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Does allelopathy involve in the association pattern of *Trifolium resupinatum*?

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Abstract

Indoor experiments demonstrated that allelopathic potential of rosette and flowering plants of qort is an important factor explaining the growth reduction of its associated species. Aqueous tissue extracts of flowering plants exhibited strong inhibitory effects on the germination percentage and radicle growth rate of the tested species as compared with those of vegetative plants. Under laboratory conditions, this inhibition was in agreement with toxicity assessments of soil samples collected from the rhizosphere of *T. resupinatum* L., where shoot and root dry mass of the tested species were significantly reduced. Detoxification of allelochemicals by presence of activated carbon can eliminate the inhibitory effects of the different extracts. This technique clarifies the occurrence of allelopathic interference by qort on seed germination and seedling growth, and hence suspects the allelopathic potential of qort in the growth reduction of associate species under field conditions along with competition.

Additional key words: allelochemicals, competition, growth parameters, *Melilotus indicus*, *Portulaca oleracea*, qort, soil residuals.

Introduction

Changes in the floristic composition of vegetation usually result from the destruction of all or some of the existing biomass by pathogens, herbivores, or man; changes in the different physical or chemical conditions of the habitat that favour the growth of some over others; interaction among the species, or the invasion and establishment of new species. These factors directly influence germination, growth, and survivorship, and constrain the number and distribution of recruits to plant population (Van der Valk 1981, Sharitz and Huenneke 1990).

It is the task of plant ecologists to investigate the ability of vegetation to use the environmental resources for survival, growth and development, and to understand interrelationships between the species. Plant-plant interference may involve not only

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competition for water, light and nutrients, but also allelopathic processes in which toxic organic compounds are released into the environment (*e.g.* Rice 1984).

Trifolium species are native in Mediterranean, Central Europe, Western and Central Asia and introduced into many temperate regions of the world. Some of them are cultivated as forage and others are wild. *Trifolium resupinatum* L. (qort) is a weed, one of 22 species that comprise this genus in Egypt (Täckholm 1974). It is a prostrate, rosette-forming, glabrous annual herb with an erect, much branched stem from the base (Boulos and El-Hadidi 1984, Boulos 1995). It is recorded as an associated species in most plant communities of different habitat types (gardens, orchards, canal banks and fallow lands). Under suitable environmental conditions, it flourishes and has the potential to compete aggressively with other species. So, it dominates a well developed community (El-Khatib, unpublished). Within the different stands of *T. resupinatum* community, *Portulaca oleracea* and *Melilotus indicus* were found to be the rarely present species with weak performance and reduced growth. These species were, therefore, selected to assess the allelopathic potential of qort and to describe the role of allelopathy in the distribution and association pattern of plant species.

Materials and methods

Tissue extract bioassays: Each of the above and below-ground tissues of rosette and flowering plants of *Trifolium resupinatum* L. were collected and placed in ice box. 50 g of each tissue type was mixed with 1000 cm^3 deionized water to prepare 5 % (m/v) aqueous extracts according to Wardle *et al.* (1992). The purified extracts were adjusted to pH = 6.8 with 1 M HCl (Rice 1972). Finely powdered activated charcoal (2 g) was added to 100 cm^3 of each of the extract solutions and 100 cm^3 of deionized water that latter being used as a control, in order to eliminate selectively allelopathic interference in the seed germination. After 12 h of thoroughly stirring and subsequent filtration, the carbon treated extract had lost its characteristic colour and smell. All the bioassay experiments were conducted under growth chamber conditions at temperature of 22 ± 2 °C with 170 µmol m^{-2} s^{-1} photon flux density during a 11-h photoperiod to assess allelopathic potential of the material against seeds collected from wild populations of its associated species, namely *Portulaca oleracea* and *Melilotus indicus.*

The allelopathic effects of these extracts on speed of germination and radicle elongation of the test species were determined. Four cm^3 of each extract were added to 4 replicates 9-cm Petri dishes, each dish containing 15 seeds of test species on two pieces of *Whatman No. 1* filter paper. Dishes were maintained under controlled environment. One cm^3 of deionized water was added to each dish, as needed, to prevent desiccation. Percentage germination was recorded (a seed was recorded as germinated if the radicle was equal to or greater than the length of the seed coat) for two weeks. Germination index, S, was calculated as described by Khandakar and Bradbeer (1983):

$$S = \{ N_1/1 + N_2/2 + N_3/3 + ... N_n/n\} \times 100/1$$

where: N_1, N_2, N_3, ...N_n = proportion of seeds which germinate on day 1, 2, 3, ...n

following setup of the experiment. This has an advantage over percentage germination because it is usually more sensitive as an indicator of allelopathic effects (Wardle *et al.* 1991). This experiment was repeated, but with five pregerminated seeds of each test species placed in each Petri dish; the length of each seedling radicle was then measured after 1 week.

Soil bioassays: Two plants of *T. resupinatum* were excavated in the field and the soil carefully removed from the root zone (12 - 20 cm, depth). Two replicate control soil samples were taken from an adjacent areas with sparse growth of *P. oleracea* and *M. indicus*. These samples were prepared to soil bioassays, and analyze for texture, calcium carbonate, organic matter, moisture content, soil reaction, and electric conductivity. All soil analyses procedures were according to the U.S. Salinity Laboratory Staff (1954) and Jackson (1967). Subsamples of each soil sample were placed in plastic pots, into which 20 seeds of the test species were placed. Percentage emergence and speed of emergence index (E) were measured after two weeks. Five vigorous seedlings were randomly selected in each pot and the rest were removed. These seedlings were left to grow for a further one month and harvested to determine shoot and root dry mass. During the experimental period, soil were daily irrigated by deionized water to reach full field capacity (26 %). All the measurements and experimental conditions were as described for the tissue extract bioassays.

Statistical analysis: All data on germination and radicle growth were subject to standard analysis of variance using the *Statgraphics* statistical analysis software (*STATG 1991*). Comparisons of the main effects were performed using the Least Significance Difference with aqueous extracts and soil residual bioassays, and *F*-test with soil characteristics. A significance level of $P < 0.05$ and 0.01 were used for all statistical procedures.

Results and discussion

Results of the bioassay experiments (Fig. 1*a.b.c*) reveal that, when data of all species was pooled together, the different tissue extracts (without carbon treatment) negatively affected germination percentage, speed of germination and radicle growth rate of the two test species. The extracts of flowering plants were considerably more inhibitory than those of the rosette plants, and their above ground tissues were more inhibitory than the below-ground tissues at equivalent concentrations. This is in agreement with the suggestion of Wardle (1993) and the results of Ballester *et al.* (1979) who reported that allelopathic potential of some plant species increases as they pass from their vegetative to flowering phases. The inhibition of seed germination may be due to the inhibitory effect of allelochemicals, in the tissue extracts, on the production of hormones-induced growth or altered enzyme activity which affect the mobilization of storage compounds during germination. Although enzyme activity was not investigated in this work, an indirect association between lower seed germination and allelopathic inhibition may be the consequence of the

inhibition of water uptake (most of seeds used not swelled) and enzymes activity. At the seedling stage, the susceptibility to these allelochemicals may be increased, so the radicle growth rate was strongly inhibited. Besides inhibiting radicle elongation, other morphological abnormalities occurred: many of the extracts caused twisted radicle growth and lack of fine-root hairs. The most severely twisted roots were observed in seedling treated with extracts of flowering plants. Many workers (Leather and Einhellig 1986, Hall and Henderlong 1989, Chung and Miller 1995) reported that seedling growth is more sensitive to allelochemicals than seed germination.

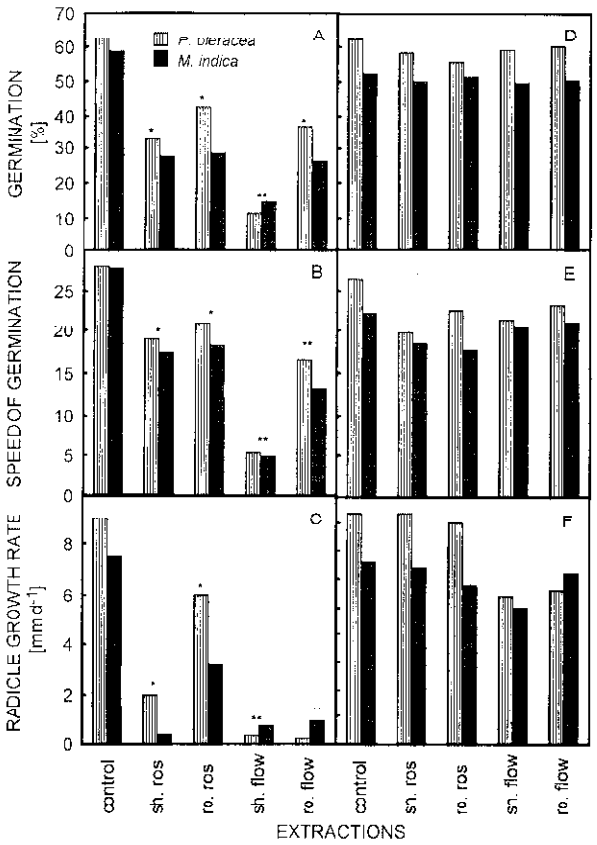

Fig. 1. Effect of different tissue extracts on percentage germination, speed of germination and radicle growth rate of *P. oleracea* and *M. indicus* in absence (*a,b,c*) or presence (*d,e,f*) of charcoal (sh.ros - shoot of rosette plants, ro.ros - root of rosette plants, sh.flow - shoot of flowering plants, ro.flow - root of flowering plants, * - L.S.D. significant at $P < 0.05$, ** - L.S.D significant at $P < 0.01$).

The both test species showed comparable responses to the various tissue extracts, but it may be considered that *M. indicus* was less influenced than *P. oleracea* (Fig. 1). These findings have particular importance to explain what is done under field conditions, where the two species in association with qort were rarely present.

Therefore, this sociological pattern may be a product of several interrelated casual factors which are not only dependent on the competitive ability of qort (which may also be, in part, dependent on the micro-environment), but also its allelopathic potential at different developmental stages.

Activated carbon, when present in the extracts (Fig. 1*d,e,f*) gave a significant increase in germination percentage, speed of germination and radicle growth rate, when compared with the pure extracts (without carbon). This may be due to adsorption of inhibitory substances by carbon. There is no reason for supposing that the carbon powder itself was an inhibitory factor in this study, since distilled water treated with carbon did not lead to any inhibition of seed germination. This is in agreement with the results of Eliasson (1959) and Zackrisson and Nilsson (1992).

Different allelochemicals in the soil samples collected from the rhizosphere of *T. resupinatum* plants showed significant inhibition on percentage of seedling emergence, speed of emergence and dry mass of the two test species (Fig. 2). These results were consistent with those of the aqueous tissue extract bioassays, and indicate that allelochemicals produced through either the annual deposition of leaf litters or living roots themselves may be released into the rhizosphere where they are adsorbed and accumulated by the soil particles, and may reduce the chance of survival of progeny of *P. oleracea* and *M. indicus*. In this context, various workers (Zinke 1962, Harris and Kimber 1983, Kershaw and Looney 1985, Pue *et al.* 1995, Inderjit and Dakshini 1995) have highlighted the significance of soil accumulated

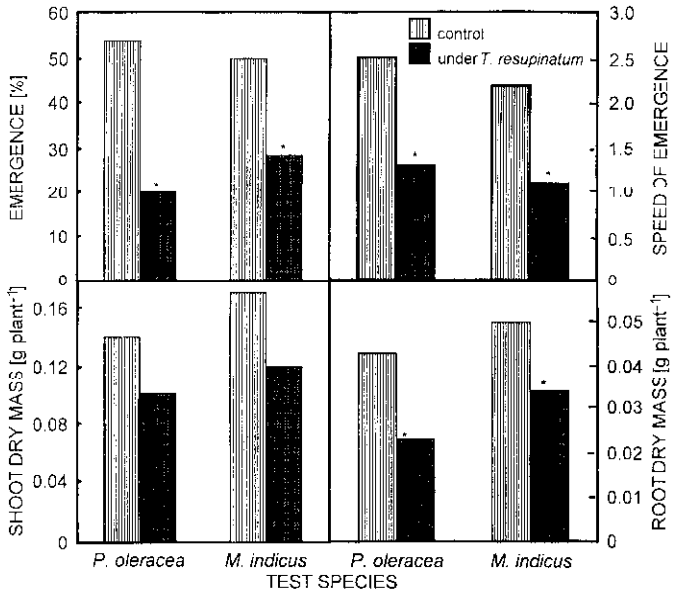

Fig. 2. Effect of soil samples on percentage of emergence, speed of emergence, and fresh and dry masses of *P. oleracea* and *M. indicus* (* - L.S.D. significant at $P < 0.05$).

allelochemicals in explaining growth and spatial arrangement of species. Although, the allelochemical pool in the soil is constantly changing through physical, chemical and biological transformation of compounds, their synergistic effects become more effective even at low concentrations.

It is important, however, to bear in mind the results of soil bioassays and the degree of similarity (hypothesis test for H0 not reject) between the edaphic factors in the different stands of *T. resupinatum* community and those of sparse growth of *P. oleracea* and *M. indicus* (Table 1) under similar micro-climatic conditions. These results suspect that other factor than either density of *T. resupinatum* (it substituted by its soil rhizosphere in the bioassay experiments and completely absent) or dissimilarity in environmental requirements (environmental pattern) may be responsible for the reduction in growth of the two test species.

Table 1. Some physico-chemical characteristics of soil samples collected from the rhizosphere of *T. resupinatum*, *P. oleracea* and *M. indicus*.

Soil factors	*T. resupinatum*	*P. oleracea*	*M. indicus*	*F*-ratio
Sand [%]	75	74	73.6	10.2
Silt [%]	6.8	8	8.2	3.4
Clay [%]	18.2	18	18.2	5
$CaCO_3$ [%]	12.2	12.1	12.2	6.3
Moisture content [%]	18.7	17.6	18.2	6.8
Organic carbon [%]	2.2	2.3	2.1	1.1
Salinity [dS m^{-1}]	20.31	20.06	0.32	0.12
pH	8	8	8	0.0

In conclusion, the controlled experiments revealed that *T. resupinatum* plants may produce allelochemicals which inhibit growth of *P. oleracea* and *M. indica.* The toxicity of these allelochemicals depends upon the tissue types and the development phase. These observations suggest the significance of the age at which plants begin to release allelochemicals. Therefore, under field conditions the reduction in growth may not be due to a deficiency in soil resources, but allelochemicals produced either by the living roots of *T. resupinatum* (true allelopathy) or by microorganisms closely associated with the roots and abundant in decaying leaves (functional allelopathy), be important in this concern. Also, growth inhibition by allelopathy would be expected to reduce the competitiveness of the inhibited plant. Although their mechanisms are distinct, allelopathy and competition are clearly related in the field (Chung and Miller 1995).

Production and mode of action of the phytotoxins produced by *T. resupinatum* plants remain unknown. However, in the present investigation most of these allelochemicals were detected by thin layer chromatograghy such as alkaloids and phenolic compounds (unpublished).

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