EVALUATING WHITE CLOVER FOR RESISTANCE TO CYLINDROCLADIUM ROOT ROT

D. S. Wofford and A. Amaya

Agronomy Department, 2183 McCarty Hall, University of Florida, Gainesville, FL 32611-0300. Florida Agricultural Experiment Station Journal Series Number

ABSTRACT

A rapid screening technique was developed for use in evaluating white clover (*Trifolium repens* L.) for resistance to Cylindrocladium root rot incited by *Cylindrocladium crotalariae*. Culture plates of the fungus were evaluated for number of infective propagules after four weeks of growth on potato dextrose agar, and no differences were detected among plates. Seedlings of 'Osceola' white clover were exposed in the greenhouse to four levels of inoculum over a six-week period, with resistance based on percent survival of uninoculated controls. It was shown that suspending 1 culture plate in 100ml of water and rating plants four weeks after inoculation was suitable for screening seedlings. A field test was conducted to verify greenhouse results. Results indicated that the greenhouse technique gave similar results, and would be sufficient to use in a breeding program to develop resistance to this disease.

KEYWORDS

White clover, disease resistance, root rot, screening technique

INTRODUCTION

White clover, *Trifolium repens* L. is botanically a perennial, however, stands in the southeastern US generally decline two or three years after establishment (Gibson and Hollowell, 1966). A major factor in stand decline is soil-borne diseases (Leath, 1985), including *Cylindrocladium* species. The soil-borne fungus *Cylindrocladium crotalariae* (Loos) Bell and Sobers has been reported as a serious pathogen on red and white clover and alfalfa in Florida (Roberts et al., 1983). The virulence of *C. crotalariae* in the field suggests that this fungus could be more serious than other soil-borne pathogens that attack these forage legumes. General symptoms in white clover include chlorosis of leaves and necrotic root and stolon tissues.

The development of resistant cultivars can be an important strategy for control of *C. crotalariae* in white clover. Recurrent selection for intrapopulation or interpopulation improvement has proven to be effective in white clover (Gibson and Cope, 1985). However, only one report on response to *C. crotalariae* in white clover is available in the literature (Wofford and Quesenberry, 1993). Additionally, a rapid screening technique must be developed in order to efficiently breed for resistance to this pathogen. The objective of this work was to develop a rapid greenhouse technique to screen white clover for resistance to *C crotolariae.*

METHODS

Cylindrocladium crotalariae was isolated from infected roots of alfalfa grown at the Agronomy Farm of the University of Florida in Gainesville. Fungal cultures used throughout this work were grown on potato dextrose agar (PDA) at 25 C under dark conditions. The first experiment was conducted to quantify the number of infective propagules and evaluate the propagule variability among culture plates. Ten four-week-old plates were separately blended for 1 minute in 200 ml of water and then diluted to 10^3. Aliquots of 0.1 ml were transferred to plates containing PDA (subplates) and gently spread on the agar surface using a glass stirrer. Three subplates were prepared from each individual plate. Subplates were maintained at room temperature for five days. Daily colony counts under a dissecting microscope were done for each subplate starting the second day. Discernible individual colony counts were possible until day four, however mycelium intermixing of close colonies occurred afterwards.

The second experiment was conducted to determine the inoculum concentration and disease incubation period necessary for accurate assessment of resistance. Seeds of 'Osceola' white clover were subjected to four different inoculum suspensions obtained by blending four-week-old PDA agar culture plates of *C. crotalariae* in different water volumes, 1 plate/100 ml, 1 plate/200 ml, 1 plate/400 ml, and 1 plate/800 ml. The experimental design was a randomized complete block with four replications. Twenty-five seeds were planted in rows 5.5 cm apart in 50 cm x 38 cm x 9 cm metal flats containing Metro-mix 200® (Grace Horticultural Products, Cambridge, MA), a commercial growing medium. Inoculations were done at the time of seeding by pouring 25 ml of fungal suspension into each row furrow. Uninoculated control flats were seeded and 25 ml of water poured into each row furrow. Results were analyzed as percent of survival relative to the uninoculated control. Weekly seedling counts were made over a six-week period.

An experiment to compare greenhouse ratings with field results was conducted using six white clover plant introduction lines previously evaluted for resistance to *C. crotalariae*. Two of these lines were considered resistant (PI 197830 and PI 201192), two moderately resistant (PI 231788 and PI 291847), and two susceptible (PI 228294 and PI 189395). One-month-old seedlings were transplanted to a field site known to be contaminated with *C. crotalariae*. Seedlings were planted in single-row plots of 12 m length with 15 plants spaced 0.80 m apart in a randomized complete block design with nine replications. To monitor disease progession, weekly chlorosis ratings were made starting 10 weeks after establishment for the following 17 weeks using the following scale: 1 = normal, 2 = moderate chlorosis, and 3 = severe chlorosis. After 30 weeks, root samples were collected from seven random plants in each row for evaluation of root rot. Root samples, including stolons, were collected using a square soil borer of 7.5 cm width and 15 cm of depth. Samples were washed and evaluated for root rot damage on a scale of 0 to 5, where 0 = no visible damage, and 5 = root completely decayed.

RESULTS AND DISCUSSION

Analysis of variance results for the experiment to quantify the number of infective propagules showed that the average number of colonies produced per plate was 9.92×10^4. No significant differences were found among plates; therefore infective propagule number is relatively constant from plate to plate. Thus, consistent inoculum concentrations can be achieved by culturing the fungus in the manner previously described for four weeks prior to use.

In the second experiment, significant differences were detected among inoculum concentrations and weeks. The number of seedlings significantly decreased through the third week after inoculation with no subsequent weekly differences detected. As inoculum density decreased, there was a significant increase in percent resistant plants from 37 to 61%. The1/100 (37% resistant) and 1/200 (41% resistant) concentrations differed from the other treatments but not from each other. Although there was no difference between the third and fourth

week incubation periods, the use of a four-week period could be beneficial in reducing escapes but is of sufficiently short duration to be useful. Therefore, a protocol using a 1/100 inoculum concentration and a four- week incubation period was selected for further work.

In the field test, plant mortality was almost nil throughout the duration of evaluation. Despite the presence of *C. crotalariae*, chlorotic symptoms were minimal during the first 11 weeks of evaluation, but increased afterwards. Overall, chlorosis differences were detected among lines, with PI 197830 having the lowest overall score. The low initial rate could be due to either unfavorable conditions for disease progression or relatively vigorous growth of the plants. Roberts (1990) reported that heavy rains followed by dry periods and temperatures around 25 C were highly conducive to infection. Although moderate rainfall was experienced during this test, there were no prolonged dry conditions. Air and soil temperatures gradually increased over time with maximum values of 23.4 and 20.8 C, respectively. Therefore, these environmental conditions were probably more favorable for plant growth than for infection.

The analysis of variance for root rot revealed a highly significant difference among lines. Overall, PI means ranged from 1.98 to 2.84, with PI's 201192, 291847, and 197830 having significantly lower root rot means than all other lines. There was a significant replication x line interaction, suggesting that the response over replications was not the same for each line. This differential response of the lines over replications may suggest a nonrandom distribution of *C. crotalariae* inoculum in the field, or it could be partially due to sampling in a highly heterozygous crop such as white clover.

The primary objective of this field test was to evaluate the relationship between greenhouse screening and field performance. The results of this evaluation showed that PI's 197830 and 201192, rated as resistant under greenhouse screening, had low root rot scores under field conditions, and greenhouse susceptible lines 228294 and 189395 had the highest root rot scores. Lines 231788 and 291847, intermediate in terms of resistance in the greenhouse, had inconsistent responses in the field. In the field, PI 231788 was more susceptible than expected and PI 291847 was more resistant than expected. However, the results of this study demonstrated that the greenhouse screening procedure gave similar results as obtained in the field for identifying the most resistant plant material. Thus, this greenhouse screening technique should be useful in a breeding program to select for improved resistance to *C. crotalariae* in white clover.

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Table 1

Means of percent resistant plants to *Cylindrocladium crotalariae* in the greenhouse and root rot scores in the field for six white

PI Line	Percent resistant[1] (%)	Root rot[2]
197830	58 a[4]	2.16 b
201192	54 ab	1.98 b
231788	30 abc	2.81 a
291847	28 abc	2.02 b
228294	16 bc	2.84 a
189395	4 c	2.62 a

[1] Percent of uninoculated control.

[2] Scale 0 to 5 (0 = none, 5 = severe).

[4] Means followed by the same letter are not significantly different at the 0.05 probability level according to DMRT.