Relationship of Wound-Induced Peroxidase Activity to Epicarp Lesion Development in Maturing Pistachio Fruit

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ABSTRACT

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Needle-puncture through the pericarp of maturing pistachio (*Pistacia vera*) fruit induced symptoms similar to those observed in fruit with epicarp lesion after insect feeding. Mechanical or insect wounds through the pericarp markedly stimulated peroxidase activity (as much as 3- to 10-fold relative to unwounded fruit) in the fruits of cultivars Kerman and Trabonella. The induced peroxidase activity was apparent histochemically within 24 hr of wounding, before symptom development, and at a distance several millimeters from the wound site. Penetration of the inner surface of the endocarp was essential for stimulation of peroxidase activity and lesion development. Both events were inducible during April and May but that capability diminished after the onset of endocarp lignification. Wounding induced at least three cathodic isoperoxidases not present in unwounded fruit. Gallic acid, methyl gallate, and other gallotannins were the principal phenols present in ethanol extracts of the pericarp and their concentrations declined early in the season. The data indicate that injury to the endocarp is essential for symptom expression and are consistent with the hypothesis that epicarp lesions result from the wound-induced peroxidation of components in the pericarp.

Epicarp lesion is a fruit disorder of the pistachio tree (*Pistacia vera* L.) characterized by necrotic, often zonate, areas in the pericarp (3,4,20). Affected tissues appear initially as light brown diffuse areas, which rapidly become dark brown to black in color. Symptomatic fruit usually abscise, especially when lesions develop early in the season. The disorder is widespread in orchards in California and Arizona and may cause estimated losses as high as 30% in some areas (4).

Bolkan et al (3,4) reported that feeding by the leaf-footed bug, *Leptoglossus clypealis* Heidemann, induced symptoms in the fruit typical of epicarp lesion. This key observation led to further studies by Uyemoto et al (27–29) and Rice et al (19) in which the range of insects capable of inducing epicarp lesion was extended to include another coreid, *Leptoglossus occidentalis* Heidemann, the mirids, *Calocoris norvegicus* (Gmelin) and *Lygus hesperus* Knight, and several members in the family Pentatomidae (stinkbugs). All of these insects occur in California pistachio orchards and all feed on pistachio fruits.

Previous research indicated that wounding with a needle was sufficient to elicit symptoms identical to insect-induced epicarp lesion, provided the wound penetrated the entire pericarp (8) and was not restricted to the epicarp and mesocarp tissue layers (25,26,28). With the onset of lignification (mid- to late-May), fruits become increasingly resistant to epicarp lesion development (28). The results from fruits wounded mechanically correlate well with observations in the field of natural epicarp lesion incidence. Only insects capable of penetrating the endocarp will induce fruit symptoms.

Because of the increasing importance of pistachio culture in California agriculture (11) and significant impact on pistachio production by epicarp lesion, kernel necrosis, and another fruit disorder of uncertain etiology, stylar-end necrosis (20), understanding the regulation of metabolism that contributes to the physiological browning and necrosis observed in these disorders is of great interest. It is well established that peroxidase activity in many plants is stimulated by wounding and other stresses (5,6,16). Borchert (5) reported the formation of isoperoxidases in wounded potato tuber and distinguished isoperoxidases in suberizing cells from those in dividing cells (6). Lagrimini and Rothstein (14) recently reported the formation in tobacco pith of two new isoperoxidases in response to wounding. Brief immersion of cucumber seedlings to heat-induced (50 C) resistance to *Cladosporium cucumerinum* Ell. & Arth. and enhanced activity of certain isoperoxidases (23) and peroxidase activity was stimulated in other plants by pathogens (16). The oxidation of phenols and other components by peroxidases may generate products toxic to the cell and contribute to the darkening of plant tissues.

The objective of this study was to determine the relationship between wounding, peroxidase activity, and epicarp lesion development in maturing pistachio fruit. Studies were performed with Kerman, the most popular cultivar for commercial production and traditionally considered the most susceptible to epicarp lesion (11), and cultivar Trabonella in which the disorder is less common. We identified the major free phenols in pericarp tissues and report the induction of specific isoperoxidases after wounding Kerman fruit.

MATERIALS AND METHODS

General. All experiments were conducted with fruits from mature pistachio trees at the University of California Wolfskill Experiment Station, Winters, CA. Fruit clusters for use in experiments were selected in April 1985 and covered with nylon netting to exclude insects (3). Fruits were wounded using carbon steel insect mounting pins that were approximately 150 microns in diameter at the tip. The greatest width at the tip of the stylet bundle of a leaf-footed bug (Fig. 1C) was determined by scanning electron microscopy to be approximately 50.8 microns (Fig. 1D). Epicarp lesion symptoms were elicited on clusters attached to the tree by puncturing each fruit through the pericarp four times on each side of the fruit. Fruits were wounded periodically during April to June 1985 and collected 24–72 hr after wounding. Wounded and unwounded fruits were transported on ice to the laboratory for biochemical studies.

To determine the effect of insect feeding on peroxidase activity, five *L. clypealis* individuals (starved for 24 hr) were caged in a large

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fruit cluster on an orchard tree. After 5 hr, the insects were removed. Fruits exhibiting wounds and symptoms were collected 48 and 72 hr later and assayed for peroxidase. Unwounded fruit were also collected for assay.

Pericarp firmness was determined as described previously (28) by using a U.C. Firmness Tester (Western Industrial Supply, San Francisco, CA) equipped with a Hunter Spring Force Gauge, Series L (Ametek Company, Hatfield, PA) and an 18-gauge needle. Ten to 15 fruits selected at random per collection date were measured (in kilogram force) and values averaged. Additionally, reaction of the endocarp with phloroglucinol-HCl was used as an indicator for the onset of lignification (29).

All enzymes and inhibitors used in our work were purchased from Sigma Chemical Co.

Histochemical methods. Fruits were collected at 0, 24, 48, and 72 hr after wounding and sections made of the injured area using a razor blade. Unwounded fruit were similarly sectioned and served as controls. Peroxidase activity was detected after addition of 0.56% guaiacol in 0.1 M K-phosphate, pH 5.8, and 0.6% H_2O_2, tannins were stained with 1% $FeCl_3$ in 0.5 N HCl (1), and polyphenoloxidase activity was detected with 0.4% aqueous solutions of L-3,4-dihydroxyphenylalanine (L-dopa) or catechol (15).

Peroxidase and polyphenoloxidase activity assays. Pericarp tissue (6 g per sample) from wounded and unwounded fruit were comminuted in 15 ml of extraction buffer using a mortar and pestle. The extraction buffer contained 0.1 M K-phosphate buffer, pH 5.8, 1 M NaCl and insoluble polyvinylpolypyrrolidone (50 mg/ml). The extract was stirred for 1 hr in an ice bath and then filtered with Miracloth (Calbiochem). The filtered extract was further clarified by centrifugation (27,000 *g* for 20 min). Protein content of the supernatant was determined by the method of Bradford (7).

Peroxidase activity was measured in the supernatant by determining the change in absorbance at 470 nm in a reaction mixture containing 0.6% H_2O_2 (0.5 ml), 0.56% guaiacol (0.5 ml, Sigma), and 0.3 ml of diluted extract. Activity was measured from initial rates and extracts were diluted to give a change A_{470nm} per minute between 0.1–0.2 (21).

Polyphenoloxidase activity was also determined in the pericarp extracts using either L-dopa or catechol as substrates (15). The reaction mixture contained 2 μmol substrate and extract (100 μg of protein equivalents) in 1 ml 0.1 M K-phosphate buffer, pH 5.8. Activities were determined from the change in absorbance at 500 nm.

Polyacrylamide gel electrophoresis of pistachio extracts. Cathodic isoperoxidases in Kerman fruit extracts were separated in a gel containing 6% acrylamide (Kodak), 0.2% *bis*-acrylamide (Bio-Rad Laboratories), 0.2 M K-acetate buffer, pH 4.5, 0.1% TEMED, 0.08% ammonium persulfate, and 1 μg/ml of riboflavin. Fruit extracts were concentrated with a Centricom-10 ultrafiltration tube (10,000 m.w. cutoff, Amicon), then diluted in a 1:1 mixture of 50 mM K-acetate, pH 6.1, and glycerol. An aliquot of the extract was placed in wells in a stacking gel consisting of

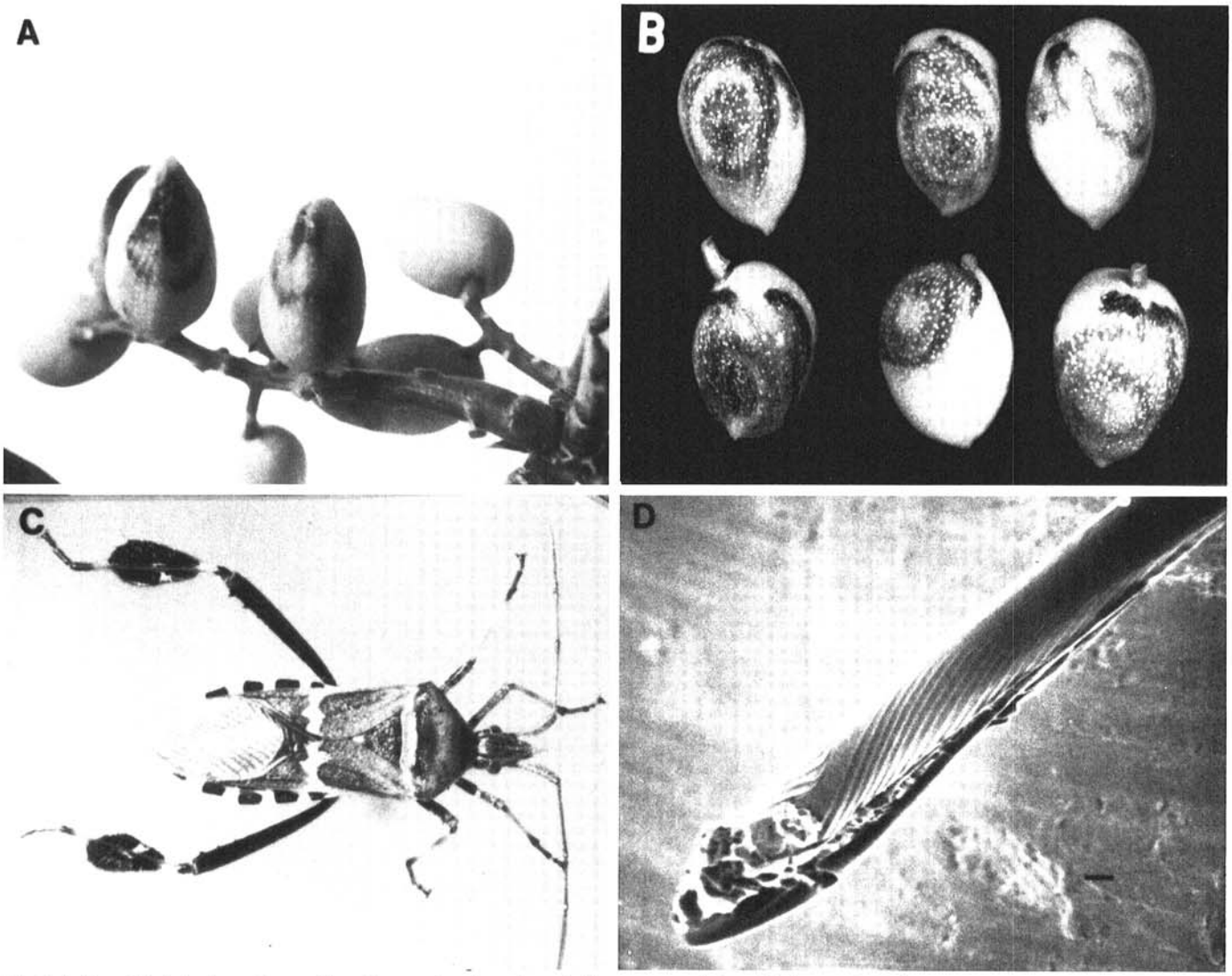

Fig. 1. A, Pistachio fruit cluster from cultivar Kerman showing epicarp lesion symptoms induced by insects. **B,** Lesions produced in Kerman fruit 72 hr after wounding with a needle. **C,** *Leptoglossus clypealis* adult. **D,** Scanning electron micrograph of stylet bundle from *L. clypealis*. Bar = 10 μm.

0.125 M K-acetate buffer, pH 6.1, 3.75% acrylamide, 0.375% *bis*-acrylamide, 0.125% TEMED, 0.025% ammonium persulfate, and 2.5 μg/ml of riboflavin. The upper reservoir buffer contained 50 mM β-alanine titrated to pH 4.5 with acetic acid, and the lower reservoir buffer contained 20 mM K-acetate, pH 4.5. The gel slab (13 cm $\times$ 1.5 mm thick) was developed at a constant 20V/cm, 50 mA. Extracts from wounded and unwounded fruit were adjusted to the same total protein concentration and approximately 50 μg of protein were placed in each well.

At the completion of the electrophoretic run, the gel was removed and placed for 30 min in a solution of 1 mM *o*-dianisidine in 0.1 M Na-acetate buffer, pH 4.5. The gel was then placed in 1% H_2O_2 for about 10 min until peroxidase bands appeared. It was then rinsed with distilled water and fixed with a methanolic solution containing 7% glacial acetic acid and 0.5% glycerol.

Extraction and characterization of pericarp phenols. Pistachio fruits, collected at regular intervals throughout April to July, were frozen at −20 C until extraction. In a preliminary experiment, we found that freezing the fruit before extraction did not affect the phenolic pattern observed by thin-layer chromatography (TLC) in comparison with fruit extracted immediately after collection from the field. The methods used for extraction were similar to those described by Bohm and Towers (2). For each sampling date, three 5-g samples of healthy pericarp tissue were homogenized in 25 ml of hot 95% ethanol with a Polytron tissue homogenizer (Kinematica). Each extract was refluxed for 2 hr, then filtered with Whatman No. 1 paper. The filtrate was concentrated in vacuo by rotary evaporation and the concentrate diluted with 5 ml of water. After acidifying the extract to approximately pH 3 with HCl, it was washed with three 5-ml aliquots of diethyl ether. The ether phases were combined and concentrated under N_2. The free phenolic extract was then diluted with 95% ethanol.

Total phenols were quantitated by the Folin-Ciocalteau method (12) using gallic acid as a standard. The principal phenolic components were separated on 250μ silica gel HL or HLF thin-layer plates (Analtech) developed in a mixture of toluene:pyridine:methanol (70:15:15, v/v). Individual phenols were visualized and characterized in part by their cochromatography with authentic standards, appearance under UV light, and reactions with acidic $FeCl_3$, diazotized *p*-nitroaniline, and vanillin-HCl (1). An extract was hydrolyzed in 1 N methanolic-HCl at 100 C for 18 hr, then analyzed by TLC to also confirm the presence of gallotannins.

RESULTS

Induction of epicarp lesion and stimulation of peroxidase activity by wounding and leaf-footed bugs. Needle-puncture elicited within 48–72 hr symptoms typical of insect-induced epicarp lesion (Fig. 1A) in both Kerman (Fig. 1B) and Trabonella fruit. Within 24 hr of wounding and before appearance of external symptoms, the endocarp tissue in the vicinity and several millimeters from the wound in Kerman fruit reacted strongly with guaiacol/H_2O_2 (Fig. 2). The endocarp in unwounded fruit did not react with the reagent as strongly or as rapidly. However, mesocarp and epicarp tissue in both wounded and unwounded fruit reacted with the reagent. In Trabonella fruit, injured mesocarp and epicarp tissues reacted more strongly than in the unwounded controls. However, the endocarps in both wounded and unwounded fruit appeared to have similarly high peroxidase activities (Fig. 3).

Large resin ducts (Figs. 2 and 3) abundant in the mesocarp layer of the fruit wall, reacted very strongly with the peroxidase reagent. Tannins primarily were localized in cells surrounding the resin ducts in unwounded fruit (not shown). Except for the tissue immediately aligning the wound, polyphenoloxidase activity was not detected histochemically in wounded or unwounded fruit.

For fruit collections made to early June, peroxidase activities in pericarp extracts from wounded Kerman and Trabonella fruit increased concomitantly with lesion development (Fig. 4). However, the ratio of peroxidase activities (72-hr samples) in wounded to unwounded fruit declined during fruit maturation and shell hardening in both cultivars (Fig. 5). The peroxidase activity in extracts from unwounded Trabonella fruit increased during fruit maturation, whereas the peroxidase activity in unwounded Kerman fruit remained fairly constant during the sampling period. Feeding injury to fruit by *L. clypealis* induced epicarp lesion and also enhanced peroxidase activity (Table 1).

Effect of wound depth on epicarp lesion development and peroxidase activity. As reported previously (25,28), penetration of the entire pericarp was essential for symptom development (Table 2). Stimulation of peroxidase activity was only observed in fruit in which the wound penetrated the entire pericarp. Several samples for each wound treatment were examined with a dissecting scope to confirm the depth of the wound.

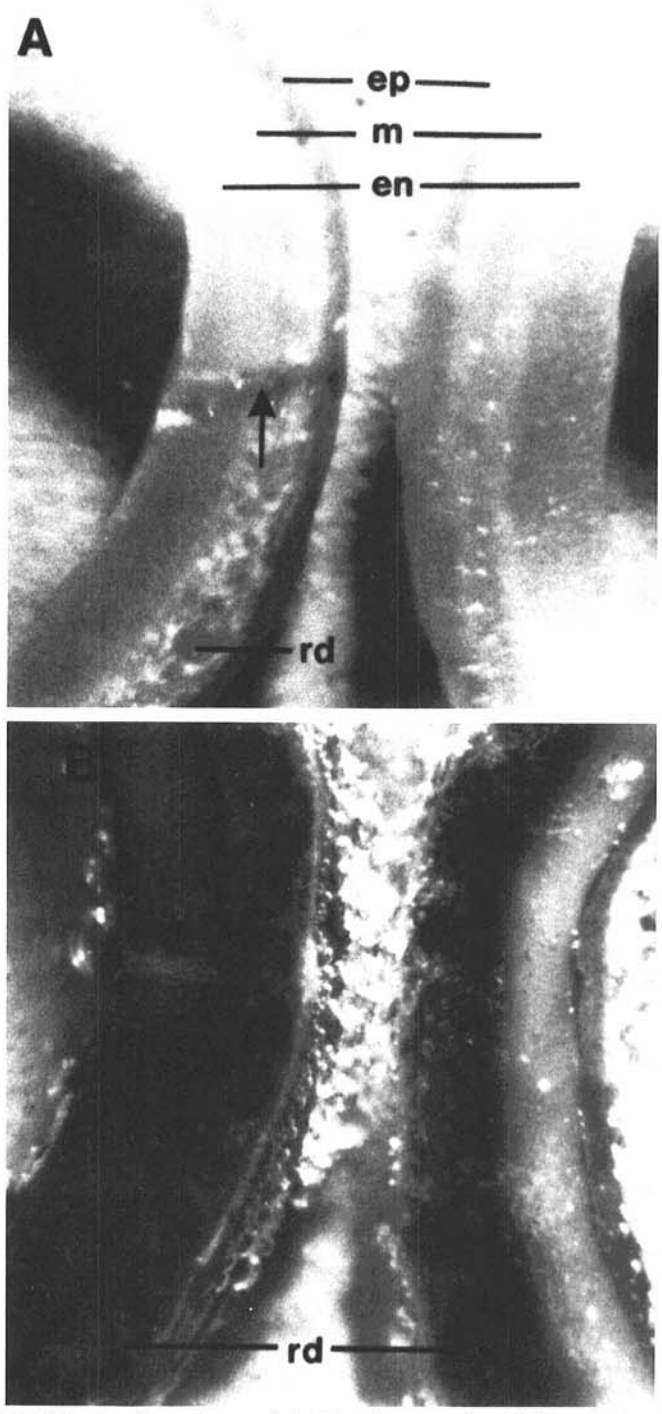

Fig. 2. Sections from Kerman fruit 24 hr after wounding. **A,** Unstained and **B,** Stained for peroxidase. Section from wounded fruit on left, unwounded on right in each photograph. Pericarp consists of the endocarp (EN), mesocarp (M) containing large resin ducts and accessory cells (RD), and epicarp (EP) tissue layers. Wound sites (arrow) are indicated.

Effect of horseradish peroxidase, polyphenoloxidase, and inhibitors on wounded fruit. The effect of wound depth on lesion development was exploited to further assess the role of peroxidase. Several drops of a 1 unit per milliliter aqueous solution of horseradish peroxidase (Type I, RZ = 0.6, EC 1.11.1.7) and an aqueous solution of mushroom polyphenoloxidase (1 unit per milliliter, assayed with L-dopa as substrate, EC 1.14.18.1) were applied separately to fruits that had been wounded to the depth of the mesocarp layer. The peroxidase solution was assayed with guaiacol as substrate and 1 unit per milliliter corresponded approximately to the activity present in extracts of wounded fruit (500–1,000 A_{470nm} min^{-1} mg^{-1}). Peroxidase but not polyphenoloxidase elicited lesion symptoms in the pericarp tissue similar to those observed in fruit affected by epicarp lesion (Fig. 6 and Table 3).

Several inhibitors of peroxidase and polyphenoloxidase were tested for their effect on lesion development in fruit that had been wounded through the endocarp layer. A few drops of an aqueous solution of catalase (1 unit per milliliter) or of one of the inhibitors (10 mM) were applied to the wounds. Catalase, which inhibits peroxidase by removal of H_2O_2, inhibited or delayed symptom development (Table 4). Diethyldithiocarbamic acid and phenylthiourea, inhibitors of polyphenoloxidase but not of peroxidase, did not affect lesion development.

Induction of isoperoxidases. Wounding induced the formation of at least three cathodic isoperoxidases in pericarp tissue not present in unwounded fruit (Fig. 7). In addition, there appeared to be an intensification of the broad band near the origin (anode in Fig. 7). There was a faint band (third isoperoxidase from the anode in the diagram in Fig. 7) present in the gel patterns of extracts from both wounded and unwounded fruit, but this is not apparent in the

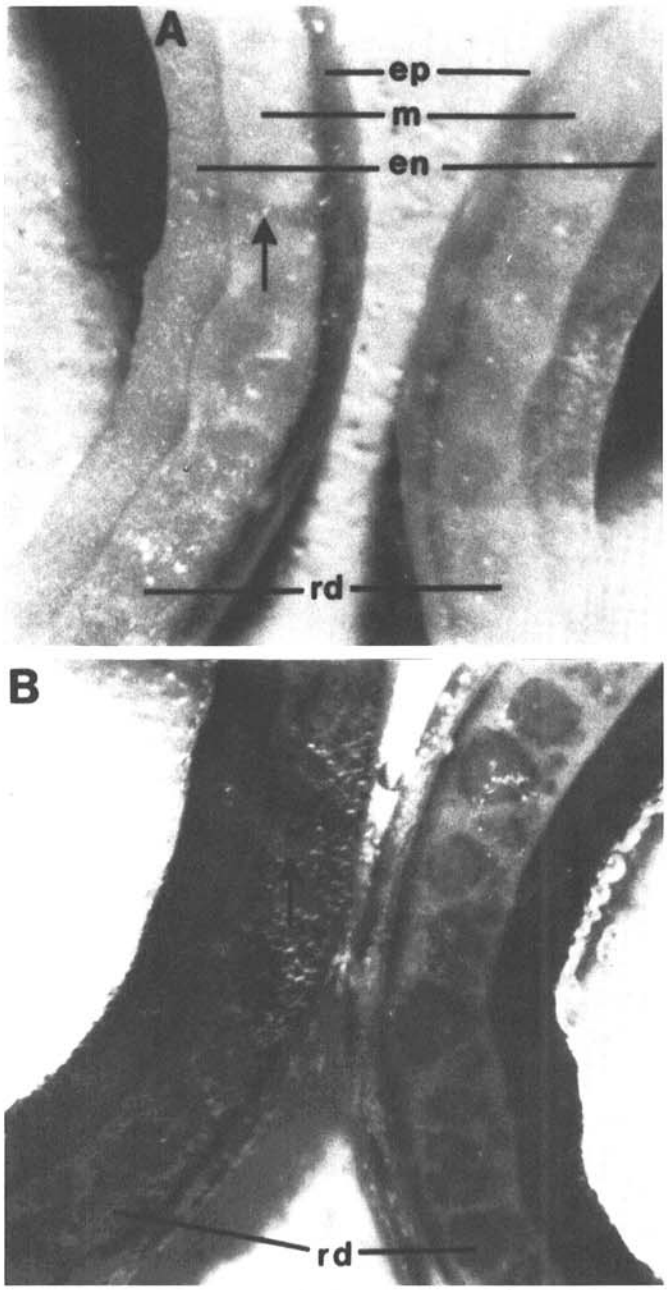

Fig. 3. Sections from Trabonella fruit 24 hr after wounding. **A,** Unstained and **B,** Stained for peroxidase. Section from wounded fruit on left, unwounded on right in each photograph. Pericarp consists of the endocarp (EN), mesocarp (M) containing large resin ducts and accessory cells (RD), and epicarp (EP) tissue layers. Wound sites (arrow) are indicated.

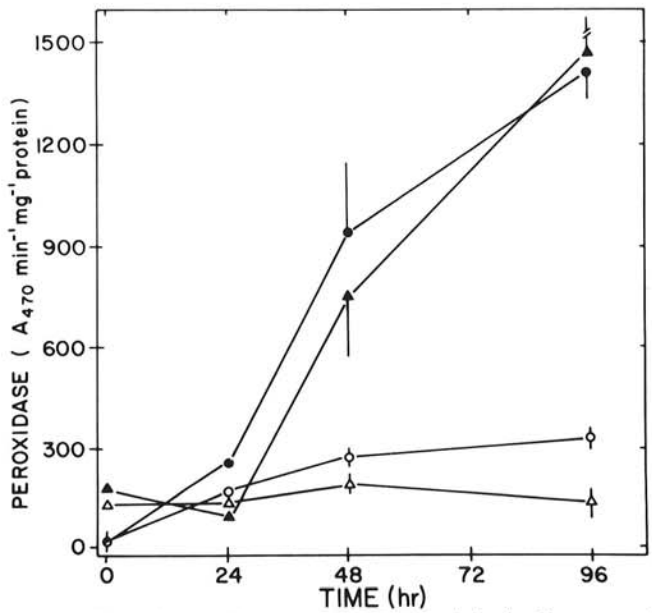

Fig. 4. Effect of wounding on peroxidase activity in Kerman and Trabonella fruit during lesion development, 25–29 April 1985. —▲— = Kerman wounded; —●— = Trabonella wounded; —△— = Kerman unwounded: —○— = Trabonella unwounded. Each value is the average and standard error of two extracts.

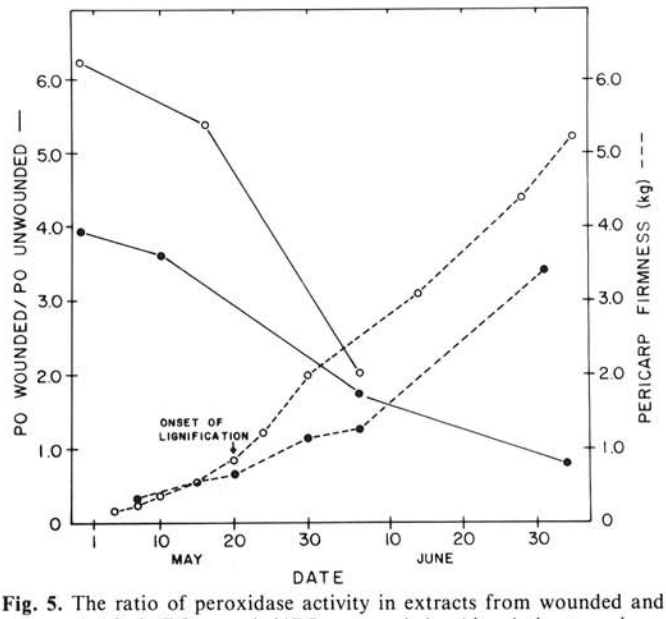

Fig. 5. The ratio of peroxidase activity in extracts from wounded and unwounded fruit (PO wounded/ PO unwounded, —) in relation to pericarp firmness (---) during maturation. Kerman (○), Trabonella (●). Peroxidase ratios were derived from activities 72 hr after wounding in two extracts per treatment for each cultivar. Each value for pericarp firmness is the mean of 10–15 fruit.

photograph of the gel. In a preliminary experiment, anodic peroxidases were also examined using the system described by Keleti and Lederer (13). No clear differences between wounded and unwounded fruit were detected in the anodic isoperoxidase patterns although further work is necessary to unequivocally establish this.

Characterization and quantitation of pistachio pericarp phenols. The composition of the major phenols from the pericarp tissues of both Kerman and Trabonella fruit appeared to be nearly identical and were extremely rich in gallotannins (Fig. 8). This was indicated by their intense blue-violet reaction with ferric chloride and dark blue fluorescence under UV light (22). The fluorescence of these compounds was not appreciably affected after exposure to ammonia vapor and were light brown after spraying with diazotized *p*-nitroaniline. The principal phenols cochromatographed with gallic acid ($R_f = 0.33$) and methyl gallate ($R_f = 0.5$). More complex gallotannins were evident ($R_f = 0.13$ and 0.03) and were hydrolyzed by methanolic-HCl. As expected, there appeared to be an increase in methyl gallate after hydrolysis (Fig. 8). Catechol tannins were not detected after spraying with vanillin-HCl.

TABLE 1. The effect of injury during feeding by insects on peroxidase activity in pistachio fruit pericarp tissue

Cultivar and treatment	Peroxidase (ΔA_{470nm} min^{-1} mg^{-1} protein)[a]	
	48 hr	72 hr
Kerman[b]		
Unwounded	216 ± 3	167 ± 1
Wounded	535 ± 87	697 ± 82
Trabonella[c]		
Unwounded	···	339 ± 74
Wounded	···	1,173 ± 34

[a] Each value is the mean and standard error of peroxidase activities in two extracts.
[b] Five *Leptoglossus clypealis* individuals allowed to feed on fruit clusters for 5 hr, removed, and 48 or 72 hr later pericarp tissues from symptomatic fruit extracted and evaluated for peroxidase activity. Experiment initiated on 20 May 1985.
[c] Naturally affected fruit collected from unbagged clusters exhibiting symptoms similar to fruit 72 hr after artificial wounding. Experiment initiated on 15 May 1985.

TABLE 2. The effect of wound depth on epicarp lesion development and peroxidase activity in cultivar Kerman pistachio fruit

Wound depth	Fruit with lesions[a] (%)	Peroxidase activity[b] (Δ A_{470nm} min^{-1} mg^{-1})
Control (unwounded)	0	132 ± 4 y
Shallow to mesocarp	0	109 ± 16 y
Shallow to endocarp	1	169 ± 22 y
Deep through endocarp	72	713 ± 117 z

[a] Values are the mean of three replications, 15–30 fruit per replicate. Experiment initiated on 16 May 1985.
[b] Values are the mean and standard error of activities in two extracts of fruit pericarp tissue. Means followed by the same letter are not significantly different, Duncan's multiple range test ($P = 0.05$).

TABLE 3. The effect of horseradish peroxidase and polyphenoloxidase on lesion development in shallow-wounded cultivar Kerman pistachio fruit[a]

Treatment	Fruit with lesions[b]	
	48 hr	72 hr
Water	2 ± 3 y	4 ± 4 y
Peroxidase (1 unit/ml)	16 ± 6 z	20 ± 10 z
Polyphenoloxidase (1 unit/ml)	0 y	2 ± 4 y

[a] Experiment initiated on 1 May 1985.
[b] Each value is the mean and standard deviation of three replications with approximately 10–30 fruit per replicate. Values followed by the same letter within a column are not significantly different, Duncan's multiple range test ($P = 0.05$). Lesions evaluated 48 and 72 hr after treatment.

An unidentified component ($R_f = 0.63$) was present in high amounts in fruit extracts. This compound fluoresced bright blue under longwave UV light, yielded a rose color after spraying with ferric chloride, and was bright yellow after spraying with diazotized *p*-nitroaniline. It had absorption maxima at 259 nm and 295 nm. Freezing and thawing the fruit separated the epicarp and mesocarp from the endocarp. Using this technique, we found the unknown to be primarily restricted to the epicarp and mesocarp tissues. This component has properties similar to some coumarins but did not cochromatograph with scopoletin or any other available standards.

Total phenol concentrations were higher in both Trabonella and

TABLE 4. The effect of inhibitors of peroxidase and polyphenoloxidase on lesion development in deep-wounded Kerman fruit[a]

Treatment	Fruit with lesions[b] (%)	
	48 hr	72 hr
Water	84 ± 14 y	86 ± 12
Peroxidase inhibitor		
Catalase (1 unit/ml)	43 ± 2 z	77 ± 12
Polyphenoloxidase inhibitors		
Diethyldithiocarbamic acid (10 mM)	91 ± 8 y	94 ± 6
Phenylthiourea (10 mM)	89 ± 1 y	91 ± 8

[a] Experiment initiated on 1 May 1985.
[b] Each value is the mean and standard deviation of three replications with approximately 10–30 fruit per replicate. Values followed by the same letter are not significantly different, Duncan's multiple range test ($P = 0.05$). Lesion development evaluated 48 and 72 hr after treatment. Values at 72 hr after treatment were not significantly different.

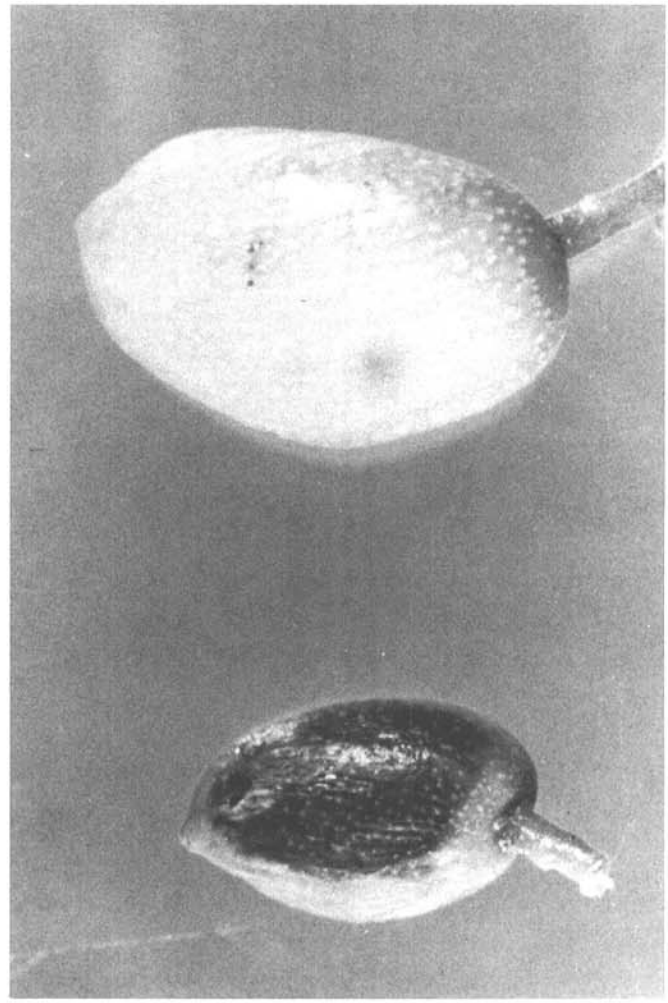

Fig. 6. The effect of horseradish peroxidase on Kerman fruit. Bottom, shallow wound plus enzyme; Top, shallow wound only.

Kerman fruit in April but declined and remained relatively constant during May and June (Fig. 9). Thin-layer analysis of the Kerman fruit extracts indicated that the concentration of gallotannins was greater in April than in May. The concentration of the unknown did not appear to change during fruit maturation, hence no further work was performed to characterize it.

Horseradish peroxidase (100 units per milliliter) and H_2O_2 or concentrated crude enzyme extracts from wounded fruit and H_2O_2 rapidly degraded (within 15 min) the gallotannins and unidentified component in the pericarp phenol extracts (approximately 1 mg of gallic acid equivalents) and produced brown-colored oxidation products in the reaction mixture. Autoclaving the enzyme preparations for 90 min eliminated their activity for the pericarp phenols. However, comparison by TLC of phenol extracts from healthy and symptomatic fruit did not reveal marked differences in gallotannin levels (data not shown).

DISCUSSION

Our results indicate that wounding pistachio fruit by needle puncture stimulates peroxidase activity in a manner similar to insect injury. Enzyme activity in both pistachio cultivars was detected histochemically within 24 hr of injury and before symptom development. However, the histochemical observations suggest a slightly different pattern between these cultivars. In Kerman fruit, a difference between wounded and unwounded fruit in the reaction with guaiacol/H_2O_2 was observed primarily in the endocarp. In Trabonella fruit, wound-induced peroxidase activity was apparent in the mesocarp and epicarp but the endocarp in both treatments reacted strongly with the reagent. Nevertheless, the enhanced enzyme activities were similar in fruit extracts from both cultivars during symptom development (Fig. 4).

In addition to the above findings, an important role for peroxidase in epicarp lesion development was suggested by the effect of externally introduced horseradish peroxidase into the epicarp-mesocarp layers on symptom expression, partial suppression of symptom expression in immature fruit with catalase, and the requirement of endocarp puncture for lesion development and stimulation of peroxidase. There was no evidence for the participation of polyphenoloxidase in epicarp lesion.

The decline in incidence of epicarp lesion in late May and June was related to lignification of the endocarp (28). The markedly diminished wound-induced peroxidase activity after the onset of lignification (Fig. 5) suggests another developmental change in maturing fruit that may contribute to this decline. The contribution, if any, of gallotannins or their oxidation products to symptom development remains to be established. Nevertheless, in April we observed that small, immature fruit expressed symptoms very rapidly and a single puncture caused the entire fruit to blacken and abscise. The levels of gallotannins were highest in these fruit (Fig. 9).

On other crop plants, feeding by the mirid, *Cyrtopeltis tenuis*

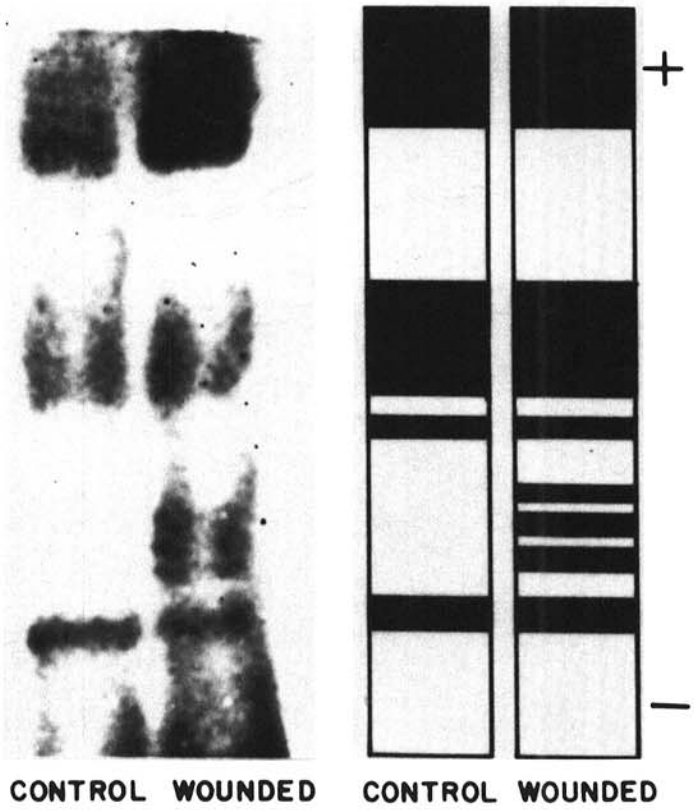

Fig. 7. Cathodic PAGE peroxidase isozyme profiles in extracts from wounded and unwounded (control) Kerman fruit. Left, photograph of gel stained for peroxidase activity as described in materials and methods. Right, diagramatic representation. (See text for further explanation.)

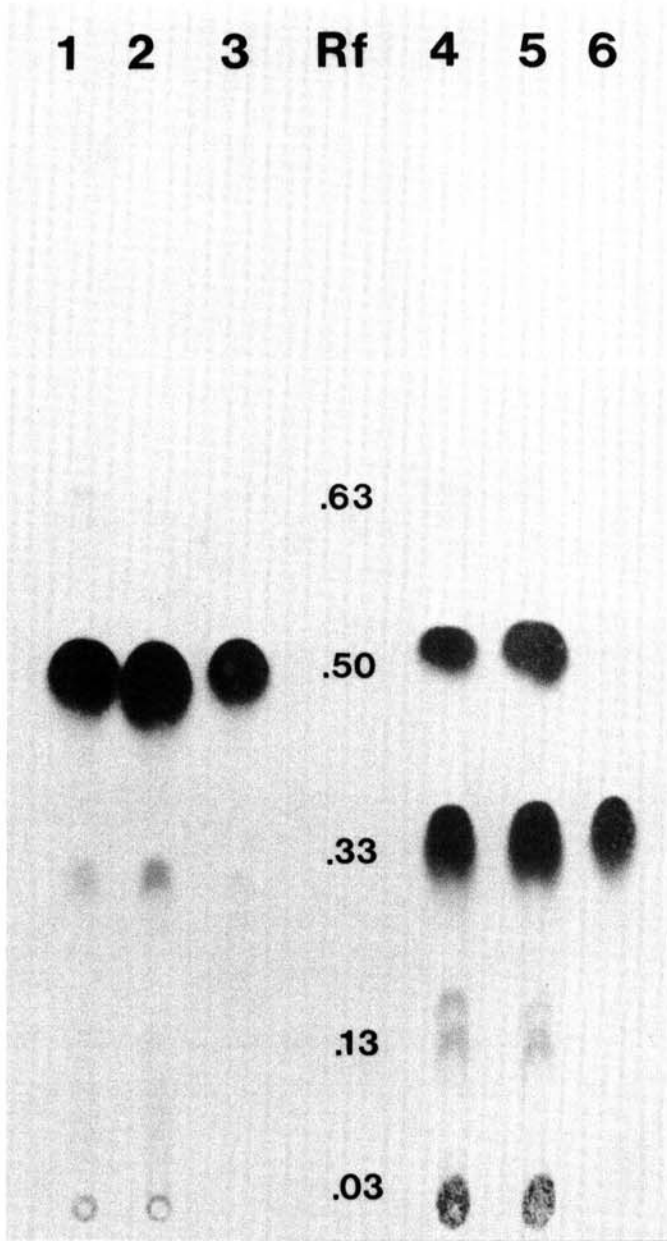

Fig. 8. Thin layer chromatogram oversprayed with acidic $FeCl_3$ of ethanol extracts from Kerman and Trabonella fruit. Lane 1, methanolyzed extract from Trabonella fruit; lane 2, methanolyzed extract from Kerman fruit; lane 3, methyl gallate; lane 4, free phenols from Trabonella fruit; lane 5, free phenols from Kerman fruit; and lane 6, gallic acid.

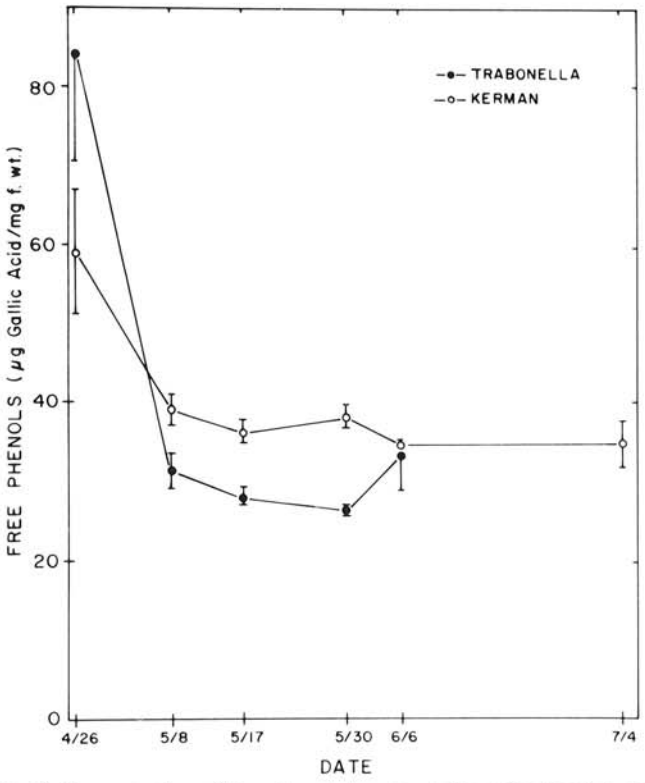

Fig. 9. Concentration of free phenols in extracts from Kerman (○) and Trabonella (●) pericarp tissue during early fruit development. Each value is the mean and standard error of three extracts.

(Reut.), produced necrotic lesions, enhanced oxidative enzyme activity (both peroxidase and polyphenol oxidase), and induced formation of three isoperoxidases in injured stem tissues of tomato (17,18). Similar observations for wounds by other hemipterous insects after feeding on plants have been reported (10,24). Mechanical injury to cotton buds mimicked the feeding damage caused by *Lygus hesperus* and the damage was most severe in young buds (24). In several of these studies, the authors presented evidence suggesting a contribution of salivary enzymes (e.g., polygalacturonase) to lesion development. We did not detect polygalacturonase activity in extracts of whole individuals or salivary glands of *L. clypealis* (*unpublished*). Although pectinase was reported to induce epicarp lesion symptoms (3), data of Uyemoto et al (26) and this report indicate that insect salivary enzymes are, at best, only indirectly involved in this fruit disorder.

The histochemical observations and depth of wounding experiments suggest that cells several millimeters from the wound site respond to a signal generated by the wounded cells of the endocarp. The nature of such a signal is unknown, although ethylene is a candidate because this hormone stimulates peroxidase activity in plants and is produced in wounded tissue (21). Future research on the mechanisms of epicarp lesion development should focus on the detailed cytology of the host response to wounding, the nature of the wound-induced signal, and the location and substrate specificity of the induced peroxidases. It will also be of interest to determine if differences exist among pistachio cultivars in their susceptibility to epicarp lesion and if these differences are reflected in the induced isoperoxidase patterns. Although current efforts on reducing epicarp lesion are focused on insect control (9), consideration of the sensitivity of fruit to wound-induced lesion development could be of value in pistachio cultivar improvement programs. The results of our study provide a basis for examining the relationship of induced peroxidases to stylar-end necrosis and other physiological browning disorders in pistachio fruit and may assist in identifying stresses which contribute to them.

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