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Autotoxic inhibition of seed germination by *Typha latifolia*: an evaluation

James B. Grace
Department of Botany and Microbiology, University of Arkansas, Fayetteville, AR 72701, USA

Summary. Seeds of *Typha latifolia* were exposed to leaf extracts, leaf pieces, and soil water from adult plants of *T. latifolia*. In liquid culture, only extracts of concentration greater than or equal to 3% (dry weight to volume) inhibited seed germination. Adding soil to the liquid cultures increased the inhibition of seed germination by extracts. The inhibitory effects of extracts were correlated with the development of water molds in the cultures; prior to the development of water molds, even 15% extracts were not inhibitory to germination. In contrast to results from extracts; neither 3% concentrations of senesced leaf pieces nor soil surface water from one year old pots of *T. latifolia* had any detrimental effects on germination. Seeds sown into pots containing established *T. latifolia* germinated as well as seeds sown into control pots. These results do not support the contention that *T. latifolia* inhibits the germination of its seeds by the release of allelopathic substances.

Introduction

A large number of species, terrestrial as well as aquatic, require a disturbance to regenerate. One common group of emergent wetland plants reported to require disturbance are species in the genus *Typha* L. Seedlings are reported to be rare in undisturbed stands (McNaughton 1968; van der Valk and Davis 1978; Grace and Wetzel 1981a; 1981b; but see Sharma and Gopel 1978). However, because of the small size of seeds, it is unclear whether the lack of regeneration from seed is the result of an inhibition of germination or because of seedling mortality. McNaughton (1968) reported that extracts of *Typha latifolia* L. inhibited germination completely; however, he found soil water to affect seedling growth more than germination. Van der Valk and Davis (1976, 1978), based on seed bank studies, provide support for McNaughton's studies by suggesting that there may be an allelopathic effect of *Typha glauca* litter on the germination of its own seeds. In contrast, Sharma and Gopel (1978) conducted experiments similar to those of McNaughton (1968) using *Typha angustata* Bory and Chaub. and *T. elephantina* Roxb., but found no evidence for inhibition of germination. Unfortunately, for at least some of the experiments, their methods were sufficiently different from those of McNaughton as to make comparisons difficult (in particular, they used much weaker extract concentrations). Finally, Bonasera et al. (1979) found *Typha latifolia* to have little effect on the germinatin of *Latuca sativa*, *Cucumbis sativus*, *Raphanus sativus* and *Lycopersicon esculentum*.

The primary objectives of this paper are as follows:

1. To reexamine the effects of litter extracts on germination in *Typha latifolia*. In particular to determine the effect of extract concentration on the results.
2. To determine the time-course of germination in the presence of extracts to see if seeds germinate after extracts decompose.
3. To examine the effects of *T. latifolia* litter and water from *T. latifolia* pots on the germination of its seeds.
4. To determine if *T. latifolia* seeds sown into tubs containing *T. latifolia* plants would germinate as well as seeds sown into control tubs containing no plants.

Methods

Seeds for all experiments were collected in Washington County, Arkansas, USA. Mature inflorescences were dried and stored at room temperature for approximately six to twelve months prior to use. Mature, viable seeds were obtained by the method of McNaughton (1968). Germinability was typically 90–100%.

Experiment I examined the effects of leaf extracts and leaf pieces on germination either with or without soil in the medium. The treatments were as follows: leaf extracts of 3%, 1.5%, 0.75%, 0.375%, and 0.18%; 0.75 grams dry mass of senesced leaf pieces of T. latifolia; tap water as control. Tap water was used rather than distilled water to provide a more natural chemical environment for seedling growth. Earlier studies revealed no difference in germination with distilled or tap water. However, seedling survival and growth was slightly better in tap water than in distilled water.

All treatments were added to 250 ml glass jars either with or without 50 ml of sterilized top soil (pH 6.5–6.9). Extracts were prepared according to the method of McNaughton (1968) by blending senesced leaf tissue of *T. latifolia* with tap water at high speed for 30 s. Raw extract was squeezed through several layers of cheesecloth, suction filtered through Whatman qualitative filter paper, and centrifuged at 37,000 *g* for 10 min (to clarify the extract). Extract concentrations were prepared on a dry mass to volume basis and were stored at 5° C until used (usually one day, never more than one week) (for pH of extracts see Table 1). Three grams of leaf pieces 3–4 cm long were placed on

Table 1. PH of various concentrations of leaf extract

Extract concentration	pH
Tap Water	8.35
0.18%	7.90
0.375	7.80
0.75	7.45
1.50	7.10
3.0	6.55
15.0	5.30

the bottom of the jar, covered with one layer of Whatman qualitative filter paper, and either weighted down with a piece of wire followed by another piece of filter paper (liquid culture), or covered with 50 ml soil (soil culture). Jars contained 100 mls water yielding a 3% dryweight per volume concentration.

Each treatment was replicated five times and each jar was sown with 10 seeds. Liquid levels of 3 cm were maintained daily by the addition of distilled water to compensate for evaporation. Aqueous phase (either extract or water) was replaced on days ten and twenty of the experiment. Liquid treatments were allowed to proceed for twenty-three days at which time germination had stopped. Soil treatments were allowed to proceed for an additional 10 days in order to permit seedling establishment.

Experiment II examined the effects of soil water, either with or without *T. latifolia* plants growing in the soil, on seed germination. Surface water from tubs of soil (silty-clay soil pH 6.5–6.9 organic matter content = 6.5%) was added to jars containing ten seeds each. Soil was either free of plants or contained one-year-old *T. latifolia* plants. Jars were either with or without 50 ml sterilized potting soil and were maintained with a 3 cm (75 ml) aqueous layer for thirty-three days. Five replicate jars were used for each treatment and germination was monitored daily.

Experiment III was conducted to determine the correlation between water mold growth in leaf extracts and the ability of the extracts to inhibit seed germination. Three-percent extract was prepared as for experiment I. Three cm of extract were added to ten 250 ml jars on day one of the experiment. Ten seeds were added to each of two replicate jars on days 0, 1, 2, 4, and 6. Light penetration through the extract was measured daily for all jars using a LiCor Quantum Sensor (responsive in the range of 0.4–0.7 μm). Because of the extreme turbidity that quickly developed as water molds grew, germination could only be determined when the experiment was terminated after eleven days. In addition to the above experiment with 3% extract, seeds were exposed to 15% extract to examine the effects on germination.

Experiment IV was conducted to determine if established plants of *T. latifolia* growing in the greenhouse would affect the germination of *T. latifolia* seeds in comparison to control soil without plants. Seven-liter tubs containing only top soil (silty-clay, pH 6.5–6.9, organic matter 6.5%) were used as controls. The effects of plants (the " + plants" treatment) was determined by allowing *T. latifolia* to grow in soil for approximately one year prior to use in this experiment (average 4.7 shoots per tub). Four replicate tubs were used for each treatment. Soil used in the control tubs was the same soil in which *T. latifolia* had been planted and had been stored dry during the twelve month interval from

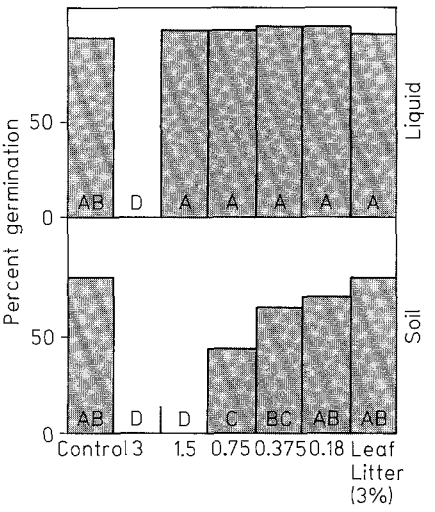

Fig. 1. The effects of leaf litter extracts and unextracted leaf litter on germination in liquid and soil culture. Treatments designated by the same letter are not significantly different at the 0.05 level based on Duncan's test

the *T. latifolia* planting to the seeding. Two 7.5 cm-diameter plastic chambers were placed in each tub. The chambers consisted of a plastic ring with fine mesh cotton fabric covering the bottom opening and were placed on the surface of the soil. The fabric was used to prevent seed burial and loss while still permitting the seed to be exposed to the soil surface.

Statistical analyses were performed using the Statistical Analysis System (Helwig and Council 1979). All percentage data were transformed prior to analysis and tested for normality. The transformation used was the arcsin of the square root as recommended by Sokal and Rohlf (1969). Data presented are detransformed.

Results

Only the 3% extract had a significant depressing effect on germination in liquid culture (Fig. 1); germination was between 94 and 100% for all other treatments. Soil culture, in contrast, had significantly less germination overall than liquid culture (based on Analysis of Variance, $P < 0.0001$). Controls in both liquid and soil cultures were not significantly different, indicating that the soil interacted synergistically with the extract to reduce germination. In the presence of soil, 0.75% and 1.5% concentrations inhibited germination.

The progression of germination through time was not the same for both control and 1.5% extract treatments in liquid culture even though the final percent germination was the same (Fig. 2). Germination in controls was 80% completed by day four whereas germination in 1.5% extract was depressed from day four to day six with another burst of germination on day seven. Actually, germination continued beyond day ten in the extract treatment and was recorded but these values are not presented in the time course because of the confounding effects of replacing the old extract with new on day ten.

Results from experiment II (the effects of soil water) showed a slight but significantly lower germination in soil culture (mean = 94%) than in liquid culture (mean = 99%). No differences in germination were found between controls

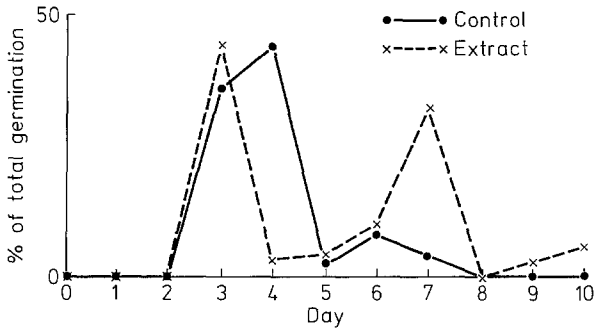

Fig. 2. The time course of germination in control (tap water) and 1.5% extract-liquid culture for the first 10 days of the experiment

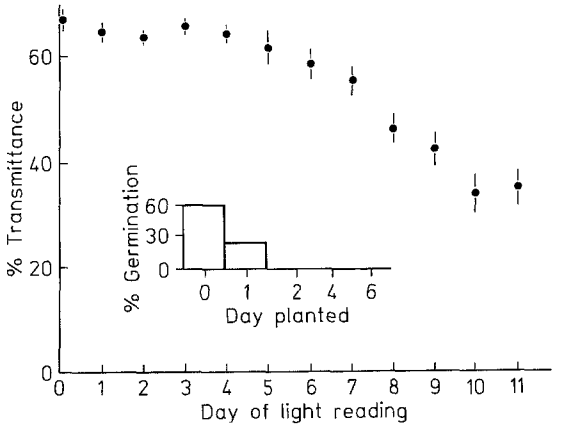

Fig. 3. Time course of light transmission through 3% extracts. Vertical bars are one standard error. Insert Figure is the germination for seeds in the experiment depending on the day planted

and the " + plants" treatment for either liquid or soil culture.

Figure 3 presents the time course of extract (3%) transparency over an eleven day period. Light penetration was not significantly reduced until the seventh day. In general, variance in light penetration between replicates increased through time. As had been observed earlier, the development of water molds is somewhat variable. Nonetheless, by day ten the extracts contained sufficient water molds to reduce light penetration from 67% to 36% (Fig. 3). Because of the turbidity which developed in the extract, it was not possible to observe the occurrence of germination during the course of the experiment. Based on the results after eleven days (Fig. 3, insert) germination was progressively inhibited as the water mold populations increased. Seeds planted on day zero had 60% germination compared to 25% for day one and 0% for subsequent days.

When seeds were planted into tubs of soil either with or without adult *T. latifolia* plants there was no significant effect on germination. In both controls and tubs containing plants, germination percentages were high (86% and 82% respectively).

Discussion

Only extract concentrations of 3% or greater were found to inhibit germination in liquid culture and only because of the presence of water molds. This finding is in agreement with the results reported by McNaughton (1968), who used 7.5% extracts, and also with those of Sharma and Gopel (1978), who used extracts of less than 3% (since they used 10% fresh weight to volume this estimate is based on the assumption that dry weight of plant material is $<1/3$ of fresh weight). However, since Sharma and Gopel (1978) used different species of *Typha* in their studies it is not safe to assume that a 3% extract would be inhibitory for their species.

The addition of soil to the cultures increased the extract effect on germination (Fig. 1). The effect appears to be synergistic since control values for germination were the same either with or without soil but the extract effects were much greater when soil was present. Rice (1979) suggests that synergistic effects would be expected when multiple allelopathic agents are present. It was noticed in my studies that treatments containing soil developed a heavier surface film, probably of organic matter, than liquid cultures. However, the exact cause of the interaction is unknown at present.

Large amounts of water mold developed in the extracts as experiments progressed and molds were most abundant in the most concentrated extracts. Figure 3 documents the development of molds by the increased turbidity of the extract. Although the rate of development of water molds was fairly variable in the several experiments conducted with extracts, in experiment III it was seven days before light transmission was significantly reduced by mold growth (however, mold growth was visually apparent by the third day). As shown in Fig. 2, most of the *T. latifolia* seeds under these experimental conditions would be expected to germinate by the fourth day so apparently the extract became inhibitory to germination prior to having a significantly reduced light intensity. It is unlikely that light intensity *per se* contributed to the inhibition of germination since transmission was still above 50% by day seven (see Sifton 1959 and Sharma and Gopel 1979 for data on light intensity effects on *Typha* germination). These results clearly indicate that the extract only became inhibitory after mold populations were well established. Germination experiments which I have conducted using 15% concentrations of extracts also showed that the extract by itself would not prevent germination and only became inhibitory after mold developed.

Microbial involvement in allelopathy has received considerable attention (Rice 1974, 1979; Richards 1976). The potential for organic matter to stimulate microbially based allelopathy is clearly of importance (Turner 1971; Kaminsky 1981). However, it is unclear how results from prepared extracts, especially when the inhibitory effects are of microbial origin, relate to allelopathy in natural conditions.

The progression of germination through time (Fig. 2 and examination of time-course data from other treatments in experiment I) indicated a delay in germination due to the extract rather than an irreversible toxic effect. Examination of the data showed that seeds in 1.5% extract continued to germinate after old extract was replaced with fresh, clearly indicating that seeds were merely being temporarily inhibited. The mechanism whereby seeds are inhibited from germinating is not known in this case but high osmotic pressure (Uhvits 1946, phenolic compounds (Mayer and Evenari 1952, 1953) and various metabolic inhibitors (Mayer and Poljakoff-Mayber 1975) are capable of reversibly inhibiting germination.

In contrast to the extract results, neither leaf litter (3% equivalent) nor soil surface water had any effect on germi-

nation in either liquid or soil culture. Water molds were not apparent in either of these experiments. The reasons for these differences between extracts, unextracted litter and soil surface water are not known but it is likely that the release of organic and inorganic compounds is both qualitatively and quantitatively different in the two treatments. For example, studies by Bokhari (1978) with prairie grasses revealed that extracts of living tissues were more inhibitory to germination than litter extracts and that living tissues were more toxic at earlier phenological stages.

The lack of inhibition of seed germination in the presence of established plants (experiment IV) is in strong contrast with the reported absence of *Typha* seedlings in nature (van der Valk and Davis 1976). Several factors could contribute to this apparent discrepancy. Even though the plants in the test tubs appeared to have reached a steady state with respect to number of shoots, the accumulation of litter was not great and the tubs must be considered to represent an early stage of stand development. Diurnal light measurements revealed that light intensity at the ground surface in the tubs with plants (81% of full sunlight) was high compared to natural stands (as low as 1%, Dykyjová and Kvêt 1978). Further, the use of cloth-bottom chambers reduced the possibility that sedimentation, algal growths and the activities of benthic invertebrates (all of which could conceivable be affected by the presence of established plants) would bury the seeds and prevent germination. Finally, because the early seedlings of *Typha* are so small it would be very difficult to observe them within dense stands of vegetation. Their reported absence in nature could be due to a suppression of seedling growth and survival rather than inhibited germination.

In conclusion, the results presented in this paper do not support the hypothesis that *Typha* seeds are inhibited from germinating by allelopathic substances. High concentrations of litter extracts will inhibit germination temporarily when water mold populations are allowed to develop; however, more natural assays such as intact leaf litter and soil surface water from established plants, do not show inhibition. Actually, the data of McNaughton (1968) suggest allelopathy to be more likely against seedlings than against seeds. That McNaughton clearly recognized this point is shown by his reliance on the seedling inhibition data in his arguments about autotoxicity.

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