Paper Chromatographic Determination of Phenolic Compounds Occurring in the Leaf, Bark and Root of *Prunus avium* and *P. mahaleb*[1]

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Abstract. Methanol extracts of fresh tissues of 'Mazzard' (*Prunus avium* L.) and 'Mahaleb' (*P. mahaleb* L.) were examined for phenolic composition. The 2 sweet cherry rootstocks differed in 3 phenolic groups: phenolic acids, coumarins, and flavonoids. 'Mazzard' contained 5 acids; p-coumaric, o-coumaric, caffeic, p-coumarylquinic and chlorogenic, whereas 'Mahaleb' contained mostly o-coumaric acid. 'Mahaleb' tissues were rich in coumarin and herniarin, but these were absent in 'Mazzard'. Three flavonoids; dihydrowogonin, kaempferol and quercetin, were found in 'Mazzard'. 'Mahaleb' contained only kaempferol. These differences in phenolic composition between the 2 rootstocks seemed related to graft-incompatibility.

'Mazzard' and 'Mahaleb' are used as rootstocks for commercial sweet cherry. Sweet cherry cultivars budded on 'Mazzard' seedlings do not show any symptoms of graft-incompatibility; however, those budded on 'Mahaleb' tend to do so in the 4th to 6th year (14, 28).

Graft-incompatibilities have been recognized in other commercial fruit crops (23). Several groups of compounds have been suggested as the causal agents of abnormal graft unions; cyanogenic glucosides (15), alkaloids (22), proteins (5), amino acids (35) and phenolic compounds (3, 15, 33).

Phenolic compounds are ubiquitous and yet specific in higher plants. Many phenolic compounds are toxic and their inhibitory roles have been shown in germination, shoot and root growth (7, 16, 25). The compounds also have been implicated in growth regulation via the IAA oxidase system (10). Mentzer et al. (21) found 12 flavonoid compounds in ether extracts of wild cherry heartwood. Chopin et al. (4) identified dihydrowogonin from cherry heartwood extract. Bate-Smith (2), in discussion of the taxonomic significance of phenolic compounds, noted differences in leaf phenolic compositions of 2 rootstocks.

This study was initiated to determine if the phenolic compounds in 'Mazzard' and 'Mahaleb' were the same, or if different, how they may influence graft behavior.

Materials and Methods

Greenhouse grown 'Mazzard' and 'Mahaleb' seedlings (3 years old) were harvested, separated into leaves, stem bark, and root bark and were frozen in liquid N until analysis. Samples of each plant part (100 g) were macerated in absolute methanol (400 ml) and further extracted with 5 x 200 ml absolute methanol. Aliquots of extracts (15 g.f.w.) were hydrolysed with 1 N HCl on a boiling water bath. The hydrolysates dissolved in methanol (5 ml) were used for paper and thin-layer chromatography.

Two-dimensional paper chromatography was used to separate phenolic compounds. Fifty microliters (150 mg f.w.) of extract was spotted on the upper left hand corner of Whatman No. 1 filter paper (46 x 57 cm) for chromatography. The first solvent was n-butanol: acetic acid:water, 6:1:2 (BAW), which filtered the long direction of the paper for 17 hr at room temperature. The air-dried paper was run for 4 hr in 2% acetic acid (HOAc) in the short direction. The air-dried chromatograms were examined under ultraviolet light before and after exposure to ammonia vapor. Replicate chromatograms were treated with 6 different reagents; $FeCl_3$-$K_3Fe(CN)_6$, diazotized p-nitroaniline, Hoepfner's reagent, 2 N NaOH plus DPNA, $NaBH_4$-HCl and vanilin-HCl (27, 31).

In order to obtain pure phenolic compounds, band application was used. The crude methanol extract was applied as a line on Whatman No. 3 filter paper. The chromatograms were developed with 2% HOAc and examined under ultraviolet radiation (UVSL-25). The distinctive color bands were marked, cut out, and eluted by shaking with 3 x 100 ml 95% ethanol. After vacuum evaporation, the residue was rechromatographed 2-dimensionally on Whatman No. 1 filter paper in BAW (1st direction) and followed by 2% acetic acid (2nd direction). The spots which reacted with $FeCl_3$-$K_3Fe(CN)_6$ on parallel chromatograms were cut out and eluted with 95% ethanol (3 x 100 ml). The eluates were used for determinations of Rf values by comparison to standard phenolic compounds in 3 different solvents; BAW, 2% HOAc, and butanol:pyridine:water, 10:3:3.

Five ul aliquots of the purified compounds were also spotted on Eastman thin-layer plates (Silcagel G) and run in 1 direction in the following solvent systems: benzene:methanol:acetic acid, 45:8:4 (BMA), 11% methanol in $CHCl_3$ and toluene:ethylacetate:formic acid, 5:4:1 (TEF).

Absorption spectra of the purified compounds in 95% ethanol were determined on a Beckman DB spectrophotometer.

Visual estimation of the relative amounts of phenolic compounds was made. The scale was based on the area and intensity of the individual spots on the 2-dimensional paper chromatogram after it was sprayed with $FeCl_3$-$K_3Fe(CN)_6$. For example, a spot of o-coumaric acid of 'Mazzard' leaf extract was arbitrarily assigned a value of 5 and other spots were rated 1 to 12 in relation to the size of the 'Mazzard' leaf o-coumaric acid spot (Fig. 1).

Results

1. *Leaf phenolic compounds.* Paper chromatograms of hydrolysed extracts from 'Mazzard' and 'Mahaleb' leaves showed presence of phenolics. Most of these compounds moved rapidly in BAW (6:1:2), suggesting that they were aglycones (Fig. 1, top). Fourteen spots were distinguishable on chromatograms of 'Mazzard' leaf extracts after spraying with $FeCl_3$-$K_3Fe(CN)_6$ reagent. In 'Mahaleb' extracts, 7 spots were noted. All compounds were colorless under visible light; however, some fluoresced under ultraviolet light (Table 1).

Chromatogram spot characteristics:

Spot 1: This compound, occurring in both species, appeared bright white under ultraviolet light. In the presence of ammonia, it fluoresced bright yellow. The compound reacted with diazotized p-nitro aniline to form a purple color, and turned a bright yellow on treatment with Hoepfner reagent. The Rf values in both thin layer and paper chromatography were comparable to those of standard o-coumaric acid. The absorption spectrum was similar to that of standard o-coumaric acid. The compound 1 was tentatively identified as o-coumaric acid (Tables 1, 2, 3).

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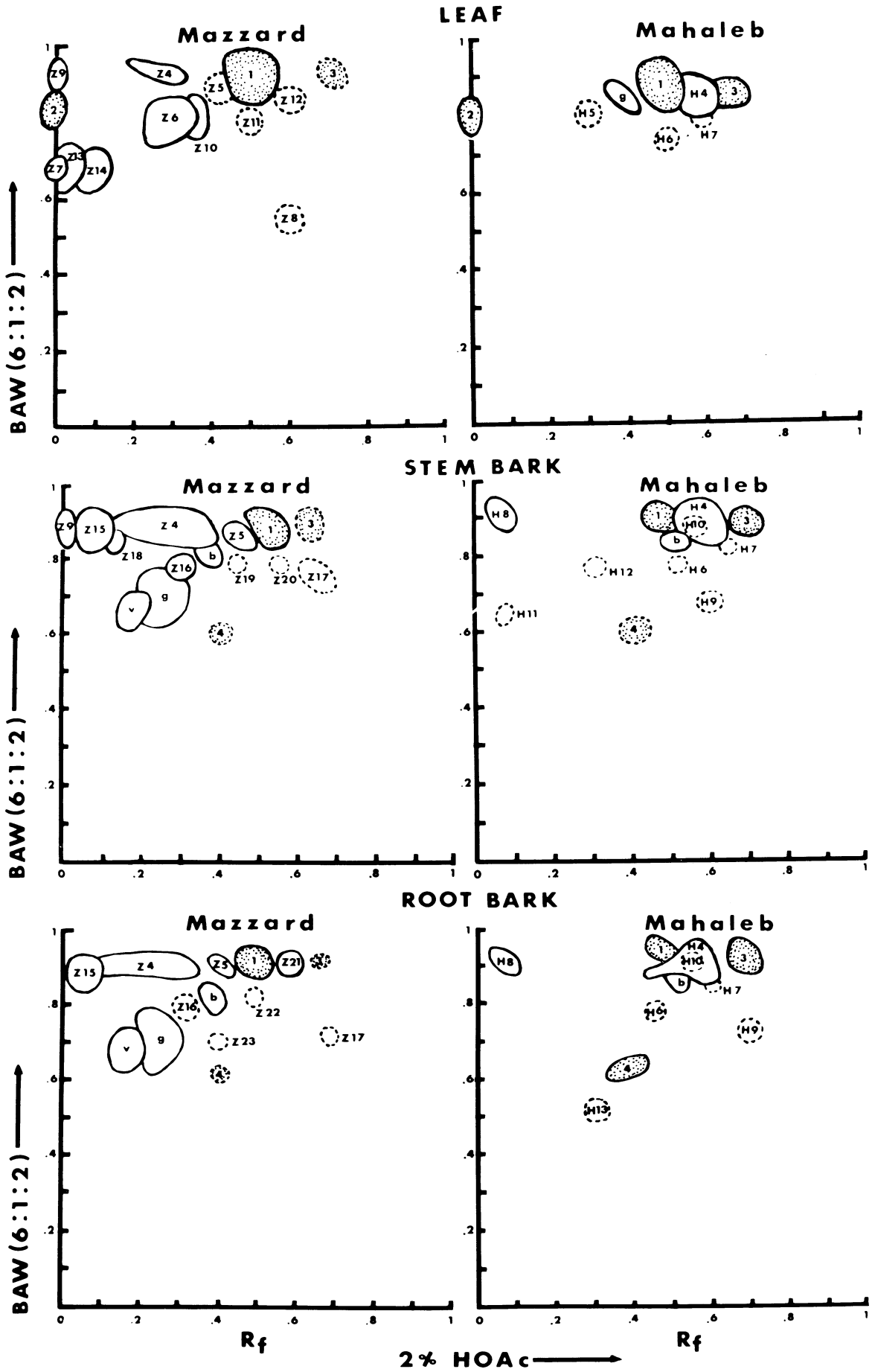

Fig. 1. Paper chromatograms of hydrolysed phenolic compounds extracted from (top) leaves, (center) stem bark, and (bottom) root bark of 'Mazzard' and 'Mahaleb' seedlings. Spots with solid lines were more intense than those with broken lines, and the shaded spots were common to both species. Those prefixed with Z or H were restricted to 'Mazzard' and 'Mahaleb' respectively.

Table 1. Color reactions of phenolic compounds extracted from Mazzard and Mahaleb leaves, hydrolysed and purified by paper chromatography. Color characteristics were compared with those of authentic compounds.[z]

Chromatogram spot no.	Tentative compound identification	UV light			Visible light			
			$+NH_3$	2N NaOH	DPNA	$FeCl_3$ $K_3Fe(CN)_6$	2N NaOH DPNA	Hoepfner
Mazzard leaves								
1	o-coumaric acid	stW	stYfl	stYGfl	Y-Pu	B	Pu	stY
2	kaempferol	Y	O	Yfl	Y	B	Y	lBr
3	coumarin	(D)	(D)	YGfl	lPu	B	Pu	pY
Z4	dihydrowogonin	pD	pD	lY	stY	B	lY	C
Z5	p-coumaric acid	C	stV	V	lY-Gray	lB	C	C
Z6	caffeic acid	B	B	W	lT-lBr	B	lBr	stT
Z7	quercetin	Y	O	Yfl	O	B	!Y	lY
Z8	chlorogenic acid	B	G	G	fBr	B	lBr	Y
Z9	unknown	pBr	pBr	pBr	Y	B	pBr	lBr
Z10	unknown	B	C	C	lBr-lY	pB	C	C
Z11	unknown	V	C	C	C	B	C	Y
Z12	unknown	B	B	C	C	B	C	C
Z13	unknown	pB	G	lW	C	B	C	lY
Z14	unknown	pB	G	lW	C	B	pY	lY
Mahaleb leaves								
1	o-coumaric acid	stW	stYfl	stYGfl	y-Pu	B	C	y
2	Kaempferol	Y	O	stY	Y	B	Y	C
3	Coumarin	(D)	(D)	stYGfl	lPu	B	lR-V	C
H4	Herniarin	V	V	stBfl	Y-Gray	C	stV	C
H5	unknown	B	B	C	lPu	B	C	C
H6	unknown	C	C	C	C	B	C	pY
H7	unknown	C	C	C	C	B	C	C
Reference compounds								
	o-coumaric	W	GY	stYGfl	Pu	B	Pu	Y
	kaempferol	Y	stY	Yfl	Y	B	Y	G
	coumarin	(D)	(D)	stYGfl	lPu	C	Pu	C
	dihydrowogonin	C	C	lY	stY	B	lY	C
	p-coumaric acid	C	stV	stV	Y-lGray	B	lV	stY
	caffeic acid	B	B	fwB	lT-lBr	B	lBr	T-Br
	quercetin	Y	Y	Yfl	10	B	fY	lY
	chlorogenic acid	B	G	fG	lT-Br	B	lBr	stO
	herniarin	V	V	stBfl	C	C	stV	C
	ferulic acid	B	B	stB	P	B	T-lB	Y

[z] Key: B = Blue; Br = Brown; C = Colorless; D = Dark; G = Green; O = Orange; Pu = Purple; R = Red; T = Tan; Y = Yellow; W = White; p = Pale; l = light; br = bright; st = strong; fl = fluorescence.

Spot 2: This compound occurred in leaves of both species. It appeared yellow under ultraviolet light, changing to orange on fuming with ammonia. It moved rapidly in BAW (Rf .80) but not in 2% acetic acid (Rf .00). This is characteristic of planar flavonoid aglycones (flavone and flavonol) (27). Its Rf values and ultraviolet absorption spectrum were similar to those of kaempferol and tentatively identified as kaempferol.

Spot 3: Compound 3 also was common to both leaf tissues, but the concentration of the compound was higher in 'Mahaleb' than in 'Mazzard' (Table 4). It was colorless under long and dark under short ultraviolet light, with or without ammonia. When sprayed with 2N NaOH, it fluoresced a bright yellow-green, a typical color reaction of coumarin. Subsequent treatment with DPNA produced the characteristic purple color obtained with coumarin (32). Rf values and spectral characteristics were similar to those of coumarin. The compound was identified as coumarin (Table 4).

Spot Z4: Compound Z4 occurred only in 'Mazzard' leaf extracts and was colorless under ultraviolet light with or without ammonia. A distinctive yellow color was obtained when the compound was sprayed with diazotized p-nitro aniline reagent. The Rf values of the compound in both thin layer and paper chromatography were comparable to those of dihydrowogonin, and therefore, Z4 was tentatively identified as dihydrowogonin.

Spot Z5: This compound found only in 'Mazzard' extracts showed no color under ultraviolet light in the absence of ammonia, but fluoresced bright violet in the presence of ammonia. Although Rf values were comparable with those of standard p-coumaric acid, the ultraviolet absorption spectrum differed. The compound apparently was too low in concentration to be detected spectrophotometrically.

Spot Z6: This compound, unique to 'Mazzard' tissues, fluoresced bright blue under ultraviolet with or without ammonia. With Hoepfner reagent it gave a bright tan color; with DPNA, a light tan later turning to light brown. Rf values of the compound in 6 different solvent systems were similar to those of standard caffeic acid, as were spectral characteristics with maxima at 320 and 243 and minima at 260 and 230 mμ. It was thus tentatively identified as caffeic acid.

Spot Z7: The compound occurred in 'Mazzard' and showed flavonoid characteristics in its color reactions. The compound fluoresced yellow under ultraviolet light. Its Rf values and absorption spectra agreed with those of the standard compound quercetin and so it was tentatively identified as quercetin.

Spot Z8: The material occurred only in 'Mazzard', and it fluoresced blue under ultraviolet light. When the chromatogram was fumed with ammonia, the color changed to green, which is typical of chlorogenic acid. Its spectral characteristics, however, differed from those of chlorogenic acid. The low concentration present in the hydrolyzed extract resulted in poor recovery during purification and thus the absorption spectrum was poor. Consequently, based only on the color reactions and Rf values it appeared that Z8 was chlorogenic acid.

Spot H4: This compound was distinctive in 'Mahaleb' tissue. It fluoresced bright violet under ultraviolet light, changing bright blue after spraying with 2N NaOH. Subsequent treatment with DPNA gave a bright violet color under visible light. These are typical color reactions of herniarin (7 methoxy coumarin) (32). The Rf values of the

Table 2. Rf values of phenolic compounds extracted from 'Mazzard' and 'Mahaleb' leaves, hydrolysed and separated by 2 dimensional paper chromatography. The compounds were rechromatographed together with reference compounds in 6 different solvent systems.[z]

Compound	Tentative identification	Rf Values[y]					
		Thin layer			Paper		
		BMA	M-C	TEF	BAW	2%HOAc	BPW
Mazzard leaf							
1	o-coumaric acid	.39	.50	s	.84	.59	.88
2	kaempferol	.29	.36	.21	.80	.00	.88
3	coumarin	.62	.53	.56	.88	.75	.87*
Z4	dihydrowogonin	.60	.57	.56	.86	.0–.42	.90
Z5	p-coumaric acid	.41	.44	s	.84	.51	.86*
Z6	caffeic acid	.27	.27	s	.72	.40	.78
Z7	quercetin	.12	—	—	.65	.00	—
Z8	chlorogenic acid	.02	.00	.01	.48	.65	.25
Mahaleb leaf							
1	o-coumaric acid	.43	.47	s	.84	.57	.88
2	kaempferol	.36	—	.19	.80	.00	.88
3	coumarin	.62	.48	.60	.88	.73	.87*
H4	herniarin	.62	.48	.60	.89	.61	.86*
Reference compounds							
	o-coumaric acid	.43	.45	s	.85	.61	.88
	kaempferol	.30	.37	.21	.81	.00	.87
	coumarin	.63	.54	.61	.89	.77	.87
	dihydrowogonin	.61	.57	.57	.87	.0–.42	.90
	p-coumaric acid	.42	.40	s	.85	.54	.86
	caffeic acid	.26	.29	s	.73	.41	.79
	quercetin	.16	.20	.08	.65	.00	.83
	chlorogenic acid	.02	.00	.01	.48	.71	.25
	herniarin	.63	.50	.60	.89	.64	.86
	ferulic acid	.45	.50	s	.82	.59	.78

[z] BMA (benzene:methanol:acetic acid, 45:8:4); M-C (11% methanol in $CHCl_3$); TEF (toluene:ethyl acetate:formic acid, 5:4:1); BAW (buthanol:acetic acid:water, 6:1:2); 2% HOAc (2% acetic acid); BPW (buthanol:pyridine:water, 10:3:3); s, streak.

[y] Each value is the average of 3 determinations. Some Rf* values were very close and difficult to ascertain true identification.

Table 3. Spectral characteristics of partially purified compounds from 'Mazzard' and 'Mahaleb' leaves, compared with those of reference compounds.

Compound number	Tentative identification	Ultraviolet absorption	
		max (mμ)	min (mμ)
'Mazzard' leaf			
1	o-coumaric acid	320,271	300,244
2	kaempferol	368,268	280,240
3	coumarin	276	252
Z4	dihydrowogonin	332,290	320,256
Z5	p-coumaric acid	270	248
Z6	caffeic acid	320,243	260,230
Z7	quercetin	370,256	286,240
Z8	chlorogenic acid	327	265
'Mahaleb' leaf			
1	o-coumaric acid	325,274	300,246
2	kaempferol	368,260	314,000
3	coumarin	310,275	300,244
H4	herniarin	320	260
Reference compounds			
	o-coumaric acid	325,274	300,243
	kaempferol	369,268	290,240
	coumarin	312,276	298,242
	dihydrowogonin	336,290	320,254
	p-coumaric acid	310,226	246
	caffeic acid	326,243	264,230
	quercetin	372,256	286,238
	chlorogenic acid	332,244	268
	herniarin	320	260

compound in 6 different solvent systems were comparable with those of standard herniarin, as was the absorption spectrum (maximum at 320 mμ and minimum at 260 mμ). The compound was tentatively identified as herniarin.

In summary, 3 compounds occurred in both 'Mazzard' and 'Mahaleb' leaves; o-coumaric acid, kaempferol, and coumarin. The first 2 compounds appeared similar in both 'Mazzard' and 'Mahaleb'. These determinations indicate that 'Mazzard' leaves contained several phenolic compounds including caffeic acid dihydrowogonin, quercetin, chlorogenic acid, and p-coumaric acid. The 'Mahaleb' leaves contained 4 major phenolic compounds, 3 of these being o-coumaric acid, kaempferol, and coumarin. Herniarin, specific to 'Mahaleb', was the dominant compound that appeared on 2-dimensional paper chromatograms.

2. *Stem and root phenolic compounds:* Bark tissues, taken from stem and root, exhibited a similarity in phenolic composition (Fig. 1, center and bottom).

Among the 13 phenolic compounds that appeared on chromatograms of 'Mazzard' stem extracts, only 3 corresponded to those found in 'Mahaleb' roots, namely: spots 1, 3, and 4. On the basis of color reaction and Rf values, Spots 1 and 3 were tentatively identified as o-coumaric acid and coumarin as shown in the analysis of leaf phenolics. Spot, 4, however, was absent in leaf tissues and identified as follows: It was colorless under ultraviolet light with or without ammonia, but gave a pink color with vanilin-HCl reagent, which is specific for flavonoids with phloroglucinol nucleus such as catechin and leucoanthocyanidins (32). The absorption spectrum of the compound had the characteristics of d-catechin with maximum absorption at 280 mμ and minimum at 250 mμ (17). Rf values were

Table 4. Relative comparison of identified phenolic compounds in extracts of leaf, stem and root of 'Mazzard' and 'Mahaleb' seedlings as determined by paper chromatography.

Spot no.	Compound[z]	'Mazzard' Leaf	'Mazzard' Stem	'Mazzard' Root	'Mahaleb' Leaf	'Mahaleb' Stem	'Mahaleb' Root
Phenolic acids							
1	o-coumaric acid	+++++	+++	+++	+++++	+	+
Z5	p-coumaric acid	++	+++	+++	0	0	0
Z6	caffeic acid	+++++	0	0	0	0	0
Z8	chlorogenic acid	++	0	0	0	0	0
Coumarins							
3	coumarin	+	+	+	+++++	+++	+++
H4	herniarin	0	0	0	++++++++++	++++++++++	++++++++++
Flavonoids							
2	kaempferol	++++	0	0	+++	0	0
4	d-catechin	0	++	+	0	++	+++
Z4	dihydrowogonin	++++	++++++++++	++++++++++	0	0	0
Z7	quercetin	++	0	0	0	0	0

[z] + = relative visual amounts phenolic compounds present. 0 = no phenolic compounds present.

comparable with those of catechin reported by Luh et al. (19). Therefore, compound 4 was tentatively identified as d-catechin.

Some of the 'Mazzard' stem phenolics exhibited color reactions and Rf values identical with those of 'Mazzard' leaf phenolic compounds. Those were Spot Z4 (dihydrowogonin), Z5 (p-coumaric acid), and Z9. 'Mahaleb' stem also contained phenolic compounds present in the 'Mahaleb' leaf tissues–namely H4 (herniarin), H6, and H7 (Table 1).

Tissues of the rootstocks differed as to the type of phenolic compounds present. 'Mazzard' stem and root contained cinnamic acids and flavanones (p-coumaric acid, o-coumaric acid, and dihydrowogonin) while 'Mahaleb' was found to have the coumarins in these tissues.

Some of the phenolic compounds present in leaves were absent in stem and root extracts, such as caffeic acid, chlorogenic acid, kaempferol, and quercetin (Table 4). D-catechin appeared only in stem and roots. Dihydrowogonin was present in all 'Mazzard' tissues tested. P-coumaric acid was present in leaves, stem, and roots of 'Mazzard'. Both 'Mazzard' and 'Mahaleb' rootstocks contained o-coumaric acid and coumarin in all the tissues tested, and herniarin was specific to 'Mahaleb'.

Summary and Discussion

'Mahaleb' rootstocks, which show graft-incompatibility symptoms with certain sweet cherry cultivars, apparently differ both in phenolic composition and number of phenolics from 'Mazzard' rootstocks which do not show such incompatibility. For example, in unhydrolyzed tissues as determined by paper chromatography, 31 compounds were present in 'Mazzard' and only 22 in 'Mahaleb'. When sweet cherries are grafted on 'Mahaleb' rootstocks, the 'Mahaleb' tissues may not have an efficient system for utilizing the phenolic compounds formed in sweet cherry cultivars, thus interfering with the physiological processes (25, 30). 'Mahaleb' roots may also be more sensitive to sweet cherry phenolic compounds, accumulated in the soil as a result of leaching or decomposition of leaf tissues. Genetically 'Mazzard' is more closely related to sweet cherry cultivars than to 'Mahaleb'.

Qualitative differences were particularly noted in phenolic acids, coumarins, and flavonoids, and these are described as follows:

Phenolic acids. 'Mazzard' tissues contained hydroxy cinnamic acid derivatives including p-coumaric, o-coumaric, caffeic, p-coumaryl quinic, and chlorogenic acids, whereas 'Mahaleb' tissues contained only o-coumaric acid. Bate-Smith (2) in his survey of leaf phenolic compounds in *Prunus*, found p-coumaric, o-coumaric, and caffeic acids in 'Mazzard'. However, in 'Mahaleb', o-coumaric acid was the only phenolic acid identified, although he questioned the presence of p-coumaric acid.

The 2 rootstocks may have different biosynthetic schemes of phenolic acids by differing in their hydroxylation pattern of cinnamic acid, which is known to be an important precursor of phenolic acids in higher plants (24, 29). 'Mahaleb' tissues, which contain coumarin and herniarin, apparently hydroxylate only the ortho position of cinnamic acid, and hence p-coumaric and caffeic acids are lacking. 'Mazzard' tissues, however, appear to be able to hydroxylate the ortho, meta, and para positions of the benzene ring of cinnamic acid.

The esters of p-coumaric and caffeic acids may regulate levels of IAA in plant tissues by influencing its decarboxylation. Tomaszewski and Thimann (34) showed that polyphenols such as chlorogenic acid and caffeic acid reduced the inactivation of IAA, whereas monophenols such as p-hydroxy benzoic acid and p-coumaric acid increased the inactivation. In 'Mazzard', caffeic acid was mainly located in leaf tissues, whereas p-coumaric acid was present in stem and root bark and in leaf tissues. Thus, translocation of p-coumaric acid from a sweet cherry scion to a 'Mahaleb' stock could conceivably favor decarboxylation of IAA and thereby reduce the vigor of the rootstock.

Coumarins. The 2 rootstocks differed markedly in coumarins. All

'Mahaleb' tissues analyzed contained both coumarin and herniarin, whereas 'Mazzard' contained no herniarin but showed coumarin activity in leaf tissues. Favre-Bonvin et al. (8) considered that *P. avium* was incapable of synthesizing coumarin. However, o-coumaric acid can easily be transformed to coumarin in acid solution or on exposure to light (2). Therefore, the coumarin observed in 'Mazzard' may have been an artifact formed during extraction.

Inhibitory activity of coumarins was found in germination and root growth, and in mitosis of barley and wheat (12, 20). Similarly, this suggests a role for those compounds in that coumarin may play a role in graft-incompatibility of *P. avium* on *P. mahaleb*. This is unlikely, because the major sites of synthesis of coumarins are the aerial parts, especially in young leaves (8, 9, 13). No coumarins were found in rootstock of 4-year old grafted *P. avium* on *P. mahaleb*, which grew vigorously without symptoms of incompatibility (8, 11).

At low concentrations, coumarins stimulated plant growth, indicating that they may be essential for normal growth (20). Their disappearance from root tissues after several years may be related to loss of vigor of the tree. Coumarin derivatives such as scopolin and scopoletin are known to inhibit IAA-oxidase (1, 26). Thus, the absence of coumarins in 'Mahaleb' rootstocks may allow the destruction of IAA. Alternatively, their absence may limit lignin synthesis, leading to incompatibility. Kosuge and Conn (18) reported that labelled coumarin was rapidly transformed into β-glucosides of o-hydroxy cinnamic acids. The latter are known to be incorporated into lignin (6).

Flavonoids. 'Mazzard' tissues contained 3 flavonoids; dihydrowogonin, kaempferol, and quercetin. The latter 2 were found only in leaf tissues. Kaempferol also occurred in 'Mahaleb' leaf tissues, but quercetin was absent. Bate-Smith (2) found both quercetin and kaempferol in hydrolysates of 'Mahaleb' leaves. The concentration of quercetin in leaves of the 'Mahaleb' seedlings we used may have been too low to be detectable, or occurrence may depend upon tree age and graft combination. Mature sweet cherry trees are known to contain various other flavonoids in heartwood tissue (4, 12).

Different flavonoids act either as synergists or antagonists to IAA *in vitro*. For example, kaempferol conjugates promote IAA oxidation while quercetin conjugates inhibit its oxidation (10). The difference in flavonoid composition in 'Mazzard' and 'Mahaleb' suggests that they may differ in rate of decarboxylation of IAA. The activity of flavonoids in IAA decarboxylation appears to depend on the hydroxylation pattern in the B ring. The effect of dihydrowogonin on IAA oxidase is difficult to estimate because of the lack of a hydroxyl group in the B ring. However, the extensive occurrence of dihydrowogonin in 'Mazzard' tissues and its absence in 'Mahaleb' suggest that it may be an important factor in graft-incompatibility.

Literature Cited

1. Andreae, W. A. 1952. Effect of scopoletin on indoleacetic acid metabolism. *Nature* 170:83–84.
2. 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.
3. Buchloh, G. 1960. The lignification in stock-scion junctions and its relation to compatibility. p67–71. *In:* Phenolics in plants in health and disease, ed. by J. B. Pridham. Pergamon Press, London.
4. Chopin, J., D. Molho, H. Pacheco, C. Mentzer and G. Grenier. 1957. Structure of a new flavone derivative isolated from cherry wood (*Prunus avium*). *Bu. Soc. Chim. Biol.* 46:192–220.
5. Crane, M. B. 1945. Origin of viruses. *Nature* 155:115–116.
6. El-Basyouni, S. Z. and A. C. Neish. 1966. Occurrence of metabolically active bound forms of cinnamic acid and its phenolic derivatives in acetone powders of wheat and barley plants. *Phytochemistry* 5:683–691.
7. Evenari, M. 1949. Germination inhibitors. *Bot. Rev.* 15:153–194.
8. Favre-Bonvin, J., M. Massias and C. Mentzer. 1966. Modifications biochimiques consecutives au greffage interspecifique dans legenre "Prunus". *Bul. Soc. Chim. Biol.* 48:1359–1365.
9. ———, ———, ———, and J. Massicat. 1968. Sites de biosynthese des coumarines chez *Prunus mahaleb*. Etude par greffage et administration dun precurseur marquie. *Phytochemistry* 7:1555–1560.
10. Galston, A. W. 1969. Flavonoids and photomorphogenesis in peas. p. 193–204. *In:* Prespectives in phytochemistry, ed. by J. B. Harbone and T. Swain. Acad. Press, London and NY.
11. Garner, R. J. 1967. The cherry rootstock position today. *E. Malling Res. Sta. Ann. Rept.* 1966. 50:212–215.
12. Goodwin, R. H. and C. Taves. 1950. The effect of coumarin derivatives on the growth of Avena roots. *Amer. J. Bot.* 37:224–231.
13. Gortz, H. F. and F. A. Haskins. 1962. Translocation of coumarin across a graft union in sweet clover. *Crop Sci.* 2:255–257.
14. Grubb, N. H. 1938. Resume of the cherry rootstock investigation. *E. Malling Res. Sta. Ann. Rept. Sup. Misc. Pub.* 22:41–44.
15. Gur, A., R. M. Samish and E. Lifshitz. 1968. The role of the cyanogenic glucoside of the quince rootstocks. *Hort. Res.* 8:113–134.
16. Hemberg, T. 1961. Biogenesis of inhibitors. *Encyclopedia of Plant Physiology*. 14:1162–1184.
17. Jurd, L. 1962. Spectral properties of flavonoid compounds. p. 107–155. *In:* The chemistry of flavonoid compounds, ed. by T. A. Geissman. The McMillan Co. NY.
18. Kosuge, T. and E. E. Conn. 1961. Metabolism of aromatic compounds in higher plants. *J. Biol. Chem.* 236:1617–1621.
19. Luh, D. S., E. T. Hsu and K. Stachowicz. 1967. Polyphenolic compounds in canned cling peaches. *J. Food Sci.* 32:251–258.
20. Mayer, A. M. and A. Poljakoff-Mayber. 1961. Coumarins and their role in growth and germination. p. 735–749. *In:* Plant growth regulation. Fourth International Conference on Plant Growth Regulation. The Iowa State University Press, Ames.
21. Mentzer, C., H. Pacheco and A. Ville. 1954. Indetermine chimique des extracts de bois. IV. Noveaux derives flavoniques du coeur de merisier (*P. avium*). *Bul. Chim. Biol.* 36:1137–1150.
22. Mockaitis, J. M. 1969. Compatibility of certain grafts in the genus Ipomea. *Flora* 159:477–483.
23. Mosse, B. 1962. Graft incompatibility in fruit trees. Tech. Comm. No. 28. Commonwealth Bureau of Horticultural and Plantation Crops. E. Malling, Kent, England. 36p.
24. Neish, A. C. 1961. Formation of m- and p-coumaric acids by enzymatic deamination of the corresponding isomer of tyrosine. *Phytochemistry* 1:1–24.
25. Pradham, J. B. 1960. The formation and possible function of phenolic glycosides. p. 9–15. *In:* Phenolics in plants in health and disease, ed. by J. B. Pridham. Pergamon Press, Oxford.
26. Schaeffer, G. W., J. G. Buta and F. Sharpe. 1967. Scopoletin and polyphenol induced lag in peroxidase catalyzed oxidation of indole-3-acetic acid. *Physiol. Plant.* 20:342–347.
27. Seikel, M. K. 1964. Isolation and identification of phenolic compounds in biological materials. p. 33–72. *In:* Biochemistry of phenolic compounds, ed. by J. B. Harborne. Acad. Press. London and NY.
28. Simons, R. K. and R. F. Carlson. 1968. Characteristics and propagation of rootstocks for deciduous fruits in the north central region, 1966–1967. *HortScience* 3:221–224.
29. Steck, W. 1968. Metabolism of cinnamic acid in plants: Chlorogenic acid formation. *Phytochemistry* 7:1711–1717.
30. Stenlid, G. 1963. The effects of flavonoid compounds on oxidative phosporylation and on the enzymatic destruction of indoleacetic acid. *Physiol. Plant.* 16:110–120.
31. Swain, T. 1953. The identification of coumarins and related compounds by filter paper chromatography. *Biochem. J.* 53:200–207.
32. ——— and H. E. Hillis. 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constitutents. *J. Sci. Food Agr.* 10:63–68.
33. Thiel, K. 1954. Untersuchugen zur Frage der Unverträglichkeit bei Birnenedelsorten auf Quitte A (Cydonia EMA). *Gartenbauwiss.* 1:127–159.
34. Tomaswewski, M. and K. V. Thimann. 1966. Interactions of phenolic acids, metallic ions and chelation agents on auxin induced growth. *Plant Physiol.* 41:1443–1454.
35. Tschiersch, B. 1963. Canavanin als Ursache der Inkompatibilität von Canavalia-Pfropfungen. *Flora* 153:73–86.