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PHENOLIC COMPOUNDS OF THE GENUS *PYRUS**

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Abstract—The progeny from intraspecific crosses made within *Pyrus betulaefolia, P pashia, P elaeagrifolia, P amygdaliformis, P communis, P calleryana, P fauriei and P ussuriensis* have been screened for disease resistance (chiefly fireblight, *Erwinia amylovora* (Burr) Winslow *et al*, but some for the woolly pear aphid, *Eriosoma pyricola* Baker and Davidson, and crown gall, *Agrobacterium tumefaciens* (E F Smith and Town, Con.) and the presence of phenolics In some instances the presence of certain phenolics appears to coincide with disease resistance but the overall conclusion is that there is no actual connection between phenolics and disease resistance

INTRODUCTION

THE RELATIONSHIP between phenolic compounds and disease resistance in plants has long been the subject of controversy, perpetuated by many mutually inconsistent results, for the background to the subject reference is made to a number of recent reviews [1–6] Many reports of relationships between phenolics and disease resistance come to grief, not upon the manner of obtaining the experimental data, but upon the unjustified generalizations based on a few particular instances. In nature there exists a balance between host plant and parasite and a particular plant is referred to as either 'resistant' or 'susceptible' depending upon the state of this balance The term 'immune' is reserved for resistance in an absolute sense Many workers have failed to realize that disease resistance is normally the prevailing condition in the plant kingdom; that marked disease susceptibility is an abnormal condition perpetuated largely by cultivation and that the object of investigation should be the susceptible individual as viewed against a general background of resistance. In addition, many workers persist in the search for single factors which might be responsible for resistance or susceptibility when it would appear more reasonable to look for differences in the overall biochemical organisation and control of the primary metabolic pathways

In the case of fireblight it should be made clear that this disease evolved independently of the pear species The pathogen originated in North America, where no pear species occur naturally, thus resistance in *Pyrus* is fortuitous and the mode of resistance might be different

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[1] J B PRIDHAM (editor), *Phenolics in Plants in Health and Disease* Pergamon Press, Oxford (1960)
[2] J KUĆ, in *Perspectives of Biochemical Plant Pathology* (edited by S Rich), p 20, The Connecticut Agricultural Experimental Station Bulletin No 663 (1963)
[3] I A M CRUICKSHANK and DAWN R PERRIN, in *Biochemistry of Phenolic Compounds* (edited by J B Harborne), Chap 13, Academic Press, New York (1964)
[4] R N GOODMAN, Z KIRÁLY and M ZAITLIN, *The Biochemistry and Physiology of Infectious Plant Disease* Van Nostrand, Amsterdam (1967)
[5] J B HARBORNE, *Comparative Biochemistry of the Flavonoids*, Chap 9, Academic Press, New York (1967)
[6] TSUNE KOSUGE, *Ann Rev Phytopathol* 195 (1969)

among different species [7,8] In its native state, the pathogen lives on the hawthorn which belongs to the same subfamily as *Pyrus*, i.e. *Pomoideae*.

If in a particular instance a correlation has been found between the presence of a phenolic compound and resistance to a plant pathogen, this could be explained as follows: (a) a cause and effect relationship, i e the presence of the phenolic somehow influences the metabolic processes involved during host-parasite interaction, or (b) the gene(s) which are responsible for disease resistance are also responsible for the appearance of the phenolic compound which is itself irrelevant to the disease resistance, or (c) the gene(s) responsible for disease resistance reside upon the same chromosome as the gene(s) responsible for the appearance of the phenolic compound, or (d) the two respective sets of genes reside upon separate chromosomes and are capable of segregation by the appropriate breeding methods

It is the purpose of the present publication to examine the phenolics of individuals originating from separate seedlings derived from the same controlled intra-specific crosses In the progeny from these crosses, segregation of the genes for resistance and susceptibility for certain diseases has been achieved and examination of these individuals should be of value in deciding if there are correlations between phenolics and disease resistance without the complication of comparing resistant plants of one species with susceptible ones of a different species

RESULTS

The progeny from intra-specific crosses obtained within eight different *Pyrus* species were examined for leaf phenolics and in a few selected instances the young bark phenolics were also examined, Tables 1–5 summarize the results of the leaf phenolics survey for *P betulaefolia P. pashia, P. elaeagrifolia, P amygdaliformis* and *P communis* in which some differences were found Table 6 gives the results of a survey of the phenolics of the young

TABLE 1 OCCURRENCE OF PHENOLICS IN THE LEAVES OF *P betulaefolia* SEEDLINGS SELECTED FOR SUSCEPTIBILITY AND RESISTANCE TO FIREBLIGHT

Phenolics showing variation between seedlings	Fireblight susceptible*							Fireblight resistant†				
	1	2	3	4	5	6	7	8	9	10	11	12
Quercetin 3-triglycosides	+	0	0	(+)	0	0	++	+	+	+	+	+
Epicatechin	0	0	0	0	0	0	++	+(+)	++	+(+)	+(+)	++
Isochlorogenic acid	0	0	0	0	0	0	t	+	++	+	+	+
Chlorogenic acid	++	++	++	++	++	++	t	++	++	++	++	++
Neochlorogenic acid	0	0	0	0	0	0	t	t	t	t	t	t
Caffeoylcalleryanin	++	+	+	++	(+)	++	0	0	0	0	0	0
p-Coumaroylcalleryanin?	t	0	0	+	0	t	0	0	0	0	0	0
p-Coumaroylarbutin	+(+)	+(+)	+(+)	+	+(+)	+(+)	0	0	0	0	0	0
Acetylarbutin	++	++	++	++	++	++	0	(+)	+	+	(+)	(+)

Phenolics present in all seedlings quercetin 3-monoglycosides ++, quercetin 3-diglycosides ++, *p*-coumaroylquinic acid t, arbutin + → ++, hydroquinone ++, caffeoylarbutin t → +

Scoring code 0 = absent, t = trace amount, + = small amount, ++ = moderate amount, +++ = large amount, ( ) = reservations regarding enclosed symbol, score on low side

* Seedlings 1 → 6 Italy, seedling 7 Reimer

† Seedlings 8 → 12 Reimer

[7] F C REIMER, *Blight Resistance in Pears and Characteristics of Pear Species and Stocks* Oregon Agr Exp Sta Bull 214, 99 pp (1925)

[8] H R CAMERON, M N WESTWOOD and P B LOMBARD, *Phytopath* **59**, 1813 (1969)

TABLE 2 OCCURRENCE OF PHENOLICS IN THE LEAVES OF *P pashia* SEEDLINGS SELECTED FOR SUSCEPTIBILITY AND RESISTANCE TO FIREBLIGHT AND ROOT APHID

Phenolics showing variation between seedlings	Fireblight susceptible†					Fireblight resistant†					Root aphid‡ Sus	Root aphid‡ Res
	1	2	3	4	5	6	7	8	9	10	11	12
Apigenin 7-glucoside	0	0	0	0	0	+	0	+	+	0	0	0
Luteolin 7-glucoside	(t)	0	t	t	(t)	+(+)	t	+	+	t	0	(t)
Luteolin 7-rhamnoglucoside	(t)	0	t	t	(t)	+	t	t	+	t	0	0
Luteolin 4′-glucoside	0	0	0	0	0	++	0	++	++	0	+	++
Apigenin 4′-glucoside	0	0	0	0	0	+(+)	0	+	++	0	+	++
Apigenin 7,4′-diglucoside?	0	0	0	0	0	+	0	t	+	0	t	++
Chrysoeriol 7-glucoside	0	0	0	t	t	0	(t)	0	0	0	0	0
Quercetin 3-monoglycosides	++	++	++	++	+(+)	+	+(+)	0	0	+(+)	0	++
Quercetin 3-diglycosides	++	+	++	++	++	(+)	+(+)	0	+	++	0	+(+)
Epicatachin	0	0	0	0	0	0	0	0	0	0	0	0
Catechin	0	0	0	0	0	0	0	0	0	0	0	++
Caffeoylcalleryanin	+(+)	0	+	t	0	++	t	t	++	0	0	+(+)
Caffeoylarbutin	++	++	++	++	++	+	++	+	t	++	t	++(+)
p-Coumaroylarbutin	++	++	++	++	++	0	++	0	0	++	0	0
Arbutin	++	++	++	t	++	+	+	+	++	t	t	+++
Acetylarbutin	0	0	0	0	0	+(+)	0	++	+ +	0	++	++

Phenolics present in all seedlings isochlorogenic acid ++ → +++, chlorogenic acid ++, neochlorogenic acid t, *p*-coumaroylquinic acid t, unidentified cinnamic acid deriv +, hydroquinone ++

Scoring code see Table 1

† Seedlings 1 → 10 QZ

‡ Seedlings 11 → 12 283

bark, buds and leaves of five *P. communis* cultivars, four of which are reported to be susceptible and one resistant to fireblight Most of the specimens examined for phenolics have already been screened for resistance to the woolly pear aphid (root aphid),[9] the pear psylla[10] and fireblight.[8]

P. betulaefolia

For this species, not all of the progeny came from the same intra specific cross; all of the fireblight resistant seedlings (Nos 8–12) and only one of the fireblight susceptible seedlings (No 7) originated from one cross (Reimer) whilst the remaining susceptible seedlings (Nos 1–6) came from a separate cross (Italy) It is clear that there is no sharp differentiation between resistant and susceptible races on the basis of leaf phenolics It does seem, however, that the presence of caffeoylcalleryanin and *p*-coumaroylarbutin is characteristic of the Italy progeny whilst the presence of epicatechin, isochlorogenic acid and neochlorogenic acid is characteristic of the Reimer progeny. The fact that these two 'chemical races' almost

[9] M. N WESTWOOD and P H WESTIGARD, *Proc. Am Soc Hort Sci* **94**, 91 (1969)

[10] P H WESTIGARD, M N WESTWOOD and P B LOMBARD, *J Am Soc. Hort Sci* **95**, 34 (1970)

Table 3 Occurrence of phenolics in the leaves of *P elaeagrifolia* seedlings selected for susceptibility and resistance to fireblight

Phenolics showing variation between seedlings	Fireblight susceptible†			Fireblight resistant†		
	1	2	3	4	5	6
Quercetin 3-triglycosides	0	0	0	+(+)	+(+)	+(+)
Catechin	0	0	(t)	+	+	0
Isochlorogenic acid	t	t	t	+	+	+
Caffeoylarbutin	0	0	0	t	t	t
Arbutin	(+)	+	++	t	++	++
Acetylarbutin	t	t	++	++	+(+)	+

Phenolics present in all seedlings quercetin 3-monoglycosides +, quercetin 3-diglycosides ++, chlorogenic acid +(+), neochlorogenic acid t, *p*-coumaroylquinic acid t, 4-allylphenol? (+), hydroquinone ++

Scoring code see Table 1

† Seedlings 1 → 6 Olez II

coincide with the fireblight resistant and susceptible races would seem to be merely a chance association

P. pashia

Of ten seedlings from the same cross (QZ) one half were fireblight susceptible and the other half fireblight resistant; here the flavone glycosides are more in evidence in the resistant than the susceptible seedlings and acetylarbutin, although absent from all susceptible seedlings, is present in three of the five resistant seedlings Examination of the young bark phenolics of one susceptible and one resistant seedling showed that the leaf differences tended to be paralleled in the bark A separate intraspecific cross (283) provided two seedlings, one susceptible and the other resistant to root aphid, here the differences in leaf phenolics were quite marked, with a much higher concentration of phenolics in the resistant seedling

Table 4 Occurrence of phenolics in the leaves of *P amygdaliformis* seedlings selected for susceptibility and resistance to fireblight

Phenolics showing variation between seedlings	Fireblight susceptible†				Fireblight resistant†		
	1	2	3	4	5	6	7
Isochlorogenic acid	++	++	+(+)	+	++	++	t
Caffeoylarbutin	0	0	t	t	+(+)	++	t
Arbutin	+	++	t	++	t	++	t

Phenolics present in all seedlings quercetin 3-monoglycosides +(+) → ++, quercetin 3-diglycosides +(+) → ++, chlorogenic acid ++, neochlorogenic acid t, *p*-coumaroylquinic acid t, acetylarbutin ++, 4-allylphenol? (+), hydroquinone ++

Scoring code see Table 1.

† Seedlings 1 → 7 Greece

TABLE 5 OCCURRENCE OF PHENOLICS IN THE LEAVES OF *P communis* SEEDLINGS† SELECTED FOR SUSCEPTIBILITY AND RESISTANCE TO FIREBLIGHT, ROOT APHID AND LEAF GALL

Phenolics showing variation between seedlings	Fireblight			Fireblight						Aphid and gall	Aphid	Gall
	Sus	Res		Sus.		Res				Sus	Res	Res
	1	2	3	4	5	6	7	8	9	10	11	12
Quercetin 3-diglycosides	+++	++	+(+)	+(+)	++	++	++	++	++	++	+	+++
Epicatechin	(t)	0	+	0	0	0	0	0	+	+(+)	+	t
Catechin	0	0	+	0	0	0	0	0	+	+	+	+
Isochlorogenic acid	++	+	++	+(+)	+	+(+)	++	+	+	++	++	++
Caffeoylarbutin	+	t	t	+(+)	+	+	++	+(+)	(+)	+	t	+++

Phenolics present in all seedlings quercetin 3-monoglycosides + → ++, chlorogenic acid ++ → +++ neochlorogenic acid t, *p*-coumaroylquinic acid t, arbutin ++, hydroquinone ++, acetylarbutin ++

Scoring code see Table 1

† Seedlings 1 → 3. OHXF 1, 117 and 226 respectively, Seedlings 4 → 9 Olez V, Seedlings 10 → 12 OPR 1, OPR 70 and Olez III respectively

P. elaeagrifolia

Three fireblight susceptible and three fireblight resistant seedlings were obtained from the one cross (Olez II) and here quercetin 3-triglycosides were found only in the resistant seedlings Otherwise the variation in phenolics did not appear to be connected with resistance or susceptibility.

P. amygdaliformis

Four fireblight susceptible and three fireblight resistant seedlings were obtained from the one cross (Greece) and here the variation in phenolics did not appear to be connected with resistance or susceptibility. Examination of the young bark led to the same conclusion

TABLE 6 OCCURRENCE OF PHENOLICS IN BARK, BUDS AND LEAVES OF *P communis* CULTIVARS

Phenolics showing variation between cultivars	Fireblight susceptible				Fireblight resistant			
	Williams†				Old home			
	Bark March	Buds March	Leaves June	Leaves Oct	Bark March	Buds March	Leaves June	Leaves Oct.
Quercetin 3-monoglycosides	0	t	++	++	+	+	++	++
Quercetin 3-diglycosides	t	++	+++	+++	+	++	+++	++(+)
Epicatechin	++	++	++	++	++	++	++	++
Catechin	+++	++	++	+(+)	++	++	+	+
Isochlorogenic acid	+	++(+)	++	+++	++	++	++	++(+)
Caffeoylcalleryanin	t	0	0	+	++	0	0	t
Caffeoylarbutin	0	0	t	+	0	0	t	++
Chlorogenic acid	++	++	+++	++(+)	++	++	+++	++
Arbutin	++	++	++(+)	++	++	++	+++	++
Acetylarbutin	t	+	+(+)	+(+)	t	++	++	+(+)
Hydroquinone	+	++	0	0	+	++	+	0

Phenolics present in all cultivars neochlorogenic acid t, *p*-coumaroylquinic acid t

Scoring code see Table 1

† Very similar results were obtained with Conference, Bristol Cross and Comice

Examination of both leaf buds and flower buds, sampled simultaneously from the same tree of Williams and Conference showed no significant differences in phenolics content

P. communis

Two fireblight susceptible and four fireblight resistant seedlings were obtained from one cross (Olez V) and the variation in leaf and young bark phenolics appeared to be unconnected with susceptibility or resistance. Further intraspecific crosses gave seedlings, one susceptible to both root aphid and crown gall, one resistant to root aphid and the third to crown gall, there was no relationship between phenolics and root aphid resistance but there was much more caffeoylarbutin in the gall resistant than in the gall susceptible seedling.

P. communis cultivars

The buds, young bark (i.e. white phloem + green cortex + cork) and leaves of five *P. communis* cultivars: Williams, Conference, Bristol Cross (i.e. Williams × Conference) and Comice (all susceptible to fireblight) and Old Home (resistant to fireblight) were analyzed for phenolics. No obvious correlation could be found between bud or leaf phenolics on the one hand and susceptibility or resistance to fireblight. However, in the young bark, caffeoylcalleryanin was found in substantial amount in Old Home, somewhat less in Bristol Cross, even less in Conference and only trace amounts in Comice and Williams. All other bark phenolics appeared to be completely uncorrelated with fireblight resistance or susceptibility. It is of interest to note that caffeoylcalleryanin could not be found in any of the June leaf samplings; traces of this phenolic appearing only in the October leaf samplings. Caffeoylarbutin was found in trace amounts in all June leaf samplings and showed substantial increase in concentration in October only in the cases of Bristol Cross and Old Home. The quercetin 3-mono and diglycoside complexes tended to be present in greater amounts in the leaves than in the young bark and buds whilst catechin, epicatechin, isochlorogenic, chlorogenic and *p*-coumaroylquinic acids and arbutin appeared to occur uniformly throughout all tissues. The quercetin 3-diglycoside complex appeared to be absent from both leaf samplings of Comice but present in the other four cultivars. In the case of Williams and Conference, examination of both leaf and flower buds from the same tree at the same time showed no significant differences in phenolics.

P. calleryana

Of eight seedlings from the same cross (SN) six were found to be fireblight susceptible and two fireblight resistant. Two different crosses (CP 5-69 and CP 11-51) gave two individuals respectively susceptible and resistant to root aphid. All ten seedlings proved to be identical in their leaf phenolics and examination of the young bark of one fireblight susceptible seedling and one fireblight resistant seedling also showed no differences in phenolics.

P. fauriei

Two seedlings, one resistant and the other susceptible to root aphid, obtained from the same cross (1), showed no differences in their leaf and young bark phenolics.

P. ussuriensis

Two seedlings, one resistant and the other susceptible to root aphid, obtained from the same cross (300) showed no differences in their leaf phenolics. However, examination of the young barks showed that the level of caffeoylarbutin was noticeably higher in the susceptible seedling.

DISCUSSION

The overall impression from the results is that there is no direct connection between phenolic constituents of *Pyrus* and disease resistance It is possible that the selective presence of certain phenolics in either resistant or susceptible plants might be a reflection of some modifications in the primary metabolic pathways which would in turn be connected with resistance/susceptibility, even this appears doubtful however The most reasonable conclusion is that any apparent connection is purely fortuitous The results do indicate, however, that under certain circumstances the occurrence of phenolics might be used to differentiate between resistant and susceptible individuals and to make probable predictions as regards the resistance or susceptibility of untested individuals The fact that the presence of phenolics is irrelevant to the susceptibility or resistance of plants to disease is in itself of considerable significance for evolutionary and taxonomic studies, if certain phenolic constituents are more or less immune from the effects of direct natural selection then they can be relied upon to reflect evolutionary and phenetic relationships in a fairly unprejudiced manner. In a preliminary computer-generated classification of the genus *Pyrus*,[11] *P regelii*, *P. calleryana*, *P Koehnei* and *P. dimorphophylla* separate off from the rest of the genus on the basis of overall phenetic resemblance *P. regelii* is the only *Pyrus* species which has lobed adult leaves and the remaining three species are highly distinctive in a chemical sense[12–14] by their possession of high concentrations of C_6–C_1 phenolic acids Since lobed leaves, under certain circumstances, are considered by taxonomists to be primitive,[15] it is tempting to so describe the accumulation of C_6–C_1 phenolic acids It is of interest to note here that whilst *P. Koehnei* and *P. dimorphophylla* have lobed juvenile leaves, *P calleryana* does not.[16] Rubtsov's report[17] that the juvenile leaves of all pear species are lobed is apparently in error.

EXPERIMENTAL

Pyrus *material* Most specimens were obtained from Oregon State University, as budding wood and propagated at Long Ashton in the spring of 1970 The following were received as leaf samples during July 1970, direct from Oregon *Pyrus betulaefolia* (from Reimer Cross) fireblight susceptible 7 and *P calleryana* (CP5-69 cross) aphid susceptible The 5 *P communis* cultivars were mature trees growing at Long Ashton The Reimer type of *P betulaefolia* was from seeds collected in North China The Italy seedlings were from seed grown in semi-isolation in Bologna, Italy The SN *P calleryana* were seedlings grown from seed produced in isolation at Winchester, Tennessee All other species were from seeds grown on wild trees in their native habitats *P pashia* (India), *P fauriei* (Korea), *P amygdaliformis* (Greece), *P ussuriensis* (Manchuria), *P elaeagrifolia* (Turkey) and *P communis* (Turkey) The seedlings are authentic as described for each species and thus are intraspecific crosses

Sampling 2 g of leaf from each specimen (sampled in June 1970) was extracted with 10 ml EtOH by brief boiling followed by disintegration The young bark of a limited range of specimens (sampled in Jan and July 1971) was extracted in a similar manner.

Paper chromatography 0 25 ml of the EtOH extracts (representing 50 mg tissue) was spotted onto large paper sheets for two-dimensional paper chromatography as described in previous papers [12–14]

Detection and identification of phenolics By *UV* inspection, diazonium and Gibbs reagents as previously described [12–14] The intensities of the chromatogram spots were visually graded as follows 0 = absent, t = trace amount only (diazo/Gibbs colour only just visible), + = small amount (weak but noticeable diazo/Gibbs colour), ++ = moderate amount (strong diazo/Gibbs colour), +++ = large amount (exceptionally strong diazo/Gibbs colour), ( ) = reservations regarding enclosed symbol, score on low side

[11] J S Challice, *Rep Long Ashton Res Stn for 1970*
[12] J S Challice and A H Williams, *Phytochem* **7**, 119 (1968)
[13] J S Challice and A H Williams, *Phytochem* **7**, 1781 (1968)
[14] J S Challice and A H Williams, *Phytochem* **9**, 1271 (1970)
[15] A L Takhtajan, *Essays on the Evolutionary Morphology of Plants* Leningrad 1954 Trans by the Amer. Inst. Biol Sciences, Washington (1959)
[16] M N Westwood, unpublished data
[17] G A Rubtsov, *Dok S S S R* **30**, 79 (1941)

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