Breeding Italian Annual Ryegrass for Tolerance to *Pythium ultimum*

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BREEDING ITALIAN ANNUAL RYEGRASS FOR TOLERANCE TO *PYTHIUM ULTIMUM*

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Abstract

This study was conducted in an attempt to improve tolerance of annual ryegrass to *Pythium ultimum* Trow. Improved tolerance of ryegrass as measured in laboratory experiments was confirmed in greenhouse studies. This tolerance was defined as improved emergence and increased root length of seedlings in the presence of *P. ultimum*, using rolled germination paper in the laboratory, and sand in the greenhouse. Further experimentation will be required to determine usefulness of this selected trait under field conditions.

Keywords: Italian annual ryegrass, breeding, *Lolium multiflorum*, *Pythium ultimum*

Introduction

Annual ryegrass (*Lolium multiflorum* Lam.) is planted in the fall (October to November) in the Southern USA. Environmental conditions may include periods of high rainfall, especially along the Gulf Coast. When periods of excess rainfall occur, especially during the seedling stage or during the growing season, root diseases can reduce forage production. In preliminary laboratory studies, we determined that *Pythium ultimum* was destructive and could kill some

germinating seed. The main effects of *P. ultimum* on ryegrass are root tip destruction and root stunting of geminating seedlings.

Material and Methods

Research in this study reports on a laboratory and two greenhouse experiments. In the laboratory, ryegrass seeds were placed on top of three stacked strips of moistened germination paper (9 x 38 cm) 1 cm from the edge. Three additional strips of moistened paper were placed on top of the seeds and the whole stack was rolled up and put into a quart (946-ml) glass jar. Plastic food wrap was used to cover the top of the jar and secured with the "ring" lid. Thirty to forty seeds were placed in each stack of six paper strips. *P. ultimum* cultures were grown on potato dextrose agar amended with antibiotics for 2 to 4 days before excising seven 4 x 4 mm pieces and placing 1 cm below seed line equally spaced apart. Control treatments did not receive any agar pieces. All jars were placed in an incubator for one week at 25/15°C with 10-hour days. After one week, paper strips were unrolled and root lengths measured and recorded.

For greenhouse studies, *P. ultimum* inoculum was prepared by filling 115-ml jars with 100-g sand, 2.5-g cornmeal, and 25-ml d-H_20. Jars were loosely capped and autoclaved for 1 hr. After cooling to room temperature, 4 agar plugs from *P. ultimum* cultures were mixed into the sand-cornmeal mixture aseptically. Sand cultures were allowed to grow for 4 days before use. Before planting, inoculum (sand-cornmeal cultures) from a single jar was divided into four "doses" and one dose was mixed into the top 2 cm of each treated cup before sowing seed. Control cups did not receive any *P. ultimum* inoculum. Plants were fertilized as needed.

In greenhouse study A, seed of 'TAM 90', (a popular ryegrass cultivar in Texas, Nelson et al., 1992), Syn. 1, and Syn. 2 were planted into a sand: peat mixture (V= 9:1) in plastic cups (500 ml) with a small hole punched through the bottom. The study started 13 Dec. 1999 and was

terminated 7 Feb. 2000. In greenhouse study B, seed of TAM 90 and Syn. 2 population were planted into sand only to increase the ease of washing soil from the roots. Temperatures were ambient and ranged from 15 to 23°C. Study B started 17 Feb. and was terminated 21 Mar. 2000. At the termination of each study, soil was washed off the roots and plant measurements were taken on number of germinated plants, root length, number of tillers/plant, and dry weight/shoot. Random complete blocks were used with ten replications (cups) per block. We used 2-blocks for controls; 5-blocks for *P. ultimum* treatments. In all studies, data were analyzed with SAS and mean separations compared using Fisher's protected LSD at 0.05.

Selection process:

1. From a population of TAM 90, 40 of the most resistant plants out of 313 infected plants were selected to produce the Syn. 1 generation.
2. From the Syn.1 population, 47/188 infected plants were selected to produce the Syn. 2 population.
3. Approximately 500 Syn. 2 plants were transplanted into an isolation block in the field. About 33 % of the plants were allowed to cross-pollinate and produce seed of Syn. 3. The rest of the plants were rouged out due to lack of vigor or tillering, small plant size, or susceptibility to crown rust.

Results and Discussion

Laboratory Study: Exposure to *P. ultimum* during germination did not affect number of germinated seed (data not shown). Mean root length reduction due to *P. ultimum* was 50% for TAM 90 seedlings vs. healthy controls (Fig. 1). In the first cycle of selection for tolerance, root lengths of infected Syn.1 seedlings were reduced 28%. Root lengths of infected Syn. 3 seedlings

were only 6% shorter than healthy controls. This indicates that we were successful in selecting genotypes that were more tolerant to *P. ultimum* than TAM 90 under the conditions of the screening protocol.

Greenhouse Study: In study A, we could not confirm the results of the laboratory study. There was no significant improvement of either the inoculated Syn. 1 or Syn. 2 seedlings compared to TAM 90 in root length, number of tillers, or in weight of shoots (data not shown). There may be some bias for TAM 90 seed in this experiment due to the fact that TAM 90 seed was produced in Oregon and seed size was larger than seed of Syn. 1 or 2, which was produced in growth chambers. This situation may have provided TAM 90 seedlings with an advantage. Also in the greenhouse, soil temperatures remained warm and the sandy soil was well drained. There were no other pathogens present and *P. ultimum* likes cold, wet soil.

In greenhouse study B, emergence of plants exposed to *P. ultimum* was 40% and 60% for TAM 90 and Syn. 2, respectively. Emergence of control plants was 85% for TAM 90 and 90% for Syn. 2. The Syn. 2 germplasm showed improved tolerance to *P. ultimum* compared to the original population of TAM 90. Mean root lengths of TAM 90 and Syn. 2 are shown in Fig. 2. While both populations were affected by *P. ultimum*, the degree of root length reduction was greater for TAM 90 (72%) than Syn. 2 (43%), when compared to their respective controls. Root lengths of TAM 90 and Syn. 2 control plants were not significantly different from each other. Syn. 2 plants were slightly smaller; however, this may be a reflection of smaller seed size. Syn. 2 seed was produced in our growth chambers while TAM 90 seed was obtained commercially.

These studies support our conclusion that it is possible to select for tolerance to *P. ultimum* in annual ryegrass. After three cycles of selection, root disease was reduced and root growth minimally affected. Further research will include selecting for forage production in Syn. 3 germplasm and exploring the effects of *P. ultimum* on plant growth over the growing season.

References

Nelson, L. R., Rouquette Jr., F. M., Evers, G. W. (1992). Registration of 'TAM 90' annual ryegrass. Crop Sci. **32**: 828.

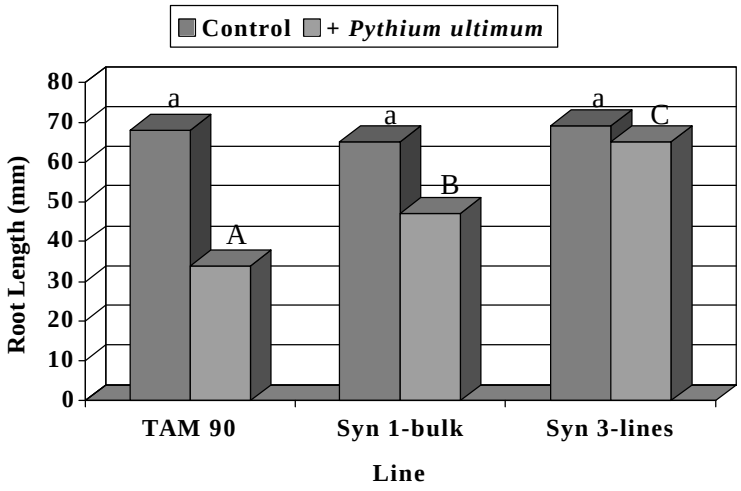

Figure 1- Mean root lengths of TAM 90 and two populations of annual ryegrassselected for tolerance to *Pythium ultimum* during germination in laboratory.

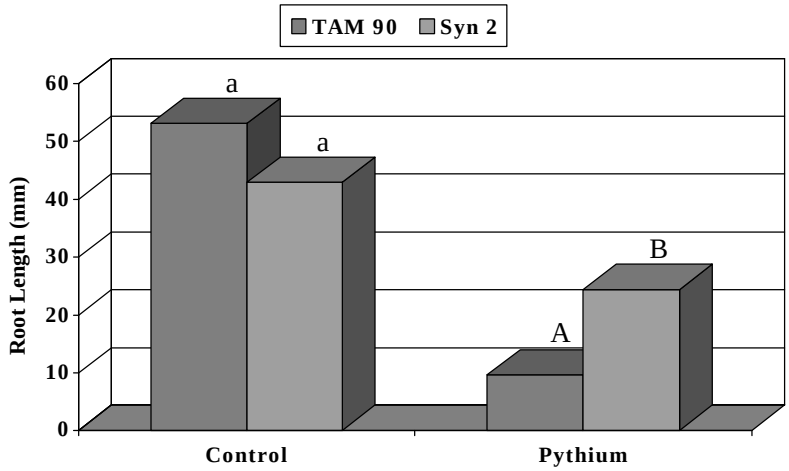

Figure 2 - Root lengths of TAM 90 and Syn 2 ryegrass after 5 weeks growth when exposed to *Pythium ultimum* during germination in greenhouse.