Applications of 200 and 500 ppm CPTA at pH 9 to 'Hamlin' orange induced significant increases in carotenoid levels (Fig. 3), but there was no response to 50 ppm. No consistent difference was shown between applications made before or after the 3-day degreening period. Since all samples were washed and waxed on December 4, color development after that date showed that changes were not inhibited by the wax coating. Application of CPTA at pH 7.3 (data not shown) gave significantly lower carotenoid levels and less uniform color. Applications at pH 8 were intermediate.

Observed responses to CPTA support reports of altered carotenoid synthesis patterns in other citrus cultivars (1, 3, 4). However, the effects of GA, ethylene and storage suggest that induced carotenoid changes are limited by the same factors affecting natural synthesis patterns. Although GA has been reported to have no effect on the CPTA response (1), our tests suggest that endogenous growth regulators, including GA, may have a controlling influence on the response, particularly at the start of the season. These responses indicate that CPTA may provide a useful tool to study carotenoid synthesis control patterns.

Some increase in response to CPTA with ethylene is to be expected, since ethylene increases carotenoid synthesis (2, 6). A similar effect of 2-chloroethylphosphonic acid on the response to CPTA has been reported (1). In our tests, the ethylene- and non-ethylene-treated samples could not be run at the same temp. Since carotenoid synthesis is favored by lower temp (5), and since observed color changes were rapid at both 21 and 29°C, this temp difference would not seriously affect the results.

Emphasis in our tests was on the nature and magnitude of the response of eitrus fruits to CPTA. The increased carotenoid levels shown have no practical value except in 'Hamlin' oranges. The other types of citrus either are preferred with a yellow color (grapefruit and lemon), or normally have an adequate carotenoid level (tangerine). Although improved color of oranges was obtained even in the presence of a wax coating, 2 problems are evident. First, the inability of CPTA to override the natural controlling factors in carotenoid synthesis, limits its effectiveness during the early part of the season when improved color is most desirable. Second, only moderate increases in pigment levels will improve color, and these are difficult to control. The distinctive red color induced by CPTA is not normal to citrus, and therefore, more complete responses are undesirable. Although its practical value seems to be limited, the use of CPTA for studies on the mechanism of control of carotenoid synthesis appears promising.

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Incidence of Blackline in *Juglans regia* L. Propagated on Various Rootstock Species[1]

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Abstract. In a 16-year field study, all graft combinations of Persian walnut, *Juglans regia* scions with other rootstock species were susceptible to blackline. 'Sinensis #5', a selection of *J. regia*, did not exhibit symptoms of blackline when used as an interstock. In no case was blackline observed in graft combinations between *J. regia*.

Blackline is a graft union disorder which occurs between scions of *Juglans regia* and the commonly used rootstocks *J. hindsii* (northern California black walnut) and 'Paradox' (*J. hindsii* x *J. regia*). The disorder usually occurs in mature trees which are more than 20 yr old. Blackline is expressed as a thin layer of dead cambial cells at the graft union. Beginning at one site in the union, blackline progresses around the trunk at the rate of about 3 inches per year (1). With time, it also moves downward into the rootstock. Sprouts from the rootstock commonly develop a few years after the start of blackline.

The first reported case of blackline in the United States was in Oregon in 1924 (2). A few years later, this same disorder was found in the Central Coast County of Contra Costa in California (3). Since then its occurrence has become more widespread, but it is not clear whether the incidence of blackline has actually increased or techniques for positive identification have been improved, or both.

Even though blackline has decimated orchards in some districts, it has been given little attention by researchers, partly because numerous affected orchards were destroyed and the land used for dwellings and industry. As a result, the economic effect of this disorder was greatly mitigated. In 1959, Serr and Forde (1) described the nature of blackline at the union, its incidence in commercial orchards, age of the trees affected, rate of its advance in the graft union, and some possible factors favorable to its occurrence, i.e., rootstock, cultivars, soil, and climate. Their report was summation of work in grower orchards where blackline was prominent. Within the parameters measured by Serr and Forde, it was evident that the effect of blackline was devastating, and the cause unknown.

We examined numerous trees of selected species and cultivars for blackline during a 16-year period in an attempt to find resistance to this disorder.

Materials and Methods

Previous studies indicated that the greatest incidence of blackline in California was in the Central Coast area. For that reason, the trees used in this study were planted at the San Jose field station in 1952, well within the area of greatest incidence. Further information indicated that trees which were planted close together matured earlier and had earlier symptoms of blackline. Therefore, the planting was designed in a 1.2 × 4.5M pattern. Materials used in the various graft combinations are indicated in each table.

Graft unions were inspected annually by making V-shaped cuts through the bark down to the cambium. Any blackline discovered was marked with a nail on each end of its development. Wherever

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interstocks of several scions were grafted on a single stock, each union was examined (Fig. 1). Figure 1 was designed to graphically represent the trees from which data for Tables 1, 2, and 3 were taken. At the onset of this experiment it was decided that if a given graft combination incurred blackline that it would be labeled susceptible and those trees removed from the plot as convenient.

Results

Single scion on rootstock. All combinations of *J. regia* cv. Payne grafted on any of several rootstocks had blackline symptoms by the 13th year after grafting (Table 1). Observe the location of the graft for data in Table 1 illustrated in Fig. 1. After the initial appearance of symptoms within any graft combination, additional trees usually developed the disorder as the years passed. One of the scion selections labelled 'BL Payne' was taken from a tree with symptoms of blackline. This combination did not result in either earlier expression or increased incidence of blackline (Table 1).

Interstocks. With 'Payne' as the scion, different interstocks were grafted to seedlings of *J. Hindsii*, selections of *Pterocarya stenoptera* DC (wing nut), and 'Paradox'. In every instance but one, blackline occurred in all combinations involving 'Payne' and another species, hybrid selection or *J. regia* selection (Table 2). Observe the location of the grafts for data in Table 2, illustrated in Fig. 1. The exception was the combination of *J. regia* on *J. regia*, 'Sinensis #5' interstock and *J. hindsii* rootstock. At the end of the 16th year, there were no blackline symptoms between 'Sinensis #5' and *J. Hindsii*. In all other combinations on *J. hindsii*, the occurrence of blackline was quite specific between *J. regia* and the non-*regia* graft partner and not between the non-*regia* intermediate and the *J. hindsii* rootstock (Table 2). When 'Paradox' was the rootstock and 'Haig' the intermediate, blackline did occur between the stock and intermediate.

Multiple grafts. Multiple grafts and/or combinations of interstocks on the same tree, did not hinder the development of blackline (Table 3). Observe the location of the grafts for data in Table 3, illustrated in Fig. 1. Use of scions from trees having symptoms of blackline (BL *J. hindsii*) did not result in earlier blackline symptoms, It is noteworthy that blackline did result between 'Payne' and *J. hindsii* intermediate on *J. regia* rootstock.

Discussion

All intermediate graft combinations with *J. regia* as the scion had early symptoms of blackline, except the combination of 'Sinensis #5' as an interstock and *J. hindsii* as a rootstock. Of further interest is that no species other than *J. regia* expressed symptoms of blackline with any of the rootstock combinations. Thus, in these tests, the blackline symptomology was confined to *J. regia* with other graft partners. It is important to note that no case of blackline occurred with *J. regia* as scion and *J. regia* as rootstock, except when *J. hindsii* was intermediate between *J. regia* stock and scion. It seems that solution to the blackline problem probably should include the use of *J. regia* as a rootstock. However, in California, *J. regia* is little used as a rootstock because of its purported susceptibility to oak root fungus and nematodes, and its poor performance in alkaline soil.

It is particularly interesting that use of grafting materials from trees infected with blackline did not result in earlier onset of blackline symptoms (Tables 1, 3). If this disorder is transmissible via a graft component, one would have expected early, severe symptoms.

In none of the various graft combinations reported did more than 50% of the trees have symptoms of blackline by the end of the test period. It might be argued that the symptomless trees could be resistant to blackline. However, in most instances, there was a gradual increase in the number of trees developing symptoms of blackline.

We conclude that *Juglans regia* should be investigated as a possible rootstock, as combinations of *J. regia* grafted to *J. regia* appear immune to blackline. Further, the major rootstocks used in California, *J. hindsii* and 'Paradox', are both susceptible to blackline (1). Additional field testing could be done using 'Sinensis #5' as an interstock, and an attempt to develop rooted cuttings for its use as a rootstock should be initiated. A more worthwhile approach might be the inclusion of 'Sinensis #5' in breeding programs for the development of new walnut cultivars.

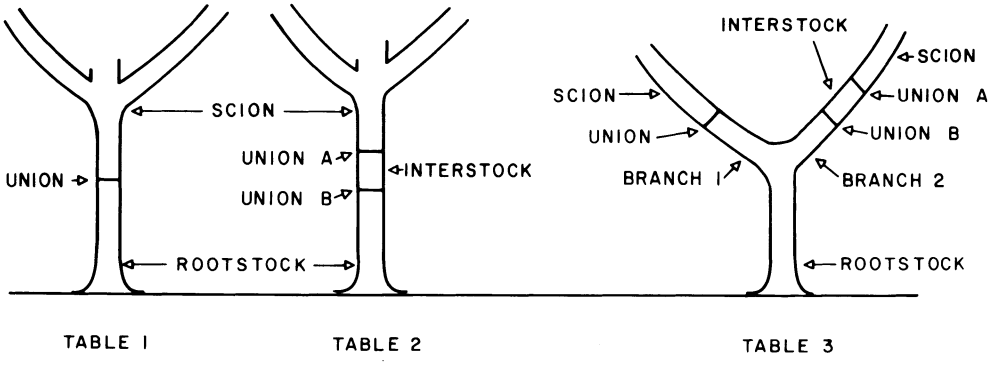

Fig. 1. Location of each union examined for Tables 1, 2, and 3.

Table 1. Age and percent of graft unions with symptoms of blackline.

Scion/Rootstock	No. of trees	Tree Age 9	10	11	12	13	14	15	16
		(Percent of unions affected by blackline)							
B.L. Payne[z]/J. hindsii	50	0	0	0	0	2	10	14	16
Payne[y]/J. hindsii	70	0	0	5	7	8	14	18	21
Payne/Paradox[x] seedlings	50	0	0	6	14	24	23	30	30
Payne/P. stenoptera	30	0	10	20	23	23	27	30	30
Payne/Royal[w]	20	0	20	TR[v]					

[z] B.L. Payne = scion from tree with blackline.
[y] Payne = Payne scion from tree free of blackline symptoms at Davis.
[x] Paradox seedlings = cross between *J. regia* and *J. hindsii*.
[w] Royal = *J. hindsii* × *J. nigra*.
[v] T.R = trees removed from plot.

Table 2. Age and percent of graft unions between the scion and interstock or interstock and rootstock showing symptoms of blackline.

Union A Scion/Interstock	Union B /Rootstock	No. of Trees	Union	Tree Age 8	9	10	11	12	13	14	15	16
				Percent of unions affected by blackline								
Payne/Burbank Paradox[z]	/J. hindsii	50	A	0	0	4	8	12	14	20	22	TR[t]
			B	0	0	0	0	0	0	0	0	
Payne/B.K. Paradox[y]	/J. hindsii	50	A	0	0	2	6	14	20	24	24	TR
			B	0	0	0	0	0	0	0	0	
Payne/C.C. Persian[x]	/J. hindsii	50	A	0	0	0	0	0	0	0	0	0
			B	0	0	0	2	8	12	14	14	18
Payne/J. sieboldiana[w]	/J. hindsii	25	A	0	0	8	8	12	12	20	TR	
			B	0	0	0	0	0	0	0		
Payne/India Nol[v]	/J. hindsii	25	A	0	0	0	0	0	0	0	0	TR
			B	0	0	0	0	0	4	8	8	
Payne/Placentia[u]	/J. hindsii	50	A	0	0	0	0	0	0	0	0	0
			B	0	0	0	2	2	2	4	8	8
Payne/Haig[t]	/J. hindsii	20	A	0	0	5	10	15	20	20	25	TR
			B	0	0	0	0	0	0	0	0	
Payne/Sorrentina[s]	/P. stenoptera	20	A	0	0	0	0	0	0	0	0	0
			B	0	0	0	10	10	10	10	10	10
Payne/Waterloo[r]	/P. stenoptera	50	A	0	0	0	0	0	0	0	0	0
			B	0	0	0	10	15	20	25	30	30
Payne/Haig	/Paradox sdlg.		A	0	0	0	0	0	0	0	0	TR
			B	0	0	4	8	12	18	22	22	
Payne/Myrtleford[q]	/P. stenoptera	20	A	0	0	0	0	0	0	0	TR	
			B	0	0	0	0	0	5	15		
Payne/P. stenoptera[p]	/P. stenoptera sdlgs.	20	A	10	30	40	40	40	50	TR		
			B	0	0	0	0	0	0			
Payne/J. formosana[o]	/J. hindsii		A	0	5	15	15	15	15	TR		
			B	0	0	0	0	0	0			
Payne/Sinensis #5[n]	/J. hindsii	20	A	0	0	0	0	0	0	0	0	
			B	0	0	0	0	0	0	0	0	0
Payne/Mexican Black[m]	/J. hindsii	20	A	0	0	0	5	10	10	20	TR	
			B	0	0	0	0	0	0	0		
Payne/J. rupestris	/J. hindsii	20	A	0	0	0	5	5	15	25	TR	
			B	0	0	0	0	0	0	0		

[z] Burbank Paradox = large tree in Burbank garden, Santa Rosa
[y] B.K. Paradox = East Paradox tree Bowman-Kuhn Ranch near Wayne Station, Santa Clara Co.
[x] C.C. Persian = old *J. regia* cultivar from Contra Costa Co.
[w] J. sieboldiana = Japanese walnut
[v] India Nol = very hard shell *J. regia* grown from a seed from India.
[u] Placentia = Santa Barbara soft-shell cultivar; *J. regia*
[t] Haig = (Paradox back cross to Franquette)? Cross by Dr. Haig of Stockton.
[s] Sorrentina = old Italian cultivar of *J. regia*.
[r] Waterloo = California cultivar of *J. regia*.
[q] Myrtleford = cultivar from Australia; *J. regia*.
[p] *P. stenoptera* 8-12 = Row 8, tree 12; Solano rootstock block; Payne grew fairly well on 8-12.
[o] *J. formosana* = Formosan walnut
[n] Sinensis #5 = cultivar of *J. regia* grown from seed E. F. Serr obtained from Japan. Seed came from a cultivar called 'Sinensis' in Japan.
[m] Mexican Black = grew from seed from a tree near Guadalajara, Mexico. Species probably is *J. major* var. glabrota. Some botanists call it *J. glabrota*.
[t] TR = trees removed from plot.

Table 3. Age and percent of graft unions between scion and interstock or interstock and rootstock having symptoms of blackline.

Branch	Union A Scion/Interstock	Union B /Rootstock	No. of trees	Union	Tree Age 9	10	11	12	13	14	15	16
					Percent of unions affected by blackline							
1	Payne/no interstock	*/J. hindsii*	50	A	0	0	2	4	4	4		
2	Payne/B.L.J. hindsii[z]	*J. hindsii*		A	0	0	2	6	6	6		TR[y]
				B	0	0	0	0	0	0		
1	Payne/no interstock	*/J. regia*	50	A	0	0	0	0	0			
2	Payne/B.L.J. hindsii	*/J. regia*		A	0	2	2	2	2		TR	
				B	0	0	0	0	0			

[z] B.L. *J. hindsii* = scion from tree with blackline.
[y] TR = trees removed from plot.

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Phenolic Content During Healing of 'Valencia' Orange Peel Under High Humidity[1]

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Abstract. Free phenolic constituents increase more than 2-fold in injured peel of oranges (*Citrus sinensis* Osbeck) cv. Valencia after 48 hr at 30°C and 96 to 98% relative humidity (rh). Concomitantly, conjugated phenolic compounds decrease to near depletion as a heavily lignified layer forms on injured cells. At 5°C all wound healing processes slow down. Infection of injured tissue by *Penicillium digitatum* Sacc. at 27°C inhibits lignin synthesis and the disappearance of conjugated phenolic compounds, but does not interfere with the usual increase in free phenolics. Mycelium of *P. digitatum* contributes little to the level of phenolic compounds of decayed fruit tissue. Extracts of free phenolic substances from healed tissue do not exhibit fungistatic activity on *P. digitatum* spores. Lignin formation provides a mechanical barrier which retards or inhibits penetration of injured tissue by *P. digitatum*.

In 1948, Hopkins and Loucks (9) reported that citrus fruit degreened with ethylene at 86°F (30°C) and 90% rh for 60 to 72 hr exhibited less green mold (caused by *Penicillium digitatum* Sacc.) than nondegreened fruit. Recently, Brown (3) confirmed these findings and noted that under such degreening conditions a layer of lignified tissue is formed on injured flavedo.

Since lignin constitutes the major portion of the post-wounding scar tissue (3) and phenylpropanoid compounds are known to be the building blocks of lignin (16), changes in phenolic content of injured 'Valencia' orange flavedo as influenced by temp, rh, and the decay organism *P. digitatum* were studied.

Materials and Methods

Mature, fully colored 'Valencia' orange (*Citrus sinensis* Osbeck) fruit were collected from trees on rough lemon rootstock. They were washed, dried, sorted for uniformity of size and external appearance, and randomly divided into as many groups as required in each experiment. In all trials, 20 and 1 or 2 g of flavedo tissue were collected at 24-hr intervals for phenolic substances and dry wt determinations, respectively. Histological evaluation of lignification was made on tissue sections stained with phloroglucinol-HCl (3).

Effect of humidity on wound healing and phenolic content of 'Valencia' orange flavedo. Two samples, each consisting of 120 fruit, were injured by rubbing fruit surfaces in 4 locations along the equatorial plane against #60 coarse sandpaper. One sample was stored at 30 ± 2°C and 96 to 98% rh; the other sample was held at similar temp, but at ambient humidity (55–75% rh). Both temp and rh were continuously monitored throughout the duration of the experiment.

Temperature effect on phenolic content of injured 'Valencia' orange flavedo. 'Valencia' oranges were injured as above, divided into 2 lots (80 fruit per lot), and placed at 90 to 96% rh. One lot was held at 5 ± 2°C, the other at 30 ± 2°C for 72 hr.

Effect of inoculation with Penicillium digitatum and storage temperature on the amount of phenolic compounds in injured 'Valencia' orange flavedo. 'Valencia' oranges were injured, divided into 2 lots, one of which was inoculated with dry spores of *P. digitatum* (3); the other group was not inoculated. All fruit were stored at 30 ± 2°C and 96 to 98% rh. The same experiment was repeated with fruit held at 25 ± 2°C and 96 to 98% rh. Fruit were also checked for decay.

Concn of phenolic compounds in in vitro-grown mycelium of P. digitatum. In order to determine the extent of fungal contribution to phenolic content of infected tissue, twelve 30 ml centrifuged orange juice samples were inoculated with *P. digitatum* spores and held in a shaker at 25°C (78°F) for 5 days. Nineteen g of mycelial tissue was collected, washed thoroughly with distilled water, and extracted for free and conjugated phenolic substances.

Effect of free phenolic extracts on germination of spores of P. digitatum. Spores of *P. digitatum* were germinated in the presence of free phenolics on a medium composed of 10 ml filtered and centrifuged orange juice, 1 g dextrose, 1 g yeast extract, and 30 g Bacto agar in one l of distilled water. A 10-ml aliquot of sterile medium was poured into a petri dish and cut after solidification to form 4 islands 7 mm in diam. An aliquot containing 10 µl of a free phenolic extract in 95% ethanol was placed on each of the 4 islands in a dish. After evaporation of ethanol, spores of *P. digitatum* suspended in 1000 ppm Tween 20 were placed on each island and incubated for 18 hr at 25°C. They were then killed with cotton blue-lactophenol. Percent germination was determined using 50 spores in each island.

Extraction of free and total phenolic constituents from injured 'Valencia' orange flavedo. The method used was a modified version of those described by Ibrahim et al. (10) and Biehn et al. (2). Tissue (20 g) was homogenized in a Lourdes mixer with 200 ml boiling 95% ethanol. The homogenate was boiled for 1 hr in a water bath and filtered on Whatman no. 42 filter paper. The filtrate was divided into 2 equal portions, each of which was evaporated to dryness under vacuum at 45° ± 2°C.

a. Extraction of free phenolic constituents. The dry residue remaining after evaporation of 1 of the ethanol fractions was suspended in 200 ml boiling distilled water and extracted twice with 0.5 vol n-hexane to remove peel oil and carotenoid pigments. Active acidity of the aqueous layer was then reduced to pH 2 using 2N HCl and extracted 3 times with 0.33 vol distilled ethyl acetate. Ethyl acetate extracts were combined and evaporated to dryness. The dry residue was subjected to a stream of N to remove residual ethyl acetate and then dissolved in 10 ml 95% ethanol.

b. Extraction of total phenolic constituents. The other half of dried ethanolic filtrate was dissolved in 50 ml boiling distilled water and subjected to 2 successive washes with equal volumes of n-hexane. The remaining aqueous layer was evaporated to dryness at 48 ± 2°C and

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