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A Survey of Old-field Herbs for Susceptibility to Phenolic Compounds

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페놀화합물에 대한 묵밭 草本植物의 感受性

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ABSTRACT

Phenolic compounds, p-coumaric and p-hydroxybenzoic acids, known as inhibitors for development and growth of many pioneer species on early stage of succession were used for the test of susceptibility in various herbs collected from abandoned agricultural fields in the vicinity of Amherst(U. S. A). The percent inhibition was generally greater for p-coumaric acid than for p-hydroxybenzoic acid. Concentrations of 5×10^{-5} and 5×10^{-4} M generally had no significant effects, but at 5×10^{-3} M was inhibitory to germination and growth of tested species. And the percent inhibition caused by the two phenolics was correlated ($r=.843$, $p<.01$). Also the indices of resistance for germination and elongation were significant ($r=.695$, $p<.01$) in this study. While *Cirsium* and *Lepidium* invading species of early stage of succession were sharply susceptible for toxic activity by phenolic acids.

INTRODUCTION

Chemical interactions among plants have frequently been hypothesized to explain patterns of vegetation dynamics and plant distribution(Muller, 1974; Rice, 1984, 1979; Fisher, 1977; Horsley, 1977). In particular the invasion of early successional communities by late successional species has often been attributed to "allelopathy." Keever(1950) showed that *Erigeron canadense* seedlings were inhibited by decaying roots of the same species, and proposed that this auto-toxicity might play a minor role in the subsequent invasion by *Aster* plants in Noth Carolina. Many researchers found that a numbe of early successional species in Oklahoma were inhibitory to each other and to themselves in bioassays, while *Aristida oligantha,* a late successional species, was resistant (Abdul-Wahab and Rice, 1967; Wilson and Rice, 1968; Parenti and Rice, 1969; Neill and Rice, 1971).

Likewise in New Jersey, *Ambrosia artemisiifolia* and *Raphanus raphanistrum* appeared to inhibit themselves and each other more than they inhibited the later successional species, *Aster pilosus* and *Hieracium pratense*(Jackson and Willemsen, 1976).

There is some evidence that phenolic compounds are the allelopathic agents involved in these successional changes. Adul-Wahab and Rice (1967) and Parenti and Rice (1969) found such phenolics as p-coumaric, p-hydroxybenzoic, chlorogenic and sulfosalicylic acids in extracts of *Sorghum halepense* and *Digitaria sanguinalis*. Jackson and Willemsen(1976) tentatively identified chlorogenic and caffeic acids in extracts of both *Ambrosia artemisiifolia* and *Raphanus raphanistrum*. Furthermore, Olmsted and Rice(1970) concluded that the late successional grass *Aristida oligantha* was indeed more resistant to phenolic compounds than two early sucessional species.

The purposes of this inestigation were to gather information on the susceptibility of various species to the toxicity of phenolic compounds, and also to test the hypothesis that early successional species tend to more susceptible to phenolics than late successional species.

MATERIALS AND METHODS

Seeds of 27 herbaceous species were collected from abandoned agricultural fields in the vicinity of Amherst, Massachusetts. Out of them, nine species (*Achillea millefolium, Agropyron repens, Amaranthus retroflexus, Cirsium vulgare, Convolvulus arvensis, Phalaris arundinaceae, Phleum pratense, Setaria glauca and Solidago gaminifolia*) germinated in the dark, six others (*Daucus carota, Lepidium virginicum, Potentilla argentea, Silene cucubalus, Solanum dulcamara* and *Verbascum thapsis*) required light for germination, and one (*Asclepias syriaca*) required stratification.

Germination and radicle elongation. Seeds of these 16 species were placed in petri plates, 10 seeds per plate, with two layers of germination blotter (Anchor Paper Co., St. Paul, Minn.). To each plate was added 11 ml of a phenolic solution(or distilled water in the case of the control). p-Coumaric and p-hydroxybenzoic acids were diluted to concentrations of 5×10^{-3}, 5×10^{-4} and 5×10^{-5} M for these solutions. These phenolics were chosen because they have known toxicity against plants (Schreiner and Reed. 1908; Börner, 1959; Wang *et al.*, 1967; Olmsted and Rice, 1970) and because they have been isolated from soil as well as from plants (Whitehead, 1964; Guenzi and McCalla, 1966; Abdul-Wahab and Rice, 1967; Wang *et al.*, 1967; Shindo *et al.*, 1979). There were four replicate plates for each phenolic treatment, and eight for the control.

The plates were put into a growth chamber giving alternating temperatures (30℃ for 16 hr, 20℃ for 8 hr) for 7 to 21 days, dependig upon the time required for germination for each species. At the end of this time the germinated seeds were counted and measured(hypocotyl plus radicle, hereafter called "elongation").

An index of resistance was calculated for each species by expressing the average gemination for the two 5x 10^{-3} M phenolic treatment as a percent of the control.

Growth. This experiment was designed to determine the effects of phenolics on growth of various species in a sand medium. Eight of the species above, and additionally *Agrostis alba* and *Dactylis glomerata*, were planted in square plastic pots(10 ml on a side) containing fine grained quartz sand. The pots were watered from the base with tap water twice per day for 2-3 weeks. After this time the seedlings were well established, and the nutrient and phenolic treatments were initiated. Hoagland's nutrient solution(Hoagland and Arnon, 1950) was added to the pots once per day. p-Coumaric and p-hydroxybenzoic acid, at 1 x 10^{-3} and 1 x 10^{-4} M, were added to the nutrient solution for the treatment pots, while the control pots received only nutrient solution. The amount of solution administered each day was sufficient so that much drained out the base of the pot; this insured that evaporation was not causing a gradual accumulation of solutes in the pots.

The plants were harvested 39 to 45 days after planting, depending on the species rates of germination and growth. They were washed free of sand, oven-dried at 80℃ for 36 hr, and weighed.

RESULTS AND DISCUSSION

Germination percentage was generally not significantly different from the control for phenolic concentrations of 5 x 10^{-5} and 5 x 10^{-4} M (Fig. 1). At 5 x 10^{-3} M, p-coumaric acid inhibited significantly the germination of most species and p-hydroxybenzoic acid of a few. *Agropyron* did not germinate at all with 5 x 10^{-3} M p-coumaric acid, and *Cirsium* and *Lepidium* were also drastically inhibited. The percent inhibition was generally greater for p-coumaric acid than for p-hydroxybenzoic acid, but the two were correlated ($r=.737$, $p<.01$). An index of resistance was calculated for each species (Fig. 1).

Elongation showed similar responses to gemination (Fig. 2). Concentrations of 5 x 10^{-5} and 5 x 10^{-4} M generally had no significant effects (but see *Lepidium, Asclepias, Solanum*). At 5 x 10^{-3} M, p-coumaric acid was always significantly inhibitory to elongation, and p-hydroxybenzoic acid was often so. Again the percent inhibition caused by the phenolics was correlated ($r=.843$, $p<.01$). An index of resistance was again calculated (Fig. 2) based on the species responses to phenolics at 5 x 10^{-3} M.

Germination responses were also corrrelated with elongation responses for these sixteen species (Fig. 3). The indices of resistance for germination and elongation were significantly correlated ($r=.695$, $p<.01$). Species like *Lepidium* and *Cirsium* were usually susceptible, while others like *Verbascum* and *Solidago* were usually resistant.

Growth in dry weight of ten species tested was heavily inhibited by two kinds of phenolics, p-coumaric and p-hydroxybenzoic acid, especially in *Agrostis, Verbascum* and *Cirsium* (Fig.4). The lowest index of resistance was found in *Amaranthus,* while the highest

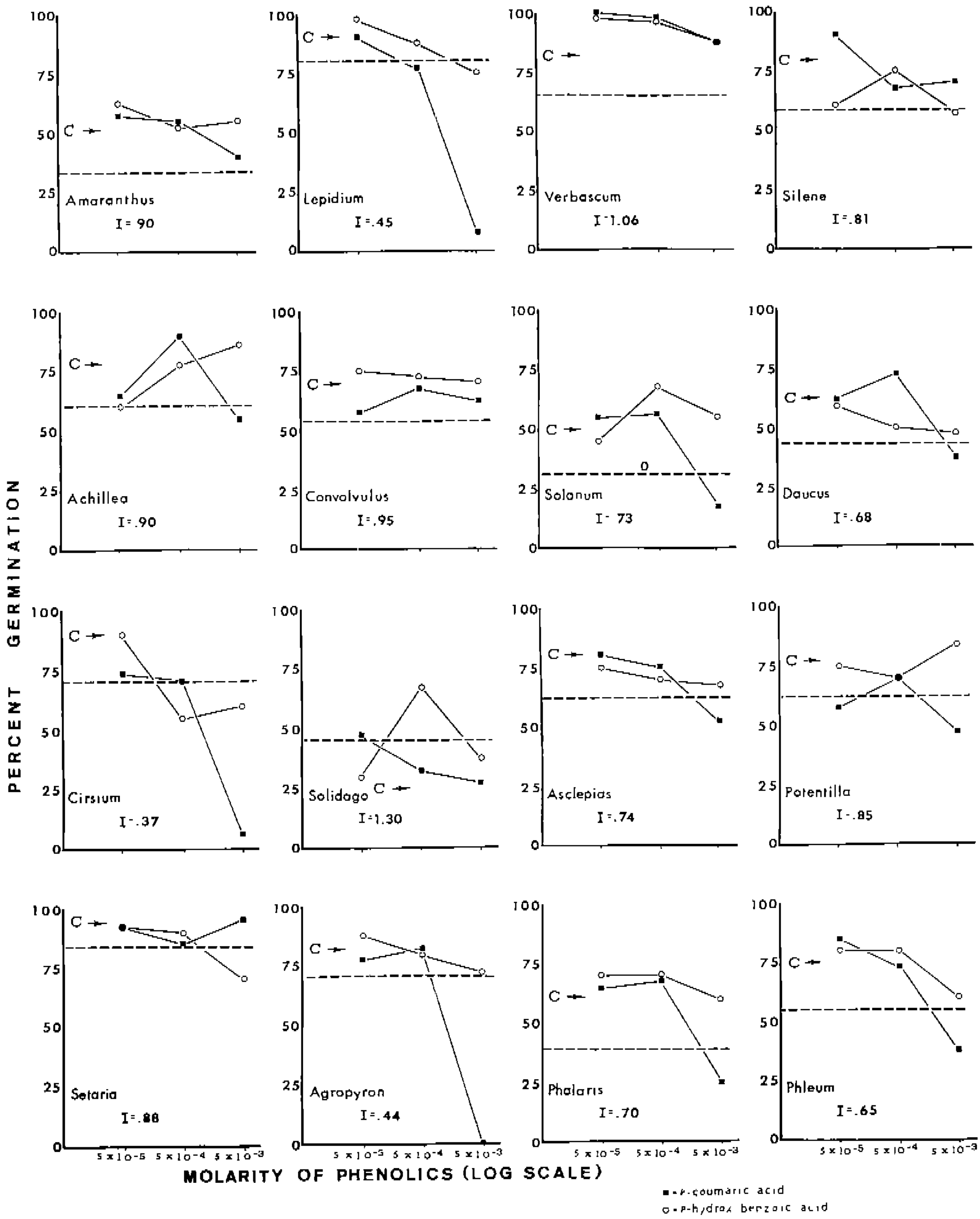

Fig. 1. Relationships between percent germination and concentrations of phenolics in 16 species. Svmbol "C" stands for mean value level of percent germination in the control. Dotted line: the lowest level of percent germination in the contol. I=index of resistance(see text).

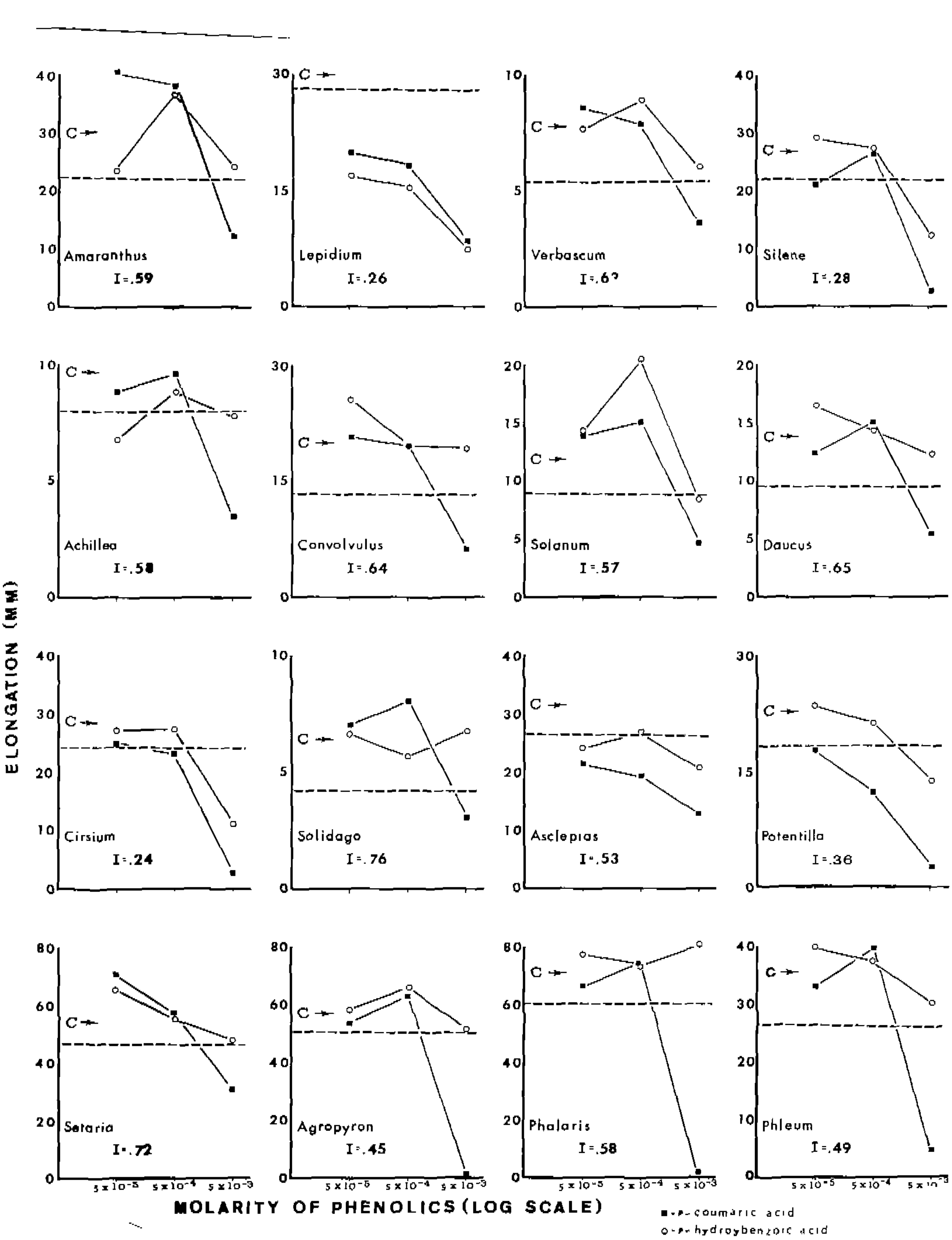

Fig. 2. Relationships between elongation and concentrations of phenolics in 16 species. For the details of caption see Fig. 1.

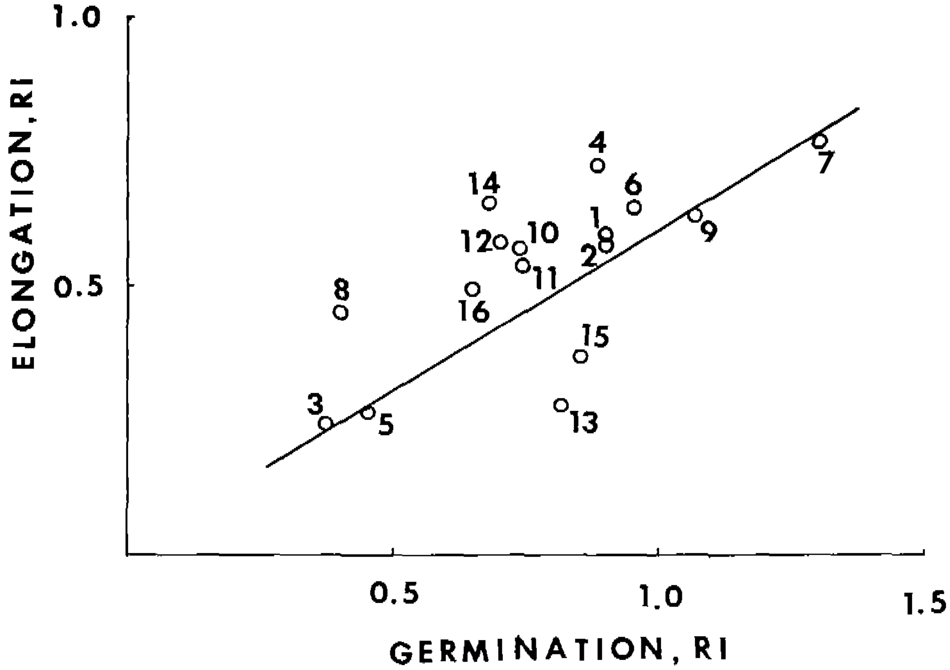

Fig. 3. Relationships between two values of resistance index (RI) from percent germination and elongation. For numerals see Table 1.

one in *Daucus* (Table 1). In general the inhibition effects of dry weight showed lower values for p-coumaric acid than for p-hydroxybenzoic acid. Based on the index of resistance, dry weight growth responses were correlated with elongation responses for eight species, dividing into *Cirsium*(elongation susceptible) and *Amaranthus*(dry weight susceptible) group on two kind of line (Fig. 5).

There are a lot of reports relating phenolic acids to seed germination, shoot elongation, total seedling growth, etc.(Kapustka and Rice, 1976; Olmsted and Rice, 1970; Rasmussen and Einhellig, 1977; Rice *et al.*, 1980; Tinnin and Muller, 1972). Among phenolic compounds cinnamic acid and coumarins are thought to be the most important phytotoxins in temperate ecosystems(Horsley, 1977).

By the results of our experiments, it is varied esponding to germination and seedling elongation in different concentration of phenolics, e.g., the heaviest inhibition of them was shown in 5 x 10^{-3} M, next one did in 5 x 10^{-4}M, but they were not much inhibitory in 5 x 10^{-5} M. Terefore, between 5 x 10^{-3} and 5 x 10^{-4} M of p-coumaric and p-hydroxybenzoic acids solution is considered to be correspond to critical inhibition concentration in this study. And also pioneer species of early stage of succession, *Cirsium* and *Lepidium*, were very keenly susceptible, namely these species showed to be inhibitory in seed germination, seedling elongation and dry weight.

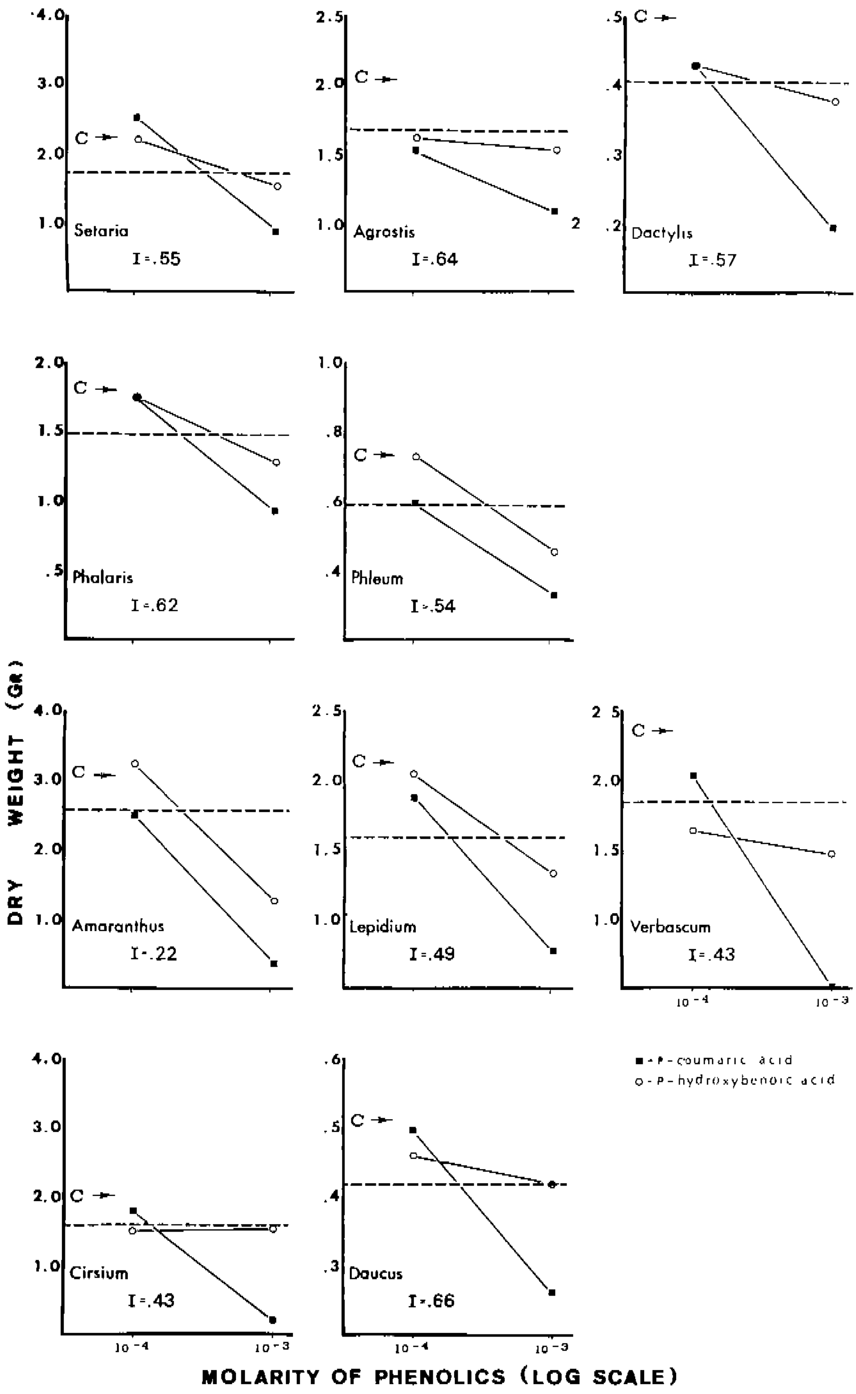

Fig. 4. Relationships between dry weight growth and concentrations of phenolics in 10 species. For the details of caption see Fig. 1.

Table 1. The values of resistance index obtained from three different dimension: percent germination, elongation and dry weight

No.	Plant name	Percent germination	Elongation	Dry weight
1	*Amaranthus retroflexus*	0.90	0.59	0.22
2	*Achillea millefolium*	0.90	0.58	-
3	*Cirsium vulgare*	0.37	0.24	0.43
4	*Setaria glauca*	0.88	0.72	0.55
5	*Lepidium virginicum*	0.45	0.26	0.49
6	*Convulvulus arvensis*	0.95	0.64	-
7	*Solidago graminifolia*	1.30	0.76	-
8	*Agropyron repens*	0.44	0.45	-
9	*Verbascum thapsis*	1.06	0.63	0.43
10	*Solanum dulcamara*	0.73	0.57	-
11	*Asclepias syriaca*	0.74	0.53	-
12	*Phalaris arundinaceae*	0.70	0.58	0.62
13	*Silene cucubalus*	0.81	0.28	-
14	*Daucus carota*	0.68	0.65	0.66
15	*Potentilla argentea*	0.85	0.36	-
16	*Phleum pratense*	0.65	0.49	0.54
17	*Agrostis alba*	-	-	0.64
18	*Dactylis glomerata*	-	-	0.57

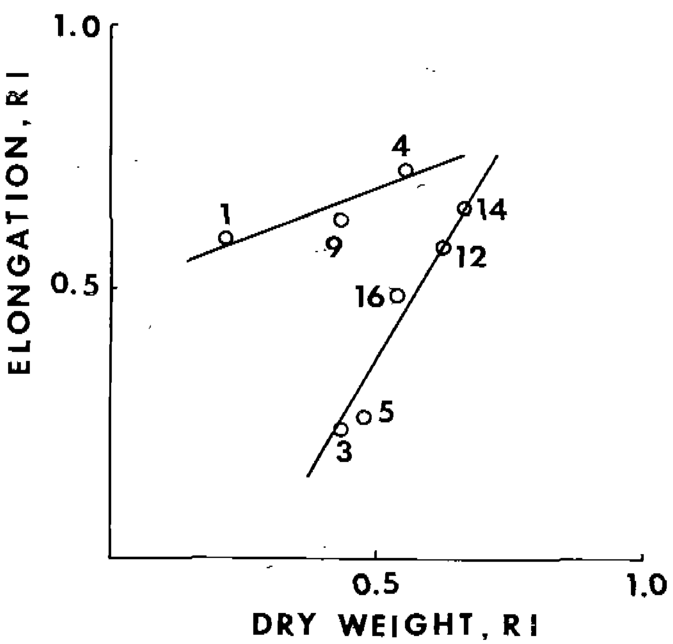

Fig. 5. Relationships between two values of resistance index (RI) from dy weight and elongation. For numerals see Table 1.

摘 要

北美 Massachusetts주에 있는 묵밭에 나는 各 屬의 草本植物 27種을 골라 實驗室에서 5×10^{3} M~5×10^{-5} M 사이의 濃度別 p-coumaric acid와 p-hydroxybenzoic acid가 各 植物의 發芽와 生長에 미치는 影響을 實驗하였다. 그 中 實驗에 성공한 18種에서 얻은 結果로부터 5×10^{-4} M과 5×10^{-5} M 濃度에서는 대부분의 植物이 對照區와 有意한 差異를 나타내지 않았으나 5×10^{-3} M 濃度에서는 심한 抑制를 나타냈으며, 5×10^{-4} M과 5×10^{-3} M 사이에 臨界濃度가 있음을 밝혔다. 또 이러한 現象은 p-hydroxybenzoic acid보다 p-coumaric acid에서 더 뚜렷하였다. 發芽, 伸長 및 乾量生長에 있어 대체로 遷移의 初期段階에 나는 種인 *Cirsium*과 *Lepidium* 등이 後期段階에 나는 植物種보다 더욱 뚜렷하게 抑制됨으로써 前者의 種間競爭에 중요한 役割을 하는 것으로 해석된다. 또 抵抗指數(resistance index, 5×10^{3} M의 phenolics에 대한 種의 感應)는 種에 따라 달랐지만 發芽率, 伸長, 乾物生長에서 얻은 指數値 사이에는 直線的 相關이 있었다.

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