Quantification of Lignin Formation in Almond Bark in Response to Wounding and Infection by *Phytophthora* Species

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ABSTRACT

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A quantitative assay for lignin using thioglycolic acid was adapted for investigating lignification in inner bark tissue of almond trees in response to wounding and infection by several *Phytophthora* species. One-day-old wounds had 71% more ligninthioglycolic acid (LTGA) than unwounded tissue. LTGA of wounded tissue increased linearly ($r^2 = 0.95$) over the 3-wk period following wounding. Cupric oxide oxidation of LTGA from 2-wk-old wounds confirmed that lignin was produced in response to wounding. The lignin content in variously aged wounds was negatively correlated ($r = -1.00$) to the length of cankers resulting after inoculation of similarly aged wounds with *P. syringae*. Substantially more LTGA was detected in the tissue of cankers caused by *P. syringae* than in nearby healthy tissue. Wounds inoculated with *P. syringae*, *P. hibernalis*, and *P. cactorum* had substantially more LTGA 3 days after inoculation than uninoculated wounds, whereas wounds inoculated with *P. infestans*, a nonpathogen of almond, had the same amount of lignin as the uninoculated wounds. The deposition of lignin in almond inner bark appeared to be a response to wounding and infection by pathogens.

In studies of fungal canker diseases of almond trees, *Prunus dulcis* (Miller) D. A. Webb, it was observed that pruning wounds eventually become immune to infection by *Phytophthora syringae* (Kleb.) Kleb. (5,8). During preliminary histological studies with almond inner bark, we observed a general dieback of the cells near the wound surface soon after wounding, followed by the formation of a lignified zone and later a suberized wound periderm extending from the outer bark to the xylem. The formation of lignin and of a wound periderm around tree bark wounds has been reported in several histological studies (4,8,14,16). Vance et al have suggested that lignin formation is a mechanism of resistance to ingress by plant pathogens (23). The suberized periderm formed around bark injuries appears to provide a barrier to infection by canker pathogens (4,14,20).

During initial investigations of the role of lignification in development of resistance, we found it difficult to quantify wound-induced lignin formation using histological techniques and were concerned that other phenolics besides lignin might be stained. A routine assay was needed that would be quantitative and more specific for lignin and permit the analysis of a large number of samples. An important long-established method of isolating and characterizing lignin from wood uses thioglycolic acid (TGA) (9). TGA reacts readily with the α-alkoxy function in lignin to give ligninthioglycolic acid (LTGA) derivatives (24), and the LTGA can be extracted from the tissue with NaOH. This method has been adapted to quantify lignin in tissue cultures (25) and potato tubers (11).

The objectives of our research were to adapt the TGA assay for determination of lignin in inner bark tissue of almond trees and to use the assay to determine the time course of wound-induced lignification. Additionally, we used the assay to investigate the formation of lignin in response to selected species of *Phytophthora*.

MATERIALS AND METHODS

Potted almond trees (cv. Nonpareil) one and two years old were purchased from a local nursery and maintained in a lathhouse until needed for experiments. The diameters of the stems of these trees at the positions of the wound treatments were 1.5–2.0 cm. Six-year-old, 11-year-old, and 25-year-old almond trees (cv. Nonpareil) in orchards were used for the field experiments. Branches used for wound treatments were approximately 1–2 cm in diameter. All fungi used were maintained on amended lima bean agar (ALBA) (6) or V-8 agar at 15 C. For inoculations, the fungi were grown on cornmeal agar (CMA) or ALBA. All experiments using potted trees were performed in a growth chamber with a 12-hr light period (10 Wm^{-2}) kept at 25 C unless otherwise noted. In all experiments, after wounds had aged, whole branches were cut out and kept in an ice chest during transport to the laboratory, and the wounded tissues were prepared for analysis the same day. In experiments in which the lignification response of corkborer wounds was studied, wounds were made with a corkborer with a diameter of 5 or 6 mm, and a 5-mm-wide ring was dissected from around the wound with a larger diameter corkborer.

TGA assay. Unless indicated otherwise, the following procedure was used for the experiments. The dead outer bark was carefully peeled or cut away from the inner bark using forceps and a razor blade, and a dissecting microscope was used to verify complete separation. The inner bark tissue was separated from the xylem and extracted in four changes of methanol over 24 to 48 hr. The tissue samples were dried overnight in a vacuum desiccator and then weighed. To each tissue sample, 5 ml of 2 N HCl containing 0.5 ml of TGA (Sigma Chemical Co., St. Louis, MO) was added, and the tubes were capped and placed in an oven at 95 C for 4 hr. The acid was drained off, and the samples were rinsed in 5 ml of deionized water. The samples were then extracted with 5 ml of 0.5 N NaOH for 18 hr at room temperature. The NaOH extracts were decanted into 12-ml conical centrifuge tubes. The samples were rinsed with 5 ml of deionized water, which was then added to the NaOH extract. The extracts were acidified with 1 ml of concentrated HCl and placed in an oven at 5 C for 4 hr. The precipitate was collected by centrifugation for 10 min in a clinical centrifuge at approximately 850 *g*. The acidic supernatant was drained off, and the pellet was resuspended in 0.5 N NaOH. The resuspensions were centrifuged to remove any undissolved matter. The absorbances of the supernatants were then measured at 280 nm (A280) with a spectrophotometer. The A280 was used as a measurement of relative lignification (11,25) or of the concentration of LTGA by using the absorptivity value of 11.9 g^{-1} L cm obtained below.

Development of the TGA assay for use with the inner bark. Branches on orchard trees were wounded in March and April 1985 by making cross-sectional cuts through internodes with pruning shears. Six weeks later, 2–4 × 4 mm areas were removed from next to the wound surface and from unwounded tissue. In the first experiment (March), the outer bark was not removed in half of the samples. In the second experiment (April), to test if an NaOH pretreatment affected the A280 of the LTGA extracts, half of the samples after methanol extraction and drying were pretreated by immersing them in 5 ml of 0.5 N NaOH for 24 hr and then adding 1.25 ml of 2 N HCl. The liquid was poured off, and the samples were rinsed twice with deionized water. The standard TGA assay outlined above was followed in the remainder of the procedure in both experiments. There were three and four replications per treatment, respectively, in the first and second experiments.

Determination of the absorptivities of LTGA from almond tissues. The absorptivities of LTGA derived from the xylem, the outer periderm (outer bark), and wounded inner bark were determined to obtain standard curves. The outer periderm (approximately 800 mg dry weight per replication) and xylem (approximately 200 mg dry weight per replication) were obtained from branches from orchard trees, with three replications for each tissue.

Wounded inner bark was obtained from tissues surrounding corkborer wounds that had aged 4 wk. There were three replications, each containing inner bark tissue from 15 corkborer wounds. The standard TGA assay was followed except that the quantities of reagents were adjusted proportionally to account for the larger tissue samples. Also, before the acid was precipitated, the solution was filtered to remove contaminating tissue fragments. After the acid precipitation, the samples were centrifuged (15 min at 5,800 *g*), washed in deionized water, and centrifuged two more times. The resulting LTGA pellet was dried 3 hr in a 60 C oven and stored in a vacuum desiccator. A portion of the purified LTGA was weighed and dissolved in 0.5 N NaOH, and the A280 was read for various dilutions to obtain the standard curve and the absorptivity.

Cupric oxide oxidation of the LTGA. The main stems of four 2-yr-old potted trees were wounded by making cross-sectional cuts with pruning shears. Two weeks later the inner bark from around the wounded surface and from unwounded tissue was removed from each tree (approximately 30–80 mg ofmethanol-extracted dry weight per sample). To the LTGA obtained from these tissues, 1.5 ml of 3 N NaOH and 265 mg of $CuSO \cdot H_2O$ were added, and the mixture was placed in a Parr 4749 acid digestion bomb (Parr Instrument Co., Moline, IL) in a 180 C oven for 2.5 hr (11). When cool, the contents were transferred with the aid of deionized water to conical centrifuge tubes. After centrifuging for 10 min in a clinical centrifuge, the supernatant was transferred to a test tube. The solids were resuspended in 1.5 ml of 0.5 N NaOH and centrifuged as before, and the supernatant was added to the previous supernatant. The pH of the supernatant fraction was adjusted to about 3 with 6 N HCl and then extracted with three half-volumes of diethyl ether. The ether extracts were partitioned against equal volumes of freshly prepared 1% NaHC03. The ether was evaporated under nitrogen, and the residue was dissolved in 0.2 ml of methanol. The samples and *p*-hydroxybenzaldehyde, syringaldehyde, and vanillin standards were spotted on 250 μm HLF silica gel TLC plates (Analtech, Newark, DE) and developed in chloroform:acetone (94:6, by volume). The aldehydes were located with UV irradiation. The silica gel with fluorescent spots corresponding to the standards was scraped off and extracted in 1 ml of chloroform:methanol (2:1, by volume), and the extract was filtered through glass wool. The silica gel was reextracted three more times in 1 ml of chloroform:methanol and filtered. The filtrates were combined and evaporated to dryness under a stream of nitrogen, and residues were dissolved in 0.02% KOH in 95% ethanol. The amounts of the aldehydes and the UV spectra were determined spectrophotometrically (15).

Time course of lignification in wounds in potted and orchard trees. Cuts were made through bark to the xylem of 10 2-yr-old potted trees with a 6-mm-diameter corkborer at various times and were distributed over 30 cm of the trunk. The inner bark tissue surrounding the aged (1–9 days old) and fresh wounds was removed with a larger corkborer, and the lignin content of these tissues was determined.

In the orchard experiments, wounds were made in a similar fashion in branches in late September and early October 1985. Wounds were made at various times on each of eight branches distributed randomly over a half-meter section of the branch. Tissues surrounding the various aged (2–21 days old) wounds were removed with a corkborer, and the inner bark was assayed for lignin. The average temperature during the course of the experiment was 20.4 C, and the daily average ranged from 14.7 to 25.3 C.

Correlation of lignification and resistance. Each week for 3 wk two corkborer wounds were made in each of 10 1-yr-old potted trees kept in an 18 C growth chamber. Half of the wounds were inoculated with 5-mm-diameter CMA disks of *P. syringae* and wrapped with Parafilm, and the other wounds were analyzed by the TGA assay. After 4 wk, the amount of discoloration due to canker formation was measured and the TGA assay was performed on the inner bark surrounding the wound.

Effect of *Phytophthora* species on lignification. In an experiment to investigate the effect of infection by *Phytophthora* species on lignification in bark wounds, 1-yr-old potted trees in an 18 C growth chamber were wounded with a corkborer. Agar plugs from colonies of *P. syringae* (from an almond pruning wound canker), *P. hibernalis* (from orange), *P. cactorum* (from almond crown rot), and *P. infestans* (from potato) were placed in fresh wounds and wrapped loosely with parafilm. To determine if the type of medium influenced the host response, mycelial plugs from two media, ALBA and CMA, were used for inoculation. Controls were uninoculated wounds treated with sterile media and uninoculated wounds with no media. All of the different treatments were applied to each of five trees resulting in five replications per treatment. After 3 days, the area surrounding the wound was removed and assayed for lignin.

To investigate the deposition of lignin in the inner bark in relation to the canker margin, wounds in seven 1-yr-old potted trees were inoculated with mycelial plugs of *P. syringae* and kept in an 18 C growth chamber. After 6 wk, various parts of the canker and the healthy tissue were removed with a 6-mm-diameter corkborer and the TGA assay was performed on the inner bark tissues.

RESULTS

Development of TGA assay for use with the inner bark. The amount of LTGA per wound was 0.70 mg around 6-wk-old wounds with outer bark, 0.21 mg for 6-wk-old wounds with outer bark removed, 0.45 mg for fresh wounds with bark, and 0.03 mg for fresh wounds with bark removed. The mean dry weight of the wounded tissue samples was 6.9 mg. In the factorial analysis of variance, there was significantly more LTGA around wounds with outer bark (0.58 mg) than around wounds with outer bark removed (0.12 mg) ($P < 0.001$) and around 6-wk-old wounds (0.46 mg) than around fresh wounds (0.23 mg) ($P < 0.01$).

The pretreatment of inner bark tissue with NaOH did not significantly decrease the LTGA detected. For the 6-wk-old wounds, the mean weight of LTGA per wound was 0.25 mg with the NaOH pretreatment and 0.23 mg without the pretreatment. The mean dry weight of the wounded tissue samples was 26 mg.

A high correlation ($r^2 = 0.999$) was found between concentration of LTGA and absorbance (Fig. 1). The absorptivity was determined to be 11.9, 9.4, and 8.4 g^{-1} L cm for the LTGA derived from wounded inner bark, xylem, and outer bark, respectively. The percentage yield (dry weight LTGA/methanol-extracted dry weight samples × 100) was 4, 15, and 31% for wounded inner bark, xylem, and outer bark, respectively.

Cupric oxide oxidation yielded more of the three phenolic aldehydes (*p*-hydroxybenzaldehyde, vanillin, and syringaldehyde) from the LTGA from 2-wk-old wounded tissue than from unwounded tissue (Table 1). All three aldehydes were detected in all unwounded tissues.

Time course of lignification. In potted trees, 1-day-old wounds had 71% more LTGA than fresh wounds ($P < 0.001$ by paired t-test). The amount of LTGA per wound increased linearly ($r^2 = 0.98$) over the 9-day period (Fig. 2). The methanol-extracted dry weight of the wounded tissue increased 34% over a 9-day period and this increase was linear ($r^2 = 0.9$)(Fig. 2). The variable, LTGA per milligram dry weight of inner bark tissue, also increased linearly ($r^2 = 0.99$).

In orchard trees, 2-day-old wounds had 191% more LTGA than fresh wounds ($P < 0.01$ by paired t-test). The amount of LTGA increased linearly ($r^2 = 0.95$) over the 3-wk observation period (Fig. 3). The methanol-extracted dry weight of the wounded tissue increased 136% over a 3-wk period and this increase was linear ($r^2 = 0.92$) (Fig. 3).

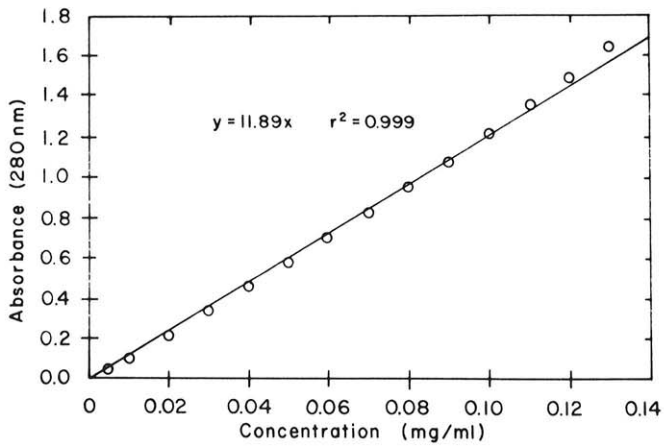

Fig. 1. Standard curve for ligninthioglycolic acid (LTGA) from 4-wk-old bark wounds in orchard almond trees. The regression formula was derived using only the data from LTGA concentrations of 0.01 to 0.08 mg/ml and was restricted so that the intercept was zero. Each point represents the mean absorbance from the data from branches on three trees.

TABLE 1. Cupric oxide oxidation products of ligninthioglycolic acid extracted from 2-wk-old wounded and unwounded almond inner bark tissue cell walls[a]

Treatment	Oxidation products[b]		
	p-Hydroxybenzaldehyde	Vanillin	Syringaldehyde
Wounded	38.8[c]	55.8[c]	20.0[d]
Unwounded	12.5	19.3	3.0

[a] The main stems of 2-yr-old potted trees were wounded by making cross-sectional cuts with pruning shears, and the trees were then kept at 25 C.
[b] Aldehyde μg/g methanol-extracted dry weight.
[c] Wounded tissue had significantly ($P < 0.05$) more oxidation product than unwounded tissue according to a paired t-test.

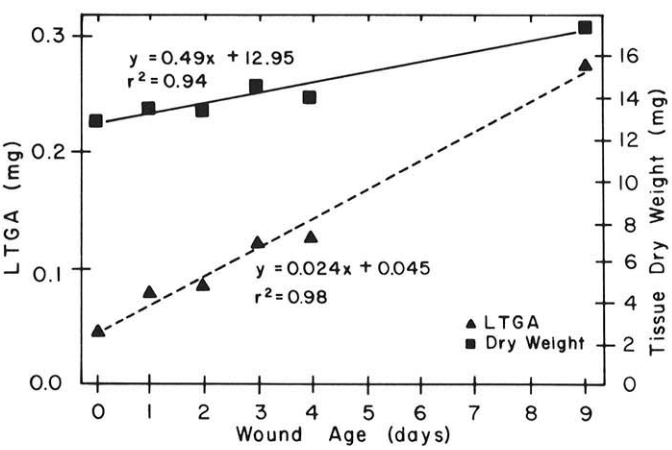

Fig. 2. Amount of ligninthioglycolic acid (LTGA) and methanol-extracted dry weight of bark wounds of various ages in 10 replications of potted almond trees kept in a 25 C growth chamber.

Correlation of lignification and resistance. The lignin content of variously aged wounds before inoculation was negatively correlated ($r = -1.00$) to the mean length of cankers resulting from infection by *P. syringae* of similarly aged wounds (Fig. 4). Four weeks after inoculation, the amount of LTGA per wound was 0.77, 1.12, 0.93, and 0.75 mg for inoculated wounds less than 1 wk old and 1-, 2-, and 3-wk-old wounds, respectively ($LSD_{0.05} = 0.21$ and uninoculated controls had 0.31 of mg LTGA). The amount of LTGA of inoculated wounds did not correspond to canker length. For example, there was little difference in LTGA between inoculated new wounds and 3-wk-old wounds, although the former had canker lengths of 42.1 mm while the latter had cankers of only 5.6 mm.

Effect of fungi on lignification. The amount of LTGA detected in 3-day-old wounds increased after inoculation with three *Phytophthora* species (*P. syringae*, *P. hibernalis*, and *P. cactorum*), but LTGA did not increase when wounds were inoculated with *P. infestans* (Table 2). A greater amount ($P = 0.0001$) of LTGA was detected in these wounds when disks of ALBA medium were used for inoculation than when disks of CMA medium were used. However, the amount of LTGA did not differ with each medium in trees inoculated with *P infestans* and in the uninoculated controls (Table 2). More LTGA ($P = 0.002$ by paired t-test) was detected in wounds with the uninoculated ALBA

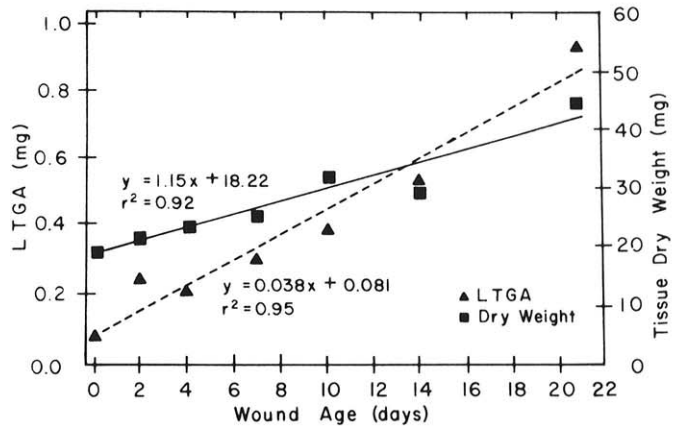

Fig. 3. Amount of ligninthioglycolic acid (LTGA) and methanol-extracted dry weight of 6-mm-diameter bark wounds of various ages in almond orchard trees. The wounds were made in late September and early October 1985 with eight replications (average temperature was 20.4 C).

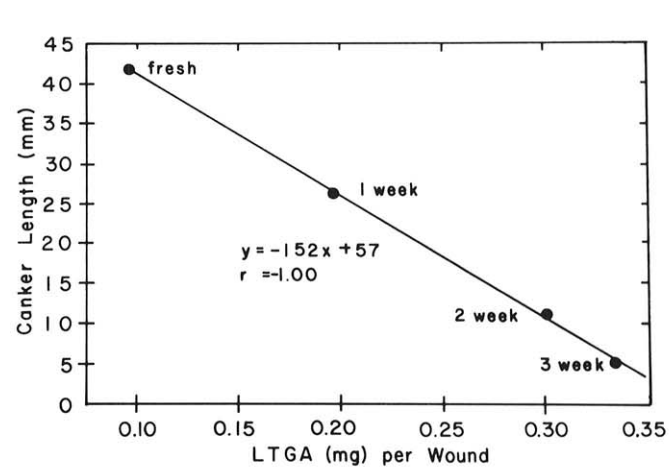

Fig. 4. Correlation of ligninthioglycolic acid (LTGA) in wounded almond bark and length of 4-wk-old cankers resulting from inoculation of similarly aged bark wounds with *Phytophthora syringae*. The ages of the wounds at the time of inoculation are given next to the data points. Potted trees were wounded and kept at 18 C. Each data point represents the mean of 10 replications.

treatment than when no medium and no Parafilm wrapping was used with the wounds. There was an almost significant ($P = 0.052$) difference between the uninoculated CMA treatment and the no medium and no Parafilm wrapping treatment.

There was substantially more LTGA ($P = 0.0001$) within the discolored tissue of the canker than in the healthy appearing tissue beyond the canker margin (Fig. 5). The disks of healthy tissue 0.3 cm (0.084 mg LTGA) and 0.9 cm (0.071 mg) from the canker margin had more LTGA ($LSD_{0.05} = 0.017$) than the tissue 15 cm away (0.053 mg).

DISCUSSION

The formation of LTGA has been suggested as a criterion for the presence of genuine lignin (9). Cupric oxide oxidation of lignins yields the phenolic aldehydes *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde, with the amount of each depending on the amount of the corresponding phenylpropanol precursor for the lignin (7). The detection of these phenolic aldehydes in LTGA in this study indicated that lignin was indeed being measured in the TGA assay. The fact that more of these phenolic aldehydes were detected in wounded tissue (Table 1) further supports the contention that genuine lignin is formed in almond bark in response to wounding.

When the TGA assay was performed on the outer bark of almond trees, substantial amounts of LTGA were detected. Since suberin from many plants contains a ligninlike phenolic component (13), this result suggests that in the TGA assay care should be taken to separate the inner bark from the outer bark which is highly suberized and the xylem which is highly lignified. However, the base pretreatment of the tissues with NaOH to remove nonlignin phenolic esters was unnecessary and indicated that these compounds were not contributing to the absorbance of the LTGA extracts.

TABLE 2. Ligninthioglycolic acid (LTGA) detected 3 days after inoculation with various *Phytophthora* species[a]

	LTGA (mg/wound)		
	Inoculation medium[b]		
Phytophthora species	CMA	ALBA	Mean
P. syringae	0.62	1.31	0.96
P. hibernalis	0.47	1.02	0.75
P. cactorum	0.38	0.73	0.56
P. infestans	0.27	0.28	0.28
Uninoculated[c]	0.29	0.27	0.28
		$LSD_{0.05}$	0.16
Mean	0.41	0.72	

[a] The inner bark of potted almonds was wounded and inoculated, and the plants were maintained in an 18 C growth chamber.
[b] CMA = cornmeal agar; ALBA = amended lima bean agar.
[c] The amount of LTGA detected was 0.21 mg when wounds aged without any media and 0.16 mg for unwounded inner bark tissue.

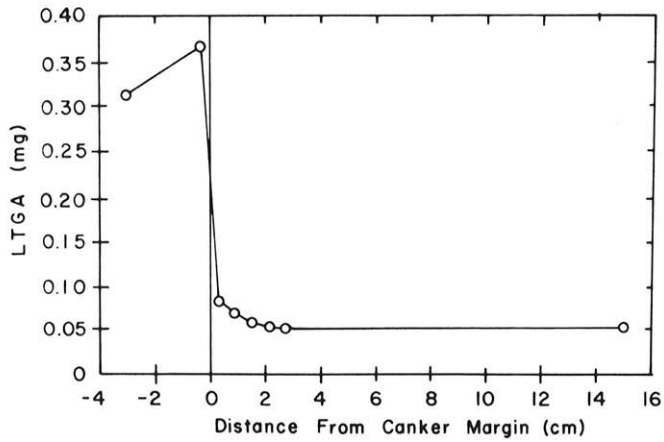

Fig. 5. The amount of ligninthioglycolic acid (LTGA) in healthy almond bark and in bark infected with *Phytophthora syringae* near the canker margin in potted trees kept at 18 C. Distances less than zero are part of the canker, and positive distances have healthy appearing bark.

The absorptivity of LTGA at 280 nm was different depending on the source of the lignin. The absorptivity ranged from 8.4 g^{-1} L cm for the outer bark to 11.9 g^{-1} L cm for wounded inner bark. Hardwood milled lignin for different species showed a wide range of absorptivities at 280 nm from 7.9 to 14.8 g^{-1} L cm, depending on the methoxyl:carbon ratio of the lignin (18). Even though absorptivity varies, the TGA assay for lignin can be used for determining relative amounts of lignin.

LTGA increased linearly as the bark wounds aged in both potted and orchard trees (Figs. 2 and 3). There are several possible sources of this LTGA in wounded inner bark tissue. We observed some lignified bast fibers in sections of unwounded tissue which may have yielded the LTGA detected in unwounded tissue. Additionally, the strongly lignified zone of cells and suberized wound periderm observed in histological studies (Doster and Bostock, *unpublished*; 8,16) probably contributed to the LTGA content of wounded almond bark tissues. Similar histological observations have been made for wounded bark tissue of other species of trees (4,14). The ligno-suberized outer periderm of almond trees increased the amount of LTGA detected. Thus, it seems likely that the ligno-suberized wound periderm also increased the amount of LTGA detected. It is also possible that some lignification occurred near the almond bark wound surface, because a response to wounding for some plant tissues is to lay down lignin near the wound surface (17).

The advantages of the TGA assay for lignin in almond inner bark were that it was quantitative and specific for bound phenolic polymers such as lignin, sensitive to small amounts of lignin, and easy to use with many replications of small tissue samples. However, the assay did not distinguish between LTGA derived from the bast fibers, the lignified zone, or the ligno-suberized wound periderm. Histological techniques allow the localization of the lignin and suberin in the tissue, although they are not quantitative, sensitive, or convenient. Therefore, histological techniques complement the use of the TGA assay in research into the development of resistance of the bark to pathogens: Photometric techniques have been used to quantify the amount of lignin and suberin in wounded tree bark (1), but it is not known how these techniques compare to the TGA aasay. The TGA assay could be used to compare the wound responses of various cultivars, the effect of various compounds on the response of treated wounds, and the effect of treatments such as temperature on lignification wounds.

The dry weights of methanol-extracted wounded tissue increased linearly (Figs. 2 and 3). This increase could be due to deposition of compounds such as lignin in cell walls or to newly formed cells derived from the phellogen of the developing wound periderm. The variable LTGA per dry weight would underestimate the degree of lignification around the wound because the dry weight of a wound also increases with age.

Observations on the development of the resistance of almond bark wounds to *Ceratocystis fimbriata* Ellis & Halst. (16) and *P. syringae* (Fig. 4) showed that fresh wounds are very susceptible and that soon after wounding susceptibility decreased until wounds became immune to infection. In this study, LTGA increased soon after wounding (1 day in potted trees and 2 days in orchard trees) and continued to increase over the time periods observed. Two-day-old wounds inoculated with *C. fimbriata* resulted in smaller cankers and 14-day-old wounds were highly resistant (16). It was also observed that material reactive with phloroglucinol-HCl, a reagent commonly used for the histological detection of lignin (12), was not apparent until 4–6 days after wounding (16). Our results indicate that the TGA assay can detect an increase in lignin (LTGA) 1–2 days after wounding and thus may be more sensitive than histochemical reagents. It has been observed that *Valsa ceratosperma* (Tode ex Fr.) Maire in apple bark (20) and *Leucostoma* spp. in peach bark (2) are able to penetrate the wound

periderm in several weak places. Therefore, in individual cases, even when there is a well-established ligno-suberized barrier, a canker can form when the fungus penetrates a weak point in the barrier. However, there was an excellent negative correlation ($r = -1.00$) between LTGA detected in aged almond wounds and canker length resulting from inoculation with *P. syringae* of similarly aged wounds (Fig. 4). Furthermore, increases in LTGA as almond bark wounds aged (observed in present study) corresponded to increases in resistance to the wound pathogen *C. fimbriata* (16), supporting the hypothesis that lignin or lignin-suberin compounds contributes to resistance of these wounds to infection by canker pathogens.

In this study, lignin was elicited by three pathogenic *Phytophthora* species in a susceptible almond cultivar. There was substantially more LTGA in almond bark wounds inoculated with *Phytophthora* species than in uninoculated wounds (Table 2), in necrotic almond bark tissue associated with a canker caused by *P. syringae* than in healthy tissue (Fig. 5), and in inoculated wounds of ages ranging from 0 to 3 wk than in uninoculated wounds (correlation study). Increased lignification was detected in susceptible potato tubers in response to infection by *P. infestans*, although the rate and extent of lignification was greater in resistant potato cultivars (10). The results of the present study suggest that the rate of lignin deposition once these aggressive pathogens are established is insufficient to prevent canker expansion. However, in susceptible almond cultivars, lignification elicited by pathogens in the genus *Phytophthora* may still slow canker expansion in the bark because wound-induced lignification in almond bark corresponded to slower expansion of cankers caused by *P. syringae* (Fig. 4). As has been observed with poplar trees and *Cytospora chrysosperma* (Pers.) F. (3), host anatomical responses associated with wounding and pathogen infection were similar. However, with sweet potato roots, which have been investigated more thoroughly, differences in respiration, phytoalexin, polyphenol and lignin formation, and coumarin synthesis have been noted between the plant's response to wounding and to infection by *C. fimbriata* (22).

The role of lignin in the development of resistance in bark wounds to canker pathogens is still unclear even though there is a good correlation between increase in LTGA and development of wound resistance. Since canker pathogens usually need wounds before infection can occur (19), the intact suberized outer periderm is an effective barrier to fungal ingress, which would indicate that the similar appearing wound periderm would function likewise as a barrier. Periderms have been observed to restrict the expansion of cankers caused by *Phytophthora cinnamomi* Rands in eucalyptus trees (21) and by *P. syringae* in almond trees (Doster and Bostock, *unpublished*). Phenolic polymers could inhibit canker expansion in several different ways, but it is believed that lignin increases the resistance of host cell walls to compressive forces and to the enzymes of the pathogen (23). Whether or not cankers develop in wounded bark may depend on the rate and extent of phenolic polymerization versus the rate of pathogen ingress into the tissue.

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