P M, Dohrmann, U and Hertel, R (1977) *Plant Physiol* **60**, 585
157 Hatzios, K K and Penner, D (1982) *Metabolism of Herbicides in Higher Plants* Burguess, Minneapolis, Minnesota
158. Castelfranco, P, Foy, C L and Deutsch, D G (1961) *Weeds* **9**, 580
159 Hurter, J (1966) *Experientia* **22**, 741
160 Shimabukuro, R H (1967) *Plant Physiol* **42**, 1269
161 Shimabukuro, R H (1968) *Plant Physiol* **43**, 1925
162 Shimabukuro, R H, Swanson, H R and Walsch, W C (1970) *Plant Physiol* **46**, 103
163 Montogomery, M L and Freed, V H (1964) *J Agric Food Chem* **12**, 11
164 Malan, C, Visser, J H and van de Venter, H A (1986) *S Afr J Plant Soil* **3**, 115
165 Malan, C, Visser, J H and van de Venter, H A (1987) *S Afr J Plant Soil* **4**, 7
166 Palmer, R D and Grogan, C O (1965) *Weeds* **13**, 219
167 Malan, C and le Court de Billot, M R (1986) *S. Afr J Plant Soil* **3**, 205
168 Malan, C, Visser, J H and van de Venter, H A (1984) *S Afr J Plant Soil* **1**, 103
169 Andersen, R N (1964) *Weeds* **12**, 60
170 Roth, W and Kunsli, E (1961) *Experientia* **17**, 312
171 Hamilton, R H and Moreland, D E (1962) *Science* **135**, 373
172 Tipton, C L, Husted, R R and Tsao, F H-C (1971) *J Agric Food Chem* **19**, 484
173 Nakano, N I, Smissman, E E and Schowen, R L (1973) *J Org Chem* **38**, 4396
174 McWhorter, C G and Holstun, Jr, J T (1961) *Weeds* **9**, 592
175 Ioannou, Y M and Dauterman, W C (1979) *Pest. Biochem Physiol* **10**, 212
176 Ioannou, Y M, Dauterman, W C and Tucker, W P (1980) *Phytochemistry* **19**, 1607
177 Hashimoto, Y, Shudo, K, Okamoto, T, Nagao, M, Takahashi, Y and Sugimura, T (1979) *Mutation Res* **66**, 191
178 Yahagi, T, Nagao, M, Seino, Y Matsushima, T, Sugimura, T and Okada, M (1977) *Mutation Res* **48**, 121
179 Hofman, J and Masojidkova (1973) *Phytochemistry* **12**, 207
180 Tipton, C L, Klun, J A, Husted, R R and Pierson, M D (1967) *Biochemistry* **6**, 2866
181 Oliva, L, Addeo, F, Chioccara, F and Prota, G (1978) *Ann Fac Sci Agrar Univ Stu Nap, Portici* **12**, 78
182 Gahagan, H E and Mumma, R O (1967) *Phytochemistry* **6**, 1441
183 Hofman, J, Hofmanova, O and Hanus, V (1969) *Tetrahedron Letters* 5001
184 Hofman, J, Hofmanova, O and Hanus, V (1970) *Tetrahedron Letters* 3213
185 Virtanen, A I and Hietala, P K (1955) *Acta Chem Scand* **9**, 1543
186 Koyama, T (1955) *J Pharm Soc Jpn* **75**, 702