A GROWTH CHAMBER STUDY ON THE EFFECTS OF METRIBUZIN ON THE RHIZOBIUM-LENTIL SYMBIOSIS

S.L.Sprout[1], L.M. Nelson[2], and J.J. Germida[1]

1. Saskatchewan Institute of Pedology, and
2. Plant Biotechnology Institute

Introduction:

In Saskatchewan, lentils (Lens culinaris L.) have been grown since 1970 (Evans and Slinkard, 1975) and production has increased substantially since then (from virtually zero in 1970 to 150,000 ha in 1986 [Saskatchewan Agriculture, 1986]). One advantage of growing lentils is their capacity for symbiotic nitrogen fixation. Lentils, when properly inoculated with Rhizobium leguminosarum, can meet up to 25% of their nitrogen requirement through nitrogen fixation (Slinkard and Dribnenki, 1984). Thus, legume crops decrease the cost to farmers that nitrogen fertilization would entail.

Crop yield can be significantly decreased by weeds through competition for nutrients. Herbicides are applied to a wide variety of crops to control weed growth. However, the application of herbicides may be detrimental with respect to nitrogen fixation. Little information is available on the effects of herbicide application on lentils. However, studies on other legume crops provide contradictory conclusions. Due to the various results obtained, it is important to consider the possible deleterious effects herbicides, such as metribuzin, have on the plant, the legume-Rhizobium symbiosis, or directly on the Rhizobium species.

This study evaluated the effects of the herbicide, metribuzin, on the lentil Lens culinaris cv. Eston)-Rhizobium symbiosis under growth chamber conditions.

Materials and Methods:

Eight Rhizobium leguminosarum strains were investigated: 92A3, 175P1, TA101, 128C54, 128C84, B13, 175K2, 175M1. The two former strains are used commercially as inoculum for lentil, whereas the latter six are the best of 150 strains assayed (based on shoot dry weight and nitrogenase activity) in a laboratory study of lentil.

One hundred and five Leonard jars (Vincent, 1970) were prepared using a combination of Turface [montmorillonite clay] (29.4% w/w), silica sand (68.6% w/w), and $CaCO_3$ (2.0% w/w). Each jar received N-free plant nutrient solution in the lower compartment, and the entire jar was sterilized for 45 minutes.

The lentil seeds were surface sterilized and then four seeds were planted in each Leonard jar at a depth of 2 cm. Each seed was inoculated with 1.0 ml of Rhizobium culture. The inoculum was grown in 50 ml of liquid Burton's medium on a rotary shaker at 28°C for 72 hours. The number of colony forming units (CFU)/ml were determined to ensure comparable inoculum levels. Using the spread plate method, the colony forming units(CFU)/ml were determined to be between 4 - 9 x 10^8/ml for each strain.

The Leonard jars were placed in a growth room (day/night temperatures of 20/15°C with 16 hour day:8 hour night regime) for the duration of the experiment. After five days, the seeds were thinned to two seedlings per jar. The treatments were:

A) each strain only (controls)
B) each strain and herbicide applied to leaves at the recommended field rate
C) each strain and herbicide applied to leaves at 2x the recommended field rate

Each treatment was conducted with five replicates (two plants per replicate). The herbicide was sprayed on the leaves of each plant (2-3 leaf stage) at 1x and 2x the recommended field rate. A plexiglass chamber (14 x 14 x 40 cm) was used for spraying. A spray nozzle was located on the top of the chamber and was connected by tubing to a beaker containing the metribuzin solution. An exhaust fan was located on the side which was vented into a fume hood to remove mist between spraying. Two heights were used for spraying, 17 cm and 24 cm below the spray nozzle. The closer height was used for this experiment but problems with uneven application resulted in utilization of the lower level (24 cm) in subsequent experiments. The plants were harvested four weeks after seeding.

After harvesting, the parameters measured were root fresh weight, shoot dry weight, number of nodules, and nitrogenase activity (using the acetylene reduction assay [ARA]). For the acetylene reduction assay, samples were taken at six different time intervals (10, 15, 20, 30, 45, and 60 minutes). When the samples were converted to a per hour basis, there were decreasing amounts of ethylene detected. Therefore, only the 10 and 15 minute samples were used for analyses. Two-way analyses of variance (randomized design) were run on the data obtained.

Results and Discussion:

From the data obtained, three trends were apparent. The majority of Rhizobium strains tested (128C84, 92A3, 175P1, B13, 175K2, 175M1) showed decreases in the parameters measured with increasing metribuzin application rates. However, many parameters remained constant with strain TA101 inoculated plants, and parameters tended to increase with strain 128C54, when metribuzin was applied. To represent these observed trends, the data presented will be for strains 128C54, TA101, and 128C84.

Both Rhizobium strain and metribuzin application rate significantly affected shoot dry weight (Figure 1) and root

fresh weight (Figure 2). In general, in plants inoculated with most strains, both root fresh weight and shoot dry weight decreased with the application of metribuzin. However, with strain 128C54 root and shoot weight increased slightly when metribuzin was applied. There was a significant interaction both for root fresh weight and shoot dry weight.

There was a significant difference in number of nodules due to strain, but not due to treatment (significant at 8.3% probability level) (Figure 3). In plants inoculated with the majority of strains, nodule numbers decreased with application of metribuzin. With strain 128C54 nodule numbers increased, whereas with strain TA101 there were consistent numbers of nodules with increasing metribuzin treatment. However, an average standard error of between 10-20 nodules means there may be essentially no differences between controls and treatments in some strains.

On a per plant basis, the metribuzin treated plants had lower acetylene reduction activity. When the numbers were converted to a per gram root fresh weight basis, there was a statistically significant difference due to the Rhizobium strain, but not due to metribuzin treatment. There was no significant interaction.

Some of the variability in these results obtained could be due to the plants being too close to the sprayer when the herbicide was applied. It was subsequently found that increasing the distance between the plant and sprayer nozzle allowed the herbicide mist to disperse resulting in spray over a larger area. This would enable more even distribution and coverage of the plants by the herbicide.

A subsequent growth chamber study, using the increased spray distance, gave similar results for strain 128C84. However, plants inoculated with strain 128C54 had no changes in parameters when metribuzin was applied. With strain TA101, the trend was the same but the amounts were substantially higher in all parameters tested. This may be due to the different spraying distance used, but further study is necessary to determine which observations are correct.

Some of the data obtained are similar to results of other workers. Bertholet and Clark (1985) found significant decreases in plant dry weight, and only obtained reductions in nodulation and nitrogen fixation if plants were injured when metribuzin was applied to faba bean. Germida et al. (1986) found that metribuzin could affect nitrogen fixation under certain conditions, but these effects were likely due to effects on plant growth rather than nitrogen fixation per se. There were no reports where the application of metribuzin caused an increase or no change in parameters measured. However, commercial inoculum was normally used, and the commercial strains used in this study also caused a decrease in plant weight and nodulation of lentil plants.

Further research is necessary to determine if the results obtained in this growth chamber study are due to plant and/or microbial effects. To determine which effects are involved, experiments planned include - comparison of inoculated plants with non-inoculated plants grown in nitrate nutrient solution to

see if similar results are obtained when plants are sprayed; application of ^{14}C-metribuzin to the leaves to determine if any of this herbicide is translocated to the nodules; and growth of Rhizobium strains on media containing various concentrations of metribuzin to determine if the herbicide inhibits these microorganisms.

Literature cited:

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Slinkard, A.E., and Dribnenki, P.C. 1984. Inoculation of pulse crops. Univ. Sask. Pub. 381. Agdex 149/23. Saskatoon.

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Figure 1. Effect of metribuzin on shoot dry wt

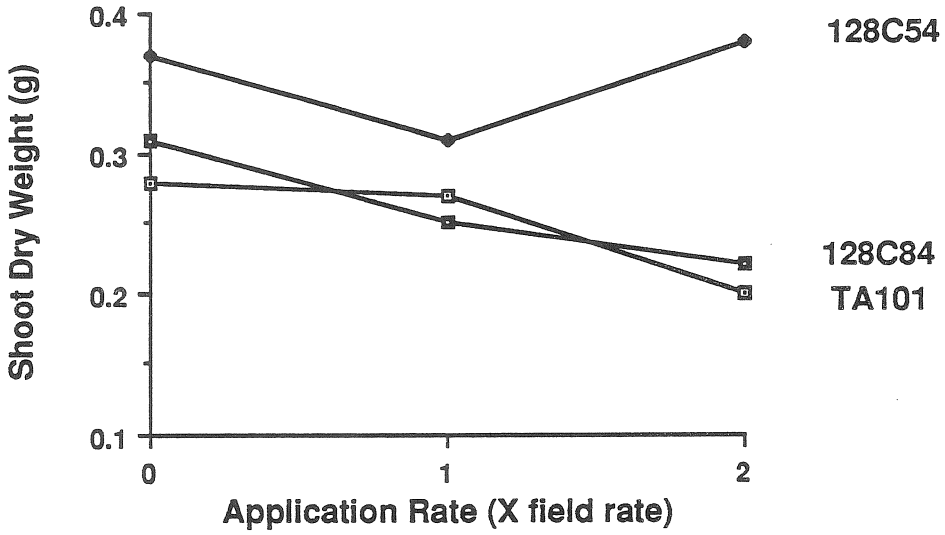

Figure 2. Effect of metribuzin on root fresh weight

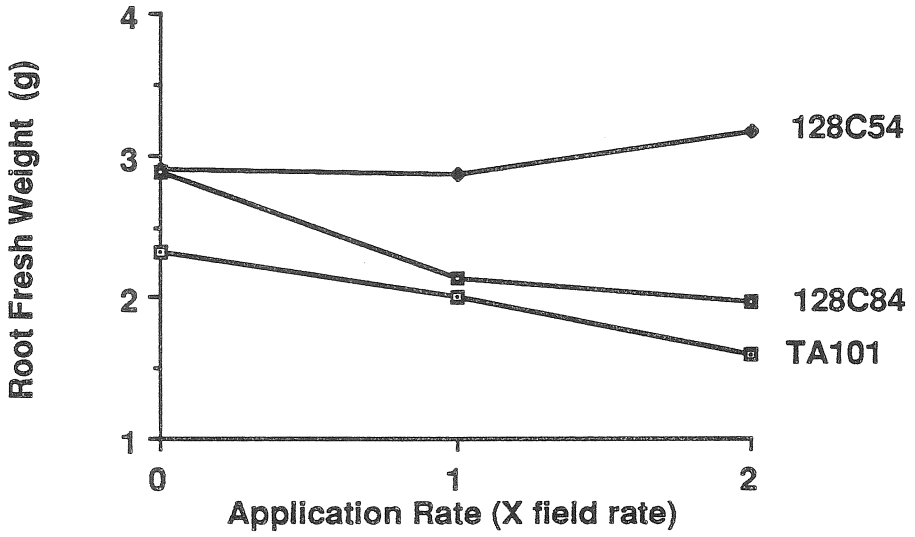

Figure 3. Effect of metribuzin on nodule number