Effects of Planting Dates on Expression of Phytophthora Root Rot Resistance in Alfalfa

D. R. PORTER, Graduate Research Assistant, and J. L. CADDEL, Associate Professor, Agronomy Department, and L. L. SINGLETON, Associate Professor, Department of Plant Pathology, Oklahoma State University, Stillwater 74078

ABSTRACT

Porter, D. R., Caddel, J. L., and Singleton, L. L. 1986. Effects of planting dates on expression of Phytophthora root rot resistance in alfalfa. Plant Disease 70:655-657.

Four field studies were conducted to identify efficient screening techniques to maximize the expression of disease resistance and minimize the time required for one cycle of selection for resistance to Phytophthora root rot (PRR) caused by *Phytophthora megasperma* f. sp. *medicaginis* in alfalfa (*Medicago sativa*) under Oklahoma conditions. In 1981 and 1982, the effects of six and eight screening dates, respectively, from 2 November through 15 July, on severity of PRR symptoms of fall-planted alfalfa were evaluated. Disease severity indices and percent resistant plants of two PRR-resistant and two PRR-susceptible cultivars maintained under saturated soil conditions were used as indicators of screening effectiveness. Results indicated effective screening can be obtained by mid-May provided soil temperatures exceed 12 C before screening. In two separate studies, the effects of six spring planting dates (5 March through 13 May) were evaluated with an August screening in 1982 and 1983. However, none of the spring planting dates clearly allowed for differentiation between susceptible and resistant cultivars.

Phytophthora root rot (PRR) of alfalfa (*Medicago sativa* L.) occurs in nearly every area of the world where alfalfa is grown (10). PRR is caused by *Phytophthora megasperma* f. sp. *medicaginis* (*P. m.* f. sp. *medicaginis*) (10,12), and it has been cited as a major factor in stand decline of alfalfa (4,6,7,10).

Resistance of alfalfa to *P. m.* f. sp. *medicaginis* was first reported in 1966 (5). Resistance can be increased in breeding strains by recurrent cycles of mass selection (i.e., plant/select resistant material/intercross selections/harvest seed/repeat cycle). Starting populations with fewer than 10% resistant plants have been increased to 63% resistant plants after three cycles of selection (8). In field trials, plant losses of PRR-resistant cultivars averaged only 21% compared with 44% for susceptible cultivars, and yield reductions were 21 and 55%, respectively (6).

It is known that water saturation of soil predisposes alfalfa to infection by *P. m.* f. sp. *medicaginis* (13,15) and promotes optimum disease development (17). A standard field test to characterize resistance in alfalfa cultivars to PRR was developed (1,9) in which 75 viable seeds were planted in early May in soil infested with *P. m.* f. sp. *medicaginis*. After 1 mo, saturated soil conditions were maintained for 2–3 wk. To attain a desired severity of PRR for effective screening, additional periods of soil saturation could be imposed if needed. At the end of a test cycle, plants were dug retaining as much taproot as possible. Roots were washed and severity of PRR determined using a classification scale of 1–6 described by Frosheiser and Barnes (8,9). Bray and Irwin (2) modified this test by using an August planting and delayed screening until September of the following year.

In Oklahoma, fall planting of alfalfa is preferred to spring planting because of higher plant emergence and lower plant mortality after emergence (3). Conditions are such in Oklahoma that spring planting with fall evaluation of PRR severity does not always coincide with favorable moisture and temperature regimes for effective screening. PRR resistance screening has been inefficient in Oklahoma because of the lengthy screening period (10 mo, September planting/July evaluation). Completion of one selection cycle using the present screening technique requires as long as 2 yr.

The purpose of this research was to evaluate the effect of planting dates on severity of PRR expression in alfalfa for effective resistance screening in this region.

Journal Article 4900, Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater.

Accepted for publication 3 February 1986 (submitted for electronic processing).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Fall planting/spring evaluation. In study 1, 100 viable seeds of each of two known PRR-resistant (Agate and Apollo) and two PRR-susceptible (Arc and Vernal) cultivars were planted 1 October 1981 on the Agronomy Research Station, Stillwater, OK. In study 2, Apollo and Vernal were replaced with WL-318 (resistant) and Saranac (susceptible) and 150 viable seeds of each cultivar were planted 6 September 1982. In both studies, seed was planted in 2-m rows (one cultivar per row) with 30-cm spacing between rows. The soil was a Port loam naturally infested with *P. m.* f. sp. *medicaginis* as confirmed by seedling baiting techniques from diseased alfalfa root tissue (14) and other observed morphological and sporulation traits (12). Rainfall combined with supplemental irrigation (daily to runoff if needed) kept the soil at or near saturation during November, December, and March. Plants were dug and roots evaluated for PRR severity on 1 and 22 April, 13 May, 3 and 24 June, and 15 July in both years. Two additional evaluation dates, 2 November and 20 December, were employed during study 2.

Spring planting/fall evaluation. In the spring of 1982 and 1983, 150 viable seeds of each of two known PRR-resistant cultivars (Agate and WL-318) and two PRR-susceptible cultivars (Arc and Saranac) were planted in 2-m rows (one cultivar per row) in an area adjacent to the previous study. Planting dates were 5 and 17 March; 2, 16, and 29 April; and 13 May during 1982 and 1983. All treatments were irrigated to maintain a saturated soil from 13 June to 17 August of 1982 and 1983. Plants were clipped and roots dug and evaluated for PRR severity on 24 August of 1982 and 1983.

General procedures. In all field experiments, split-plot designs were used for all studies, with screening (or planting) dates as main plots in randomized complete blocks and four cultivars as subplots with six replicates (16). In all studies, overhead irrigation was applied immediately after each planting. In each case, postemergence plant counts were made 2 wk after planting, providing the base number of plants for calculating disease severity indices (DSIs) and percent resistant plants when surviving plants were evaluated later for PRR symptoms. Evaluation of individual plant roots for PRR severity was based on a scale of 1–6, where 1 = no symptoms and 6 = dead plants; plants in classes 1 and 2 were considered resistant (8,9). An average DSI was calculated for each subplot as follows: DSI = [summation (class no. × % in class)]/100, to provide a weighted

average of all plants. Significant differences between resistant and susceptible cultivars for PRR severity, as expressed in DSIs and percent resistant plants (classes 1 and 2), were used as indicators of screening effectiveness.

RESULTS

Fall planting/spring evaluation. At all 1982 evaluation dates (Fig. 1), both DSIs and percent resistant plants differed significantly ($P < 0.05$) between the resistant and susceptible cultivars sown in October 1981. DSIs generally increased and percent resistant plants decreased with later spring evaluations, apparently coinciding with increased PRR activity as mean soil temperatures increased (Fig. 2). Because all dates of screenings allowed for differentiation between resistant and susceptible cultivars in 1982, two additional earlier fall screening dates (2 November and 20 December) were added to the study sown in 1982. However, in spring 1983, significant differentiation between resistant and susceptible cultivars for DSI and percent resistant plants did not occur until 13 May (Fig. 3). In both years, the DSIs for the cultivars were similar in magnitude to those obtained in other studies (8). No significant ($P > 0.05$) date × cultivar interaction was detected in either year.

As shown in Fig. 2 in 1982, March soil temperatures were at or above 12 C beginning about 2 wk before the first evaluation date (1 April). In 1983, however, soil temperatures did not exceed or approach 12 C until about 2 wk before the third evaluation date (13 May). Wilkinson and Millar (17) reported *P. m.* f. sp. *medicaginis* activity only after soil temperatures reached 15 C in spring, with an increase in activity at 18–20 C and no activity below 12 C in fall. Our findings agree with theirs. The differences in effective screening dates in 1982 (1 April) and in 1983 (13 May) apparently coincide with the predisposing effects of the necessary soil temperature on infection prior to the screening dates. Soil temperatures in 1983 were cooler (< 10 C), thus suppressing *P. m.* f. sp. *medicaginis* activity.

Spring planting/fall evaluation. Field tests in 1982 comparing several spring planting dates (March through May) with an August evaluation resulted in no consistent significant differentiation between resistant and susceptible cultivars (Fig. 4). In 1982, only the third planting date (2 April) resulted in significant differentiation in DSIs and percent resistant plants between resistant and susceptible cultivars with an August evaluation. Also in 1983 (Fig. 5), DSIs presented no clear differentiation between resistant and susceptible cultivars. Although, percent resistant plants did allow for significant differentiation between the resistant and susceptible cultivars on 2 and 29 April planting dates. In both years, there was no significant cultivar × date interaction. The results from the date of planting studies suggest that

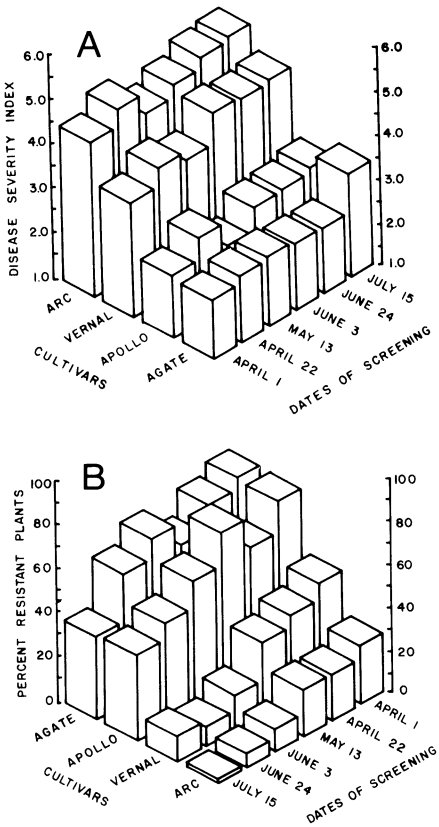

Fig. 1. Phytophthora root rot (PRR) rating: **(A)** average disease severity index and **(B)** percent resistant plants for two resistant (Agate and Apollo) and two susceptible (Arc and Vernal) alfalfa cultivars planted in October 1981 and screened for PRR symptoms at six dates in 1982. LSD (0.05) = 0.52 and 13.7, respectively, for cultivars at each date.

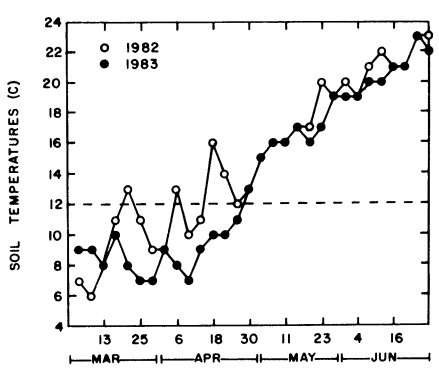

Fig. 2. Mean weekly soil temperatures (C) at a 15-cm depth from 1 March through 30 June of 1982 and 1983.

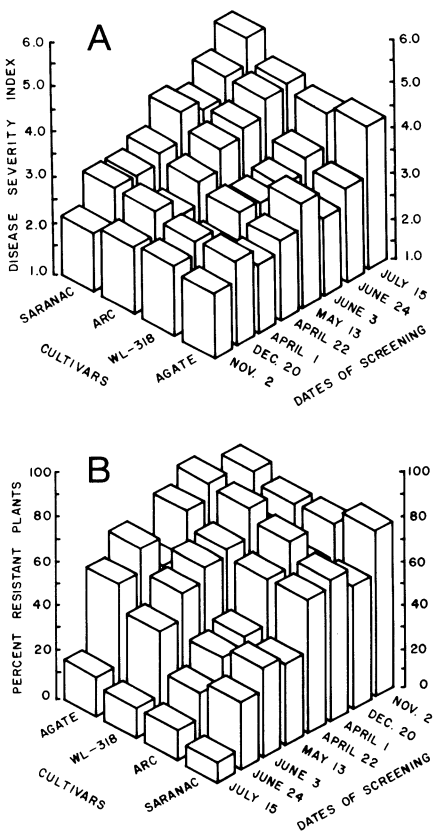

Fig. 3. Phytophthora root rot (PRR) rating: **(A)** average disease severity index and **(B)** percent resistant plants for two resistant (Agate and WL-318) and two susceptible (Arc and Saranac) alfalfa cultivars planted September 1982 and screened for PRR symptoms at six dates in 1982–1983. LSD (0.05) = 0.48 and 10.8, respectively, for cultivars at each date.

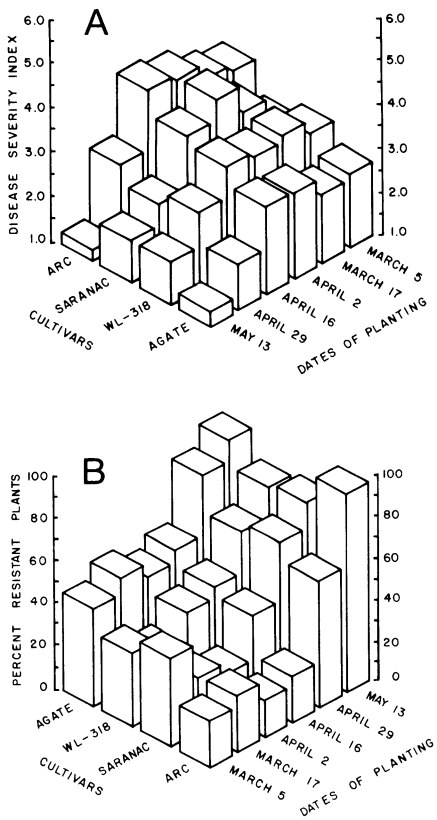

Fig. 4. Phytophthora root rot (PRR) rating: **(A)** average disease severity index and **(B)** percent resistant plants for two resistant (Agate and WL-318) and two susceptible (Arc and Saranac) alfalfa cultivars screened for PRR symptoms in August 1982 resulting from six spring planting dates in 1982. LSD (0.05) = 0.64 and 16.9, respectively, for cultivars at each date.

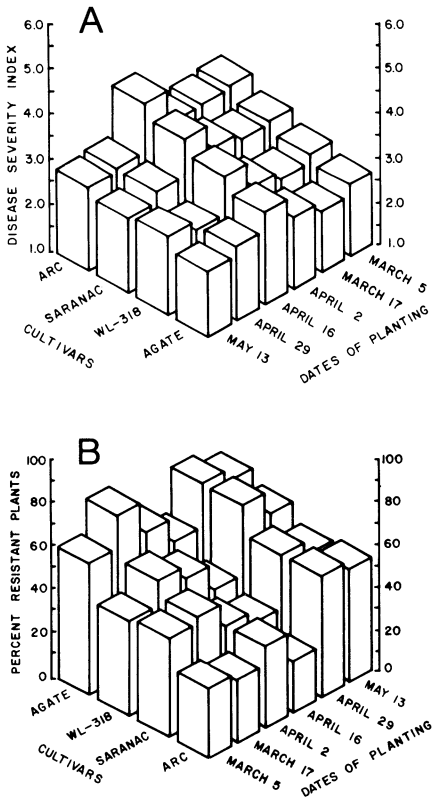

Fig. 5. Phytophthora root rot (PRR) rating: **(A)** average disease severity index and **(B)** percent resistant plants for two resistant (Agate and WL-318) and two susceptible (Arc and Saranac) alfalfa cultivars screened for PRR symptoms in August 1983 resulting from six planting dates in 1983. LSD (0.05) = 0.43 and 10.7, respectively, for cultivars at each date.

there might be an optimum time period in April for planting alfalfa cultivars for effective PRR screening in August. However, the inconsistencies in the data suggest that predicting the optimum planting date in a given year would be difficult if not impossible. Also, stand establishment with spring planting is poor in this area. The average number of plants per row (of 150 initially seeded) for both years with spring planting (2 wk after planting) were as follows: 29 on 5 March, 18 on 17 March, 47 on 2 April, 61 on 16 April, 33 on 29 April, and 28 on 13 May. By contrast, stand establishment usually exceeded 70% with the October plantings.

DISCUSSION

These studies show that screening alfalfa for resistance to PRR can be effective in Oklahoma. The most important modifications from the field method reported by Barnes et al (1,9) include establishing screening nurseries in late summer or fall and monitoring soil temperatures during the following spring. Expression of PRR was inhibited by soil temperatures below 12 C even with frequent irrigation to maintain the soil in a saturated state. It appears that disease symptoms can only be induced in fall-sown alfalfa after soils warm up in April or May.

With fall planting, use of either an April or May screening date as dictated by soil temperature to maximize the expression of disease resistance will facilitate completion of one cycle of selection for PRR resistance per year. Breeding material can be planted in September or October and screened in April/May after soil temperatures reach 12 C or higher for at least 2 wk before evaluation. Selections can then be transplanted and grown in a greenhouse for intercrossing in July and August, seed harvested, and planted to begin another selection cycle in September.

Spring planting in Oklahoma does not appear to be a consistently useful method for PRR evaluation because of poor stand establishment caused by preemergence and postemergence damping-off. More research is needed to identify the problems associated with spring sowing. As suggested by our results and by Havey and Grau (11), it seems likely that other seedling pathogens in addition to *P. m.* f. sp. *medicaginis* may be responsible for some of these problems.

This research shows that the field screening portion of breeding for PRR resistance can be reduced from the 10–12 mo previously used to as little as 7 mo.

LITERATURE CITED

1. Barnes, D. K., Frosheiser, F. I., Sorensen, E. L., Elgin, J. H., Jr., Nielson, M. W., Lehman, W. F., Leath, K. T., Ratcliffe, R. H., and Buker, R. J. 1974. Standard test to characterize pest resistance in alfalfa varieties. U.S. Dep. Agric. ARS NC-19. 23 pp.
2. Bray, R. A., and Irwin, J. A. G. 1978. Selection for resistance to *Phytophthora megasperma* var. *sojae* in Hunter River lucerne. Aust. J. Exp. Agric. Anim. Husb. 18:708-713.
3. El-Tomi, O. A. 1982. Influence of sowing dates on establishment of alfalfa in Oklahoma. M.S. thesis. Oklahoma State University, Stillwater. 50 pp.
4. Erwin, D. C. 1954. Root rot of alfalfa caused by *Phytophthora cryptogea*. Phytopathology 44:700-704.
5. Erwin, D. C. 1966. Varietal reaction of alfalfa to *Phytophthora megasperma* and variation in virulence of the causal fungus. Phytopathology 56:653-657.
6. Faris, M. A., and Sabo, F. E. 1981. Effect of *Phytophthora megasperma* on yield and survival of resistant and susceptible alfalfa cultivars. Can. J. Plant Sci. 61:955-960.
7. Frosheiser, F. I. 1980. Conquering Phytophthora root rot with resistant alfalfa cultivars. Plant Dis. 64:909-912.
8. Frosheiser, F. I., and Barnes, D. K. 1973. Field and greenhouse selection for Phytophthora root rot resistance in alfalfa. Crop Sci. 13:735-738.
9. Froshier, F. I., and Barnes, D. K. 1984. Phytophthora root rot resistance. Page 25 in: Standard Tests to Characterize Pest Resistance in Alfalfa Cultivars. J. H. Elgin, Jr., ed. U.S. Dep. Agric. Misc. Publ. 1434. 44 pp.
10. Graham, J. H., Stuteville, D. L., Frosheiser, F. I., and Erwin, D. C. 1979. Phytophthora root rot. Pages 29-30 in: A Compendium of Alfalfa Diseases. American Phytopathological Society, St. Paul, MN. 65 pp.
11. Havey, M. J., and Grau, C. R. 1985. Decline of established alfalfa in soil naturally infested with *Phytophthora megasperma* f. sp. *medicaginis* and level of correlation by seedling assay. Plant Dis. 69:221-224.
12. Kuan, T.-L., and Erwin, D. C. 1980. Formae speciales differentiation of *Phytophthora megasperma* isolates from soybean and alfalfa. Phytopathology 70:333-338.
13. Kuan, T. L., and Erwin, D. C. 1980. Predisposition effect of water saturation of soil on Phytophthora root rot of alfalfa. Phytopathology 70:981-986.
14. Marks, G. C., and Mitchell, J. E. 1970. Detection, isolation, and pathogenicity of *Phytophthora megasperma* from soils and estimation of inoculum levels. Phytopathology 60:1687-1690.
15. Pulli, S. K., and Tesar, M. B. 1975. Phytophthora root rot in seeding-year alfalfa as affected by management practices inducing stress. Crop Sci. 15:861-864.
16. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York. 481 pp.
17. Wilkinson, H. T., and Millar, R. L. 1982. Effects of soil temperature and moisture on activity of *Phytophthora megasperma* f. sp. *medicaginis* and alfalfa root rot in the field. Phytopathology 72:790-793.