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# ALLELOPATHIC CONSTITUENTS OF THE CHAPARRAL SHRUB *ADENOSTOMA FASCICULATUM**

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**Abstract**—Leachates of intact leaves of *A. fasciculatum* were highly toxic to the growth of seedlings o *Bromus rigidus*. When analyzed by paper chromatography, these leachates were found to contain nine identifiable phenolic compounds. These included the major toxins, but an unidentified, non-phenolic substance was also toxic. The soil beneath the shrubs was extracted and shown to contain five of the nine phenolics present on the leaves, as well as the unidentified toxin.

## INTRODUCTION

AS A MAJOR component of the chaparral vegetation of California, *Adenostoma fasciculatum* H.&A. (Rosaceae) dominates extensive portions of this Mediterranean-climate area. Earlier work[1] has shown that this shrub is strongly allelopathic, producing substances toxic to the germination and early growth of herbaceous species in its vicinity. This phenomenon is manifested as a nearly complete lack of herbaceous plants in mature stands of *A. fasciculatum*. Following fire, which destroys the aerial portions of the shrubs, the herbs germinate and grow prolifically for a few seasons, being suppressed again only when the shrub tops have regenerated. A toxic material washes from the leafy tops of the shrubs with rain water (or fog drip) and is active in the upper layers of the soil, suppressing the herbs. It has further been shown that competitive effects cannot account for this phenomenon.[1]

Other workers have reported similar or related phenomena in other plants, often including the chemical identity of the toxins involved. Funke[2] found that the shrub *Artemisia absinthium* was toxic to other plants in its vicinity, and that the toxic principle, transported by rainwash, was the sesquiterpene lactone absinthin. Annual sunflower, *Helianthus annuus*, has been shown to have marked allelopathic effects as an early component of old-field succession. This species releases ecologically effective toxins from the leaves as leachate and from the roots as exudate. The toxic constituents include chlorogenic and isochlorogenic acid, scopolin, and an α-naphthol derivative.[3] Muller and his co-workers demonstrated that *Salvia leucophylla* and two other *Salvia* species produce allelopathic effects by a different mechanism. These shrubs volatilize inhibitory terpenes, including α-pinene, camphene, β-pinene, dipentene, cineole, and camphor, which are toxic to nearby seeds and seedlings after adsorption on soil colloids.[4–6]

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[1] J. K. MCPHERSON and C. H. MULLER, *Ecol. Monogr.* **39**, 177 (1969).
[2] G. L. FUNKE, *Blumea* **5**, 281 (1943).
[3] R. E. WILSON and E. L. RICE, *Bull. Torrey Bot. Club* **95**, 432 (1968).
[4] C. H. MULLER, W. H. MULLER and B. L. HAINES, *Science* **143**, 471 (1964).
[5] W. H. MULLER and C. H. MULLER, *Bull. Torrey Bot. Club* **91**, 327 (1964).
[6] C. H. MULLER and R. DEL MORAL, *Bull. Torrey Bot. Club* **93**, 130 (1966).

The original paper on *A. fasciculatum*[1] reported only preliminary work on the chemistry of the toxins, and no chemical identities. This paper presents further work on the subject, including the names of several toxic substances in the leaf leachate and from the soil beneath the shrubs.

## MEASUREMENTS AND EXPERIMENTS

### *Leaf Leachate*

Leafy branches of *A. fasciculatum* were collected in the Santa Ynez Mountains of southern Santa Barbara County, California. These branches were returned to the laboratory, spread on a large, shallow polyethylene funnel, sprayed with distilled water, and the resulting leachate collected. An effort was made to keep the volume of leafy branches constant between batches, and the same amount of water was used each time. More precise quantification, such as by weight, was made difficult by the variation in the amount of stem material present. It was considered important to allow these leafy branches to remain intact, so that the field situation would be simulated as closely as possible. This leachate was vacuum filtered and used in both the bioassays and chromatographic processes described below.

Bioassays of the leachate were conducted to confirm its toxicity. The method used was a modification of that described by McPherson and Muller.[1] The results of consecutive experiments using different lots of leachate are presented in Table 1.

TABLE 1. RESULTS OF BIOASSAYS OF VARIOUSLY CONCENTRATED LEAF LEACHATE

| | 2 July expt | | | | 6 July expt | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 1× | 5× | 10× | Control | 1× | 5× | 10× |
| Osmotic concn (mosmols) | 0 | 1 | 28 | 71 | 0 | 6 | 34 | 70 |
| Replicate means | 17·3 | 12·1 | 6·0 | 2·6 | 17·6 | 12·1 | 8·6 | 3·1 |
| | 17·0 | 12·1 | 5·8 | 2·4 | 14·9 | 13·8 | 6·7 | 4·1 |
| | 16·9 | 13·5 | 7·5 | 3·7 | 13·8 | 11·6 | 7·7 | 3·4 |
| Grand mean | 17·1 | 12·3* | 6·1* | 2·9* | 15 7 | 12·7† | 7·7* | 3·3* |
| Per cent of control | | 72 | 36 | 17 | | 80 | 49 | 21 |
| Osmotic toxicity, % of control‡ | | 99 | 90 | 70 | | 96 | 87 | 70 |

Values are means of radicle lengths in mm of *Bromus rigidus* grown in sand after 48 hr at 26°

* Significantly different from control at 1% level, T-test.

† Significantly different from control at 5% level, T-test.

‡ Bell and Muller (1970), unpublished data, based upon mannitol equivalents of corresponding leachate osmolalities.

The earlier work is clearly confirmed by these data; in fact there is somewhat greater inhibition shown in the present results. We attribute this to the use of sand in the bioassay, a technique which puts the entire seed and its emerging radicle in contact with the toxin-bearing medium. This was not the case with the earlier sponge-filter paper technique. We also feel that the sand technique is a closer imitation of the natural process, in which seeds are buried in the soil and litter. Furthermore, the osmotic potential of the leachate can account for only a minor portion of the growth reduction.

TABLE 2. $R_f$ VALUES AND COLOUR RESPONSES OF PHENOLIC COMPOUNDS FROM *Adenostoma fasciculatum* LEAF LEACHATE COMPARED WITH THOSE OF KNOWN COMPOUNDS

| | $R_f$ values, 3 MM paper | | | | |
|---|---|---|---|---|---|
| | 2% AA | | | BAW | |
| | In original mixture | Alone after elution | Known compound | Alone after elution | Known compound |
| Ferulic acid | 0·34 | 0·37 | 0·35 | 0·92 | 0·89 |
| *p*-Coumaric acid | * | 0·41 | 0·43 | 0·93 | 0·91 |
| Phloridzin | 0·38 | 0·42 | 0·39 | 0·80 | 0·88 |
| Syringic acid | 0·47 | 0·51 | 0·53 | 0·91 | 0·91 |
| Umbelliferone | 0·58 | 0·57 | 0·52 | 0·91 | 0·94 |
| Vanillic acid | 0·53 | 0·56 | 0·56 | 0·93 | 0·92 |
| *p*-Hydroxybenzoic acid | * | 0·63 | 0·63 | 0·95 | 0·92 |
| Ferulic acid | * | 0·67 | 0·64 | 0·92 | 0·89 |
| *p*-Coumaric acid | * | 0·72 | 0·71 | 0·93 | 0·91 |
| Hydroquinone | 0·71 | 0·74 | 0·75 | 0·92 | 0·88 |
| Arbutin | 0·79 | 0·81 | 0·76 | 0·92 | 0·93 |

| | Colour response | | | | | |
|---|---|---|---|---|---|---|
| | UV | | DPN | | DQC | |
| | Leachate compound | Known compound | Leachate compound | Known compound | Leachate compound | Known compound |
| Ferulic acid | bl | bl | sky-bl | sky-bl | dk-bl | dk-bl |
| *p*-Coumaric acid | abs | abs | dk-bl | dk-bl | bl to yel | bl to yel |
| Phloridzin | * | * | or-yel | or-yel | bl-gy | bl-gy |
| Syringic acid | lp | p | in-gr | gr | pu-bl | pu-bl |
| Umbelliferone | bl | bl | tn | tn | vi | vi |
| Vanillic acid | * | * | rs | rs | sky-bl | sky-bl |
| *p*-Hydroxybenzoic acid | abs | abs | r | r | sky-bl | sky-bl |
| Ferulic acid | bl | bl | sky-bl | sky-bl | dk-bl | dk-bl |
| *p*-Coumaric acid | abs | abs | dk-bl | dk-bl | bl to yel | bl to yel |
| Hydroquinone | abs | abs | br | br | br to dk-br | br to dk-br |
| Arbutin | bl | bl | bl-vi | bl-vi | sky-bl | sky-bl |

$R_f$ values are means of 3–5 runs.

* Not detected. Key to colours: abs = absorbs, bl = blue, br = brown, dk = dark, gr = green, gy = gray, in = indistinct, l = light, or = orange, p = pink, pu = purple, r = red, rs = rose, tn = tan, vi = violet, yel = yellow.

### *Identity of Toxins*

The leaf leachate was studied using paper chromatography. Detailed results are presented in Table 2. The phenolic compounds phloridzin, *p*-coumaric acid, ferulic acid, syringic acid, umbelliferone, vanillic acid, *p*-hydroxybenzoic acid, hydroquinone, and arbutin, were present in the leachate of *A. fasciculatum* leaves, a material produced in an ecologically relevant way.

*Chromatogram Bioassays*

The question of the effectiveness of these phenolics as toxins remained. To answer this question, we cut freshly developed 2% AA strip chromatograms of the ether fraction of leaf leachate into segments, guided by the appearance of spots under UV and by DPN or DQC-sprayed duplicates. These segments were placed in Petri dishes, moistened with distilled water, planted with ten lettuce seeds each, closed, and incubated for 48 hr at 26°. Control strips were cut into identical segments, using developed but unspotted paper, and were planted in the same way. Tests and controls were triplicated.

The results of this bioassay (Table 3) show complete suppression of germination and growth between about $R_f$ 0·48 and 0·79. This region corresponds to the area where most of the phenolic compounds are found. An area of milder toxicity exists between 0·33 and 0·48, the locale of phloridzin, *p*-coumaric acid, and syringic acid. Segments 0·0 (including the origin) through 0·33 exhibit no toxicity, but 0·79 through 1·0 show considerable toxicity, which is unrelated to any apparent phenolic compound.

TABLE 3. GERMINATION AND GROWTH OF LETTUCE SEEDS PLANTED ON CHROMATOGRAM SEGMENTS TO DETERMINE THE PHYTOTOXICITY OF VARIOUS COMPONENTS OF THE LEAF LEACHATE OF *Adenostoma fasciculatum*

| Compound | Spot centered on $R_f$ | Mean radicle growth (mm) | | | Mean germination (%) | | |
|---|---|---|---|---|---|---|---|
| | | Control | Test | % of control | Control | Test | % of control |
| | 0·06 | 11·9 | 12·6 | 105 | 97 | 90 | 93 |
| | 0·16 | 14·1 | 10·3 | 72 | 93 | 83 | 89 |
| | 0·24 | 11·7 | 12·4 | 105 | 93 | 93 | 100 |
| | 0·31 | 12·1 | 11·4 | 93 | 93 | 97 | 104 |
| Phloridzin | 0·35 | 17·9 | 7·8 | 42 | 100 | 93 | 93 |
| Ferulic acid* / *p*-Coumaric acid | 0·40 | 14·1 | 7·9 | 55 | 97 | 90 | 93 |
| Syringic acid | 0·43 | 14·2 | 5·4 | 38 | 100 | 76 | 76 |
| | 0·46 | 13·0 | 4·4 | 33 | 90 | 43 | 48 |
| Vanillic acid | 0·49 | 14·8 | 0 | 0 | 83 | 0 | 0 |
| Umbelliferone | 0·54 | 12·6 | 0 | 0 | 93 | 0 | 0 |
| *p*-Hydroxybenzoic acid | 0·60 | 13·1 | 0 | 0 | 97 | 0 | 0 |
| Ferulic acid | 0·63 | 13·2 | 0 | 0 | 83 | 0 | 0 |
| Hydroquinone | 0 68 | 13·3 | 0 | 0 | 90 | 0 | 0 |
| *p*-Coumaric acid | 0·72 | 15·2 | 0 | 0 | 100 | 0 | 0 |
| Arbutin† | 0·76 | 13·4 | 0 | 0 | 100 | 0 | 0 |
| | 0·82 | 11·7 | 6·0 | 46 | 97 | 20 | 20 |
| | 0·93 | 11·0 | 5·2 | 51 | 93 | 3 | 3 |

Developed in 2% AA.

* Not detected, but presumed to be in this area on basis of earlier chromatographic work.

† The toxicity shown here probably does not result from arbutin, but from hydroquinone formed by hydrolysis of arbutin during the bioassay.

*Non-phenolic Toxicity*

To determine if all of the toxicity could be attributed to the phenolic compounds, an experiment was undertaken in which the phenolics were removed from leachate by extraction with a base. Detailed procedure is given below.

The results, shown in Table 4, indicate that considerable toxicity remains when the phenolics are removed from the system. In chromatogram bioassays both germination and growth are suppressed on $R_f$ segments 0·6 through 1·0, and, while germination is unaffected, growth is reduced in segments 0·3 through 0·6 as well. We cannot further characterize these non-phenolic toxins at present, but hope that they can be identified later.

TABLE 4. RESULTS OF LETTUCE SEED BIOASSAY OF CHROMATOGRAM SEGMENTS, LEACHATES BASE-EXTRACTED TO REMOVE PHENOLICS

| | Mean* growth (mm) | | | Mean* germination (%) | | |
|---|---|---|---|---|---|---|
| $R_f$ segment | Control | Test | % of control | Control | Test | % of control |
| 0·0–0·1 | 14·2 | 12·1 | 85 | 93 | 87 | 94 |
| 0·1–0·2 | 13·8 | 11·7 | 85 | 100 | 93 | 93 |
| 0·2–0·3 | 12·3 | 9·5† | 77 | 97 | 80 | 86 |
| 0·3–0·4 | 13·4 | 7·7† | 57 | 93 | 93 | 100 |
| 0·4–0·5 | 12·6 | 7·3† | 57 | 87 | 87 | 100 |
| 0·5–0·6 | 15·3 | 6·6† | 42 | 93 | 87 | 94 |
| 0·6–0·7 | 15·3 | 7·5† | 49 | 97 | 73‡ | 75 |
| 0·7–0·8 | 14·9 | 6·0† | 40 | 97 | 70 | 72 |
| 0·8–0·9 | 16·0 | 4·6† | 28 | 90 | 40‡ | 44 |
| 0·9–1·0 | 13·2 | 5·0† | 37 | 90 | 13† | 14 |

Developed in 2% AA.

* Three replications.
† Significantly different from control at 1% level, T-test.
‡ Significantly different from control at 5% level, T-test.

*Soil Phenolics*

Soil was collected from several sites; (a) under *A. fasciculatum* shrubs, (b) in their immediate vicinity but not under the crowns, and (c) in the vicinity of shrub stands (on the same soil) but outside their influence. These soil collections were extracted using a modification of the method of Wang *et al.*[7]

TABLE 5. $R_f$ VALUES AND ESTIMATED RELATIVE QUANTITIES OF PHENOLIC COMPOUNDS FROM SOILS IN AND NEAR *Adenostoma fasciculatum* STANDS

| | Origin of soil and sample number | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Away from shrubs* | | Opening in shrub stand | | Under shrubs, litter covered | | | $R_f$ |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| Ferulic acid | + | − | + | ++ | − | ++ | ++++ | 0·30 |
| *p*-Coumaric acid | − | − | − | − | +++ | ++ | +++ | 0·38 |
| Syringic acid | − | − | − | − | ++ | − | ++++ | 0·47 |
| Vanillic acid | ++ | + | − | + | ++++ | +++ | ++++ | 0·54 |
| *p*-Hydroxybenzoic acid | ++ | + | − | + | ++++ | +++ | ++++ | 0·59 |
| Ferulic acid | + | − | + | ++ | + | − | ++ | 0·64 |
| *p*-Coumaric acid | − | − | − | − | ++ | + | ++ | 0·67 |

Chromatograms developed in 2% AA.

* Sparse cover of other plant species.

[7] T. S. C. WANG, T. K. YANG and T. T. CHUANG, *Soil Sci.* **103**, 239 (1967).

TABLE 6. GERMINATION AND GROWTH OF LETTUCE SEEDS PLANTED ON CHROMATOGRAM SEGMENTS TO DETERMINE THE PHYTOTOXICITY OF VARIOUS COMPONENTS OF EXTRACTS OF SOIL FROM OPENINGS IN SHRUB STANDS (4), AND BENEATH SHRUB CROWNS (7)

| Compound | Spot centered on $R_f$ | Mean radicle growth (mm) | | | | |
|---|---|---|---|---|---|---|
| | | Control | 4 | % of control | 7 | % of control |
| | 0·06 | 12·3 | 12·1 | 98 | 12 6 | 102 |
| | 0·16 | 11·5 | 10·5 | 91 | 10 9 | 95 |
| | 0·23 | 12 5 | 10 6 | 85 | 11·3 | 91 |
| Ferulic acid | 0·29 | 11·3 | 11·1 | 98 | 12·0 | 106 |
| *p*-Coumaric acid | 0·35 | 12·4 | 10·9 | 88 | 11·6 | 93 |
| Syringic acid | 0·42 | 11·7 | 11·2 | 96 | 11·1 | 95 |
| Vanillic acid | 0·49 | 11·3 | 11·1 | 98 | 9·3 | 82† |
| *p*-Hydroxybenzoic acid | 0·55 | 11·5 | 11 0 | 96 | 10·0 | 87 |
| | 0 60 | 13·6 | 10·0 | 73† | 11·0 | 81† |
| | 0·65 | 10·0 | 13·2 | 132 | 9·6 | 96 |
| | 0·71 | 10·4 | 10·8 | 104 | 10 0 | 96 |
| | 0·78 | 11·1 | 10·1 | 91 | 7·2 | 65* |
| | 0·86 | 10·5 | 6·1 | 57* | 1 3 | 13* |
| | 0·95 | 9·4 | 7·9 | 84 | 7·7 | 82† |

| Compound | Spot centered on $R_f$ | Mean germination (%) | | | | |
|---|---|---|---|---|---|---|
| | | Control | 4 | % of control | 7 | % of control |
| | 0 06 | 93 | 93 | 100 | 90 | 97 |
| | 0 16 | 87 | 90 | 103 | 90 | 103 |
| | 0·23 | 93 | 97 | 104 | 93 | 100 |
| Ferulic acid | 0 29 | 93 | 100 | 107 | 97 | 104 |
| *p*-Coumaric acid | 0 35 | 90 | 90 | 100 | 97 | 108 |
| Syringic acid | 0 42 | 87 | 100 | 115 | 83 | 95 |
| Vanillic acid | 0·49 | 100 | 93 | 93 | 100 | 100 |
| *p*-Hydroxybenzoic acid | 0 55 | 100 | 100 | 100 | 97 | 97 |
| | 0·60 | 97 | 97 | 100 | 100 | 103 |
| | 0 65 | 97 | 93 | 96 | 87 | 90 |
| | 0 71 | 97 | 93 | 96 | 87 | 90 |
| | 0·78 | 100 | 97 | 97 | 70 | 70† |
| | 0 86 | 87 | 93 | 107 | 10 | 11* |
| | 0 95 | 80 | 67 | 84 | 90 | 112 |

Controls are identical segments from unspotted, developed paper. Developed in 2% AA.

* Significantly different from control at 1% level, T-test.

† Significantly different from control at 5% level, T-test

Identification of soil phenolic compounds was accomplished using UV light, DPN and DQC spraying, and comparison with known compounds as before. We also estimated (Table 5) the relative quantities of the substances by comparing the intensity of their responses to UV and spraying. Five of the nine compounds found in the leaf leachate were also present in the soil beneath the shrubs. Those absent were phloridzin, umbelliferone, hydroquinone, and arbutin. In soils originating away from shrub stands or in litter-free openings in the stands, phenolic compounds were irregular in occurrence and present in much smaller quantities than in soils from under the crowns of shrubs (Table 5).

*Bioassays of Soil Extract*

Using the procedures given below, we bioassayed chromatograms of representative soil extracts. Extracts of soil samples seven and four (Table 5) were chosen as representatives of the phenolic-laden soils under the shrubs and the largely phenolic-free soils, respectively. The chromatograms were again cut into identifiable spots and the between-spot paper. Germination and growth of lettuce seeds planted on the chromatogram segments were used to assay toxicity (Table 6). A duplicate experiment produced very similar results.

In that part of the chromatostrips where the phenolic compounds occurred there was no reduction of germination in either soil. Moderate reduction of radicle growth was found on two segments, those centered on $R_f$s 0·49 and 0·60, in the under-shrub soil. The other soil produced a reduction only on segments 0·78 and 0·86, a region where we detected no phenolics. Overall, there was considerably more toxicity exhibited by the under-shrub soils than by those from openings in the stand.

We removed the phenolics from a fresh lot of soil extract by the base-extraction method described below. When this material was chromatographed and bioassayed the only suppression of germination and growth occurred in $R_f$ segments centered on 0·85 (strong suppression) and 0·95 (moderate suppression). These results suggest that, though phenolics are readily detectable in the soil and produce some toxicity, other substances are also effective as toxins under the conditions of our experiments.

*Bioassay of Known Phenolics*

Using the procedures described below, we bioassayed (Table 7) commercial samples of the phenolics found in *A. fasciculatum*. Although we have not quantified the amounts of phenolics present in our leachates and extracts, the concentrations used in this experiment, 100, 200, 300, and 400 ppm, are believed to be representative of those found naturally. Only *p*-coumaric and *p*-hydroxybenzoic acids did not reduce germination of lettuce seeds to some

TABLE 7. GERMINATION AND GROWTH OF LETTUCE EXPOSED TO AQUEOUS SOLUTIONS OF COMMERCIAL STANDARD PHENOLICS AT FOUR CONCENTRATIONS

| Compound | Radicle growth, % of control (ppm) | | | | Germination, % of control (ppm) | | | |
|---|---|---|---|---|---|---|---|---|
| | 100 | 200 | 300 | 400 | 100 | 200 | 300 | 400 |
| Arbutin* | 85 | 68† | 75† | 77‡ | 89 | 78† | 82 | 97 |
| *p*-Coumaric acid | 89 | 72† | 55† | 41† | 113 | 100 | 180 | 153 |
| Ferulic acid | 83‡ | 88 | 77† | 77† | 103 | 78 | 58‡ | 63‡ |
| Hydroquinone | 55† | 36† | 22† | 16† | 93 | 72† | 85 | 48† |
| *p*-Hydroxybenzoic acid | 46† | 40† | 37† | 34† | 108 | 81 | 88 | 88 |
| Phloridzin | 81† | 76† | 63† | 71† | 83‡ | 87‡ | 90 | 87‡ |
| Syringic acid | 78† | 81† | 72† | 69† | 92 | 67‡ | 63† | 41† |
| Umbelliferone | 60† | 25† | 22† | 19† | 83‡ | 83‡ | 90 | 80† |
| Vanillic acid | 77† | 64† | 48† | 46† | 74 | 78 | 52† | 37† |

* The toxicity shown here probably does not result from arbutin, but from hydroquinone formed by hydrolysis during the bioassay.

† Significantly different from control at 1% level.

‡ Significantly different from control at 5% level.

extent, and some compounds inhibited it strongly. Growth was retarded by every phenolic tested. Most reduced growth significantly even at the lowest concentration used. The most toxic compounds generally were those whose $R_f$s are between 0·48 and 0·79 in 2% AA, a fact in agreement with the results of the bioassays of leachate chromatogram segments.

## DISCUSSION AND CONCLUSIONS

It is clear that the leaf leachate of *A. fasciculatum* was highly inhibitory to the growth of seedlings, and that this inhibition was not due to osmotic effects. Nine phenolic compounds were present in the leaf leachate and these were the source of most, but not all, of the toxicity.

Phenolic compounds, including some of those found here, have often been reported by others[7–11] to be active phytotoxins under similar circumstances.

Extracts of soil from beneath *A. fasciculatum* shrubs contained five of the nine phenolics present in the leaf leachate; however, they showed less toxicity when bioassayed. It is probable that this was caused by lower concentrations of all phenolics rather than by the absence of four of them, since there was no localization of toxicity in their $R_f$ areas in the leaf leachate chromatogram bioassays.

Upon removal of the phenolics from both leachate and extract by chemical means, some toxicity remained. The residual toxic material was present at high $R_f$s under our chromatographic conditions, was soluble in water and ether, and was not removed by washing an ether solution with a salt-saturated, aqueous NaOH solution. We cannot characterize it further at present.

These facts fit in well with the results of our[1] earlier ecological studies, when we concluded that *A. fasciculatum* produced toxins which accumulated on the leaves until washed off by rain, were actively toxic to seedlings in the soil, and were largely denatured or dispersed during the subsequent dry season.

The soil and plant material used in the present work was collected in late May and June of a dry year, no rain having fallen for at least 2 months prior to the collections. The leaves consequently had accumulated a generous amount of the toxins which enabled us readily to identify the phenolic constituents. The soil, however, showed fewer phenolics and less toxicity, presumably because the toxins had been partially denatured or dispersed and none added during the dry period. This agrees with our previous findings, i.e. without annual renewal of the toxins the allelopathic effects were much diminished.

In our earlier work we also showed, by means of soil heating experiments, that a large part of the soil toxicity could be eliminated by relatively low temperature heating treatments. We now tentatively conclude that the residual toxicity in those experiments was caused largely by the non-phenolic inhibitors reported here. Soil micro-organisms presumably would degrade these toxins, but only when the soil is moistened by the next season's rains.

Some questions remain regarding the phenomenon of allelopathy by *A. fasciculatum*, for example, the fate of phenolic toxins in the soil after their brief period of activity, the nature of the non-phenolic toxin or toxins, the exact source of the toxins within the leaves and their means of exit and deposition, and the nature of the inhibitory effect upon seeds and seedlings. It is hoped that further research will resolve some of these questions.

[8] A. S. ABDUL-WAHAB and E L. RICE, *Bull. Torrey Bot. Club* **94,** 486 (1967).
[9] U. BLUM and E. L. RICE, *Bull. Torrey Bot. Club* **96,** 531 (1969)
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## EXPERIMENTAL

*Leachate bioassays.* Plastic Petri dishes (90 × 15 mm) were partly filled with 50 g dry sand which had been screened to remove particles larger than 1·0 mm and smaller than 0·25 mm. The sand was smoothed and twenty seeds of *Bromus rigidus* planted in a radial pattern just below the surface. The sand was moistened with 10 ml $H_2O$ (controls) or leachate, which was used at original strength and concentrated *in vacuo* to 5× and 10×. Milliosmol concentration of the leachates was measured with a Fiske Osmometer, model G66. Petri dish covers were pressed on over 10 × 10 cm squares of parafilm. Both tests and controls were triplicated. The radicles were measured and means taken, considering only the germinating seeds.

*Sample preparation and chromatographic technique.* Leaf leachate, concentrated 5×, was extracted with an equal volume of ether (×3) and the ether layers combined. The ether was allowed to evaporate and the residue taken up in 3 ml of EtOH. This material was then spotted on 2 × 57 cm strips of Whatman 3 MM chromatography paper and developed in selected solvents.

Descending development of papers using 2% aq. HOAc (2% AA, v/v) yielded a series of spots when viewed in UV light and/or sprayed with diazotized *p*-nitranıline followed by 10% $Na_2CO_3$ (DPN)[12] or 0·1% ethanolic 2,6-dichloroquinone chlorimide followed by saturated sodium borax solution (DQC).[13] This was repeated several times. Several freshly developed strips were cut into segments as indicated by UV light and known $R_f$s from other, sprayed strips. These segments, plus the paper between them, were eluted separately in EtOH for 12 hr using a Bailey–Walker apparatus. The EtOH was evaporated almost to dryness and used to spot new 3 MM paper strips. These strips were developed with either 2% AA or *n*-BuOH–HOAc–$H_2O$ (4:1:5, v/v, called BAW). Strips spotted with commercial standard compounds were developed simultaneously to check the identity of compounds from *A. fasciculatum* leaf leachate.

*Removal of phenolics from leachate.* Freshly made ether extract of leaf leachate was shaken five times with small volumes of 5% NaOH saturated with NaCl to remove the phenolic compounds. The ether portion was evaporated and the residue taken up in EtOH as before. Chromatograms of base-extracted and non-extracted leachate were developed in 2% AA, viewed in UV and sprayed with DPN to check the efficiency of phenolic removal. The base-extracted material showed no phenolic response, while the non-extracted leachate exhibited all the phenolics found in previous experiments. New chromatograms of the base-extracted material were cut into 1/10 $R_f$ segments and bioassayed with lettuce seed, using developed but unspotted paper as a control; both were triplicated.

*Soil extraction.* To 100 g air dry soil was added 150 ml of 95% EtOH and sufficient 2 N NaOH to bring the pH to 11. This mixture was shaken on a mechanical shaker for 24 hr, periodically readjusting the pH to 11, and then vacuum filtered. The filtrate was reduced to about 50 ml *in vacuo* at 50–60°, centrifuged, and the supernatant brought to pH 7 with 2 N HCl. The resultant liquid was evaporated to dryness on a hot plate (60–65°), the residue ground to a powder and taken up in three washes of ether which was acidified to pH 2 with 2 N HCl. This was evaporated to dryness at room temp. in a fume hood and the residue dissolved in 3 ml of 95% EtOH. This alcohol solution was applied to 2 × 57 cm strips of 3 MM chromatography paper and developed in 2% AA.

[12] J. M. Hais and K. Macek, *Paper Chromatography*, Nakladatelatví Ceskoslovenské akademie ved, Prague (1963).

[13] A. Vázquez, J. Méndez, M. D. V. Gesto, E. Seoane and E. Vieitez, *Phytochem.* **7**, 161 (1968).