A Survey of Phytoalexin Induction in Leaves of the Rosaceae by Copper Ions

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The leaves of 130 species of Rosaceae were surveyed for phytoalexin induction. Both biotic and abiotic induction was examined and antifungal compounds were detected in 47 species. However, these compounds appeared to be constitutive metabolites, released from bound phenolic materials already present in the leaf. In *Pyrus*, hydroquinone was produced from the hydrolysis of arbutin present in the vacuole before inoculation. In most other species, the fungitoxic agents were mainly catechin-like derivatives, apparently released from the tannins present within the leaf. By contrast, the synthesis in the leaf of the characteristic biphenyl or benzofuran phytoalexins which are produced in sapwood, was confined to a very few species. The biphenyl aucuparin was identified as a phytoalexin from the leaves of *Sorbus aucuparia*.

Introduction

Phytoalexin production is now well established as one of several defence systems which provide plants with resistance to microbial infections. Several hundred phytoalexins have now been characterized from over thirty plant families (Grayer and Harborne, 1994). The response has been mainly studied using the drop diffusate technique on leaf tissue, but seed, shoot, stem and wood tissues have also been used as well as cell culture. The type of compound formed *de novo* in a plant is usually characteristic at the family level, in that isoflavanoid phytoalexins are commonly produced in legumes, sesquiterpenoids in the Solanaceae, furanocoumarins in the Umbelliferae and so on (Harborne and Turner, 1984). As many as thirty compounds may be formed in a given plant-fungal interaction, as happens in infected carnation *Dianthus caryophyllus*, tissue (Niemann, 1993).

The protective value of phytoalexin synthesis in warding off fungal pathogens has been established by the experiments of vanEtten and co-workers (1989) on the *Nectria haematococca-Pisum sativum* interaction and by the successful genetic transfer of phytoalexin production (*e.g.* stilbene synthase) from one plant, *Vitis vinifera*, to another, *Nicotiana tabacum* (Hain *et al.*, 1993). In spite of these and many other experiments, it is not yet clear how universal within the angiosperms this mechanism is. We therefore decided to survey members of the Rosaceae for phytoalexins in the leaves. Since the family is related both morphologically and chemically to the Leguminosae (Bate-Smith, 1961), which regularly produces isoflavonoids as phytoalexins (Ingham, 1981), we expected to find some similarities in response between the two families.

Relatively little is known about the disease resistance mechanisms in the Rosaceae, in spite of its economic importance as a source of many cultivated fruits. The first phytoalexin to be reported in the family was benzoic acid, which is formed in apple fruit following infection by *Nectria galligena* (Browne and Swinburne, 1971). Since then, biphenyl or benzofuran phytoalexins have been characterized from sapwood of *Cotoneaster lactea* (Burden *et al.*, 1984), *Malus pumila* (Kemp *et al.*, 1985) and *Pyrus communis* (Kemp and Burden, 1984) and from the leaves of *Eriobotrya japonica* (Mikayado *et al.*, 1985), *Photinia glabra* (Widyastuti *et al.*, 1992) and *Rhaphiolepsis umbellata* (Watanabe *et al.*, 1990). Additionally some sesquiterpenoids have been characterized as antimicrobial agents in damaged *Rosa rugosa* leaves (Hashidoko *et al.*, 1989). Also, flavan-3-ols have been reported to accumulate in fungus-infected leaves of several Rosaceae (Treutter and Feucht, 1990). The results of a representative survey of the family for phytoalexin synthesis in leaf tissue is presented here.

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Materials and Methods

Plant material

Most plant material was botanically verified from the Harris Garden, the botanical garden of the University of Reading. Several species were grown from seed in pots in the glasshouse (see Table I). In all cases, material treated with chemical insecticides and fungicides was avoided. Care was taken to use only healthy, non-damaged young leaf tissue throughout the experiment. Plant material were collected between early May and mid-August, 1992.

Fungal inoculum

Fungal species used as phytoalexin inducers (*Alternaria alternata, Aspergillus niger, Botrytis cinerea, Cladosporium herbarum, Fusarium culmorum, Geotricum candidum* and *Trichoderma viride*) were obtained from stock cultures maintained at the School of Plant Sciences, the University of Reading. They were grown on a dish containing potato-dextrose agar medium, pH 5.6. Cultures vigorously producing spores or conidia were flooded with deionized water containing 0.05% Tween-20 and gently scraped. The spore suspensions so obtained were passed through three layers of lens tissue to remove mycelial fragments and were then adjusted to a density of approx. 1.0×10^6 spores/ml with the aid of haemocytometer.

Stress treatment

1. Biotic induction

Freshly excised leaves were immediately floated on *ca.* 10 ml of deionized water, adaxial side uppermost, in a plastic Petri dish (9 cm in diam.). Fungal spore suspension droplets were placed on the leaf surface and incubated under diffused light at 20 ± 1 °C for 3 days (72–84 h). Controls received only water droplets containing 0.05% Tween-20.

2. Abiotic induction

a) A similar amount of leaf material was floated on *ca.* 10 ml of 0.1% of $CuSO_4 \cdot 5H_2O$ solution containing 0.05% Tween-20 in a plastic Petri dish (9 cm), so that the leaf area contacting with $CuSO_4$ solution became maximal. Controls were run with 0.05% Tween-20 solution; they were incubated in the same manner as described above.

b) Hammer mill ground flakes of chitin and chitosan, both of crab shell origin, were used as elicitors. Suspension of 4000 μg ml^{-1} (Keen *et al.*, 1983) of fine powder were applied on the leaf surface floating on water in Petri dish.

Fungitoxin detection

Only cupric sulphate-treated leaves and the corresponding controls were further analyzed as follows. The supporting $CuSO_4$ solution and water were decanted and extracted (×2) separately with *ca.* 2 ml EtOAc. The extracts were brought to dryness in an air flow. The residue were dissolved in a small amount of MeOH and analyzed on silica gel TLC with MeOH–$CHCl_3$ (49:1) and *n*-hexane–EtOAc–MeOH (60:40:1) (HEM) as solvent systems. At the same time, the leaves showing extensive necrosis were extracted with MeOH for overnight. This extract was concentrated and the EtOAc soluble fractions were chromatographed on TLC plates similarly. The developed TLC plates were sprayed with a dense spore suspension of *Cladosporium herbarum* in a nutrient solution (KH_2PO_4, 4.7 g; KNO_3, 2.7 g; $Na_2HPO_4 \cdot 2H_2O$, 2 g; $MgSO_4 \cdot 7H_2O$, 0.7 g, glucose, 100 g, in 1 l of tap water, pH 5.7). After three days incubation in the moist condition at 22 °C, the presence/absence of fungitoxins and their R_f values recorded.

Further characterization of induced fungitoxin

Once induced fungitoxins, *i.e.* those not detected in the control or in healthy tissue, were detected, a larger scale induction was performed. Typically a few tens of grams of fresh leaves were treated with 100 ml of 0.1% $CuSO_4 \cdot 5H_2O$ solution. After TLC purification with suitable solvent system, the UV spectra with/without shift reagents (NaOAc, NaOH) were measured. Also, some specific "phenolic" reagents such as Folin-Ciocalteu's and Gibbs were employed to determine their identity.

Results

At the beginning of the survey, spore suspensions of several pathogenic and non-pathogenic fungi were used to cause phytoalexin induction.

Table I. Production of necrosis and of antifungal agents in leaves of the Rosaceae.

Species	Necrotic reaction	Antifungal agents⁺
Subfamily Spiraeoideae		
Tribe Spiraeae		
Aruncus sylvester Kneffii	++	–
Neillia thibetica	++	–
Physocarpus malvaceus (Greene) O. Kuntze	++	B, E
Sibiraea altaiensis (Laxm.) Schneid.	+	–
Sorbaria arborea	+	–
Spiraea bella Sims	+	C, E
S. betulifolia Pallas	++	C, D, E
S. japonica L.**	+	–
S. nipponica "Tosaensis"	++	C, D, F
S. pubescens	++	–
S. thunbergii	++	A, D, F
Tribe Exochordeae		
Exochorda racemosa (Lindl.) Rehder	+	–
Subfamily Rosoideae		
Tribe Kerrieae		
Kerria japonica (L.) DC. "Picta"	–	B
K. japonica (L.) DC. "Pleniflora"	–	–
Rhodotypos scandens (Thunb.) Makino	–	–
Tribe Potentilleae		
Fragaria vesca L. var. *americana* Porter	+	–
F. virginiana Duchesne	+	–
F. × *ananassa* Duchesne	+	–
Geum macrophyllum Willd.	++	B
G. montanum L.	++	–
G. pyrenaicum Miller	++	H
G. rivale L.*	+	B
G. roylei Bolle	+	–
G. × *intermedium* Ehrh.	+	–
Potentilla anserina L.	–	–
P. argyrophylla	–	–
P. fruticosa L.	++	–
P. nepalensis Hook.*	+	B
P. reptans L.	+	C, G
P. × *russelliana* Lindley ex Sweet	++	–
Rubus fruticosus	+	–
R. idaeus L.	+	–
R. loganobaccus	+	–
R. odoratus L.	++	–
R. tricolor	+	–
Tribe Roseae		
Rosa achubrensis Chrsham.	+	–
R. agrestis Savi	+	–
R. alba L.	–	–
R. beggeriana Schrenk ex Fisch. & May	+	F
R. blanda Aiton	+	–
R. brunonii Lindley	++	–
R. celeste	+	–
R. centifolia L.	+	–
R. felicite	+	–
R. filipes	+	–
R. hugonis Hemsley	+	–
R. hugonis × *xanthina*	+	–
R. iberica	++	–
R. laevigata Michx.	+	–
R. macrophylla	+	–
R. maximowicziana Regel "Jackii"	+	–
R. mollis Sm.	+	–
R. multiflora Thunb.	++	–
R. nanothamnus × *willmottiana*	++	–
R. nutkana Presl. (*R. muricata* Greene)	+	–
R. primula Boulengep	+	–
R. primula × *hugonis*	+	–
R. primula × (*primula* × *omeiensis*)	+	–
R. roxburghii Tratt	+	–
R. rubiginosa L.	+	–
R. rugosa Thunb.	+	–
R. salicitorum Lydberg	+	–
R. sericea Lindley	++	–
R. virginiana	–	–
R. woodsii Lindley	+	–
Tribe Sanguisorbeae		
Acaena buchananii Hooker. f.*	+	–
A. hieronymi Kuntze*	–	–
A. sanguisorba Vahl.*	–	–
Alchemilla conjuncta	++	–
A. glabra	+	F, G
Sanguisorba minor L.*	+	–
S. officinalis L.*	–	–
Sarcopoterium spinosum (L.) Spach	+	–
Tribe Ulmarieae		
Filipendula hexapetala Gilib. "Flore Pleno"	+	–
F. ulmaria (L.) Maxim.*	+	–
Subfamily Prunoideae		
Osmaronia cerasiformis (Torrey & Gray) Greene	++	–
Prinsepia uniflora Batai.	+	I, K, L
Prunus armeniaca "Farmingdale"	++	J
P. autumnalis	++	K
P. avium "Plena"	++	K, L
P. cerasoides D. Don	++	O
P. divaricata Ledeb.	++	L
P. domestica L. "Plum"	++	–
P. domestica L. "Stella"	++	L
P. domestica L. "Victoria"	++	–
P. laurocerasus L.	+	–
P. lusitanica L.	+	O
P. padus L.	++	I, J, K, L
P. persica "Duke of York"	++	I, K, L

Table I. (Continued).

Species	Necrotic reaction	Antifungal agents+
P. persica var. *nectarine* "Pineapple"	++	I, K, L
P. rufa Hooker Fil.	++	I, L
P. serrula Frachet	++	K, L, M, N
P. spinosa L.	++	–
P. tenella Batsch.	++	I, L, M
P. yedoensis	+	–
P. "Ichiyo"	++	J, K, L
P. "Mikurama-Gaeshi"	++	K
P. "Taihaku"	++	K
Subfamily Maloideae		
Amelanchier alnifolia Nuttal	+	Q, S, T
A. ovalis Medickus	+	Q
Aronia arbutifolia (L.) Elliott var. *atropurpurea* Robins.	+	–
Chaenomeles cathayensis (Hemsley) Schneid.	++	T
C. japonica (Mast.) Lavallèe	+	–
Cotoneaster acutifolius Turcz.	+	–
C. divaricatus Rehder & Wilson	+	–
C. horizontalis Decne.	+	L
C. splendens Flinck & Hylmö	+	–
Crataegus monogyna Jacq.	++	–
C. pontica C. Koch	++	L
C. prunifolia (Poir.) Pers.	++	–
Cydonia oblonga Miller**	++	L, Q
Malus baccata (L.) Borkh. var. *mandshurica* Schneid.	++	–
M. coronaria	++	–
M. domestica Borkh.	++	–
M. fusca (Raf.) Schneid.	+	L
M. hupehensis (Pamp.) Rehder	++	–
M. orientalis	++	–
M. sieboldii (Regel) Rehder	++	B, P, S
M. sieversii (Ledeb) Roem	++	–
M. silvestris Miller	++	P
M. toringoides (Rehder) Hughes	++	–
Mespilus germanica L.	++	–
Photinia davidiana	–	–
Pyracantha coccinea Roemer "Lalandii"	+	W
P. coccinea "Golden Sun"	++	V
P. coccinea "Mohave"	++	V
Pyrus communis L. "Doyenne du comice"	++	S, W
P. communis L. "Glou Morceau"	++	P, R, V
P. elaeagrifolia Pallas	++	R, V
P. pyraster	++	Q, R, V
P. ussuriensis Maxim.	++	–
Sorbus aucuparia L. "Edulis"	++	U
S. commixta Hedl.	+	–
S. × *intermedia* (Ehrh.) Pers.	+	–

* Grown in glasshouse.
** Plant material provided from local gardens.
+ For properties, see Table II.

However, these spores germinated rather poorly on leaves, even after three days incubation, while those in water germinated well. Under the microscope, it was apparent that there was hardly any necrotic response in the cells underneath the droplets. On the other hand, cupric sulphate-treated leaves produced a necrotic response and released some antifungal compounds at the same time. A necrotic response was observed with most species (Table I). The identity of these active compounds is not entirely clear; however, the UV spectra strongly suggest that they are probably catechins and gallocatechins.

Plants of the subfamily Prunoideae often produce strong fungitoxins, whereas plants of the Maloideae, do this less often. The Spiraeoideae also produce fungitoxins, but the UV spectra of the predominant fungitoxins (Table II) suggest interesting differences between those of the Prunoideae and of the Maloideae. The Rosoideae seldom produce fungitoxins as stress metabolites. Most of the antifungal compounds detected responded positively to phenolic spray reagents on TLC plates. The data available including chromatographic behaviour for the fungitoxic compounds, designated A to W, are listed in Table II.

Apart from the catechin-like compounds, two distinctive antifungal compounds were identified. One is hydroquinone (compound R) from all *Pyrus* species examined. Hydroquinone is well known as the aglucone of arbutin, which is ubiquitous in *Pyrus* as a constitutive compound (Bate-Smith, 1961). The other is aucuparin (compound U) from *Sorbus aucuparia.* Although aucuparin and its derivatives were discovered first in the heartwood of this plant (Erdtman *et al.,* 1963), the leaves are apparently a new source. Aucuparin was not detected in the whole extract of healthy, non-treated leaves and appears therefore to be a true phytoalexin in the leaves of this plant.

Finally, in attempting a chemical elicitation by biological means, treatment with chitin and chitosan caused virtually no response in the host plants. Among those examined, only *Malus toringoides* showed an extensive necrotic "lesion". In this case, the flakes of chitin and chitosan became yellow, perhaps due to deposition of colouring material leaked from the leaves. A similar necrotic response and the leakage of yellow substance to the supporting water was also observed on treatment

Table II. R_f values, UV/VIS spectra and chemical nature of fungitoxins.

Compound	R_f (×100) in HEM	UV/VIS spectral maxima [nm] MeOH	+NaOH	+NaOAc	Spray reagent* F-C	Gb	pN
A	0	–					
B	26	282	–	280		maroon	
C	34	–					
D	48	268	268				
E	57	–					
F	66	268	264	264			
G	13	–				yellow	
H	84	–					
I	17	274, 319	275	274, 312	–	purple	
J	30	275	275	275	–		
K	43	270	–	268			
L	56	226, 272, 280 sh	272 sh, 279 sh	226, 272, 280 sh	±	–	purple
M	66	288, 292	–	–	±		
N	77	274	–	–			
O	91	226, 282			grey	mauve	
P	6	–			grey	grey	yellow
Q	14	224 sh	–	–	+	blue	
R+	36	227, 295	304 sh, 312, 219		+	mauve	yellow
S	43	–			–		
T	52	272	275 sh	275 sh			
U++	58	273			+		
V	63	225, 285				–	
W	74	226, 267 sh, 275, 282 sh					

* Key: F-C, Folin-Ciocalteu's reagent with ammonia vapour; Gb, Gibbs reagent; pN, diazotized *p*-nitroaniline with 20% Na_2CO_3 overspray.

\+ Identified as hydroquinone by direct comparison (UV spectra, co-TLC) with an authentic marker.

++ Identified as aucuparin by direct comparison (UV spectra, MS, co-TLC, co-PLC) with an authentic marker.

with cupric sulphate solution, but no fungitoxic compound was detected. Two *Prunus* species (*P. padus* and *P. yedoensis*) showed only a slight necrotic spot underneath the droplets, but otherwise no response.

Discussion

The results of a survey of leaves of 130 plants from the Rosaceae (12, Spiraeoideae; 62, Rosoideae; 22, Prunoideae; 34, Maloideae) revealed only one species, *Sorbus aucuparia*, that gave a genuine phytoalexin response. Instead of phytoalexin production, over a third of the species boosted the production of constitutive phenolic compounds such as catechins. Our results are in line with other reports of flavan-3-ols as major antifungal compounds in this family (Treutter and Feucht, 1990a, b). Indeed, Rosaceous plants are in general rich in tannins based on procyanidin and ellagic acid, and are recognized as a "tanniferous family", beside possessing high amounts of phenolics such as *p*-coumaric acid and caffeic acid (Bate-Smith, 1961). The toxicities of catechins and tannins against microorganisms are well documented (Scalbert, 1991).

We employed a variety of both biotic and abiotic techniques to induce phytoalexin synthesis so that our results cannot be simply due to experimental error (see also Hashidoko *et al.*, 1989). Our techniques were successful in inducing the expected phytoalexins in several non-rosaceous plants, including the pea, groundnut and rice. A similar study on Cucurbitaceae earlier revealed the lack of a phytoalexin response in that family (Deverall, 1976). It is apparent therefore that

plants in certain families do in fact lack an active phytoalexin induction system in the leaves. Macromolecular barriers, such as chitinases and other glucanohydrolyases of plant origin (Ghaouth *et al.*, 1991) which degrade fungal cell walls, may be present instead.

Overall, the resistance mechanisms of the rosaceous plants to fungal pathogens are still unclear. Possessing antifungal compounds does not simply mean that the plants are resistant (Hunter, 1971; Oydvin and Richardson, 1987; Sierotzki and Gessler, 1993). If a fungitoxin, or any other warding off agent, is involved in a plant's defence mechanism, it has to obey important criteria (Harborne, 1987). In fact, some pathogens on the rosaceous plants grow without contacting the intracellular components. They establish hyphal growth between the cuticle and epidermal cells or intercellular spaces, without penetrating the cells (Dickinson, 1982; Valsangiacomo and Gessler, 1992). This is true for *Venturia inaequalis, Diplocarpon rosae,* a black spot fungus on *Rosa* and *Taphrina deformans,* a hypertrophic fungi causing the leaf curl on *Prunus* species.

Disease resistance in plant relies on several complex mechanisms, and phytoalexin production is only a part of an integrated natural defence system. The results of our survey show that in the leaves of rosaceous plants, "phytoalexins" are not often produced as major protective compounds. This is further exemplified by the fact that when they are occasionally produced in the leaves, as they are in *Photinia glabra* (Widyastuti *et al.*, 1992), the amount formed is quite insufficient to halt the advancement of pathogens. Our survey has more recently been extended to cover fruit and sapwood tissues. Preliminary results reveal that the fruit tissue produce many phenolic antifungal compounds especially when they are immature, but these are constitutive components of healthy tissue. On the other hand, sapwood tissues respond dynamically, and a range of phytoalexin-like compounds have been detected, as will be reported later. We have also detected phytoalexin induction in the roots of a herbaceous member of the family, *Sanguisorba minor* (Kokubun *et al.*, 1994).

Bate-Smith E. C. (1961), Chromatography and taxonomy in the Rosaceae, with special reference to *Potentilla* and *Prunus.* J. Linn. Soc. Bot. **58,** 39–54.

Brown A. E. and Swinburne, T. R. (1971), Benzoic acid: an antifungal compound formed in Bramley's seedling apple fruits following infection by *Nectria galligena* Bres. Physiol. Plant Pathol. **1,** 469–475.

Burden R. S., Kemp M. S. and Wiltshire C. W. (1984), Isolation and structure determination of cotonefuran, an induced dibenzofuran from *Cotoneaster lactea.* J. Chem. Soc. Perkin Trans. I, 1445-1447.

Deverall R. J. (1976), Current perspectives in research on phytoalexins, in: Biochemical Aspects of Plant-Parasite Relationships (J. Friend and D. R. Threlfall, eds.). Academic Press, London, pp. 207–223.

Dickinson C. H. (1982), Plant pathology and plant pathogens, in: Basic Microbiology, **Vol. 6** (J. F. Wilkinson, ed.). Blackwell Scientific Publications, Oxford.

Erdtman, H., Eriksson G. and Norin T. (1963), Aucuparin and methoxyaucuparin, two phenolic biphenyl derivatives from the heartwood of *Sorbus aucuparia* (L.). Acta Chem. Scand. **17,** 1151–1156.

Ghaouth A. E., Arul J., Grenier J. and Asselin A. (1991), Glucanohydrolases and inhibitory activity to *Botrytis cinerea* in extracts from strawberry fruit. Can. J. Plant Pathol. **13,** 315–320.

Grayer R. and Harborne J. B. (1994), A survey of antifungal compounds in higher plants 1982–1993. Phytochemistry, in press.

Hain R., Reif H. J., Krause E., Langebartels R., Kindl H., Vornam B., Wiese W., Schmelzer E., Schreier P. H., Stocker R. H. and Stenzel K. (1993), Disease resistance results from foreign phytoalexin expression in a novel plant. Nature **361,** 153–156.

Harborne J. B. (1987), Natural fungitoxins, in: Biologically Active Natural Products (K. Hostettman and P. J. Lea, eds.). Ann. Proc. Phytochem. Soc. Eur. Clarendon Press, Oxford, pp. 195–211.

Harborne J. B. and Turner B. L. (1984), Plant Chemosystematics. Academic Press, London.

Hashidoko Y., Tahara S. and Mizutani J. (1989), Antimicrobial sesquiterpene from damaged *Rosa rugosa* leaves. Phytochemistry **28,** 425–430.

Hunter L. D. (1971), Is sieboldin a factor in apple scab resistance? Rep. E. Malling Res. Stn. for 1970, pp. 134–135.

Ingham J. (1981), Phytoalexin induction and its taxonomic significance in the Leguminosae, in: Advances in Legume Systematics (R. M. Polhill and P. H. Raven, eds.). HMSO, London, pp. 599–626.

Keen N. T., Yoshikawa M. and Wang M. C. (1983), Phytoalexin elicitor activity of carbohydrates from *Phytophthora megaspora* f. sp. *glycinea* and other sources. Plant Physiol. **71,** 466–471.

Kemp M. S. and Burden R. S. (1984), Isolation and structure determination of γ-pyrufuran, a third induced antifungal dibenzofuran from the wood of *Pyrus communis* L. infected with *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar. J. Chem. Soc. Perkin Trans. I, 1441–1443.

Kemp M. S., Holloway P. J. and Burden R. S. (1985), A pentacyclic triterpene induced in wood of *Malus pumila* infected with *Chondrostereum purpureum.* J. Chem. Res., 154–155.

Kokubun T., Harborne J. B. and Eagles J. (1994), 2′,6′-Dihydroxy-4′-methoxyacetophenone, a phytoalexin from the roots of *Sanguisorba minor.* Phytochemistry **35,** 331–333.

Miyakado M., Watanabe K., Ohno N., Nonaka F. and Morita A. (1985), Isolation and structural determination of eriobofuran, a new dibenzofuran phytoalexin from leaves of loquat, *Eriobotrya japonica* L. J. Pestic. Sci. **10,** 101–106.

Niemann G. J. (1993), The anthranilamide phytoalexins of the Caryophyllaceae and related compounds. Phytochemistry **34,** 319–328.

Oydvin J. and Richardson D. G. (1987), A paper chromatographic survey of the phenol content of apple peel from scab-resistant and scab-susceptible genotypes. Nowr. J. Agric. Sci. **1,** 7–14.

Scalbert A. (1991), Antimicrobial properties of tannins. Phytochemistry **30,** 3875–3883.

Sierotzki H. and Gessler C. (1993), Flavan-3-ol content and the resistance of *Malus* × *domestica* to *Venturia inaequalis* (Cke) Wint. Physiol. Mol. Plant Pathol. **42,** 291–297.

Treutter D. and Feucht W. (1990a), Accumulation of flavan-3-ols in fungus-infected leaves of Rosaceae. Z. Pflanzenkr. Pflanzenschutz **97,** 634–641.

Treutter D. and Feucht W. (1990b), The pattern of flavan-3-ols in relation to scab resistance of apple cultivars. J. Hortic. Sci. **65,** 511–517.

Valsangiacomo C. and Gessler C. (1992), Purification and characterization of an exopolygalacturonase produced by *Venturia inaequalis,* the causal agent of apple scab. Physiol. Mol. Plant Pathol. **40,** 63–77.

VanEtten H. D., Matthews D. E. and Mathews P. S. (1989), Phytoalexin detoxification: importance for pathogenicity and practical applications. Annu. Rev. Phytopath. **27,** 143–164.

Watanabe K., Widyastuti S. M. and Nonaka F. (1990). Two biphenyl compounds from *Rhaphiolepsis umbellata* as its phytoalexins. Agric. Biol. Chem. **54,** 1861–1862.

Widyastuti S. M., Nonaka F., Watanabe K., Sako N. and Tanaka K. (1992), Isolation and characterization of two aucuparin-related phytoalexins from *Photinia glabra.* Ann. Phytopath. Soc. Japan **58,** 228–233.