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Evolving Resistant Varieties to *Sclerotinia* Root and Crown Rot in Alfalfa

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Abstract: *Scleotinia* crown and stem rot is a major disease on alfalfa caused by *Sclerotinia trifoliorum* Eriks. This research was determined the response to one cycle of bidirectional selection for resistance to *Sclerotinia* crown and stem rot. Eight plants were identified as the most resistant and eight plants were identified as the most susceptible of the 100 tested plants. These plants were grown in the greenhouse, then evaluated by stem tip inoculation technique. Differences between genotypes and experiments were significant for the pathogen. Means of the three procedures on comparison, showed that the pathogen progressed more rapidly in solid plastic bags (8.85 cm) as compared to perforated plastic bags (3.18 cm) and pippet bags (3.59 cm). This selection separated resistant and susceptible populations and will lead to development of alfalfa resistant to *Sclerotinia* crown and stem rot.

Key words: Resistant varieties, alfalfa, *Sclerotinia* root rot

Introduction

Crown and stem rot caused by *Sclerotinia* species is a disease of alfalfa which affects its stand density and yield. It infects alfalfa plantation in cool season, along with other economically important forage legumes such as red clover, white clover, crimson clover and berseem clover (Pratt and Knight, 1984). *S. sclerotiorum* is one of the pathogens associated with the root rot complex of Chickpea and its occurrence is increasing in both incidence and severity on Chickpea grown in the Mediterranean region (Anonymous, 1996). The initial infection in alfalfa occurs in the fall and early winter. During the winter and early spring the fungal mycelia grow within and between plants. Patches like symptoms of dead plant parts enlarge and coalisce through spring and cause major losses in stands (Gilbert and Bennett, 1917). This disease is specially dangerous to first year stands of alfalfa sown in fall.

The evaluation of cultivars resistant to *Sclerotinia* crown and stem rot is more important as the use of fungicides is not economical nor feasible in alfalfa grown for forage (Leath *et al*., 1988). For artificial inoculation of the cultivars to be tested for resistance or susceptibility, stem tip inoculation method developed by Pratt and Rowe (1991) was adopted. Halimi *et al.* (1998) also applied stem tip inoculation procedure to evaluate alfalfa population against *Sclerotinia trifoliorum*. The research work was carried out with the objectives to assess the progression of the boundary of the necrotic lesion of alfalfa plants as influenced by resistance/susceptibility of the plant to different isolates of *Sclerotinia trifoliorum*.

Materials and Methods

Genetic materials: The research work was carried out in 1992 in Department of Agriculture, Forage Research Unit, Mississippi State, USA.

The eight plants were identified as most resistant and the eight plants were identified as most susceptible of the 100 tested were used as plant test of susceptible and resistant. For the Pathogen, (AF-2) was obtained from Dr. R.G. Pratt (Forage Research Unit, Mississippi State, MS).

Stem inoculation: Stem cuttings were selected and inoculated by applying the fungal isolates. For inoculation, pieces of absorbent cotton were hand rolled into loose balls, autoclaved and moistened with V-8 Juice (20% V/V). These cotton balls were placed in the 5 days old fungal colonies grown on corn meal agar in petri dishes. After three days, the fungal mycelia infested cotton balls were spread with tweezers and placed at the center of a 26 by 36 mm piece of masking tape. A day before inoculation, one vegetative stem from each cultivar included in the study was cut 25 cm from growing tip and placed in flask. Each plant was encased in a transparent and perforated plastic sheet (48 holes/sq inch). Disease growth in the form of progress of necrotic lesion was observed on daily basis and the increasing necrotic boundary was marked with a black marker on the plastic surface.

The disease progress was marked on daily basis upto ten days starting from July 10 to 20 and August 4 to 14, 1992. The flasks having stem cuttings were kept in a growth chamber (18 °C and mercury light). Data thus recorded was subjected to analysis of variance. The plants with shortest necrotic lesion were marked as resistant and longest necrotic area marked as susceptible.

The experiment as conducted by adapting three different methods for alfalfa stem tip inoculation. One method was by encasing the alfalfa plant stem in solid plastic bags in the month of July (Pratt and Rowe, 1991). Second method was to encase and wrape the stems in transparent and perforated plastic bags (48 holes/sq inch) in the month of August (Pratt and Rowe, 1991). Third method was to fasten the fungus mycelia wraped plant stems with pippets in the month of July (Pratt and Rowe, 1991).

Results and Discussion

After 3 days of incubation in a saturated atmosphere, symptoms of initial infection by *S. trifoliorum* species on stems were developed by wilting and gray brown discoloration of terminal leaves. Brown necrosis extending as much as 0.5 cm down the proximal stem and white mycelial growth on stem at the bottom of the tap enclosing inoculum.

Mean lengths of the necrosis induced by *S. trifoliorum* in the alfalfa lines are given in Table 1. Showing significant differences among the alfalfa plants regarding the pathogenesis. Mean lengths of necrosis induced by *S. trifoliorum* in the alfalfa in the experiments I, II and III (Table 1).

Highly significant differences among the genotypes were noted in the analysis of data. Differences between genotypes and experiments were insignificant for the pathogen. As indicated by the differences in the means of the genotypes, the pathogen progressed very rapidly in the first procedure as compared to second and third procedures. In the susceptible population (WX) the longest necrotic lesion (21.7 cm) was produced in Del 60 while the shortest lesion was produced in APO 365 (1.4 cm) (Table 1). In procedure 2, the pathogenesis progressed rapidly in BIC29 (11.8 cm) and slowly in Del 174 (1.6 cm). In procedure 3, the rate of progress of pathogenesis was slower as compared to second and first procedures. However APO 216 (5.6 cm) produced the longest necrotic lesion while in Del 174 no symptom was developed. These results are consistent with conclusions and suggestion of previous studies with *S. trifoliorum* on alfalfa by Elgin *et al.* (1968), Pierson *et al.* (1988) and Welty and Busbice (1978).

In case of resistant alfalfa population (BX) Del 190 surpassed all the other genotypes regarding pathogenesis of *S. trifoliurum* in procedure 1 (14.8 cm) along with BIC 215 (4.0 cm) in procedures 2 and Del 190 in procedure 3 (16.0 cm), while BIC 215 (0.8 cm) in

Table 1: Length (cm) of necrosis induced by *S. trifoliorum* in stems of 16 Alfalfa genotypes by using three procedures

Plant and F-value	Procedure 1 (plastic bags)	Procedure 2 (perforated bags)	Control 1	Control 2	Procedure 3 (pippet bags)	Control 3
APO 216	15.0	02.7	04.8	03.5	03.5	05.6
APO 365	01.4	03.0	03.2	03.5	03.5	02.0
ARC 429	16.5	03.7	03.2	02.5	02.5	01.2
ARC 329	08.0	02.13	02.3	03.0	03.0	02.6
BIC 270	14.0	02.8	19.8	04.5	04.5	01.3
BIC 29	01.8	11.8	02.0	15.0	15.0	02.2
DEL 60	21.7	03.5	17.0	04.1	04.1	02.8
DEL 174	14.9	01.6	01.3	01.5	01.5	00.0
APO 287	13.0	02.26	03.3	02.9	02.9	03.5
APO 145	05.9	03.0	01.8	02.3	02.2	03.2
ARC 407	02.2	01.6	01.3	03.0	03.0	01.6
ARC 290	02.86	02.9	01.3	01.6	01.6	03.0
BIC 140	04.7	02.6	02.9	02.0	02.0	02.7
BIC 215	00.8	04.0	02.3	01.7	01.7	06.5
DEL 190	14.8	02.0	20.5	02.8	02.8	16.0
DEL 301	04.0	01.5	02.2	01.9	01.9	03.3
Mean	08.85	03.18				03.59
F-Value	20.54**	20.848**				60.50**

**: P< 0.01

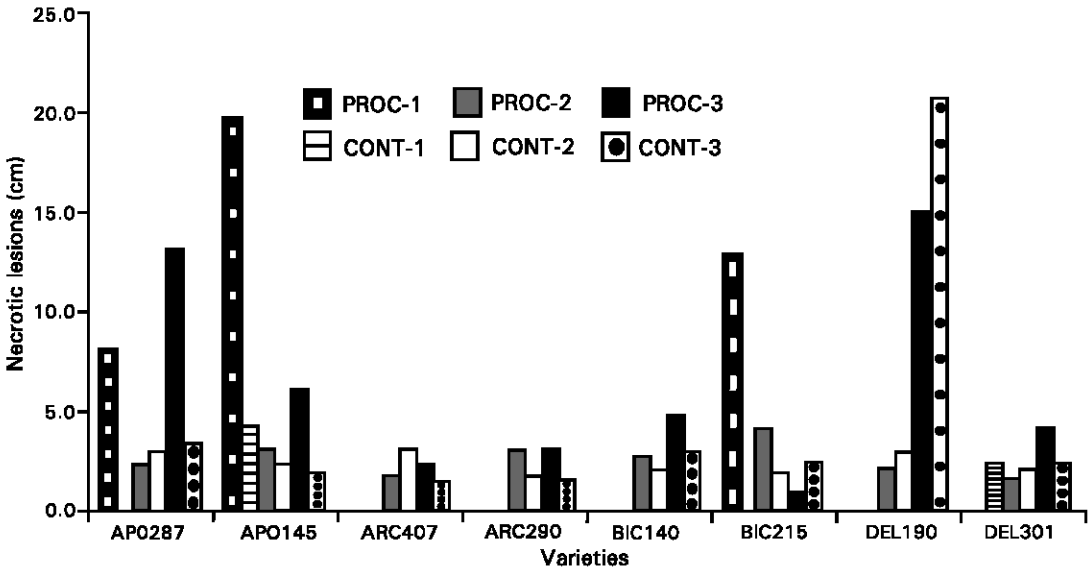

Fig. 1: Comparison of various procedures adopted for alfalfa stem tip inoculation by *S. tirfoliorum* in resistant population

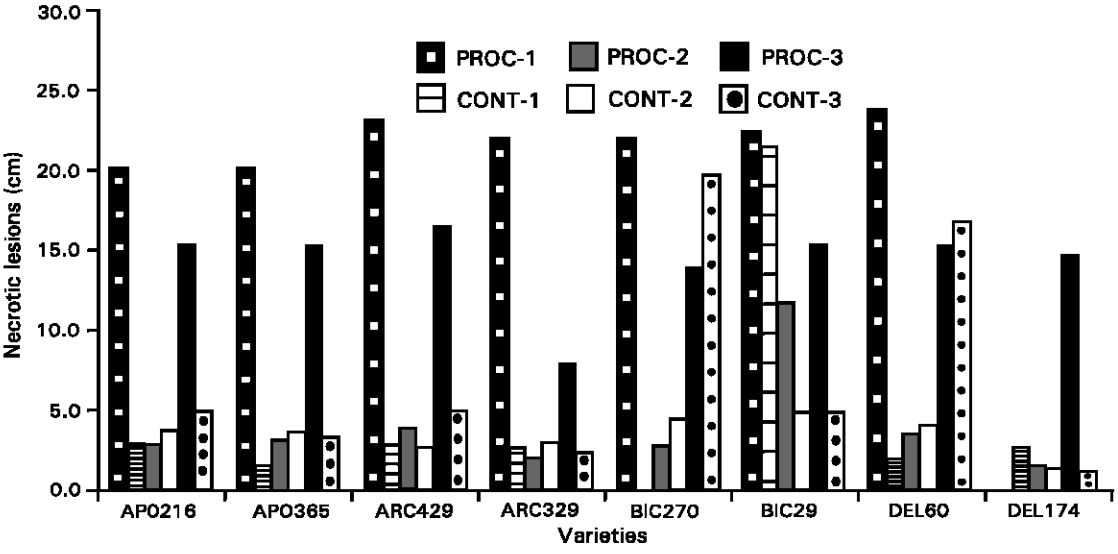

Fig. 2: Comparison of various procedures adopted for alfalfa stem tip inoculation by *S. tirfoliorum* in susceptible population
PROC = Procedure CONT = Control

procedure 1, Del 301 (1.5 cm) in procedure 2 and ARC 407 (1.6 cm) in procedure 3 were the lowest in necrotic lesion produced in the alfalfa population. These results are consistent with Halimi *et al.* (1994), who evaluated plants of four genetically different populations by adopting stem tip inoculation procedure for one cycle of divergent selection and reported that resistance to *Sclerotinia* is heritable and can be increased or decreased using the stem tip inoculation technique.

Comparison among various procedures adopted for alfalfa stem tip inoculation by *S. trifoliorum* in resistant population (Fig. 1) and comparison among various procedures adopted for alfalfa stem tip inoculation by *S. trifoliorum* in susceptible population (Fig. 2). In case of resistant population BIC 215 conferred high resistance in perforated plastic bags (Procedure 2) while Del 190 showed severe infection and in plastic bags (Procedure 1) ARC 407 was least infected while APO 145 was most infected. Pippet plants (Procedure 3) were the least infected in all cases except in Del 190 (16 cm) and BIC 215 (6.5cm).

In case of susceptible population (Fig. 2), the progress of the pathogenesis varied significantly among the three procedures. Plastic bags (procedure 2) were prominent in APO 216, ARC 429, Del 60 and Del 174 while plastic bags (procedure 1) was progressive in BIC 29, BIC 270, ARC 429 and BIC 270.

Pippet plants (procedure 3) expressed disease in BIC 270, BIC 29 more prominently while the least expression was in ARC 329 and Del 174.

Results of this study on pathogenesis of alfalfa stems by *S. trifoliorum* are generally similar to results obtained in two previous research studies conducted by Chun *et al.* (1987), who observed overall differences among cultivars in the extent of lesion development on excised soybean stems. However, high variability in stem reactions often gave different rankings of cultivars between experiments and caused a low reproducibility of results. Causes of this variability were not apparent.

Selecting plants for resistance to stem necrosis after inoculation with *S. trifoliorum* is difficult but the trait has proven heritable. With repeated tries for selecting resistance and susceptible, check germplasm will be developed. This germplasm can then be used to develop alternative selection procedures that are less demanding in time and economic resources than the stem-tip inoculation.

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