Journal of the Tennessee Academy of Science
Volume 48, Number 4, October, 1973

ALLELOPATHY IN CEDAR GLADE PLANT COMMUNITIES[1]

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Abstract

Results of a preliminary study to test for mutual allelochemic effects among dominant species of open cedar glades indicate the widespread occurrence of leachable germination inhibitors among these species. These metabolites appear capable of affecting local species distribution and, consequently, zonation in open cedar glade communities. Species differed from each other in susceptibility to extracts tested; soil microorganisms did not affect activity of metabolites, but in some instances soil itself appeared to do so; seed of certain taxa reacted differently to metabolites following storage periods of differing lengths.

Introduction

The relative simplicity of cedar glade plant communities and the sharp delimitation of zones within them (Quarterman, 1950) provide a good opportunity for investigating allelopathy on a community level and for considering its interactions with other environmental factors. Although glades occur in a humid mesothermal climate (Trewartha, 1954), with an average annual precipitation of 1168.7cm (National Oceanic and Atmospheric Administration, 1972), moisture is a critical factor in cedar glade communities (Freeman 1933; Quarterman 1950; Caudle 1968), interacting with soil depth in influencing the location of population and zone margins.

In open glades in the Central Basin of Tennessee areas with soil from zero to 5 cm deep (Zone I) are dominated in winter and spring by three annual herbs, *Arenaria patula* Michx., *Leavenworthia* spp., and *Sedum pulchellum* (L.) Scop.; in summer, there are two dominants, *Cyperus inflexus* Muhl. and *Talinum calcaricum* Ware, occurring as sporadically distributed populations. Much of the soil of Zone I is bare of higher plants during the summer but blue-green algae are present in great abundance at all times. Zone II, whose soil varies in depth from more than 5 to about 20 cm, is dominated by two grasses, *Sporobolus vaginiflorus* (Torr.) Wood and *Aristida longespica* Poir.; a legume, *Petalostemon gattingeri* (Heller) Heller; and a moss, *Pleurochaete squarrosa* (Brid.) Lindb. (Quarterman, 1950). Many other species, e.g., *Isanthus brachiatus* (L.) BSP. and *Dodecatheon media* L., occur in this zone as seasonally dominant, sporadically distributed populations.

Although soil depth with its concomitant moisture differences, and the timing of life cycles to fit the seasonal precipitation pattern (Zager, 1971) appear to be over-riding factors restricting species distribution between zones, Within zones, shoot and root competion have been shown to affect such distribution (Breeden, 1968; Caudle, 1968; Baskin and Quarterman, 1970). A degree of separation between populations of dominant species within each of the zones is evident, but mutual exclusion of species is not invariable and is not regularly associated with changes in depth of soil, suggesting that local species distribution has a more complex basis than has been demonstrated thus far. The occurrence of growth and/or germination inhibitors in several glade species has been demonstrated repeatedly and raises questions regarding the participation of these inhibitory metabolites in the species distribution patterns. Waits (1964) found that a hot-water extract of dried *Leavenworthia stylosa* shoots prevented germination of seeds of five local species of *Lesquerella* but had essentially no effect on germination of seeds of *Petalostemon gattingeri*. Seed extract of *Astragalus tennesseensis* Gray inhibited the germination of *Sporobolus vaginiflorus*, although presence of *Astragalus* extract reduced root growth in all species tested (Caudle, 1968). *Psoralea subacaulis* T. & G. (Baskin and Quarterman, 1970) inhibited the germination of *Psoralea*. This inhibitor was identified as psoralen (Baskin *et al.*, 1967). In addition, aqueous shoot extracts of five *Lesquerella* taxa endemic to the Central Basin were found to inhibit germination in several native and cultivated taxa. The present report deals with the possibility that allelopathy is one of the factors controlling local distribution of glade species within or between zones, either as a controlling factor or as a participant with edaphic and moisture conditions in such control.

The objective of the research was to test aqueous extracts of all dominant species of the two shallow soil zones (I and II) for reciprocal effects on germination. Difficulties in germinating seeds of some species precluded cross-testing all species, however, so the results presented include only some of the possible allelochemic interactions.

General Methods

Shoot material was collected in the field, usually at the time of maximum biomass of each species, air-dried, and stored in the laboratory. Aqueous extracts were made from one part by weight of ground shoot material (1/2 to 1 mm) and 10 or 20 parts respectively, of distilled water, mixed for five minutes in a Waring blender. The crude extract was strained through four layers of cheesecloth, then filtered three times through Whatman

[1]This work was supported by National Science Foundation Grant number GB-12439, for which appreciation is expressed.

#1 filter paper, and used immediately to moisten seeds for germination tests.

Seeds were collected at maturity from field populations, air-dried and stored in paper bags in an air-conditioned laboratory, and used during the following year. Seeds for Experiments I and II were collected in 1967, those for Experiment III in 1968. All seeds of a given species came from the same or adjacent populations in Davidson or Rutherford Counties (U.S.G.S., La Vergne and Murfreesboro Quadrangles).

Germination tests were conducted in petri plates on filter paper, which was moistened with 5 ml of plant extract or, in the controls, of distilled water. Three plates were set up for each test treatment. Plates were wrapped in plastic sheeting (Glad Wrap) to reduce drying and placed in lighted, programmed incubators (Hotpack) at a 12/12-hr photoperiod and at the following temperatures; *Sedum*, 10°C; *Talinum* and *Dodecatheon*, 10-27°C; *Arenaria, Leavenworthia*, 20°C; *Aristida*, 15-25°C; and *Sporobolus*, at 25°C. Results were recorded after two weeks of incubation. Number of seeds per test is indicated with results.

In Experiment I, seeds of *Talinum calcaricum* and of seeds of *Talinum calcaricum* and of *Sporobolus vaginiflorus* were pre-treated for 6 weeks and of *Dodecathon media* for 8 weeks at 4° C. *Leavenworthia stylosa* seeds were moistened with hte appropriate liquid for 14 days, results recorded, then dried for 60 days, rewet with water (Zager et al., 1971) and final results recorded two weeks later.

In Experiment II, soil collected from the top 4 decimeters of a glade in Rutherford County (U.S.G.S. Murfreesboro Quadrangle) and passed through a 7mm screen was used as a germination substrate; a portion of this soil was autoclaved at 120°C for one hour. Two grams of soil were placed in each plate, covered with a layer of filter paper and moistened with 15 ml of extract or of water. All seeds tested in this experiment were surface-sterilized with sodium hypochlorite (2.5% for 5 min.).

In Experiment III, part of the crude extract was autoclaved at 120°C for 15 minutes and part filtered through a 0.20 μ plain nalgene millipore filter; the solid components from the original filtrate were mixed with enough distilled water to make a slurry, which was poured onto discs of Whatman #1 filter paper in a Buchner funnel and evacuated until a solid residue completely covered the paper. These coated discs were used as germination substrates to simulate a little layer.

TABLE 1. *Effects of aqueous shoot extracts (1:10) on germination. Each datum is a mean of 3 observations (25 seeds/plate)*

Species tested	Zone I Sedum	Germination as % of Water Control Extracts of Shoot Material Zone II Aristida	Isanthus	Petalostemon	Pleurochaete	Sporobolus
Zone I						
Arenaria patula	40**	-	0*	0**	-	3**
Leavenworthia stylosa	70**	-	13**	4**	-	5**
Sedum pulchellum	134**	-	0**	0**	-	6**
Talinum calcaricum	-	73*	46**	46**	76*	107
Zone II						
Dodecatheon media	-	-	-	-	-	42**
Sporobolus vaginiflorus	-	66**	57**	76**	92	74**

* significant at 5% level
** significant at 1% level
\- not tested

Results

Experiment I.

Crude extracts (1:10) of *Sedum pulchellum* (Zone I), of *Aristida longespica, Pleurochaete squarrosa,* and *Sporobolus vaginiflorus* (the four general dominants of Zone II), and of one sporadically distributed Zone II dominant, *Isanthus brachiatus,* were assayed for effects on germination in four Zone I and one Zone II dominant, and in *Dodecatheon media* from a Zone II population. (See Table 1.)

Extract of *Sedum* shoots inhibited germination of its two zonal and seasonal associates but stimulated germination of its own seeds; extracts from Zone II dominants inhibited germination in most species tested from both zones. (See Table 1). These results indicate a rather general occurrence of leachable germination inhibitors among dominant species of open cedar glades effective, under the experimental conditions, upon the other dominant species tested.

Experiment II.

To investigate the importance of soil and of soil micro-organisms upon such inhibition, sterilized and unsterilized glade soil was used as germination substrate for testing the activity of autoclaved *Sedum* extract on surface-sterilized seeds of Zone I dominants. (Preliminary tests had indicated no decrease in effectiveness of *Sedum* extract following autoclaving.

TABLE 2. *Effects of glade soil and soil microorganisms on inhibition of germination by Sedum extract on Zone I dominants. Each datum is a mean of 3 observations (100 seeds/plate)*

Species	Germination as % of Water Control UGS	SGS	SSE	UGS+ SSE	SGS+ SSE
Arenaria	86.1	96.8	26.1**	67.6**	3.0**
Leavenworthia	114.3	119.0	47.6**	66.6	95.2
Sedum	100.0	85.5**	39.6**	55.1**	53.4**
Talinum	89.8	86.4*	86.4*	62.7**	59.3**

** Significant at 1% level

* Significant at 5% level

UGS - Unsterilized glade soil; SGS - Sterilized glade soil;

SSE - Sterilized Sedum extract.

Except for the inhibition of *Sedum* germination on sterilized soil, no significant differences occurred among substrates (see Table 2). Presence or absence of soil with or without its microflora made no difference in activity of the *Sedum* extract except in the case of *Leavenworthia,* to which soil both sterilized and unsterilized, afforded protection against inhibition. This result implicates the physio-chemical nature of the soil itself, rather than soil microorganisms, in explaining the protection afforded. In these tests, *Sedum* extract inhibited, rather than stimulated, germination of its own seeds, a reversal of the effect obtained in Experiment I (see

TABLE 3. *Effects of aqueous shoot extracts (1:20) of Zone II dominants on germination. Each datum is a mean of three observations (100 seeds/plate). S=Solid; C=Crude;A=Autoclaved; F=Filtered. Germination as % Control Extracts.*

Species Zone I	Aristida S	C	A	F	Pleurochaete S	C	A	F	Petalostemon S	C	A	F	Sporobolus S	C	A	F
Arenaria patula	69**	87	76**	85*	93	102	101	92*	46**	54**	11**	53**	72**	78**	70**	55**
Sedum pulchellum	86**	54**	84**	83**	106	104	100	103	49**	83**	72**	41**	68**	52**	96	81**
Zone II																
Aristida longespica	83*	97	125**	123**	84**	87**	85**	88**	69**	89*	66**	83**	80**	83*	132**	119**
Petalostemon gattingeri	91**	92**	98	93	67**	51**	88**	81**	97	97	100	98	84**	82**	100	94
Sporobolus vaginiflorus	100	101	107**	108**	96**	91**	98	102	71**	86**	97*	96*	84**	92	96	103

*=significant at 5% level
**=significant at 1% level

Table 1). A probable explanation of this discrepancy lies in the age of the seeds used; these were from the same collection (U.S.D.A. LaVergne Quadrangle, Mt. View, July 5, 1968), but had been in dry storage for six months longer. The germinability of *Sedum* seeds increases with age and they may also become less susceptible to inhibitory metabolites with time. Another possibility is that changes may occur in the extract during autoclaving.

Experiment III.

Effects of the four dominant species of Zone II were tested upon the two most readily germinable dominants of Zone I, *Arenaria* and *Sedum,* and upon the three angiosperm dominants of Zone II (Table 3). Extracts were made from one part of shoot material and 20 parts of water. Each extract was used crude, autoclaved, filtered sterile, and in solid residue deposited upon filter paper discs.

In the between-zone series, all substrates were significantly inhibitory except those from the moss *Pleurochaete* (See Table 3). Autoclaving and filtering of extracts made no important changes in degree of inhibition, indicating that the inhibitor(s) is heat stable or changes during autoclaving to other inhibitory compounds, and that microorganisms do not affect inhibition in these cases. *Arenaria* and *Sedum* can both grow in deeper soils than those of Zone I; in the field, they germinate at a time when only *Pleurochaete,* of the Zone II species, is beginning a period of active growth. It was not *Pleurochaete,* however, that inhibited germination of their seeds, but *Aristida, Petalostemon,* and *Sporobolus,* which are dying or dormant at this season. The litter deposited by these three species before and during the germination periods of *Arenaria* and of *Sedum* provides a mean by which their metabolites might prevent invasion of deeper soils by the winter annuals. In the field, *Petalostemon* more frequently occurs interspersed among Zone I species than do the other Zone II dominants because of its propensity to become established in crevices in the rock, even within Zone I. Each procumbent plant may cover an area up to 30 cm in diameter and thus may distribute its litter and/or leachates widely.

Within Zone II there was more variation among the interactions than was true of those between zones, but one or more of the species tested was inhibited by each extract used on it. *Petalostemon* had no effect upon germination of its own seeds; *Aristida* provided less effective inhibitors than did *Pleurochaete, Petalostemno,* or *Sporobolus.* Tests with extract from *Aristida* shoots in the 1:10 proportion (Table 1) had inhibited germination of *Sporobolus,* but in this series, at 1:20, it failed to do so. A possible explanation lies in the greater dilution of the metabolites. Germination conditions differed also, however, in that *Sporobolus* seeds that were tested in the 1:10 extract had been cold-treated; those in the 1:20 had not. A discrepancy between the action of *Pleurochaete* extracts upon *Sporobolus* in different experiments (Tables 1 and 3), however, may require another kind of explanation, since it had not been inhibitory at 1:10 and was significantly so at 1:20. The moss used for the 1:10 extract was gathered in the fall, that for the 1:20 in early summer, when the amounts of metabolites in the shoots might be expected to be at the highest level. The seasonal effect could be important.

Extracts from the three angiosperm dominants of Zone II have the capacity to interefere with germination of species of Zones I and II; extracts from all four Zone II dominants affected germination of the dominant angiosperms within Zone II. In general, *Petalostemon* and *Sporobolus* extracts more strongly affected germination of associated species of both zones than did those of *Aristida* or of *Pleurochaete.*

Discussion and Conclusions

The experiments reported here strongly suggest that community structure in open cedar glades is influenced by allelochemic interactions of associated species, since all extracts used inhibited germination in one or more species tested. The potential for germination inhibition is present, both within and between zones, but these effects must be investigated in the field before one can be sure that such inhibition occurs there and/or is effective in determining species distributions. Such investigations are in progress and results will be reported at a later date. Since extrapolation of results to field situations is a primary objective of the work, the