Phytotoxic Effects of Cherrybark Oak

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Abstract. Growth of natural vegetation as well as survival and growth of planted seedlings of cherrybark oak (*Quercus falcata* var. *pagodaefolia* Ell.) were much less beneath large seed trees of cherrybark oak. In greenhouse studies, germination and seedling growth were reduced in soils collected beneath cherrybark oak. Cold-water extracts of fresh, whole leaves of cherrybark oak contained a substance which inhibited growth of sweetgum (*Liquidambar styraciflua* L.) seedlings. Chromatographic and spectrophotometric analyses revealed that the primary inhibiting substance in leaf extract was salicylic acid. Leaching of this substance from oak crowns by rain presumably causes inhibition of the understory beneath cherrybark oak. ***Forest Sci. 17: 180–185.***

Additional key words. Growth regulators, *Quercus falcata* var. *pagodaefolia*, growth and development (trees), foliar analysis.

THREE years after a mixed-hardwood stand was cut on the Santee Experimental Forest in coastal South Carolina, Hook and Stubbs (1967) observed retarded development of reproduction under residual oak seed trees, particularly cherrybark oak (*Quercus falcata* var. *pagodaefolia* Ell.) (Fig. 1). Surrounding areas, including those beneath seed trees of other species, had produced the tremendous burst of vegetative growth that normally follows harvest cuttings on fertile bottomland sites. Neither light, moisture, nor previous stand conditions offered adequate explanation for the differences in understory development. Hook and Stubbs (1967) therefore suggested that root exudates or crown leachates of certain oaks might have an inhibitory effect on understory vegetation.

Production of an inhibiting substance by oak species would have important implications for the silviculture of southern hardwoods. Accordingly, the present study was begun. Preliminary studies (DeBell 1969) confirmed Hook and Stubbs' (1967) observation of reduced growth under cherrybark oak. A survey indicated lower average heights and densities for vegetation beneath seed trees of cherrybark oak than beneath those of sweetgum (*Liquidambar styraciflua* L.).

Plantings of cherrybark oak seedlings were established on cleared plots under and away from seed trees of sweetgum, loblolly pine (*Pinus taeda* L.), and cherrybark oak. First-year survival and growth were least in plantings beneath cherrybark oak. Moreover, greenhouse tests showed that germination and seedling growth of sweetgum and cherrybark oak were less in soils collected beneath cherrybark oak seed trees than in similar soils collected from the surrounding area.

Source of a Phytotoxin

Given such evidence for inhibition beneath cherrybark oak, the problem was origin and identification of the suspected toxin—whether it entered the external environment as a crown leachate, root exudate, or as stem flow bearing a bark product Cold-water extracts of leaves, bark, and roots of cherrybark oak were prepared in the following manner:

When this research was done, the author was Assoc. Plant Physiologist, Southeast. Forest Exp. Sta., USDA Forest Service, Charleston, S. C. He is now affiliated with Crown Zellerbach Corp., Camas, Wash. The manuscript is based on a doctoral dissertation submitted to the Graduate School of Arts and Sciences, Duke University, Durham, N. C. The author thanks Drs. Charles W. Ralston and Robert L. Barnes for their guidance and interest during the investigations. Manuscript received Nov. 24, 1970.

FIGURE 1. *Vegetation development beneath a sweetgum (A) as contrasted with that under a nearby cherrybark oak (B) 4 years after a seed-tree cut. Note the sharp boundary between the affected and unaffected areas (B).*

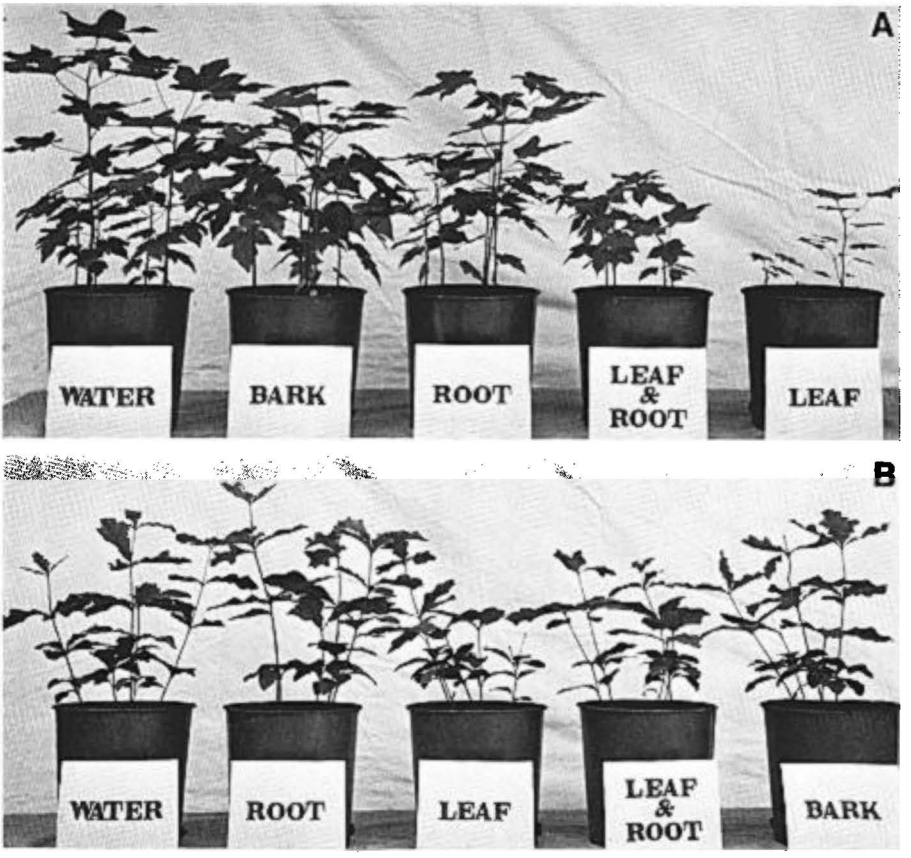

FIGURE 2. *Sweetgum (A) and cherrybark oak (B) seedlings after 4 months of treatments. Growth of sweetgum was substantially reduced in pots receiving leaf extract, but cherrybark oak was unaffected by any treatment.*

Root extract: 50 g of fresh roots (<0.3 cm diameter) and peelings from outer layers of the tap root of cherrybark oak saplings were stirred with 1,000 ml of cold, distilled water for 5 min. After standing for 65 hr, the solution was filtered and diluted with distilled water to 3,600 ml.

Leaf extract: 100 g of fresh, whole cherrybark oak leaves were stirred with 2,000 ml of cold, distilled water for 5 min. After standing for 65 hr, the solution was filtered and diluted to 3,600 ml.

Root + leaf: 1,200 ml of each of the above extracts were combined.

Bark extract: 10 g of bark ground in Wiley mill were placed in a funnel lined with Whatman No. 42 filter paper; 1,000 ml of cold, distilled water was percolated through the ground bark once and diluted to make 2,400 ml.

All solutions were filtered and diluted on Monday mornings, and held under refrigeration for use during the same week.

Cherrybark oak and sweetgum seedlings grown in pots of sterilized sand were used as assay plants. From early May to mid-September, nutrient solution was added to the pots twice a week and the extracts (100 ml) were added five times a week. Control seedlings received only distilled water and nutrient solution. Although seedling heights did not differ significantly in early May, by September sweetgum seedlings receiving the leaf extract were substantially lower in total height, internode length, number of leaves, and dry matter as compared with controls (Fig. 2). Number of nodes and root/shoot ratios

TABLE 1. Bioassay of chromatographic segments of the anionic portion of crude leaf extract of cherrybark oak. Results are average lengths of radish radicles. (In millimeters)

Segment	Control strip (solvent only)	Treated strip (solution plus solvent)	Difference (treated minus control)
1	25	21	–4
2	35	29	–6
3	26	19	–7
4	27	23	–4
5[a]	29	20	–9*
6	33	27	–6
7[b]	28	13	–15**
8	27	19	–8*
9	25	22	–3

[a] Segment contained fluorescent spot *Y* (R_F of approximately 38).

[b] Segment contained fluorescent spot *X* (R_F of approximately 57).

* Significant at 5-percent level.

** Significant at 1-percent level.

were not affected. Root and bark extracts had no significant effect on growth. Apparently, fresh leaves of cherrybark oak contain a substance which can be leached with cold water and is capable of inhibiting growth of certain species. No extract caused significant growth differences in cherrybark oak seedlings (Fig. 2), perhaps because the concentration of the diluted extracts was too low.

Identification of a Phytotoxin

Crude extract was prepared by soaking 50 g of fresh, whole leaves of cherrybark oak in 1,000 ml of distilled water for 2 to 3 days. The filtered solutions were concentrated to 200 ml and separated into several fractions with ion-exchange resins, activated charcoal, or extraction with ether at three pH levels—1.9, 6.9, and 12.0. Each fraction was tested for inhibitory activity by adding 5 ml to a 10-cm petri dish containing filter paper and 10 to 20 radish seeds. Radicle lengths were measured after 5 to 7 days to determine which fractions significantly reduced radicle elongation as compared with an untreated control. The inhibitory effects of crude extract were removed by any of the following: anionic exchange, adsorption on activated charcoal, or extraction into ether at pH 1.9. These findings indicated that the toxic compound was anionic and probably aromatic—most likely a phenolic or aromatic acid.

The anionic portion of crude leaf extract was chromatographed in a descending manner on Whatman No. 1 paper in isopropanol (200): ammonia (10): water (20) solvent. Under ultra-violet light the chromatograms revealed a fluorescent compound (*Y*) at R_F 38 and another (*X*) at R_F 57. Segments of similarly run chromatograms of the anionic portion and control segments through which solvent only had passed were bioassayed for inhibitory activity. The segment containing spot *X* proved most inhibitory to elongation of radish radicles (Table 1). Two other segments (one of which contained spot *Y*) also reduced radicle growth, but to a lesser extent. Therefore, spot *X* was assumed to be the primary inhibitor.

Few phenolic acids have R_F values in the vicinity of 57 in isopropanol : ammonia : water (IPrAm), and these can be separated by chromatographing in benzene · acetic acid : water (BzA). Therefore, the concentrated extract was chromatographed two-dimensionally in IPrAm and BzA. Examination under ultra-violet light revealed that spot *X* had R_F values of 58 in IPrAm and 82 in BzA. Because *O*-hydroxybenzoic acid (salicylic acid) has been reported as toxic to certain plants at minute concentrations and its R_F values in IPrAm and BzA are similar to those of spot *X*, synthetic salicylic acid was compared with the unknown toxic substance.

Salicylic acid and the unknown toxic substance (spot *X*) were compared by paper chromatography in two solvents and by spectrophotometric determinations of ultra-violet absorption and fluorescence spectra (Table 2). The similarities indicated that the primary inhibitory substance in leaf extracts of cherrybark oak was salicylic acid.

TABLE 2. Analytical comparison of unknown toxic substance in cherrybark oak leaf extract with synthetic salicylic acid.

Analytical technique	Salicylic acid	Unknown substance
Paper chromatography	*Average* R_F	
IPrAm solvent	57	58
BzA solvent	82	82
U-V absorption spectra	*Nanometers*	
Maximum 1 wavelength	305	305
Maximum 2 wavelength	270	270
Maximum 3 wavelength	233	230
Minimum 1 wavelength	285	290
Minimum 2 wavelength	250	248
Minimum 3 wavelength	216	214
U-V fluorescence spectra		
Fluorescence maximum	436	436

A bioassay with synthetic salicylic acid was also made. Ethanol solutions containing synthetic salicylic acid in amounts varying from 0 to 400 μg/ml were prepared, and 2-ml aliquots were pipetted onto filter papers in petri dishes. The alcohol was allowed to evaporate overnight, and the next morning radish seeds and 5 ml of distilled H_2O were placed in each dish. After 5 days, the average lengths of radish radicles ($n = 20$) were as follows:

Concn. of salicylic acid (μg/ml of H_2O)	*Radicle length (mm)*
0	45
4	44
8	26
16	18
20	21
40	12
80	4
160	2

Assuming that all salicylic acid added was solubilized, a concentration of 8 μg/ml (ppm) reduced radicle elongation to less than 58 percent of the control. The general appearance of the inhibited radicles was similar to that produced by leaf extracts of cherrybark oak containing the unknown toxic substance.

Discussion

On the basis of these results, I suggest that leaching of salicylic acid from cherrybark oak crowns by rain is the mechanism responsible for inhibition of vegetation beneath cherrybark oak. Other workers have found that salicylic acid is toxic to various plants at concentrations ranging from 5 to 10 ppm (Prill *et al.* 1949), and it has been extracted from woody cuttings of *Quercus* by Gesto *et al.* (1967). Furthermore, the reduction in growth of radish radicles induced by leaf extract was similar to that caused by synthetic salicylic acid. Most workers attribute the inhibitory effect of monophenolic acids to enhanced activity of IAA oxidase and the resulting increased breakdown of auxin in the plant. Such a mechanism would account for reduction in total height and internode elongation of sweetgum when leaf extracts were added in the greenhouse tests, as well as the lack of effect on number of nodes produced. It is possible that salicylic acid also affects other general biochemical reactions.

Though the evidence strongly suggests that salicylic acid is responsible for inhibition, the possibility exists that salicylic acid is detoxified rapidly in the soil. Although this acid has been identified in tissue extracts from a number of plants, Mojé (1966) reported that it has never been isolated from the soil. Possibly some other inhibitory substance might accumulate in soil in greater amounts, or a substance which was non-toxic in the leaf extract might be converted to a toxic compound by soil microorganisms.

The present study, as well as work by Bode (1958), Muller (1966), del Moral and Muller (1970), and Jameson (1970), indicates that phytotoxic substances may play important roles in the development of forest and range vegetation. What this phytotoxicity means in terms of practical forest management is less certain, and additional research is needed. However, the possible influences of the oak phytotoxin can not be ignored in the silviculture of mixed-hardwood forests, especially in poorly drained bottoms where oppor-

tunities for toxin removal through leaching and microbial degradation are considerably less than on well-drained, upland sites. Leaching of toxins from leaves of mature oak trees may account for poor development of seedlings and saplings in stands regenerated by selection (Hook and Stubbs 1965) and by shelterwood cutting. Because seedlings of cherrybark oak are less sensitive to the phytotoxin than are those of sweetgum—and perhaps other species—this selective effect may someday be used to modify stand composition.

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