A Two Stage Selection Procedure for Resistance to Sclerotinia in Red Clover

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Introduction

Sclerotinia crown and stem rot, caused by *Sclerotinia trifoliorum* Eriks., is one of the most destructive diseases on forage legumes in the eastern and north central U.S. and in Europe. Development of resistant germplasm in red clover (*Trifolium pratense* L.) has been difficult and slow. Until recently, the primary source of inoculum used in laboratory or greenhouse screening procedures was mycelium. However, the natural inoculum in the field is the ascospore. Recently, we developed a procedure for producing and storing ascospores in the laboratory which has allowed us to use ascospores as the source of inoculum in greenhouse screening tests (Smith et al., 1993; Marum et al., 1994). Selection for resistance in red clover using ascospores as inoculum has been slow but effective. It has been most difficult to generate uniform inoculation conditions whether using mycelium or ascospores, resulting in susceptible plants escaping infection and contributing to susceptibility in the subsequent generations. To overcome some of this procedural variability, a single leaf inoculation procedure has been proposed (Mouset-Declas et al., 1994). This becomes a very labor intensive procedure when screening thousands of plants for resistance. Therefore, a more efficient procedure is needed and this paper describes a two stage process which employs both a mass and a leaflet inoculation phase in the procedure.

Procedures

The two stage procedure involves a mass inoculation of two-week-old seedlings with ascospores followed by a replicated single leaflet inoculation of surviving seedlings.

Inoculum preparation and application. An isolate of *Sclerotina trifoliorum*, M1-B, collected from the Agricultural Research Station at Marshfield, WI was used for all procedures. Inoculum concentration for both stages was 10,000 spores ml^{-1} in a solution containing 10 g glucose l^{-1} and 3 drops of Tween 80 100 ml^{-1}.

Stage one. Mass Inoculation: Red clover seeds are sown in either sectioned or open plastic trays containing standard sterilized greenhouse soil. The trays are inserted in non-draining plastic flats such that the trays could be watered from the bottom with Hoagland's solution. Two-week-old plants were spray-inoculated with 80 ml of ascospore inoculum per pan. Pans were covered with clear plastic tops and sealed to retain 100% relative humidity. Plants are incubated at 15°C at 100% relative humidity in 12 hr day-length for 14 da. Plants are evaluated on a Disease Severity Index (DSI): 1 = healthy plant with no damage to cotyledons, 2 = slight necrosis with slight necrosis to cotyledons, 3 = moderate necrosis with death of cotyledons, 4 = severe necrosis, and 5 = dead plant.

Stage two. Leaflet Inoculation: Twenty-eight days after the mass inoculation, one leaflet from one leaf of each surviving plant is placed on moist, sterile filter paper in each of three separate 15 cm petri dishes sectioned to contain 10 to 16 leaflets per dish. One drop of ascospore inoculum at previously described spore concentration is placed in the center of each leaflet. The petri dishes are covered and sealed with parafilm and the leaflets incubated as prescribed for the seedlings in the mass inoculation. Fourteen days after inoculation leaflets are scored on a scale of 1 to 5 (1 = no necrosis, green leaflet; 5 = leaflet completely infected, light to dark brown). Plants with leaflet scores of DSI 1 or 2 are subsequently intercrossed to produce the next generation.

Results

Slight progress from selection for resistance to *S. trifoliorum* was realized in the first cycle of selection using the mass inoculation procedure

and standard phenotypic selection (Table 1). However, the process did not prove to be effective in the two subsequent cycles (C2 and C3) of selection. At this point we introduced the two stage inoculation procedure. The effectiveness of this procedure is reported in Table 2. Population R-C4 derived from plants declared as resistant by the leaflet inoculation step has significantly greater number of resistant plants than present in the MR-C4, S-C4 and C4 Mass populations. The mean DSI of population R-C4 (3.55) is significantly less than that for the other three populations and from the previous cycle of selection (C3). The response over all four cycles of selection is depicted in Figure 1.

Conclusions

The two stage ascospore inoculation procedure for identifying red clover seedlings with the resistant reaction to *S. trifoliorum* is effective and efficient. The initial mass inoculation step allows a breeder the opportunity to evaluate thousands of plants in a short period of time and the second step of leaflet inoculation effectively eliminates seedlings that have escaped infection in the earlier inoculation. Substantial progress should be realized with minimal effort using this procedure.

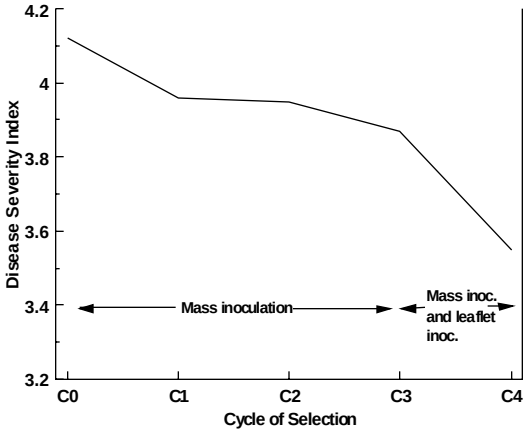

Figure 1. Response of four cycles of selection for resistance to Sclerotinia.

References

Marum, P., R.R. Smith, and C.R. Grau. 1994. Development of procedures to identify red clover resistant to *Sclerotinia trifoliorum*. Euphytica 77:257-261.

Mousset-Declas, C., G. Delclos, and G. Raynal. 1994. Production of *Sclerotinia trifoliorum* ascospores in laboratory and resistance test. *In* Proc. XIII Trifolium Conf. , R. Narasimhalu, ed. Charlottetown, Prince Edward Island, Canada. July 12-14, 1994, p. 68.

Smith, R. R., P. Marum, C.R. Grau, and D.K. Sharpee. 1993. Reaction of red clover to ascospore inoculum of *Sclerotinia trifoliorum*. Agron. Abst. 1993. p. 102.

Table 1. Response to three cycles of selection for resistance to S. *trifoliorum* in red clover using ascospores for inoculum.

	Percent plants with DSI of			
Popn.	1/2(R)	3(MR)	4/5(S)	Mean*
C0	10	10	80	4.16[b]
C1	12	12	76	3.96[a]
C2	14	13	73	3.95[a]
C3	12	18	70	3.87[a]
Arlington	8	22	70	4.14[b]

*Means followed by the same letter not significantly different at the 5% level.

Table 2. Response of populations derived from leaflet inoculation for resistance to S. *trifoliorum* in red clover.

	Percent plants with DSI of			
Popn.**	1/2(R)	3(MR)	4/5(S)	Mean*
R-C4	35	5	60	3.55[a]
MR-C4	12	10	78	4.30[c]
S-C4	8	3	89	4.55[d]
C4	24	6	70	4.00[b]
C3	19	5	76	4.11[b]
C0	10	5	85	4.41[d]

*Means followed by the same letter not significantly different at the 5% level.
**Popn. R, MR, and S are C4 popn. Derived from C3 plants determined to be resistant, moderately resistant, or susceptible by the leaflet inoculation test, respectively.