

## BIOCHEMICAL RESPONSES OF CHESTNUT OAK TO A GALLING CYNIPID

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**Abstract**—We characterized the distribution of nutritional and defensive biochemical traits in galls elicited on chestnut oak (*Quercus prinus* L.) by the gall wasp *Andricus petiolicolus* Basse (Cynipidae) in comparison with gypsy moth-wounded and unwounded leaves. Gall cortex and epidermis exhibited elevated soluble peroxidase (POX) and soluble invertase activities, and greater condensed tannin concentrations than did nutritive tissues or leaves. Nutritive tissue, on which the insect feeds, contained few polyphenols, and lower POX and invertase activities compared with other gall tissues and leaves. Elevated total POX activity arose from a complex pattern of enhanced and suppressed isoform activities in galls. Invertase enzyme activity decreased in all tissues over the course of the 7-d study, although gypsy moth wounding suppressed this decline slightly in ungalled leaves. Our results indicate that the distribution of biochemical defenses in this typical cynipid gall differs significantly from the leaf tissue from which it is formed and support a role for invertases in establishing the gall as a sink. *A. petiolicolus* larvae do not induce, and may suppress, plant defense responses in nutritive tissue, while enzymatic activity and phenolic accumulation are enhanced in gall tissues surrounding feeding sites. These patterns suggest that the gall is manipulated by the insect to enhance its food and protective value.

**Key Words**—Plant–insect interactions, gall, oak, cynipid, peroxidase, invertase, tannins.

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## INTRODUCTION

Galls are specialized plant structures formed when a galling organism alters the development of normal plant tissue (Kinsey, 1920; Felt, 1940). Oak trees support a diverse assemblage of galling insects (Dreger-Jauffret and Shorthouse, 1992), the most prevalent of which are wasps of the family Cynipidae (Kinsey, 1920; Bronner, 1983). Cynipid galls consist of an outer layer of epidermal tissue, a cortex of sclerenchyma, and one or more nutritive tissue chambers that contain and nourish the larvae.

While galls may provide diverse benefits to the insects that elicit them (Price et al., 1987), considerable evidence supports the view that the gall provides a superior food source to the insect (the "Nutrition Hypothesis" [Price et al., 1987; Bronner, 1992]). For example, galling aphids elicit proliferation of phloem elements, on which they feed (Wool et al., 1999). The plant's nutritive value to insect and microbial gallers may be enhanced further *via* formation of strong metabolic sinks in the gall (Bronner, 1977, 1983; Larson and Whitham, 1997). All galls so far examined constitute enhanced sinks for photosynthate (Inbar et al., 1997, Larson and Whitham, 1997). Sink strength is functionally linked to invertase activity in normal plant tissues (Ricardo and Ap Rees, 1970; Leigh et al., 1979; Huber, 1989; Patrick, 1990; Yelle et al., 1991; Scholes et al., 1994; Zhang et al., 1996) and bacterial galls (Weil and Rausch, 1990).

Most galls contain specialized, distinctive "nutritive tissues" on which the insect feeds and which may contain elevated or altered nutrient concentrations (Paclt and Hässler, 1967; Bronner, 1977, 1992; Schonrogge et al., 2000). Putative anti-herbivore defenses may be increased or decreased in gall tissues (Hartley, 1998; Nyman and Julkunen-Tiitto, 2000), but recent evidence from sawfly systems indicates that at least phenolic concentrations are reduced in nutritive tissues compared with the rest of the gall or other tissues (Nyman and Julkunen-Tiitto, 2000).

We studied the globular gall elicited on petioles and midribs of chestnut oak (*Quercus prinus* L.) leaves by the cynipid wasp *Andricus petiolicolus* Basse. Like other members of its genus, *A. petiolicolus* larvae chew and progressively destroy the nutritive tissue inside the gall as they develop (Kinsey, 1930; Bronner, 1983). Chewing by free-living insects induces defensive biochemical responses in many tree species (Karban and Baldwin, 1997), including chestnut oak, which responds to gypsy moth (*Lymantria dispar* L.) attack by increasing production of polyphenols in the leaves (Hunter and Schultz, 1995). Plant defense responses may also include increased activity of oxidative enzymes such as peroxidases (POXs) and polyphenol oxidase (PPO), which activate and polymerize polyphenols and are implicated in biochemical responses to insects and pathogens (Vaughn and Duke, 1984; Appel, 1993; Felton et al., 1994; Bi et al., 1997). The Nutrition Hypothesis predicts that such defensive responses should be suppressed in tissues consumed by galling insects.

We examined these aspects of the Nutrition Hypothesis by measuring the concentrations of protein, of putatively defensive phenolics, and activities of oxidative and sink-inducing enzymes in nutritive and non-nutritive tissues of *A. petiolicolus* galls as compared with galled and ungalled leaves, and with leaves wounded by a nongalling chewing insect, the gypsy moth (*Lymantria dispar* L.).

#### METHODS AND MATERIALS

*Tissue Collection.* Twenty-six *Q. prinus* L. saplings parasitized by *A. petiolicolus* were selected in late May, 1997 near State College, Centre County, PA, USA. Three to five ungalled and 3–5 galled leaves were collected from each tree on May 24 (Date 1) by plucking the leaf at the base of the petiole. Chilled galls were quickly dissected and separated into cortex, epidermis, and nutritive tissues. Ungalled leaves, galled leaf, and gall tissues were flash frozen in liquid N<sub>2</sub> and stored at –20°C. Nutritive tissue from several trees was pooled to provide enough sample for analysis. Epidermis was separated from cortex for colorimetric analyses of polyphenols. Because of assay sensitivity limitations and the small amounts of nutritive tissues, phenolic contents could not be quantified colorimetrically, so staining was used instead (below).

To assess the impact of wounding on leaf traits, on May 24 (Date 1) we confined 4–7 third-instar gypsy moth larvae within window-screen cages and allowed them to feed on the chestnut oak saplings for 7 d (until May 31, “Date 2”; see Rossiter et al., 1988). Each sapling received 5 cages, and each cage contained both galled and ungalled leaves, producing wounded and unwounded galled and ungalled leaves after 1 wk.

*Enzyme and Protein Extraction.* Frozen gall and leaf tissue was homogenized in an ice cold mortar and pestle with potassium succinate (KSuc) buffer, pH 5.5, containing 10% (v/v) glycerol and 10% (v/v) polyvinylpyrrolidone. Buffer was added to plant tissue at a ratio of 10:1 (v/w), and 10% (v/v) Triton X-100 was added at a rate of 0.8  $\mu$ l/mg tissue. Nutritive tissue samples were diluted further to obtain enough extract for analysis. All extracts were sonicated for 15 min and centrifuged at 1100  $\times$  g for 15 min at 4°C. The supernatant was removed, stored at –20°C, and later used as the source of protein and enzyme activity. All chemicals were purchased from Sigma Chemical Company, St. Louis, MO, or Bio-Rad Laboratories, Hercules, CA.

*Protein Assay.* Total protein was measured in each sample extract using the Bradford reagent method (Bio-Rad, Inc.). Bovine serum albumin was used as a standard, and standard curves were prepared with the same concentration of Triton X-100 as in the samples to account for detergent effects on the assay.

*Peroxidase: Total Soluble Activity.* POX activity was assayed by using a modified version of Bi et al. (1997) spectrophotometric procedure. Twenty  $\mu$ l of extract were added to 980  $\mu$ l of 88.2 mM H<sub>2</sub>O<sub>2</sub> and 4.6 mM guaiacol in 0.1 M KSuc,

pH 5.5, and vortexed for 5 sec. The increase in absorbance due to the formation of tetraguaiacol was monitored for 60 sec at 470 nm.

*Peroxidase: Nitrocellulose Blotting.* To determine the location of POX in gall tissue layers, sectioned galls were pressed to wet nitrocellulose, and the blot was stained for POX activity according to Spruce et al. (1987). *A. petiolicolus* galls were sliced in half with a scalpel, and the cut face was pressed to the nitrocellulose for 60 sec. After 3–5 water rinses, the nitrocellulose was stained with 20 mM guaiacol, with or without 60 mM H<sub>2</sub>O<sub>2</sub>, in 0.1 M KSuc, pH 5.5, for 2–5 min. Orange staining indicated the location of POX activity.

*Peroxidase: Tissue Staining.* POX activity within the gall tissue was examined using diaminobenzidine (DAB) as a substrate (Harris et al., 1994). Galls were sectioned into 1 mm slices and rinsed with water to remove POX that smeared across tissue layers during cutting. Tissue slices were then exposed to the following substrates (in 0.05 M Tris–HCl, pH 7.6): no substrate, 2.3 mM DAB, 2.3 mM DAB + 5.9 mM H<sub>2</sub>O<sub>2</sub>, 2.3 mM DAB + *Aspergillus niger* catalase. Slices were immersed in the substrate or control solutions for 8 min, rinsed with water, and examined for POX activity indicated by orange–brown staining.

*Peroxidase: Isoelectric Focusing.* POX isozymes were separated using polyacrylamide gel electrophoresis/isoelectric focusing (IEF) with a Model 111 mini-IEF unit (Bio-Rad, Inc.). Gels were cast using 50  $\mu$ l 3/10 ampholyte, 200  $\mu$ l 8/10 ampholyte, and 700  $\mu$ l 3/5 ampholyte, 5.05 ml H<sub>2</sub>O, 2.0 ml 24.25% acrylamide with 0.75% bis-acrylamide, and 2.0 ml 25% glycerol (v/v). Fifty  $\mu$ l of 0.44 M ammonium persulfate (w/v) and 5  $\mu$ l tetramethylethylenediamine were added to initiate gel polymerization.

One  $\mu$ l of crude extract was added to each well of a 10 well sample template and allowed to diffuse into the gel for 5 min. Isozymes were separated by stepwise increases in voltage of 100, 200, and 450 V for 15, 15, and 45 min, respectively. Following focusing, gels were soaked in 0.1 M KSuc, pH 5.5, for 10 min to equilibrate the pH throughout the gel. POX isozymes were visualized by immersing the gel in substrate solution for 30 min. The substrate solution was made by dissolving 8 mg/ml *o*-dianisidine in methanol, combining 2.5 ml of this solution with 97.5 ml 0.1 M KSuc buffer, pH 5.5, and adding 44.8  $\mu$ l 30% H<sub>2</sub>O<sub>2</sub>. Gels were then rinsed 4–6 times in KSuc buffer. Relative isozyme activity was quantified by using a Shimadzu CS-9000U dual-wavelength scanning densitometer.

*Polyphenol Oxidase: Total Soluble Activity.* We used a spectrophotometric assay for PPO activity developed by Tono and Fujita (1995) that employs caffeic acid as substrate. Ten  $\mu$ l enzyme extract were added directly to a cuvette containing 490  $\mu$ l of 0.06 mM caffeic acid in 0.1 M KSuc, pH 5.5, shaken 4 times, and the absorbance monitored at 310 nm for 30 sec.

*Polyphenol Oxidase: Nitrocellulose Blotting.* To determine the location of PPO activity in gall tissue, the same blotting procedure used for POX was repeated

with 14 mM L-DOPA in 0.1 M KSuc, pH 5.5, as a substrate. Orange staining indicated the location of PPO activity.

*Polyphenol Oxidase: Tissue Staining.* Galls were sectioned as in the POX procedure, and PPO activity was visualized in the gall by immersing tissue slices in 10 mM L-DOPA in 0.1 M KSuc (pH 5.5). Slices were immersed in the substrate solution or a control solution without L-DOPA for 45 min at room temperature.

*Soluble Invertase Activity.* Soluble invertase activity was determined by using the dinitrosalicylic acid (DNSA) method (Sumner, 1925; Arnold, 1965) with modifications by Miller (1959). Fifty  $\mu$ l of enzyme extract were added to 950  $\mu$ l 0.1 M sucrose in 0.1 M KSuc, pH 5.5 (Tang et al., 1996), and incubated for 40 min at room temperature. One ml Sumner's reagent (44 mM 3,5-DNSA, 53 mM phenol, 0.25 M NaOH, 4 mM Na<sub>2</sub>SO<sub>3</sub>) was added to the reaction mixture, and the solution was boiled for 15 min. Before cooling, 330  $\mu$ l of 1.42 M sodium potassium tartrate were added to the mixture. The absorbance was read at 560 nm on a spectrophotometer and converted to a reducing sugar concentration based on a glucose standard curve. The above procedure was repeated without the 40 min incubation in order to determine the concentration of reducing sugar already present in the enzyme extract; this amount was then subtracted from the final concentration before calculating the enzyme activity. Blanks contained all components except the enzyme extract, for which the sucrose/KSuc solution was substituted.

The results for all enzyme assays were expressed as activity per milligram total protein to reflect the amount of enzyme abundance or activity relative to other proteins, and as activity per gram fresh weight to assess the activity an herbivore or pathogen is likely to encounter in the hydrated plant tissue.

*Phenolics: Tissue Concentrations.* Leaf, gall cortex, and gall epidermis were assayed for (1) "Folin-reactive polyphenols" with the Folin-Denis assay (Appel et al., 2001), which measures the ability of extracts to reduce a mixture of phosphomolybdic and phosphotungstic acids, (2) *condensed tannins* using the Butanol-HCl assay (Appel et al., 2001), which quantifies hydrolyzed proanthocyanidin residues, and (3) *hydrolyzable tannins* with the potassium iodate method modified for quantitative use (Schultz and Baldwin, 1982), which quantifies galloyl esters. Purified chestnut oak tannins from the same trees were used as standards (Appel et al., 2001), and results are expressed as mg phenolics per mg tissue dry weight.

*Phenolics: Tissue Staining.* The location of polyphenols in gall tissue was determined by using the nitroso reaction according to Harris et al. (1994). Galls were sliced into 1 mm sections with a scalpel and immersed in equal volumes of 1.2 M NaNO<sub>3</sub>, 3.3 M urea, and 1.7 M acetic acid for 5 min before adding 2 volumes of 2 M NaOH. Sections were rinsed in water and examined immediately under a Zeiss light microscope for red staining, indicating the presence of polyphenols.

*Data Analysis.* Chemical variation among chestnut oak tissues (2 gall tissues, ungalled, galled, and gypsy moth-wounded leaves) was subjected to analysis of variance (ANOVA) with the SAS MIXED procedure (SAS Institute, Inc.,

1999). We used an incomplete block design without replication with individual trees as blocks and tissue type, wounding, and date as fixed effects. Because they were pooled from several trees, nutritive tissues were assigned unique block numbers. Differences of adjusted least square means ( $\alpha = 0.05$ ) were used to identify significant differences among the 4 tissue types and 2 dates. One-way ANOVA was used to determine significance of differences among tissues in polyphenol concentrations.

## RESULTS

*Protein Content.* Protein concentrations declined with time in ungalled leaves, but overall variation with date was not statistically significant (Table 1, Figure 1A). Protein concentration in the gall cortex was 25–50% lower than in ungalled leaves; other differences among tissues were not statistically significant (Table 1, Figure 1A). Gypsy moth wounding had no effect on protein concentration of any tissue.

*Peroxidase: Total Soluble Activity.* Total soluble POX specific activity differed significantly among tissues, but not among dates (Table 1). POX activity in gall cortex + epidermis was double the activity in leaves (galled or ungalled) and 5 times the activity in the nutritive tissue (Figure 1B). POX activity was not altered when leaves were wounded by gypsy moth larvae (Table 1, Figure 1B).

TABLE 1. ANOVA RESULTS FOR CHEMICAL VARIABLES

Variable	Effect	df (num., den.) <sup>a</sup>	F-value	P-value
Protein concentration	Wounding <sup>b</sup>	1, 96	1.70	0.195
	Tissue <sup>c</sup>	3, 96	38.37	<0.001
	Date	1, 96	2.16	0.145
	Wound × Tissue	3, 96	1.37	0.258
	Date × Tissue	3, 96	10.79	<0.001
POX <sup>d</sup> specific activity	Wounding	1, 96	0.25	0.620
	Tissue	3, 96	20.90	<0.001
	Date	1, 96	1.53	0.220
	Wound × Tissue	3, 96	0.54	0.656
	Date × Tissue	3, 96	0.93	0.432
Invertase specific activity	Wounding	1, 95	0.08	0.773
	Tissue	3, 95	54.96	<0.001
	Date	1, 95	30.68	<0.001
	Wound × Tissue	3, 95	1.23	0.302
	Date × Tissue	3, 95	0.96	0.414

<sup>a</sup>Numerator and denominator degrees of freedom for the F-test.

<sup>b</sup>Wounding consisted of 1 wk of herbivory by caged gypsy moth larvae.

<sup>c</sup>Tissues were ungalled leaf, galled leaf, gall body, and nutritive tissue.

<sup>d</sup>Peroxidase.

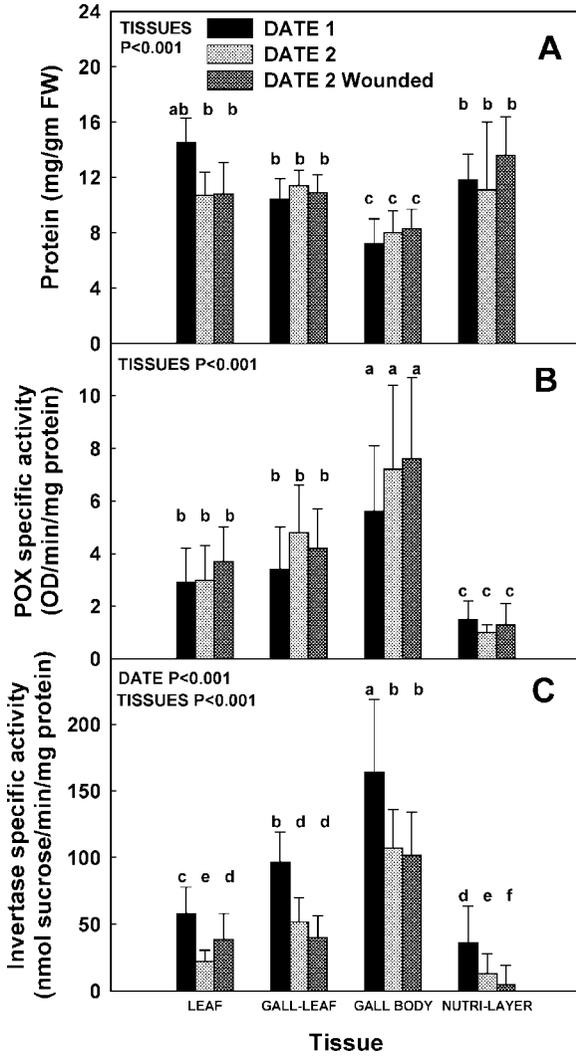


FIG. 1. Means (standard deviation) of total soluble protein concentration (A), peroxidase (POX) specific activity (B), and invertase specific activity (C), by tissue, date, and wounding treatment. Means with the same lowercase letters are not significantly different ( $P > 0.05$ ). Leaf = ungalled leaves; gall-leaf = leaf on which there was one gall; gall body = gall cortex + epidermis; nutri-layer = dissected nutritive tissue from feeding chambers.

Patterns in POX activity and statistical significance were the same when activity was expressed per gram fresh tissue (data not shown).

*Peroxidase: Nitrocellulose Blots and Tissue Staining.* Nitrocellulose blots of *A. petiolicolus* galls revealed POX activity in the gall cortex immediately surrounding nutritive tissues, but little or none directly within the nutritive tissue (Figure 2A and B). Moderate staining occurred in the gall cortex and intensified in the epidermis of the gall structure. No POX staining was observed when  $H_2O_2$  was excluded from the substrate solution as a control (not shown). Direct POX staining (Figure 2E) was generally consistent with the results of the nitrocellulose blotting. Staining within the nutritive tissue was weak (Figure 2E), while intense staining was observed around the gall epidermis (not shown) and in the cortex just outside the nutritive tissue.

*Peroxidase Isozymes.* Isozyme activities in leaves and gall cortex + epidermis were examined with IEF (Figure 2F). Activity of several isozymes appeared to be elevated in gall tissues and/or galled leaves compared to leaves on the same tree, while others were depressed. The gel resolution limited our ability to determine statistical significance of most of these differences. However, quantitative scanning densitometry indicated that gall cortex + epidermis contained significantly greater activity of one anionic and one cationic isozyme (Figure 2F, arrows) and lower activities of most other isozymes. This pattern was observed in all 3 sets of chestnut oak leaves and galls measured.

*Polyphenol Oxidase: Total Soluble Activity, Nitrocellulose Blots, and Tissue Staining.* Although our assays were able to detect PPO in extracts of other plant species under the same conditions (data not shown), our chestnut oak extract exhibited no PPO activity and actually inhibited auto-oxidation of the substrate. In contrast to the POX assay, no PPO staining of the nitrocellulose membrane was observed with L-DOPA as a substrate (not shown). No PPO staining was observed in any gall sections in the absence of  $H_2O_2$  (not shown), indicating that this enzyme is either not present or not detectable by this method in *A. petiolicolus* gall tissue.

*Soluble Invertase Activity.* Specific activity of soluble invertase differed significantly among tissues and dates (Table 1), with similar patterns observed for invertase activity per unit fresh weight (data not shown). Activity was greatest in gall cortex + epidermis and lowest in nutritive tissue on the first date; galled and ungalled leaves had intermediate activities (Figure 1C). Invertase specific activity declined over the week in all tissues, but there was some indication that gypsy moth wounding may have limited this decline in ungalled leaves (Figure 1C). A *post-hoc* Tukey analysis of wound effects supported this observation (Figure 1C). However, wounding did not have a statistically significant effect on invertase activity across all tissues and dates.

*Polyphenol Tissue Concentrations and Staining.* Colorimetric assays indicated that gall epidermis had significantly elevated condensed tannin (ANOVA,  $F_{2,41} = 20.8$ ,  $P < 0.001$ ) and nearly-significant elevated Folin-reactive contents

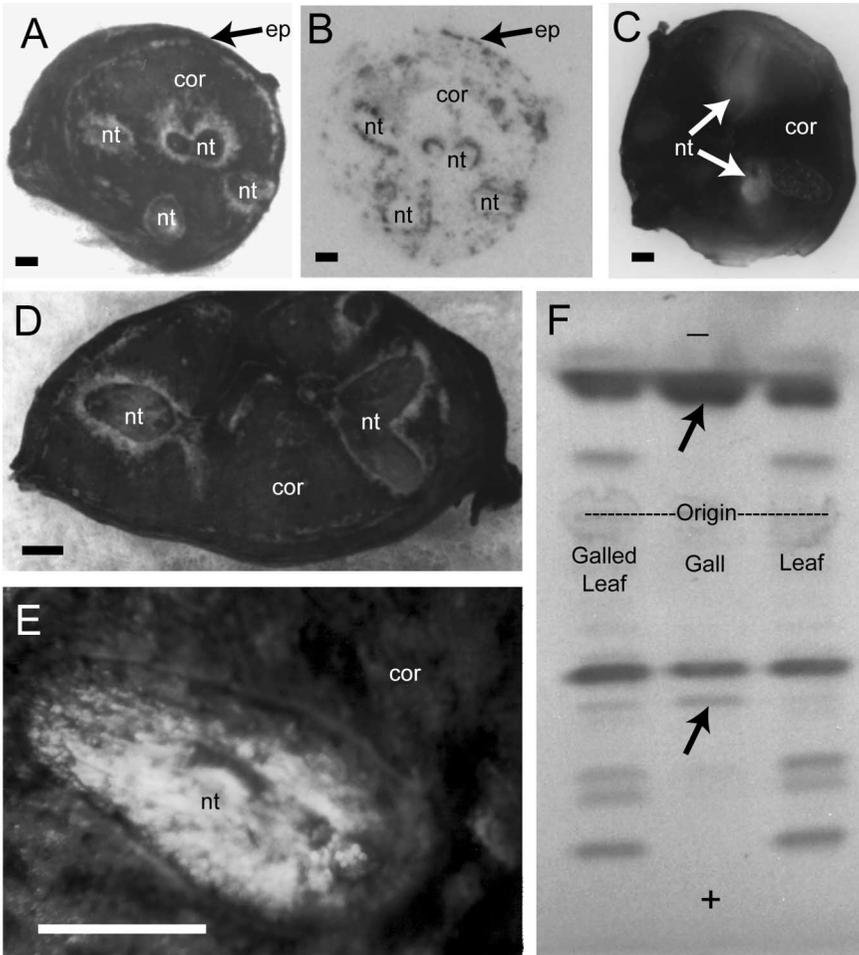


FIG. 2. Unstained *A. petiolicolus* gall section (A) with its nitrocellulose blot (B). In (B), note the absence of peroxidase (POX) staining within, and intense POX activity immediately outside of nutritive tissue (nt). ep = epidermis, cor = cortex. (C) *A. petiolicolus* gall stained for polyphenols. Staining is reduced or absent in the nutritive tissue (arrows). (D) A second unstained *A. petiolicolus* gall section with associated close-up (E) of nutritive tissue stained for POX with DAB. Note lighter color (less POX activity) in nutritive tissue compared to surrounding cortex. All scale bars = 1 mm. (F) IEF gel showing POX isozyme activities in *A. petiolicolus* galls from chestnut oak. "Galled leaf" indicates the leaf on which the gall formed, "gall" indicates gall cortex + epidermis, and "leaf" indicates a control leaf from the same tree with no galls. (-) = direction of migration for cationic POXs, (+) = direction of migration for anionic POXs relative to the origin. Arrows indicate isozymes that were significantly more active in gall tissue.

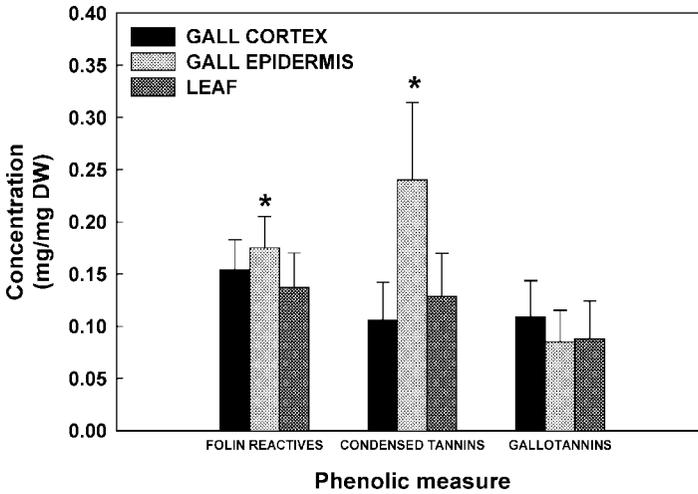


FIG. 3. Mean (standard deviation) polyphenol concentrations of gall tissues and ungalled leaf on last day of study. (\*) indicates mean differs at  $P = 0.08$  for Folin-reactives, and  $P < 0.05$  for condensed tannins.

( $F_{2,41} = 2.56$ ,  $P = 0.08$ ), while cortex phenolic concentrations were indistinguishable from those of the adjacent leaf tissue (Figure 3). Dark red staining for polyphenols with the nitroso reaction revealed lower polyphenol concentrations in the nutritive tissue than in the gall cortex or epidermis (Figure 2C). Globules of polyphenols were present in some cortical cells.

#### DISCUSSION

Our biochemical characterization of *A. petiolicolus* galls provides support for the Nutrition Hypothesis and reveals a previously undescribed shift in POX isoforms within cynipid gall tissue. The pattern of POX isoform activity in gall cortex + epidermis differed from that of adjacent leaf tissue; some isoforms were more abundant or active in galls, while others were suppressed there. Enhanced activity of particular POX isoforms is frequently associated with responses to wounding, pathogen infection and other stresses (Mäder et al., 1980; Grisebach, 1981; Christensen et al., 1998; Hiraga et al., 2000a,b). Differential activation of various isoforms has been seen in response to pathogens (Lebeda et al., 2001), ethylene and abscisic acid (Kim et al., 2000), cytokinins (Limam et al., 1998), and aphid feeding (Chaman et al., 2001), and diverse stimuli elicit differential accumulation of mRNAs for a wide range of POXs in rice (Hiraga et al., 2000). It is important to note that differences in POX isozyme activity were not detectable in the present study by the spectrophotometric assays of total POX activity.

The distribution of POX in *A. petiolicolus* galls is likely to reduce the food quality of the outer part of the galls for many herbivores and pathogens, but should improve food quality in the tissues the cynipid larva actually eats. Total POX activity (per mg protein) was elevated above that of leaves in gall cortex + epidermis, a finding similar to that of Gopichandran et al. (1992) in thrips galls. In contrast, nutritive tissue had less total POX activity than did leaf and cortex. The results from our nitrocellulose blots (Figure 2B) and direct staining for POX (Figure 2E) confirm that most of the POX activity within galls is concentrated in the cortex and epidermis, rather than the nutritive tissue where it could adversely affect the developing larvae.

Elevated POX within the gall cortex and epidermis could benefit cynipid larvae by deterring other organisms from consuming or entering the gall. POX activity can produce reactive oxygen species (ROS) such as  $\text{H}_2\text{O}_2$  and  $\text{O}^{2-}$  (Bolwell, 1996; Bi et al., 1997) and catalyzes the oxidation of *o*-dihydroxyphenolics to *o*-quinones in the presence of  $\text{H}_2\text{O}_2$  (Kahn, 1985; Bi et al., 1997). Appropriate phenolic substrates for POX are known to be present in many oak galls, particularly those of cynipids (Figure 3; Nierenstein, 1930; Cornell, 1983; Hartley, 1998), and were present at high levels in non-nutritive tissues of *A. petiolicolus* galls (Figure 2C). In the gall cortex, POXs and oxidized phenolics may toughen cell walls through lignin production and cross-linking with cell wall proteins (Gaspar et al., 1982; MacAdam et al., 1992; Lamb and Dixon, 1997).

Because both phenolic compounds and POX activity were found to be localized away from the nutritive tissue, *A. petiolicolus* larvae are likely to consume few POX-associated ROS (Tenhaken et al., 1995; Doke et al., 1996; Lamb and Dixon, 1997; Wojtaszek, 1997) harmful to herbivores (Appel, 1993; Felton et al., 1994; Bi and Felton, 1995). These gross patterns are consistent with the view that gall chemistry may be influenced by the galling insect (Hartley, 1998) to provide protection from herbivores (Janzen, 1977; Schultz, 1992), fungal entomopathogens (Taper et al., 1986; Barrett et al., 1998), or fungal endophytes that can threaten galling insects (Carroll, 1988; Wilson, 1995), while preserving or enhancing the food quality of the nutritive tissues.

Schonrogge et al. (2000, and personal communication) have suggested that cynipid galls resemble plant seeds in tissue structure and/or physiology. Patterns in POX isoform activity (Gijzen et al., 1999) and invertase activity (Wobus and Weber, 1999) seen in developing seeds resemble those we found in galls and suggest that cynipid gall development might incorporate aspects of seed development.

As in crown galls formed by *Agrobacterium* (Weil and Rausch, 1990) and galls of the aphid *Hormaphis hamamelidis* (Rehill and Schultz, 2003), our results implicate elevated invertase activity as a means by which insect galls become sinks. Galls are known to act as sinks for plant assimilates (Bronner, 1977, 1983; Larson and Whitham, 1997), and high levels of soluble invertase are associated with the establishment of sink characteristics (Patrick, 1990; Sturm and Chrispeels, 1990;

Yelle et al., 1991). Within the gall tissue, vacuolar acid invertase may hydrolyze sucrose to provide the hexoses required for elevated metabolic activity (Billet et al., 1977; Zhang et al., 1996), and invertases in the nutritive tissue are known to hydrolyze sucrose to glucose and fructose (Bronner, 1977). While gypsy moth wounding may also elicit invertase activity (Arnold and Schultz, 2002), the increase brought about by gallers was much greater than that caused by 1 wk of gypsy moth feeding in this study.

Although gall formation is associated with significant biochemical changes, not all aspects of plant biochemistry necessarily differ in gall tissues. Previous studies have found increased nitrogen and protein content (Paclt and Hässler, 1967; Schonrogge et al., 2000), extensive protein synthesis (Rohfritsch and Arnold-Rinehart, 1991), and large amounts of soluble amino compounds, ribosomes, and mRNA in the nutritive tissue of other cynipid galls (Bronner, 1977, 1992). However, nutritive tissues of *A. petiolicolus* galls had protein levels similar to those of ungalled leaves, despite having lower protein levels in the cortex. We also found that neither galled nor control chestnut oak tissues contained PPO, although we have measured vigorous PPO activity in tissues from other plant species under the same analytical conditions. Although PPO is present in a number of plants (Richard-Forget and Gauillard, 1997; Shin et al., 1997; Halder et al., 1998) and could reinforce some anti-herbivore functions of POX (Felton et al., 1994; Haruta et al., 2001), it may be absent (Grisebach, 1981) or difficult to extract from some plant tissues (Hsu et al., 1988; Burton and Kirchmann, 1997). We have found previously that PPO activity is low or absent in the leaves of chestnut oak (H. M. Appel and J. C. Schultz, unpublished data), and conclude that this enzyme either is not present in chestnut oak tissue, is present in an inactive form, or is not detectable using the methods employed.

Our data show that *Andricus* galls are biochemically distinct from their host leaves. Altered and redistributed activity of various POX isoforms, concentration of polyphenols away from feeding sites, and the increase in invertase activities in *A. petiolicolus* gall tissue relative to leaves probably combine to improve plant quality for this galling insect. It is also clear that while some responses typically induced by chewing insects are active in *A. petiolicolus* gall cortex or epidermis, they are not induced or are suppressed in the insects' food tissue; this may represent manipulation of host plant quality by the insect (Hartley, 1998; Nyman and Julkunen-Tiitto, 2000).

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