

## Tolerance of *Festuca rubra* L. to zinc in relation to mycorrhizal infection

L. Symeonidis

Department of Botany, University of Thessaloniki, GR-54006 Thessaloniki, Greece

Received May 14, 1990

**Summary.** Plant yield of mycorrhizal and non-mycorrhizal *Festuca rubra* L. was linearly decreased with increasing zinc concentrations in nutrient solution. In all cases, non-mycorrhizal plant growth was significantly greater than that of mycorrhizal plants. Zinc and phosphorus concentrations of root and shoot of mycorrhizal plants were greater in all zinc treatments while mycorrhizal plants showed equal or lower tolerance indices to zinc than non-mycorrhizal plants. Yield depressions of mycorrhizal plants may be the result of enhanced zinc and phosphorus concentrations combined with the cost for growth and maintenance of the mycorrhizal fungi.

**Key words:** *Festuca rubra* L. – Zinc tolerance – Vesicular-arbuscular mycorrhiza

### Introduction

It is well known that some of the heavy metals are essential for plant growth, but when present in excess cause phytotoxicity (Antonovics et al. 1971).

Mycorrhizal infection has been shown to increase the uptake of phosphorus (Pairunan et al. 1980; Stribley et al. 1980; Hayman 1983; Fitter 1985; Pacovksy and Fuller 1986; Baas et al. 1989; see also reviews by Hayman 1978; Smith 1980) as well as uptake of micronutrients such as copper and zinc, which are present in rather low concentrations in normal soils (Gilmore 1971; Lambert et al. 1979; Harley and Smith 1983; Killham and Firestone 1983; Tinker and Gildon 1983; Dueck et al. 1986). While enhancement of nutrient absorption will be beneficial in cases of restricted nutrient supply, it could be physiologically damaging to the host plants when they are growing with elevated amounts of heavy metals.

Bradley et al. (1982), Gildon and Tinker (1983), Brown and Wilkins (1985), Dueck et al. (1986) and Jones and Hutchinson (1986) all found that mycorrhizal infection resulted in an increased heavy metal resistance of host plants. In contrast, Killham and Firestone

(1983) found that mycorrhizal plants contain greater concentrations of heavy metal, resulting in growth depression.

The objective of the present study was to investigate the relationship between mycorrhizal infection and tolerance of *Festuca rubra* L. to zinc.

### Materials and methods

Seeds of *Festuca rubra* L., kindly supplied by Dr. G. Hendry (University of Sheffield), were germinated in petri dishes under growth-room conditions. A week after germination, seedlings were transferred in trays with autoclaved sand, where half of the seedlings were inoculated with vesicular-arbuscular mycorrhizal (VAM) fungi. Infected roots of field plants were used as inoculum. Randomly selected plants were examined 4 weeks later and all were found to be about 20% mycorrhizal. At this stage there were no differences in size or mass between mycorrhizal (M) and non-mycorrhizal (NM) plants.

All plants were transferred to 7.5-cm plastic pots containing autoclaved sand moistened with 0.1-strength Rorison nutrient solution (Hewitt 1966) with and without zinc. Zinc was added as zinc sulphate at 0, 7.5, 15 and 30 ppm zinc. There were two plants per pot and three replicates for each level of metal concentration in the M and NM conditions. Plants were allowed to grow in a growth room (20–16°C, day length 16 h) and watered twice weekly with 0.1-strength Rorison nutrient solution containing the appropriate level of zinc (Zn). After six weeks in the growth room, all plants were harvested, divided into roots and shoots, carefully washed in distilled water, oven dried in 80°C, weighed, ashed at 500°C and analyzed by atomic absorption for Zn. Phosphorus (P) was determined colorimetrically with a molybdate/antimony reagent (Stainton et al. 1977).

Root samples about 2 cm in length were stained with lactophenol/cotton blue (Phillips and Hayman 1970) to determine the extent of mycorrhizal colonization. Dry masses of roots and shoots in M and NM plants were expressed as a tolerance index (TI):  $TI = 100 \times (\text{growth in Zn})/(\text{growth in control})$ .

### Results

In Fig. 1 and Table 1 are shown the growth response of *F. rubra* L. to zinc concentrations and VA mycorrhizal infection. As the zinc concentration in the nutrient so-

lution was increased, plant yield decreased linearly in both mycorrhizal (M) and non-mycorrhizal (NM) plants. In all cases the root and shoot growth of NM plants were significantly greater than M plants. Confidence limits 95% (95% C.L.).

Zinc concentrations in root and shoot increased with increasing Zn concentration in nutrient solution (Fig. 2, Table 1) but did not differ significantly in M and NM plants. However, one can see that there was a slight increase in zinc concentrations in both root and shoot of mycorrhizal plants in each of the Zn concentrations (0, 7.5, 15 and 30 ppm) in nutrient solution.

Phosphorus content of root and shoot of M plants after Zn treatment at 0 and 7.5 ppm was significantly (95% C.L.) greater than that of NM plants. At higher Zn concentrations (15 and 30 ppm) phosphorus concentrations of root and shoot, although slightly higher in M plants, did not differ markedly between M and NM plants (Fig. 3, Table 1). Phosphorus concentration in roots and shoots of M and NM plants was negatively correlated with Zn concentration in nutrient solution, as well as with Zn concentration in these tissues in all

Zn treatments (Figs. 2 and 3, Table 1). Mycorrhizal infection (MI) was low (Table 1).

Table 2 shows the tolerance indices at all Zn concentrations in nutrient solution. Mycorrhizal plants had smaller or equal tolerance indices to non-mycorrhizal plants.

## Discussion

The reduction of biomass, as well as the increased uptake and concentration of zinc in mycorrhizal and non-mycorrhizal plants, with increasing concentrations of zinc in nutrient solution are as expected (see reviews by Baker 1987 and by Baker and Walker 1989).

The decreasing phosphorus concentration in plant tissues with increasing zinc concentrations in nutrient solution may be due to an interaction between P and Zn. Ernst (1968) found that an increase in the Zn level of nutrient solution brought about a fall in the P content and a rise in the Zn content of the shoots. Zn and P appeared to be mutually antagonistic whenever either

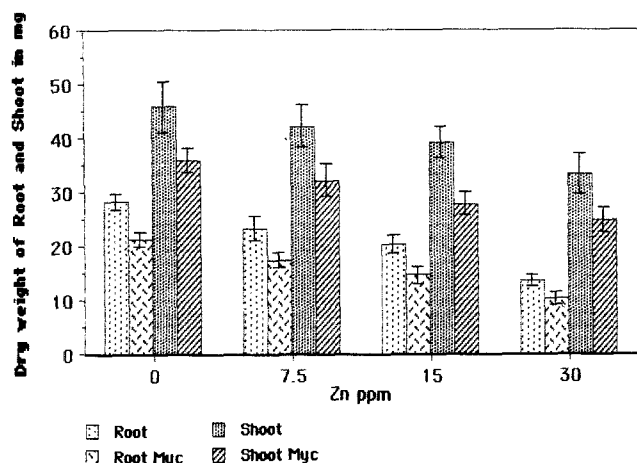


Fig. 1. Dry mass of root and shoot of mycorrhizal and non-mycorrhizal plants of *F. rubra* L. at different zinc concentrations in nutrient solution

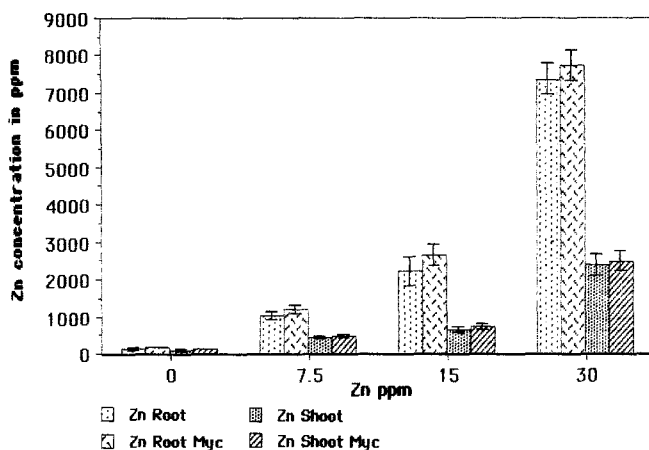


Fig. 2. Zinc concentration of root and shoot of mycorrhizal and non-mycorrhizal plants of *F. rubra* L. at different zinc concentrations in nutrient solution

Table 1. Dry mass, Zn and P concentration and mycorrhizal infection of *F. rubra* L. grown with and without mycorrhizal association at different Zn concentrations

| Zn (ppm) | Myc. | MI (%) | Dry mass (mg) |             | Zn conc (ppm) |            | P conc (ppm) |             |
|----------|------|--------|---------------|-------------|---------------|------------|--------------|-------------|
|          |      |        | Root          | Shoot       | Root          | Shoot      | Root         | Shoot       |
| 0        | —    | 0      | *28.1 ± 1.6   | *45.8 ± 4.8 | 138 ± 26      | 101 ± 21   | *1629 ± 179  | *4489 ± 267 |
|          | +    | 24.8   | *21.3 ± 1.4   | *35.9 ± 2.4 | 157 ± 31      | 107 ± 22   | *2471 ± 121  | *5172 ± 304 |
| 7.5      | —    | 0      | *23.1 ± 2.5   | *42.2 ± 4.1 | 1015 ± 131    | 437 ± 36   | *1373 ± 132  | *3334 ± 210 |
|          | +    | 26.6   | *17.3 ± 1.5   | *32.1 ± 3.2 | 1176 ± 152    | 469 ± 48   | *2015 ± 312  | *3982 ± 325 |
| 15       | —    | 0      | *20.3 ± 1.8   | *39.2 ± 3.0 | 2233 ± 367    | 635 ± 85   | 1157 ± 37    | 2068 ± 171  |
|          | +    | 23.9   | *14.6 ± 1.7   | *27.5 ± 2.6 | 2624 ± 301    | 735 ± 68   | 1179 ± 31    | 2197 ± 221  |
| 30       | —    | 0      | *13.5 ± 1.3   | *33.3 ± 3.7 | 7324 ± 481    | 2381 ± 285 | 954 ± 72     | 1404 ± 116  |
|          | +    | 22.7   | *10.3 ± 1.2   | *24.7 ± 2.3 | 7715 ± 450    | 2492 ± 290 | 1001 ± 51    | 1536 ± 141  |

Myc. indicates grown with (+) or without (—) mycorrhizal association; MI = mycorrhizal infection. An asterisk indicates results are significantly different (confidence limits 95%)  $n = 6$

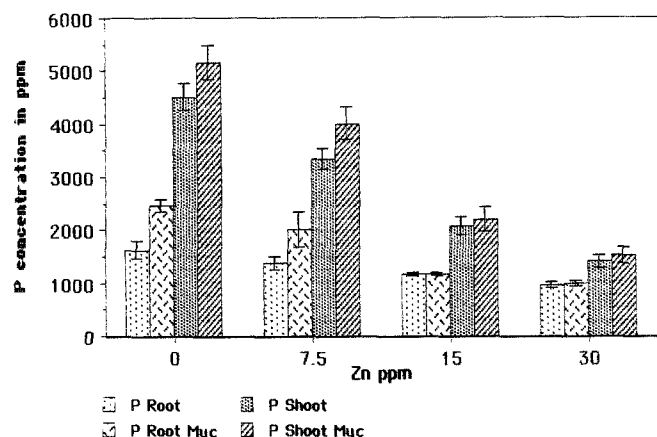


Fig. 3. Phosphorus concentration of root and shoot of mycorrhizal and non-mycorrhizal plants of *F. rubra* L. at different zinc concentrations in nutrient solution

Table 2. Tolerance indices from dry mass of roots and shoots of *F. rubra* L. grown with and without mycorrhizal association at different Zn concentrations

| Zn (ppm) | Myc. | Tolerance index (%) of plant |       |
|----------|------|------------------------------|-------|
|          |      | Root                         | Shoot |
| 7.5      | —    | 82                           | 92    |
|          | +    | 81                           | 89    |
| 15       | —    | 72                           | 85    |
|          | +    | 68                           | 77    |
| 30       | —    | 48                           | 73    |
|          | +    | 48                           | 69    |

The tolerance index was calculated as  $100 \times (\text{growth in Zn}) / (\text{growth in control})$

element exceeded some threshold value (Boawn and Leggett 1964; Olsen 1972).

Bradley et al. (1982), Brown and Wilkins (1985) and Jones and Hutchinson (1986) all found that mycorrhizal fungi can increase the tolerance of their host to some heavy metals, e.g. zinc, copper and nickel. They have explained this protective action as due to enhanced binding of heavy metals on the hyphal complexes in the mycorrhizal roots, thus facilitating exclusion of metals from the shoot and avoidance of metal toxicity.

Dueck et al. (1986) concluded that the alleviation of Zn toxicity by VAM-infection is brought about by some change in the chemical speciation of Zn during its passage through the hyphae, which may reduce its toxic effect in the host. The observed yield reduction and decreased tolerance indices to zinc of mycorrhizal plants, in contrast to the above authors, may be the result of enhanced Zn concentration in mycorrhizal plants (Gilmore 1971; Lambert et al. 1979; Killham and Firestone 1983). High levels of P may be the cause of yield reduction (Hall et al. 1977; Webb and Loneragan 1988; Baas et al. 1989).

Work by Pang and Paul (1980), Stribley et al. (1980) and Paul and Kucey (1981) indicate that as much as 10% of host photosynthate translocated below ground may be expanded in fungal growth and respiration. Similar yield reductions following VAM-infection have also been reported by Killham and Firestone (1983) as the result of enhanced metal uptake.

It is concluded that the observed growth depressions in M plants in comparison with NM plants may be associated with: (a) high phosphorus levels in M plants at least in the first two treatments (0 and 7.5 ppm zinc); (b) the enhanced zinc concentration by mycorrhizal plants, and (c) the energy cost for growth and maintenance of the mycorrhizal fungi.

**Acknowledgements.** I would like to express my thanks to Drs. D. J. Read and A. J. M. Baker for the assistance and facilities they provided me with during my stay in their laboratory (Department of Animal and Plant Sciences, University of Sheffield, UK) where the experimental part of this work was carried out.

## References

- Antonovics J, Bradshaw AD, Turner RG (1971) Heavy metal tolerance in plants. *Adv Ecol Res* 7:1–85
- Baas R, Van Dijk C, Troelstra SR (1989) Effects of rhizosphere soil, vesicular-arbuscular mycorrhizal fungi and phosphate on *Plantago major* L. ssp. *pleiosperma* Pilger. *Plant Soil* 113:59–67
- Baker AJM (1987) Metal tolerance. *New Phytol* 106 [Suppl]:93–111
- Baker AJM, Walker PL (1989) Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. *Chem Speciation Bioavailability* 1:7–17
- Boawn LC, Leggett GE (1964) Phosphorus and zinc concentrations in Russett Burbank potato tissue in relation to development of zinc-deficiency symptoms. *Soil Sci Soc Am Proc* 28:229–232
- Bradley R, Burt AJ, Read DJ (1982) The biology of mycorrhiza in the Ericaceae. XIII. The role of mycorrhizal infection in heavy metal resistance. *New Phytol* 91:197–209
- Brown MT, Wilkins DA (1985) Zinc tolerance of mycorrhizal *Betula*. *New Phytol* 99:101–106
- Dueck T, Visser P, Ernst WHO, Schat H (1986) Relationship between VA-mycorrhiza and zinc-toxicity in *Festuca rubra* L. and *Calamagrostis epigejos* (L.) Roth. In: physiological and genetic aspects of mycorrhizae Gianinazzi-Pearson V, Gianinazzi S (eds) INRA, Paris, pp. 661–663
- Ernst W (1968) Der Einfluss der Phosphatversorgung sowie die Wirkung von ionogenem und chelatisiertem Zink auf die Zink- und Phosphataufnahme einiger Schwermetallpflanzen. *Physiol Plant* 21:323–333
- Fitter AH (1985) Functioning of vesicular-arbuscular mycorrhizas under field conditions. *New Phytol* 99:257–265
- Gildon A, Tinker PB (1983) Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. I. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. *New Phytol* 95:247–261
- Gilmore AE (1971) The influence of endotrophic mycorrhizae on the growth of peach seedlings. *J Am Soc Hortic Sci* 96:35–38
- Hall IR, Scott RS, Johnstone PD (1977) Effect of vesicular-arbuscular mycorrhizae on response of 'Grasslands-Huia' and 'Tamar' white clovers to phosphorus. *N Z J Agric Res* 20:349–355
- Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London

- Hayman DS (1978) Endomycorrhizae. In: Dommergues YR, Krupa SV (eds) Interactions between nonpathogenic soil microorganisms and plants. Elsevier, Amsterdam, pp 401–442
- Hayman DS (1983) The physiology of vesicular-arbuscular endomycorrhizal symbiosis. *Can J Bot* 61:944–963
- Hewitt EJ (1966) Sand and water culture methods used in the study of plant nutrition. *Commonw Agric Bur Tech Commun* 22, 2nd ed
- Jones MD, Hutchinson TC (1986) The effect of mycorrhizal infection on the response of *Betula papyrifera* to nickel and copper. *New Phytol* 102:429–442
- Killham K, Firestone MK (1983) Vesicular-arbuscular mycorrhizal mediation of grass response to acidic and heavy metal depositions. *Plant Soil* 72:39–48
- Lambert DH, Baker DE, Cole H, Jr (1979) The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. *Soil Sci Soc Am J* 43:976–980
- Olsen SR (1972) Micronutrient interactions. In: Mortvedt JJ (ed) Micronutrients in agriculture. Soil Science Society of America, Madison, WI, pp 243–264
- Pacovsky RS, Fuller G (1986) Development of two endomycorrhizal symbioses on soybean and comparison with phosphorus fertilization. *Plant Soil* 95:361–377
- Pairunan AK, Robson AD, Abbott LK (1980) The effectiveness of vesicular-arbuscular mycorrhizas in increasing growth and phosphorus uptake of subterranean clover from phosphorus sources of different solubilities. *New Phytol* 84:327–338
- Pang PC, Paul EA (1980) Effects of vesicular-arbuscular mycorrhiza on  $^{14}\text{C}$  and  $^{15}\text{N}$  distribution in nodulated faba beans. *Can J Soil Sci* 60:241–250
- Paul EA, Kucey RMN (1981) Carbon flow in plant microbial associations. *Science* 213:473–474
- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br Mycol Soc* 55:158–160
- Smith SE (1980) Mycorrhizas of autotrophic higher plants. *Biol Rev* 55:475–510
- Stainton MP, Capel MJ, Armstrong FAJ (1977) The chemical analysis of freshwater. Fisheries and Env Canada Misc Sp Publ no 25, 2nd ed
- Stribley DP, Tinker PB, Rayner JH (1980) Relation of internal phosphorus concentration and plant weight in plants infected by vesicular-arbuscular mycorrhizas. *New Phytol* 86:261–266
- Tinker PB, Gildon A (1983) Mycorrhizal fungi and ion uptake. In: Robb DA, Pierpoint WS (eds) Metals and micronutrients: uptake and utilisation by plants. Academic Press, London, pp 21–32
- Webb MJ, Loneragan JF (1988) Effect of Zn deficiency on growth, phosphorus concentration, and phosphorus toxicity of wheat plants. *Soil Sci Soc Am J* 52:1676–1680