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Consequences of Host Plant Chemical and Physical Variability to an Associated Herbivore

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Abstract

The effects of watering and fertilizer treatments on the vigor and biochemistry of the willow, *Salix lasiolepis*, and subsequent colonization and survivorship of its gallforming herbivore, *Euura lasiolepis*, were investigated in two field experiments. Some plants received low (LW), intermediate (MW) or high (HW) levels of water as treatments, while others received no (OF), low (LF) or high (HF) fertilizer levels. In the watering experiment, plant protein concentrations decreased, while growth rate and number of galls per plant increased with increased water treatments. Plant growth proved to be the best correlate of sawfly attack. Sawfly survivorship increased slightly with greater watering, and phenol concentrations showed no pattern among treatments. In the fertilization experiment, leaf protein increased with fertilization, although shoot length, number of galls and survivorship of *E. lasiolepis* survivorship were greatest in intermediate treatment plants. In both experiments, plant growth, rather than protein or phenol levels, was the best predictor of sawfly attack and survivorship.

In a natural experiment with galls on wild plants, galled tissue had significantly greater protein concentrations and lower phenol concentrations than did ungalled tissue. We suggest that gallformers modify host plant biochemistry within willow galls, which may explain why the chemical parameters of ambient plant quality we tested were less predictive than plant growth.

Key words: *Euura lasiolepis*; Fertilizer experiment; Gallformers; Herbivores; Herbivory; *Salix lasiolepis*; Survivorship; Water experiment.

Introduction

Physical and chemical characteristics are the two plant features that herbivores interact with. The chemical basis of plant-herbivore interactions has been increasingly emphasized since the concept of coevolutionary responses to plant chemical defense has become established (Fraenkel, 1959, 1969; Ehrlich and Raven, 1964), and extensively reviewed (e.g. Harborne, 1972, 1977, 1978; Wallace and Mansell, 1976; Chapman and Bernays, 1978; Rosenthal and Janzen, 1979; Visser and Minks, 1982; Hedin, 1983). However, the physical

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attributes of plants, such as growth and structural characteristics, and their relation to herbivory have received much less attention. Because physical properties of plants have chemical bases in terms of growth regulation and chemical constituents of plant parts, physical and chemical properties may be closely correlated. Therefore, we studied relationships between physical and chemical traits in a plant species and their relative influence on host plant selection and survival of an insect herbivore.

An emphasis of plant-herbivore ecology has been that stressed plants are superior resources for herbivores because nutritional quality is improved, plant defenses are reduced, or both (e.g. White, 1974, 1976, 1978, 1984; Rhoades, 1979, 1983; Berryman, 1982; Cates et al., 1983). While the evidence is compelling, these mechanisms are not universal; many herbivores become most abundant on vigorous plants. Van Emden et al. (1969) cite many cases in which fertilizer treatments to plants increase aphid populations. Tetranychid mites frequently respond similarly (Huffaker et al., 1969; Hussey and Huffaker, 1976). Fertilizer treatments to trees increase herbivore numbers in some cases (Bakke, 1969; Shaw and Little, 1972; Onuff et al., 1977).

Plant vigor is important for many plant parasites such as gall formers, as they attack meristematic tissue (Weis et al., 1988). Growth rates of dwarf mistletoes are correlated positively with host plant growth rates (Hawksworth, 1960). Washburn and Cornell (1981) reported on a brief epidemic of the gall wasp, *Xanthoteras politum* (Bassett), that occurred on rapidly growing oak shoots sprouting after a fire. Wasp densities declined within 3 years as plants aged and exhibited reduced growth. Frankie and Morgan (1984) have shown that young oak trees that are susceptible to the gall wasp, *Disholcaspis cinerosa* (Bassett), become resistant as the trees age. According to Thoeny (pers. comm., 1984), a lepidopteran gall former on black locust attacks only juvenile plants and plant parts. The gall forming sawfly, *Euura lasiolepis* Smith, attacks the fastest growing shoots of arroyo willow (Craig et al., 1986) and water stress causes heavy sawfly mortality, due, in part, to its negative effects on plant vigor (Price, 1988; Price and Clancy, 1986). The bud galler, *Euura mucronata* (Hartig) Man. (Churchill), also attacks younger, more vigorously growing plants (Price et al., 1987a, b).

We studied the relationship between physical and chemical plant traits in vigorous and stressed willow plants and their effects on an associated herbivore. Our major aim was to find the plant character which best predicts sawfly attack and survivorship, using simple chemical tests of protein and phenol levels and measurement of a physical character, plant growth.

The arroyo willow, *Salix lasiolepis* Bentham, and a shoot galling sawfly specific to this willow, *Euura lasiolepis* Smith (Hymenoptera: Tenthredinidae) were examined. The willow grows as a shrub up to 4 meters tall, forming clones of a few m^2 to 32 m^2 in size. Aspects of clonal dynamics, sawfly attack and sawfly life history have been reported (Price and Craig, 1984; Price, 1988; Price and Clancy, 1986; Craig et al., 1986).

We analyzed phenolic glycosides in this study because they are the chemicals most strongly implicated in poplar and willow (Salicaceae) defense (Rowell-Rahier, 1984; Palo, 1984; Tahvanainen et al., 1985a, b; Markham, 1971; Pasteels et al., 1983; Smiley et al., 1985; Rowell-Rahier and Pasteels, 1982). Zucker (1982) suggested their possible importance in the defense of narrowleaf cottonwood (*Populus angustifolia* James) against a gall forming aphid, *Pemphigus betae* Doane and some generalists only use willow species without these defensive compounds (Rowell-Rahier, 1984). Phenol glycoside concentrations are known to vary with-

in willow (Horn, 1985) and poplar (Zucker, 1982) populations, and may be responsible for differential attack of plants (Similey et al., 1985).

The term "plant stress" is used here in the conventional sense (e.g. Boyer, 1982; Osmond et al., 1987) as a reduction in growth or reproductive potential from that achieved under nonlimiting conditions. In our study stress was estimated by growth relative to the best growth achieved in each experiment.

Methods

The study site was located on Museum of Northern Arizona property, near Flagstaff, Arizona (elevation 2,132 m). The two experiments were conducted at Coyote Spring, a perennial spring, with two large willows that are heavily attacked by sawflies. The native willows we used for cuttings for experiments and gall samples were located at 2 sites on Schultz Creek: Museum of Northern Arizona (MNA) and at Northland Press (NP) at the southern end of museum property.

The effects of water and fertilizer treatments on willow growth and on sawfly attack and survivorship were tested experimentally. Six willow clones were used in experiments (MNA Nos. 1–4 and NP Nos. 8, 9), representing clones which consistently sustained high or low sawfly attack (High attack: MNA 2 and 3, NP 8; Low attack: MNA 1 and 4, NP 9). One-year-old shoots were taken from each clone, rooted and planted in 18.9 litre pots filled with alluvial silty sand from MNA and NP sites. Each pot contained 4 shoots from 4 different clones. For the water treatment experiment, clones MNA Nos. 1 and 2 and NP Nos. 8 and 9 were used in each pot; for the fertilizer treatment, clones MNA No.s 1–4 were used. Plants were maintained outdoors through the summer with water applications but no fertilizer and buried in soil in October for the winter.

Potted plants were placed on platforms at the Coyote Spring site on 30 May, 1983, to intermingle with the upper canopy of the heavily attacked native clones CS Nos. 1 and 2, to expose them to colonizing sawflies. Pots were rotated every 14 days to minimize position effects on attack.

The water treatment experiment used three pots per treatment, and three treatment levels, with treatments applied between 28 May and 10 October, 1983. Springwater was applied until dishes under the pots overflowed. The low water treatment (LW) pots received water every 22 days until June 19, and every 14 days thereafter, medium water treatment (MW) pots received water every 7 days and high water treatment (HW) pots received water everyday. No fertilizer was used in these experiments.

The fertilizer experiment used 3 pots per treatment and 3 treatment levels. All pots received water daily. A fertilizer application consisted of 6.2 g Miracle Gro® dissolved in 1 liter of water. The no fertilizer treatment (OF) pots received only water, the medium treatment (LF) pots received fertilizer once per week, and the high fertilizer treatment (HF) pots received the treatment 2 times per week between 28 May and 27 June, 1983.

The number of attacks or galls per shoot was counted, and shoot length was measured on the 10 most distal shoots per plant, plus any attacked shoots on 6 July, 1983. Craig et al. (1986) have shown that gallformer colonization of willow shoots occurs at nodes and increased densities of galls on longer shoot reflect increased gall densities at individual nodes on shoots. Because the number of nodes per shoot decreases with increasing shoot length,

gallformers are not responding to an increase in oviposition sites, but rather some other feature of long shoots (Craig et al. 1986). For convenience we used number of galls per shoot as a measure of gall densities. Shoot lengths were measures of plant growth in this study. Galls were collected in December, when sawfly larvae had spun cocoons, and dissected and survivors counted (survivorship = surviving to the cocooned larval stage).

The protein and phenol concentrations of leaves were also measured to determine if they were good correlates of sawfly attack and survivorship. Leaves were sampled because they are used by sawflies in initial testing for oviposition sites and petioles of young leaves are used as oviposition sites (Price and Craig, 1984). Five young, expanded leaves were taken from 2 plants per clone per treatment in the water treatment experiment (120 samples total), and from all plants in the fertilizer treatment experiment (180 samples total) on 17 August, 1983.

Protein and phenol concentrations were also measured in developed galls and ungalled stems from wild clones. Ten galls and 10 ungalled shoots at the position at which a gall would occur, were collected from 1 clone (CS 1) in August, 1982, and each was pooled; and 15 galls and 15 ungalled shoots per clone were collected from clones MNA 2 and 3 in March, 1985, and were not pooled. The parenchymatous nutritive tissue in galls and analogous cortex tissue from ungalled shoots were extracted and analysed chemically.

Total phenols were determined using the Folin-Denis procedure as described by Zucker (1982). Since this time, Julkunen-Tiitto (1985) has recommended use of the Folin-Ciocalteu phenol reagent, however we regard our results as valid because differences between the techniques are small. Phenol concentrations are expressed as total phenol concentrations per optical density (OD) at 725 nm per mg of dry mass of plant tissue (OD_{725}).

For protein analysis, dried plant material was extracted in 1.0 ml 0.1 N NaOH in a closed tube for 3 hours, stirred vigorously for 5 minutes, set aside for an additional 3 hours, stirred for 5 minutes and left overnight. The samples were stirred for 5 minutes and then centrifuged for 3 minutes. One tenth of a ml (0.1 ml) of this supernatant was added to a test tube along with 4.9 ml of Bio-Rad® protein dye reagent prepared according to specifications. The OD (optical density) of the sample was measured at 595 nm. Bovine gamma globulin was used as a standard and its assay was linear over a 5-fold range of concentrations. Protein concentrations were expressed as percent protein in dry weight of sample.

Principal components analysis (PCA) was used to reduce dimensionality and establish overall differences due to treatments (Afifi and Clark, 1984). PCA transformed all response variables (protein and phenol concentrations, plant growth, sawfly attack) into a single variable, the first PCA axis. A split plot ANOVA was used to test for differences in the scores along the first principal component among treatments and clones (Cochran and Cox, 1957). Following this, differences in each response variable among treatments and clones were determined using split plot ANOVA. The pot represented the experimental unit or plot and each was split by the presence of 4 clones per pot; treatments and clones were the main effects. Stepwise multiple regression (Hull and Nie, 1981) was used to measure the correlation between sawfly attack and the plant variables: shoot length (growth), protein concentrations, phenol concentrations, clone and treatment. The stepwise program enters variables in descending order of importance. Sawfly survivorship data was arcsine-square root transformed and analyzed with a oneway ANOVA (sample sizes were too small to examine clonal effects on survivorship) (Zar, 1984). Data from watering and fertilization experiments were not compared because different clones were used in the two experiments.

Results

Water treatment experiment

Water treatments produced the following effects on 36 experimental plants. Split-plot ANOVA on the scores along the first principal component (all response variables transformed into one variable) indicated significant differences due to treatments ($F_{2,3} = 18.0$, $p<.005$) and clones ($F_{3,9} = 10.00$, $p<.01$).

When the variables were analyzed separately, protein concentrations decreased significantly with increasing water treatment, while phenol concentrations did not vary significantly with treatment level (Table 1). The increased concentration of protein in leaves of LW plants is a commonly observed plant response to water stress (White, 1984). Shoot lengths increased significantly with increased water treatment, indicating that water increased growth rate. The attack rate or number of galls on plants increased significantly with increased water treatment (Table 1). Multiple regression indicated that, of all variables measured, sawfly attack was most strongly and positively correlated with plant growth, while no other factors contributed significantly to the regression equation (Table 2). Increased gall densities on faster growing shoots have been shown to reflect greater densities at shoot nodes and number of nodes do not increase with shoot length (Craig et al., 1986). Significant differences occurred between clones in each analysis.

Survivorship of *E. lasiolepis* larvae did not differ significantly among water levels. There was, however, a slight trend of improved survivorship with increased water (Fig. 1A), which corresponds to host plant selection patterns of adult sawflies and plant growth patterns.

The overall pattern in this experiment was one of increased sawfly attacks on rapidly growing, well watered plants, although water stressed plants had higher protein concentrations. This suggests that growth rates of willows, rather than nutrient availability, represent the best index of a suitable resource for gall forming *E. lasiolepis*.

Table 1. Split plot ANOVA results of differences in phenol and protein concentrations, plant growth and sawfly attack among water treatments (Mean±1 S.E.).

Response variable	Water treatment level: LW	MW	HW	Probability: $p<$(df)
% total protein	11.89 (0.27)	9.70 (0.36)	8.71 (0.30)	0.025 (2, 3)
Total phenols	0.48 (0.05)	0.47 (0.04)	0.51 (0.07)	n.s. (2, 3)
Shoot length (cm)	35.30 (3.21)	47.0 (3.75)	67.30 (7.68)	0.010 (2, 6)
Sawfly attack (mean no. of galls/shoot)	2.0 (0.72)	2.0 (0.70)	6.10 (1.84)	0.050 (2, 6)

Table 2. Multiple stepwise regression equation correlating sawfly attack with protein and phenol concentrations, shoot length (growth), clone and treatment level in water experiments (df = 5, 17). Beta or slope (β), standard error of beta (S.E. of β), t test testing difference of slope from 0 (t), the significance of t (sign. of t) and the change in R^2 with addition of each variable to the regression equation are presented.

Step variable:	β	S.E. of β	t	Sign. t	Change in R^2
Shoot length	0.173	0.067	2.635	0.017	0.46
Phenols	15.300	7.978	1.918	0.072	0.51
Clone	−0.661	0.989	−0.668	0.513	0.55
Protein	1.120	1.310	0.856	0.404	0.57
Treatment	1.003	2.171	0.462	0.650	0.57

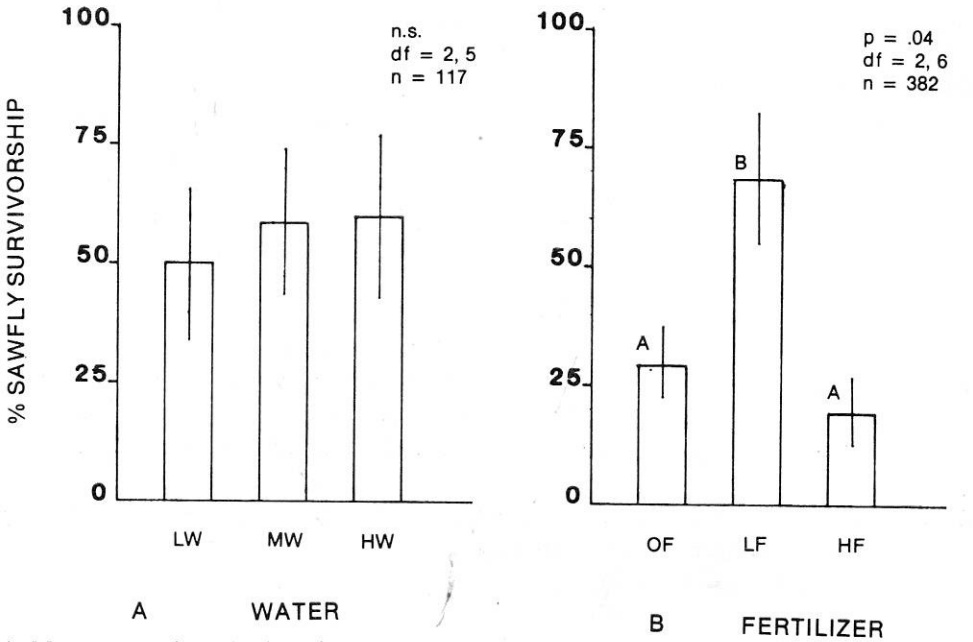

Fig. 1. Mean percent ($\bar{x}\pm 1$ S.E.) sawfly survivorship by treatment per A. water experiment and B. fertilizer experiment.

Fertilizer treatment experiment

Fertilizer treatments produced the following effects on 36 experimental plants. Split-plot ANOVA on the scores of the first principal component indicated significant differences due to treatments ($F_{2,6} = 38.43$, $p<.005$) and clones ($F_{3,18} = 5.75$, $p<.05$).

When analyzed separately, protein concentrations increased, although nonsignificantly, with increased fertilizer treatment, while phenol concentrations decreased significantly with an increase in fertilizer (Table 3). Protein and phenol concentrations were negatively correlated in this experiment ($r^2 = .41$, $p<.05$, $n = 9$). Unlike protein concentrations, shoot length or growth was significantly greater on LF or intermediate treatment plants, while it declined in plants receiving the most fertilizer (HF) (Table 3). Attack rates were not significantly different among treatments, although there was a slight trend of more galls occurring on plants receiving the intermediate fertilizer treatment (Table 3). Multiple regression determined that sawfly attack was most strongly correlated with plant growth, while no other variable contributed significantly to the regression equation (Table 4). Clonal differences were significant in each of these analyses.

Table 3. Split plot ANOVA results of differences in phenol and protein concentrations, plant growth and sawfly attack among fertilizer treatments (Mean$\pm$1 S.E.)

Response variable	Fertilizer treatment level			Probability
	OF	LF	HF	$p<$(df)
% total proteins	9.20 (0.26)	12.34 (0.59)	13.79 (0.99)	n.s. (2, 6)
Total phenols	0.45 (0.02)	0.31 (0.02)	0.20 (0.01)	0.005 (2, 6)
Shoot length (cm)	66.80 (8.60)	158.30 (9.38)	96.30 (8.50)	0.005 (2, 6)
Sawfly attack (mean no. of galls/shoot)	6.83 (2.50)	14.00 (2.50)	12.42 (3.80)	n.s. (2, 6)

Table 4. Multiple regression equation correlating sawfly attack with protein and phenol concentrations, shoot length (growth), clone and treatment level in fertilizer experiments (df = 5, 28). Beta or slope (β), standard error of beta (S.E. of β), t test testing difference of slope from 0 (t), significance of t (sign. of t), and the change in R^2 with addition of each variable to the regression equation are presented.

Step variable:	β	S.E. of β	t	Sign. t	Change in R^2
Shoot length	0.140	0.028	4.858	0.000	0.42
Clone	−2.770	1.259	−2.199	0.036	0.50
Treatment	1.986	3.289	0.604	0.551	0.50
Protein	−0.286	0.613	−0.466	0.645	0.51
Phenols	6.292	21.577	0.721	0.971	0.51

Percent survivorship of sawfly larvae was significantly greater on LF plants (Fig. 1B), which also had the longest shoot lengths. Shoot growth appears to affect the survival of sawfly eggs (Price and Clancy, 1986) and newly hatched larvae (Preszler and Price,1988). The percentage of survivorship between treatments did not differ after young larvae were established in the gall.

The results of this experiment also indicate that sawfly survivorship and to a certain extent, attack, are increased on rapidly growing plants, regardless of their biochemical status, as we have measured it.

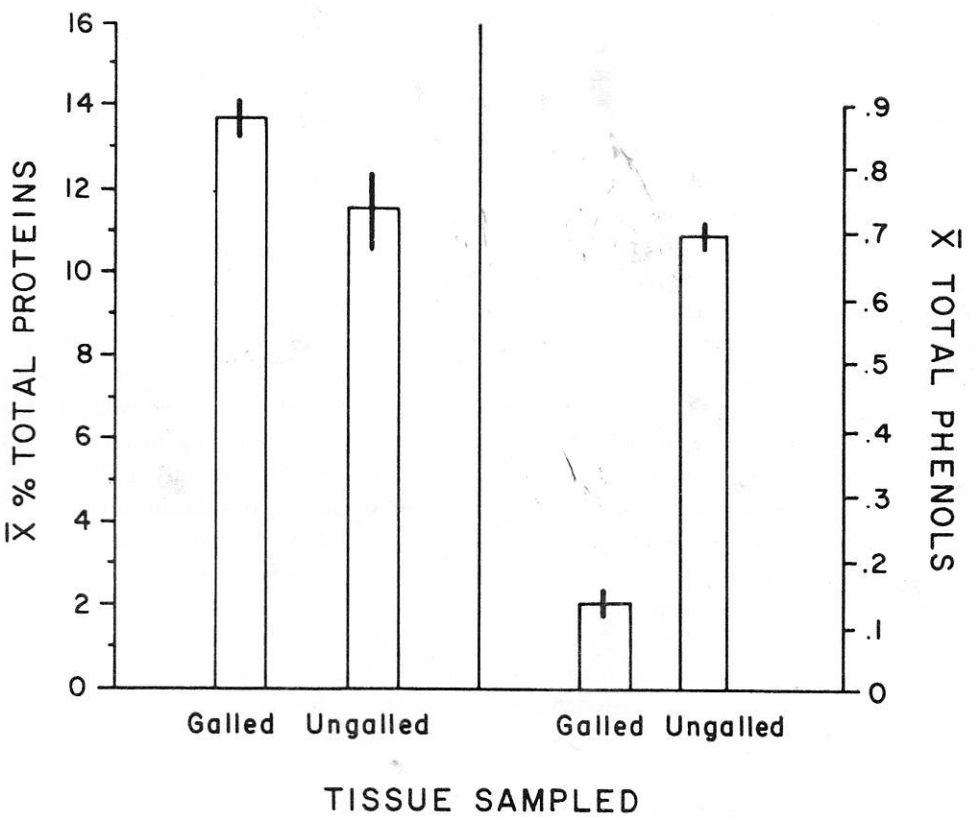

Fig. 2. Mean (±1 S.E.) protein and phenol concentrations in *E. lasiolepis* gall tissue and in ungalled tissue. Percent total soluble protein/mg dry weight plant tissue: $p<0.04$, df = 1, 47; total phenol concentration (OD_{725}): $p<0.001$, df = 1, 47.