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Resistance of Zn-accumulating plants against the disease caused by *Pythium ultimum* (Trow)

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Abstract

Some species of plant growing on calamine soils hyperaccumulate heavy metals from those soils in their tissues. This study tests the hypothesis that such metal accumulation confers a benefit to the plant by providing defense against fungal pathogens, using the Zn-hyperaccumulator *Thlaspi caerulescens* (J. & C. Presl.) and the pathogen *Pythium ultimum* (Trow).

Infection of plants by *P. ultimum* was assessed by observing symptoms of damping-off in seedlings and by microscopic observation of fungal hyphae and spores in seedling roots. Using *P. ultimum* as a test pathogen, comparison was made between the susceptibility of Zn accumulating seedlings of *T. caerulescens* with those of non-accumulating species of *T. arvense* and between seedlings of *T. caerulescens* grown from three seed collections of different zinc status. The seeds of the Zn-hyperaccumulating species germinated well up to the level of 30μg Zn ml^{-1}. The germination/damping-off rate increases/decreases with the increasing of Zn concentrations in the seeds of Zn-hyperaccumulating plants. Whereas, in non-accumulator the germination rate was decreased with increasing of Zn concentrations and not a single seed was germinated in presence of *P. ultimum*. In the three populations of *T. caerulescens* damping-off was manifested according to the concentration present in the seeds. In all of these experiments infection by *P. ultimum* was greatly reduced in the roots containing high concentration of Zn. The results confirm the hypothesis that heavy metal hyperaccumulation in these plants confers protection against attack by fungal root-pathogens.

Introduction

Some heavy metals such as zinc, copper and manganese are essential trace elements in that they are necessary for enzymatic and physiological processes occurring within plants (Hewitt, 1958; Vallee and Wacker, 1970). The amounts required are very low, and normally the trace quantities of heavy metals present in soils are sufficient for plant life. At many places in the world, elevated concentrations of heavy metals can prevent normal plant growth. Metal-enriched soils can result from the weathering of metalliferous ores in mineral outcrops or as a result of past and present human activity. Concentrations of 1-3% Zn, 0.1% Cd and 0.3% Cu are common in soils close to mineral wastes and metalliferous workings. A small number of plants endemic to metalliferous soils are capable of accumulating unusually high concentrations of potentially phytotoxic metals such as zinc, nickel, cadmium, lead, copper and cobalt in both their above and below-ground biomass. These plants have been termed as hyperaccumulators by Brooks *et al*. (1977). *Thlaspi* species from calamine (zinc/cadmium/lead-rich) soils can accumulate and tolerate zinc to more than 3%, cadmium to 0.1% and lead to 0.8% (Baker and Brooks, 1989). Hyperaccumulators of zinc, nickel, copper and cobalt have also been discovered in the metallophyte floras of the tropics and sub-tropics. Boyd and Martens (1992) have advanced five hypotheses to explain the ecological function of metal hyperaccumulation by plants. One of the hypotheses is defense against herbivores or pathogens.

The Zn-accumulator *Thlaspi caerulescens* was used in the experiments to determine the rate of infection by *P. ultimum. T. caerulescens* is strongly associated with soils contaminated with lead and zinc in Britain and NW Europe. In order to fully evaluate the properties of the Zn-accumulating species, a non-accumulating species of *Thlaspi arvense* was selected as controls. The seeds of *T. arvense* L. were collected from the non-metalliferous sites.

Materials and Methods

Seeds of Zn-accumulating species of *Thlaspi caerulescens* and of the non-accumulating species *T. arvense* were surface sterilized in 2% calcium hypochlorite solution for 20 minutes before being used in the experiments. Zinc treatment solutions were prepared from stock $ZnSO_4.7H_2O$ solution, filter-sterilized and added to give a final concentration of 0, 5, 10, 20 and 30μg Zn ml^{-1} in Rorison's nutrient solution. Five ml of each medium was added to a 9 cm sterilized disposable standard petri dish having two filter papers (Whatman No. 1) in it. Six petri dishes were used for each treatment. Three were inoculated with 4 evenly spaced disks of a 3-day old culture of *Pythium ultimum* maintained on CMA and incubated at 25 ºC. After two days there was good growth of *P. ultimum* in the petri dishes. The other 3 petri dishes for each treatment were used as an uninoculated control. Two days after inoculation, 50 sterilized seeds each of *T. caerulescens* and *T. arvense* were spread in each petri dish so that they were at an almost equal distance from each other. The dishes were incubated in the growth room at 22 ± 2 ºC under 16/8 hours light/dark for 20 days. Three ml nutrient solution amended with the before mentioned different concentrations of zinc was added every 5 days. Rate of infection (damping-off) was determined every two days after germination of seeds by counting the number of seedlings that had died. The percentage germination in inoculated samples as well as in control was determined after 3 days from the time of sowing. The infected roots and stem were examined under the microscope. Seeds of three populations of *T. caerulescens* (Clough Wood, Prayon & Whitesike mine) were treated as above without mixing the Zn solution. The metal contents of the different parts of seedlings and of ungerminated seeds of three populations were determined by atomic absorption spectrophotometry after 10 days of sowing of seeds. The efflux of Zn during germination/growth of seeds/seedlings from the three populations was calculated as following.

Efflux(μg) = [Amount in ungerminated seed (μg)]-[Amount in germinated seed + Root + Shoot (μg)]

Results

Zinc concentrations between 0-30 μg ml^{-1} did not affect the germination of the Zn-accumulator *Thlaspi caerulescens* whereas the germination rate of the non-accumulating species *T. arvense* decreased with increasing concentrations of Zn (Table 1). A comparison of infection by *P. ultimum* of the two species of *Thlaspi* showed that 100% damping-off occurred in seedlings of the non-accumulating species of *Thlaspi* in all zinc concentrations, whereas in the zinc-accumulating species symptoms of damping-off were maximal (100%) in the absence of zinc, but fell steadily with increasing concentrations of zinc in the external medium.

Ungerminated seeds collected from plants growing at Clough Wood, Prayon and Whitesike mine varied in the amounts of zinc contained (Table 2). Seeds from Clough Wood had a very high zinc concentration of 875μg g^{-1}. The zinc concentration of Prayon seed was intermediate (825μg g^{-1}). Seed collected from Whitesike mine had the lowest concentrations of zinc (615μg g^{-1}). The distribution of zinc in seedlings broadly reflected the concentrations of the metals in ungerminated seeds. Zinc concentrations in roots declined in the sequence Clough Wood > Prayon > Whitesike mine. Zinc concentrations in shoots were approximately thrice those found in roots + germinated seeds and declined in the same sequence. Table 2 shows the 215μgg^{-1} of zinc content effluxed from seedling of the Clough Wood population, 200μgg^{-1} from Prayon and 165μgg-1 from Whitesike mine. These losses represent 25%, 24% and 26% respectively of the zinc content of the orignal seeds. The results indicate that infection rate by *P. ultimum* is based on the concentrations of Zn in the seeds.

Table 1: Seed germination and % damping-off of Zn-accumulating and non-accumulating species of *Thlaspi* by *Pythium ultimum* (mean values, n = 3).

Zn concentration (μg ml^{1})	***T. caerulescens***			***T. arvense***		
	Seed germination (%)		**Damping-off (%)**	**Seed germination (%)**		**Damping-off (%)**
	Control	**Inoculated**		**Control**	**Inoculated**	
0	72 a	55 b	100 a	80 a	0	100
5	68 a	58 ab	80 b	78 a	0	100
10	67 a	56 b	61 c	51 b	0	100
20	70 a	62 a	50 c	35 c	0	100
30	68 a	65 a	28 d	15 d	0	100

Values in the same column followed by the same letter are not significantly different (P=0.05).

Table 2: Content (μgg^{-1}) of Zn in the seeds and seedlings of three populations of *Thlaspi caerulescens* (Mean values, n = 3).

Population	Root	Germinated seed	Shoot	Ungerminated seed	Amount efflux
Clough wood	70 a	85 a	505 a	875 a	215 a
Prayon	55 b	80 a	490 b	825 b	200 b
Whitesike mine	35 c	45 b	370 c	615 c	165 c

Values in the same column followed by the same letter are not significantly different (P= 0.05).

Table 3: Damping-off of seedlings of three populations of *Thlaspi caerulescens* by *Pythium ultimum.* (Mean values, n = 3)

Population	Germination (%)	Damping-off (%) after:			
		7 days	8 days	9 days	10 days
Clough wood	53	50	85	94	100
Prayon	48	62	100	100	100
Whitesike mine	40	100	100	100	100

Seedlings of *T. caerulescens* from seed collected at Whitesike mine had all succumbed to infection by *P. ultimum* seven days after sowing. At that time only 62% of seedlings from the Prayon population had damped-off and only 50% of seedlings of the Clough Wood population. Damping-off of Prayon seedlings had risen to 100% the following day (day 8), but complete damping-off of Clough Wood seedlings was not achieved until day 10 due to presence of high concentration of Zn in this population as compared to the other populations (Table 3).

Discussion

The experiments described in the present studies were designed to examine the influence of heavy metals on infection by the seedling pathogen *P. ultimum*. Analysis of seedlings of *T. caerulescens* showed that this was capable of accumulating Zn metal. The pattern of metal accumulation by this species reflected the metal content of the soil at the site from which this is originally collected.

Heavy metals may exert an effect on a potential root pathogen either in the aqueous medium surrounding the roots or within the root tissues themselves. An attempt was made to distinguish between these two possible modes of action by comparing the infection by *P. ultimum* of seedlings of two species of *Thlaspi*, *T. caerulescens* which accumulates zinc and *T. arvense* which is a non-accumulator. When grown in the absence of an external supply of zinc, 70-80% of seeds of both species germinated. Of these, 100% showed damping-off symptoms, indicating successful infection of the roots by *P. ultimum*. However, with increasing concentrations of zinc in the bathing medium, differences between the species both in germination and damping-off became more marked. Whereas germination of *T. caerulescens* seeds was unaffected by concentrations of zinc up to 30μgml^{-1}, that of *T. arvense* was progressively reduced to 15% at that concentration. Whilst all seedlings of *T. arvense* damped-off at all concentrations of zinc, the incidence of damping-off in seedlings of *T. caerulescens* fell progressively with zinc concentration to 28% at 30 μgml^{-1}. The different incidence of damping-off between the two species suggests strongly that the external zinc concentration is not the primary factor regulating successful infection. If the low incidence of damping-off of *T. caerulescens* seedlings at high zinc concentrations were due to the direct inhibition of the fungus prior to contact with the root, it would be expected that seedlings of *T. arvense* would show similarly reduced disease levels at high zinc concentrations (Boyd and Martens, 1992). That they do not suggest that the 'protective' action of zinc results from the accumulation of the metal in roots of *T. caerulescens* but not in those of *T. arvense*.

In order to investigate more directly the influence of zinc within root tissues on disease incidence, seeds from three different populations of *T. caerulescens* were selected. The soils on which these populations grew differed in their concentrations of zinc and it was found that these differences were reflected in the zinc concentrations detected in seeds and seedlings of the three populations. Zinc concentrations in root tissue were highest in Clough Wood seedlings, intermediate in Prayon seedlings and lowest in Whitesike mine seedlings. Damping-off incidence, on the other hand, was greatest in Whitesike mine and lowest in Clough Wood seedlings, suggesting that the metal concentrations in the roots conferred protection against infection by *P. ultimum*. Similar result has been interpreted by Morrison *et al*. (1979) for cobalt and copper-accumulating species which were subject to increased fungal attack when grown in non-mineralized soil. In general, seedlings become progressively able to resist infection by *P. ultimum* as they mature, largely due to the increasing proportion of their root tissues that become protected by thickened walls etc. (McClure and Robbins, 1942).

All these results support the view that zinc protects species able to accumulate Zn metal in their tissues, and that this protective role is directly related to the concentration of the metal within the tissues. However, it may be that metal ions leaking from the roots result in an elevated concentration at the root surface. Direct measurement of zinc efflux from *T. caerulescens* and calculation of zinc loss from *T. caerulescens* indicate that substantial quantities of this metal leave the root under the experimental conditions employed. This loss represents the 25% of the Zn-contents of the original seeds in the case of *T. caerulescens* (Table 2).

References

Baker AJM, Brooks RR, 1989. Terrestrial higher plants which accumulate metallic elements: a review of their distribution, ecology and photochemistry. *Biorecovery*, **1**: 81-126.

Boyd RS, Martens SN, 1992. The raison d! ^etre of metal hyperaccumulation plants. In: *The Ecology of Ultramafic (Serpentine) Soil* (Baker AJ, M Proctor J, Reeves RD, eds.), pp.279-289. Intercept, Andover.

Brooks RR, Lee J, Reevesr D, Jaffre T, 1977. Detection of nickelliferous rocks by analysis of herbarium specimens of indicator plants. *J. Geochemical Exploration*, **7**: 49-57.

Hewitt EJ, 1958. The role of mineral elements in the activity of plant enzyme systems. *Ruhland, Handbuch der Pflanzenphysiologie*, **4**: 427-481.

McClure TT, Robbins WR, 1942. Resistance of cucumber seedlings to damping-off as related to age, season of year and level of nitrogen nutrition. *Botanical Gazette*, **103**: 689-697.

Morrison RS, Brooks RR, Reeves RD, Malaisse F, 1979. Copper and cobalt uptake by mettalophytes from Zaire. *Plant and Soil*, **53**: 535-539.

Vallee BL, Wacker WEC, 1970. Metalloproteins in Nevrath, H. The proteins, vol.5, New York.