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Allelopathic Actions in the Florida Scrub Community

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Abstract. The hypothesis that allelochemicals released from members of the Florida scrub community deter the invasion of fire-prone sandhill grasses was investigated. Constituents of the endemic scrub members, *Ceratiola ericoides, Conradina canescens* and *Calamintha ashei*, were examined for their phytotoxic activity. Effects of the plant natural products on the germination and radicle growth of lettuce (*Lactuca sativa*), as well as little bluestem (*Schizachyrium scoparium*) and green sprangletop (*Leptochloa dubia*), two native grasses of the Florida sandhill community, were tested.

The data suggest that ursolic acid and other natural detergents which are released from a source plant enhance the solubilization of allelopathic lipids *via* micellization. In general, natural surfactants seem to play a significant role in the water transport of lipophilic phytotoxins to target seeds or seedlings.

The upland, well-drained sandy soil of Florida and the southeastern Coastal Plain supports two different plant communities. The sand pine scrub is characterized by sand pine (*Pinus clausa* (CHAPM. ex ENGELM.) VASAY ex SARG.) with a dense scrub layer but no herbaceous ground cover. In contrast, the sandhill or pineland is dominated by longleaf pine (*P. palustris* MILL.) with a dense herbaceous cover of grasses and forbs but few shrubs (CHAPMAN 1932, GARREN 1943, LAESSLE 1958). In 1898 Whitney stated that "it is an impressive sight to stand at the border line between the scrub and the high pine land and notice the difference in the character of the vegetation". Frequent fires of the sandhill burn through the deciduous surface fuels every 3–8 years (WILLIAMSON and BLACK 1981) and the evergreen scrub community burns about every 50 years (HARPER 1915, RICHARDSON 1977). The fire susceptibility of the two communities is remarkably different in that fires sweep through the sandhills until they reach patches or strands of scrub where they are often extinguished by drastic changes in the fuel supply. WEBBER (1935) called the scrub "a fire-fighting machine", and NASH (1895) noted over 90 years ago that "The scrub flora is entirely different from that of the high pine land, hardly a single plant

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being common to both; in fact these two floras are natural enemies and appear to be constantly fighting each other. . . "

In the absence of surface fires in the sandhills, the woody scrub species will colonize (LAESSLE 1958, VENO 1976). They grow well in the sandhills until a surface fire sweeps through the graminoid ground cover and kills the scrubs (VENO 1976, HEBB 1982). Large shrubs and especially groups of shrubs may survive, in particular if graminoid growth around them has been suppressed. Therefore, we have been investigating the hypothesis (RICHARDSON and WILLIAMSON 1988, WILLIAMSON *et al.* in press) that the scrub community produces chemicals that inhibit the growth of sandhill grasses and possibly pines which provide the fuel for surface fires.

In order to test aspects of this allelopathy/fire hypothesis, we initially focused on three early colonizers of the Florida scrub, *Ceratiola ericoides* (A. GRAY) HELLER (*Empetraceae*), *Calamintha ashei* (WEATHERBY) SHINNER (*Lamiaceae*), and *Conradina canescens* TORR. & GRAY (*Lamiaceae*). Preliminary results indicated that water-soluble allelotoxins released from the aerial parts of these scrub plants inhibit germination and growth of sandhill species (RICHARDSON and WILLIAMSON *et al.* in press). In this review, we present a summary of our chemical and bioactivity data involving allelotoxins that are released from these shrubs. Their effects on the germination and growth of little bluestem (*Schizachyrium scoparium*) (MICHX.) HASH (*Poaceae*), and *Leptochloa dubia* (H.B.K.), two native sandhill grasses, were tested. These investigations were to determine the potential of scrub plants to deter the invasion of fire-prone sandhill grasses into the scrub. Furthermore, we present evidence for the role of natural detergents as solubilization enhancers of bioactive lipids. Finally, the possible role of detergents produced by source plants in the release and transport of allelopathic lipids in rain washes from the source plant to target species are discussed.

MATERIALS AND METHODS

Bioassays

Preliminary bioassays involved application of test washes derived from leaves of *Ceratiola ericoides*, *Conradina canescens*, and *Calamintha ashei* to target seeds of three native sandhill grasses (*Andropogon gyrans* ASHE, *Leptochloa dubia*, and *Schizachyrium scoparium*) as well as *Lactuca sativa* and subsequent monitoring of germination and growth (RICHARDSON and WILLIAMSON 1988).

Germination and radicle growth assays of plant extracts and their purified constituents were carried out on *S. scoparium*, *L. dubia*, *and L. sativa* seeds by previously described methods (DE LA PEÑA 1985, FISCHER *et al.* 1988, TANRISEVER *et al.* 1988, WILLIAMSON *et al.* in press).

Chemical Analyses

Ceratiola ericoides. Constituents (flavonoids and triterpenes) for the germination and radicle growth studies were isolated by previously described methods (TANRISEVER *et al.* 1987).

Calamintha ashei and *Conradina canescens.* The isolation of the mono-, sesqui- and triterpenes from *C. ashei* used in the bioassay studies (TANRISEVER *et al.* 1988, MACIAS *et al.* 1989) as well as the methods for isolation and structure determination of the *C. canescens* terpenoids have been previously described (DE LA PEÑA 1985, WILLIAMSON *et al.* in press).

Micellization studies of scrub plant leachates and their pure constituents were performed using the WOLFF (1981) method (FISCHER *et al.* 1988).

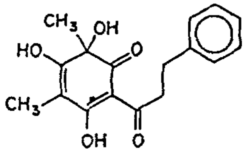
RESULTS AND DISCUSSION

Allelopathic Effects of *Ceratiola ericoides* Constituents

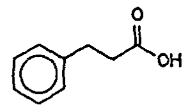
The monotypic genus *Ceratiola* represents one of only three genera of the family *Empetraceae*. It is a locally dominant endemic species of the Florida scrub community. Monthly bioassays have shown that water extracts of *Ceratiola* litter significantly inhibited germination and radicle growth of native grasses of the sandhill community but extracts of fresh leaves were only mildly active (RICHARDSON and WILLIAMSON 1988). Chemical analysis of the dichloromethane extract of ground aerial parts of *C. ericoides* provided the known flavonoids angoletin, 2′, 6′-dihydroxy-4-methoxy-3′, 5′-dimethyldihydrochalcone, 2′, 4′-dihydroxychalcone, 7-hydroxyflavanone, 8-methylpinocembrin, and 6,8-dimethylpinocembrin. Methanol extracts gave catechin, epicatechin, and the dimer epicatechin-(4$\beta\rightarrow$8; 2$\beta\rightarrow$O$\rightarrow$7)-epicatechin. In contrast, water washes of freshly harvested leaves lacked the above phenolics but yielded a novel dihydrochalcone, ceratiolin (**1**) (TANRISEVER *et al.* 1987).

Angoletin, 2′, 6′-dihydroxy-4′-methoxy-3′, 5′-dimethyldihydrochalcone, 2′, 4′-dihydroxychalcone, 6,8-dimethylpinocembrin, and 8-methylpinocembrin were tested for effects on germination and radicle growth of *L. sativa* and *S. scoparium* but only statistically insignificant inhibitions of germination were observed with the exception of 6,8-dimethylpinocembrin, which showed inhibitions of radicle growth (66 %) of *S. scoparium* (FISCHER *et al.* 1988).

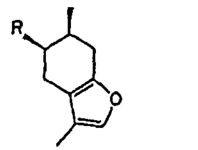
Catechin, epicatechin, and the epicatechin dimer had minor to moderate effects on the germination of *L. sativa*, and only catechin at 62 ppm exhibited a significant germination inhibition (66 % of control) on *S. scoparium*. The germination and growth inhibitions caused by the flavonoids, catechins, and the catechin dimer do not reach levels that were observed with plant leachates and active chromatographic fractions of *C. ericoides* (FISCHER *et al.* 1988).



ceratiolin (1)

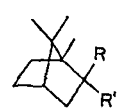


hydrocinnamic acid (2)

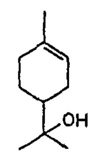


	R
epievodone (3)	H
desacetyl-calaminthone (4)	OH
calaminthone (5)	OAc

1,8-cineole (6)

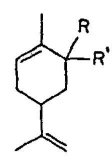


camphor (7) R=R'=O
borneol (8) R=H; R'=OH

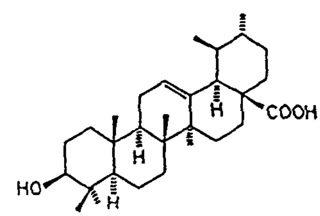


α-terpineol (9)

myrtenal (10), R=-CHO
myrtenol (11), R=-CH_2OH



carveol (12) R=H, R'=OH
carvone (13) R, R'=O



ursolic acid (14)

Fig. 1. Constituents of *Ceratiola ericoides*, *Calamintha ashei* and *Conradina canescens*. In the text only the numbers of chemical compounds are used.

Ceratiolin (**1**) was suspected to play a significant role in the allelopathic activity of *Ceratiola*. However, at 125 ppm ceratiolin had no inhibitory effect on the germination of *S. scoparium* and it promoted its radicle growth (145 % of control). Laboratory experiments had shown that ceratiolin is thermally unstable and spontaneously decomposes during storage of the plant material. Water solutions of ceratiolin, when exposed to sunlight or in the dark, after 3 days contained hydrocinnamic acid (HCA) (**2**) (TANRISEVER *et al.* 1987). Since the formation of **2** from **1** is facile in water, this decomposition must also take place under natural conditions, especially in decaying *C. ericoides* litter.

Although ceratiolin at 125 ppm showed no inhibitory activity against *S. scoparium* and *L. sativa*, HCA exhibited considerable phytotoxic effects. At concentrations of 125, 250, and 500 ppm, HCA showed significant inhibitory activity on *S. scoparium* germination (46, 32 and 17 % of control). This result may explain earlier data which showed that fresh leaf washes were considerably less active than litter washes of *C.*

ericoides. Soil under *C. ericoides* is acidic, which promotes degradation of ceratiolin into HCA. Therefore, activity is likely to be greater in the sandy soil and decomposing litter than in fresh leaf rain washes. Varying concentrations of HCA have been found in the soil under *Ceratiola* (JORDAN, unpublished). Seasonal concentration of HCA in soil under *C. ericoides* varies considerably. During 1987, no HCA was detected in the soil from January to May and October to December. However, during the months of June to September, HCA concentrations varied from trace amounts to a maximal value of 255 ppm in early September.

TABLE 1
Monoterpenes in the active diethyl ether fraction (EE), in the steam distillation extract (SD) and in the leaf soak (LS) of *Conradina canescens*† and inhibition of germination (G) and radicle lengths (RL) of *Leptochloa dubia* and *Schizachyrium scoparium* as per cent of water controls

Compound				*Leptochloa dubia*		*Schizachyrium scoparium*	
	EE	SD	LS	G	RL	G	RL
1,8-Cineole (6)	+	+	+	100	97	20**	26**
Camphor (7)	+	+	+	12**	34**	16**	61
Borneol (8)	+	—	+	0**	—	8**	47*
α-Terpineol (9)	+	+	+	0**	—	0**	—
Myrtenal (10)	+	+	—	2**	5**	8**	13**
Myrtenol (11)	+	+	—	0**	—	0**	—
Carveol (12)	+	—	—	0**	—	0**	—
Carvone (13)	+	—	—	0**	—	0**	—

† All monoterpenes were identified (GC/MS analysis) by correlation with standards.
* Treatment was significantly different from the control at $P \leq 0.05$.
** Treatment was significantly different from the control at $P \leq 0.01$.

Allelopathic Activities of *Calamintha ashei* and *Conradina canescens*

Calamintha ashei is a member of the mint family (*Labiatae*), and an endemic of the Florida scrub. Water washes of fresh *C. ashei* leaves exhibited significant inhibitory activity on *S. scoparium* germination and growth (RICHARDSON and WILLIAMSON 1988). Chemical analysis of the aerial parts of *C. ashei* provided copious amounts of ursolic acid (UA, **14**) and the sesquiterpene caryophyllene oxide as well as menthofuran, epievodone (**3**), calaminthone (**5**) and desacetylcalaminthone (**4**) (TANRISEVER *et al.* 1988, MACIAS *et al.* 1989).

A saturated aqueous solution of a chromatographic fraction of *C. ashei* containing calaminthone (**5**), epievodone (**3**), and caryophyllene oxide had no significant effects on *L. sativa* but totally inhibited germination of *S. scoparium*. In contrast, saturated aqueous solutions of the pure compounds stimulated germination of *S. scoparium*, giving 185 % of control for epievodone (**3**). When epievodone was administered as a volatile or in a saturated aqueous solution of UA (**14**), significant inhibitory effects were observed. The above bioassays gave two interesting results: 1. The high activity and specificity, as demonstrated by the terpene mixture of calaminthone, epievodone, and caryophyllene oxide, toward the monocot *S. scoparium* was contrasted by insignificant effects on the dicot *L. sativa*. 2. Distinct opposite effects between pure terpenes and their mixtures as shown by germination promotion of *S. scoparium* by individual terpenes and strong inhibitory effects exhibited by the terpene mixtures. Strong synergistic phytotoxic effects by a monoterpene mixture (camphor, pulegone, and borneol) were previously reported by ASPLUND (1969).

Water washes of fresh *C. canescens* leaves were strongly inhibitory due to the presence of a series of monoterpenes. A bioassay-guided search for active constituents of *C. canescens* gave a highly active chromatographic fraction from the diethyl ether extract (DE LA PEÑA 1985, WILLIAMSON *et al.* in press). Major *Conradina* constituents included 1,8-cineole (**6**), camphor (**7**), borneol (**8**), myrtenal (**10**), myrtenol (**11**), α-terpineol (**9**), carveol (**12**), and carvone (**13**). In the water leaf soak of fresh *C. canescens* leaves 1,8-cineole, camphor, borneol, and α-terpineol were present, the first three of which are known to be potent plant germination and growth inhibitors (FISCHER 1986).

Biological activities of saturated aqueous solutions of eight *C. canescens* monoterpenes on *L. dubia* and *S. scoparium* are summarized in Table 1. Saturated aqueous solutions of all eight monoterpenes exhibited strong phytotoxicity, with the exception of 1,8-cineole, which did not inhibit *L. dubia* (WILLIAMSON *et al.* in press).

Release Mechanisms of Allelopathic Plant Lipids

In agricultural and natural ecosystems two major release and transport mechanisms of allelopathic agents have been proposed (MULLER *et al.* 1964, MULLER 1965, RICE 1984). Low molecular mass organic acids, phenolic acids, and plant phenolics are sufficiently water-soluble to be washed off the leaf surface into the soil by dew and rain. Also, low molecular mass alcohols, aldehydes, carboxylic acids, ketones, and certain phenolics, as well as mono- and sesquiterpenes, can be released from the plant leaf surface by volatilization to be carried to the ground for absorption by lipophilic organic soil particles. However, the above mechanisms exclude the large group of nonvolatile, relatively water-insoluble plant lipids from participation in allelopathic actions. Furthermore, in the Florida scrub the "volatility mechanism" may be ineffective for several reasons. First, in the scrub, coastal winds would not allow allelopathic volatiles to be absorbed or adsorbed in the immediate vicinity of the source plant for effective allelopathic action. Second, the sandy scrub soil lacks the organic matter necessary for absorption of lipophilic volatiles. Alternatively, water dispersal of lipids could be facilitated by the involvement of natural detergents such as UA (**14**) through micellization (FISCHER *et al.* 1988, WILLIAMSON *et al.* in press). This would allow release and transport in rain washes of volatile and nonvolatile hydrophobic plant products. Therefore, we performed analyses of leaf washes for the detection of micelles (FISCHER *et al.* 1988, TANRISEVER *et al.* 1989, WILLIAMSON *et al.* in press). We used the method by WOLFF (1981) for detection of micellization which is based on the fact that below the critical micelle concentration (CMC) the fluorescence of acridine is independent of surfactant concentration, while above the CMC, quantum yields decrease drastically with increasing surfactant concentration. Acridine fluoresces only in protic media and is sufficiently hydrophobic to be located mainly within the hydrophobic interior of the micelle.

We were able to demonstrate that water leachates of *Ceratiola ericoides, Calamintha ashei,* and *Conradina canescens* form micelles (FISCHER *et al.* 1988, WILLIAMSON *et al.* in press). Since the leaf waxes of all three scrub species are rich in UA, this triterpene most likely provides the matrix for micellization. These results suggested that UA acts as a micellar host, in particular for the bioactive terpenoids in *C. ashei* and *C. canescens* rain washes. This would enhance the rate of "solubilization" of plant lipids in aqueous foliar leachates and/or aid the transport to target seeds or plants. The "synergistic" allelopathic effect of UA, as demonstrated for *Calamintha* terpenoid constituents, is presently not understood.

Ursolic acid and other natural detergents may serve several important ecological functions:

1) Fixative actions for volatile plant constituents to reduce the otherwise rapid volatilization of allelopathic volatiles.
2) Actions as detergents to enhance water dispersal of nonpolar volatile and nonvolatile plant lipids in rain washes and dew.
3) Facilitation of the transport of phytotoxins to and entry into the membranes of target seeds or seedlings.

Acknowledgement

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