Mineral Nitrogen, Manganese, and pH Influence Growth of Arrowleaf Clover in Solution Culture

H.H. Schomberg and R.W. Weaver

Introduction

In the southeastern United States where many of the soils are acidic growth of legumes may be retarded due to N deficiency since soil acidity is known to inhibit the establishment of an effective N_2-fixing symbiosis (Munns 1978). The yield of arrowleaf clover (*Trifolium vesiculosum* Savi) grown at pH 5.0 was reduced nearly 40 percent when compared to plants grown at pH 6 (Hoveland et al. 1969). Poor growth of clover on acidic soils may be due to increased availability of Al or Mn (Munns 1978; Foy 1984). Dinitrogen fixation activity of white clover was reduced by large concentrations of Mn in plant culture solutions (Vose and Jones 1963; Lowe and Holding 1970).

Coll et al. (1989) found that the yield of N_2-fixing arrowleaf clover plants was depressed more than plants dependent on mineral N in a soil having a pH of 5.6. Yield of arrowleaf clover dependent on mineral N declined only 22 percent due to reduced soil pH while the yield of plants dependent on N_2-fixation declined on the average more than 50 percent. These results were influenced by the presence of readily available Mn. Plants grown at pH 6.8, 5.6, and 5.0 contained tissue Mn concentrations of 194, 524, and 768 µg g^{-1} respectively. Plants supplied mineral N accumulated similar concentrations of Mn as plants not supplied mineral N.

The purpose of this experiment was to evaluate the influence of pH and Mn availability in solution culture on the growth of arrowleaf clover and to determine if plant response to these conditions changed when the plants were provided mineral N.

Materials and Methods

Arrowleaf clover was grown in plastic containers modified to allow for the continuous recirculation of nutrient solution from the lower 1 L pot to the upper 1 L pot. The upper pot was filled with perlite and the lower pot contained half strength Evans et al. (1982) nutrient solution. The nutrient solution circulated from the lower pot to the upper pot at the rate of 15 ml mi^{-1}. Perlite was used as a support medium because it has a low CEC and very little buffering capacity. Twenty-five arrowleaf clover seed were planted in each pot and thinned to 20 following emergence. The pots were inoculated and experimental treatments initiated at the time of planting. All pots were inoculated with 10 ml of a 3-day-old culture of *Rhizobium trifolii* strain 162 x 68 containing 10^9 bacteria ml^{-1}. The experimental treatments consisted of two N levels (0 and 14 mg N pot^{-1}), three pHs (4.5, 5.5, and 6.5), and three Mn concentrations (0, 250, and 500 µM). The N source was NH_4NO_3. Manganese was added to the nutrient solution as $MnSO_4$. The pH treatments were adjusted once daily by adding either NaOH or HC1. Nutrient solutions and N and Mn treatments were replenished on days 14 and 21. The plants were reinoculated on day 14. There were two replications of each treatment. The experiment was conducted in a glasshouse in College Station, Texas. The pots were planted and treatments initiated on 04-26-1988 and harvested 05-26-1988. The shoots were harvested and dried in a forced draft oven at 65°C for 48hours and dry weights were measured. Manganese concentration in the shoot was measured by atomic absorption spectrophotometry (Parkinson and Allen 1975).

Results and Discussion

Arrowleaf clover plants germinated in 3 to 5 days. Manganese toxicity symptoms were not observed until the first trifoliate leaves had developed 10 to 12 days after emergence of the plants. The first symptoms, yellowing of the leaf margins, were observed on plants supplied with mineral N and 500µM Mn. Plants growing without mineral N were slower to develop Mn toxicity symptoms than plants grown with mineral N. The high level of Mn severely reduced the growth of plants not supplied mineral N. Although there was little direct influence of pH on plant dry weight, plants grown at pH 6.5 developed Mn toxicity symptoms earlier than plants growing at pH 4.5 or pH 5.5.

Plant dry weight declined as the Mn concentration in the nutrient solution increased (Table 1). The 250 and 500 µM Mn treatments decreased dry matter yield 26 and 56 percent respectively, regardless of the N source. Addition of mineral N increased yields more than 140 percent at all Mn levels.

TABLE 1. SHOOT DRY WEIGHT FOR ARROWLEAF CLOVER GROWN IN SOLUTION CULTURE AT THREE pHS, TWO N TREATMENTS, AND THREE Mn CONCENTRATIONS.

		pH			
N	Mn	4.5	5.5	6.5	Mean
mg pot^{-1}	µM	mg $plant^{-1}$			
0	0	15.1	19.3	22.5	19.3
	250	11.9	15.9	14.5	14.1
	500	8.2	10.4	6.8	8.5
14	0	43.6	50.3	48.7	47.5
	250	38.0	33.3	33.0	34.7
	500	23.0	20.0	18.4	20.5

*LSD for comparison between treatments = 6.2 (α=0.05)
*LSD for comparison of the N by Mn means = 3.6 (α=0.05)

Tissue analysis of the plant material indicated that the concentration of Mn was similar for plants grown with or without mineral N. The levels of Mn in the plant tissue averaged across N treatments were 57, 1395, and 2931 $\mu g\ g^{-1}$ for the 0, 250, and 500 μM Mn treatments, respectively. Since the plants that received mineral N were larger than plants not supplied mineral N, the total amount of Mn absorbed from the nutrient solution was greater. At high tissue concentrations, Mn is believed to replace Mg in the chlorophyll molecule and thereby reduce the supply of photosynthates to the developing plant. This may have resulted in lower photosynthetic efficiency which would account for the decline in dry weight. On the other hand the high tissue Mn concentration could have indirectly affected plant growth through an inhibition of the N_2-fixation process.

The sensitivity of Arrowleaf clover to acid soil conditions is exacerbated by the presence of a toxic ion such as Mn. Arrowleaf clover growth was reduced at pH 4.5 in the absence of Mn. However, the addition of Mn to the nutrient solution further reduced the growth of arrowleaf clover. Plants grown with mineral N produced higher yields, however, the negative influence of Mn on growth of these plants was similar to that of plants dependent on N_2-fixation. Although the addition of mineral N to arrowleaf clover may not altogether alleviate the negative influence of Mn on plant growth, it can improve the plant's ability to survive a Mn induced stress.

Literature Cited

1. Coll, J.J., H.H. Schomberg, and R.W. Weaver. 1989. Effectiveness of rhizobial strains on arrowleaf clover grown in acidic soil containing manganese. Soil Biol. Biochem. (in press).
2. Foy, C.D. 1984. Physiological effects of hydrogen, aluminum, and manganese toxicities in acid soil. *In:* Soil Acidity and Liming (F. Adams, Ed). Agronomy Monograph 12. American Society of Agronomy, Madison, WI.
3. Evans, H.J., B. Koch, and R. Klucas. 1972. Preparation of nitrogenase from nodules and separation into components. Methods in Enzymology 24: 470-476.
4. Hoveland, C.S., E.L. Carden, A. Buchanan, E.M. Evans, W.B. Antony, E.L. Mayton and H.E. Burgess. 1969. Yuchi arrowleaf clover. Alabama Agricultural Experiment Station Bulletin 396, Auburn, AL.
5. Lowe, J.F. and A.J. Holding. 1970. Influence of clover source and of nutrient manganese concentration on the *Rhizobim* white clover association. *In*: White Clover Research, Occasional Symposium No. 6 British Grassland Society (J. Lowe, Ed). pp. 61-70. Imprint, Luton.
6. Munns, D.N. 1978. Soil acidity and nodulation. *In*: Mineral Nutrition of Legumes in Tropical and Subtropical Soils (C.S. Andrew and E.J. Kamprath, Eds). pp. 247-263. C.S.I.R.O., Melbourne.
7. Parkinson, J.A. and S.E. Allen. 1975. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Communications in Soil Science and Plant Analysis 6, 1-11.
8. Vose, P.B. and D.G. Jones. 1963. Interaction of manganese and calcium on nodulation and growth of *Trifolium repens*. Plant and Soil 18, 372-385.
9. Weaver, R.W. and L.R. Frederick. 1982. *Rhizobium*. *In*: Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties (A.L. Page, R.H. Miller, and D.R. Keeney, Eds) pp. 1043-1070. American Society of Agronomy, Madison, WI.