Relationship between Phytophthora root rot severity index and the percentage of resistant alfalfa plants

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Six named cultivars and several unnamed lines of alfalfa *(Medicago sativa)* were inoculated under greenhouse and field conditions with *Phytophthora megasperma* f.sp. *medicaginis,* the cause of root rot, over a 3-year period. Plants were rated for disease severity three and 12 weeks after inoculation in the greenhouse and field plots, respectively. Disease severity was divided into six categories (1 = no disease, 2 = very slight .... 6 = dead). The percentage of resistant plants (%R) was obtained by combining categories 1 and 2 and a disease severity index (DSI) was calculated from all plants in an experiment. A high degree of correlation (r = 0.78** to 0.97**) and a consistent linear relationship between %R and DSI were found in both greenhouse and field trials. Results indicated that %R values alone can be used for disease assessment to save time. The correlation between greenhouse and field tests was significant ($P \leq 0.01$) in all but one trial.

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Pendant 3 ans, six cultivars et plusieurs lignées de luzerne *(Medicago sativa)* ont été inoculés en serre et au champ avec *Phytophthora megasperma* f. sp. *medicaginis,* la cause du pourridié phytophthoréen. La gravite de la maladie chez les plantes a été évaluée trois semaines après l'inoculation dans le cas des plantes de serre et 12 semaines plus tard dans le cas des plantes au champ. Il y a six categories pour définir la gravite de la maladie (1 = aucune maladie, 2 = plante très légèrement atteinte ... 6 = plante morte). On a obtenu le pourcentage de plantes résistantes (% R) en combinant les categories 1 et 2 et calculé un indice de gravite de la maladie (IGM) à partir de toutes les plantes d'une experience. On a trouvé une correlation élevée (r = 0,78** à 0.97**) et une relation linéaire constante entre le pourcentage de plantes résistantes et l'indice de gravite de la maladie dans les essais en serre et au champ. Les résultats donnent à penser que l'on peut se servir uniquement des pourcentages de resistance pour évaluer la gravite de la maladie afin d'épargner du temps. La correlation entre les tests en serre et ceux effectués au champ était significative ($P \leq 0.01$) dans le cas de tous les essais à l'exception d'un seul.

Introduction

There is general agreement among plant pathologists and breeders that field trials are more desirable than greenhouse tests for evaluating disease resistance in field crops. For the evaluation of alfalfa *(Medicago sativa* L.) root rot caused by *Phytophthora megasperma* f.sp. *medicaginis,* Kuan and Erwin (10), Frosheiser and Barnes (5) described both field and greenhouse screening methods and reported that there was a good correlation between the two methods. The field method requires about 17 weeks to complete and has been widely used. As reviewed by Heisey (7), several greenhouse methods (6, 7, 8, 9) have been developed for screening alfalfa for Phytophthora root rot (PRR). Among these methods, however, there are considerable differences in the type of plant growth medium, containers, seedling age, amount of inoculum, and periods of incubation and soil saturation.

Since 1983 various alfalfa cultivars and breeding lines have been evaluated for resistance to PRR at Ottawa on behalf of the Ontario Forage Crop Committee that has the responsibility to recommend lines for use in Ontario (1). With respect to PRR resistance, alfalfa lines are usually classified on the basis of the percentage of resistant plants (%R) along with some information on the disease severity index (DSI). Field tests were conducted routinely using the method outlined by Frosheiser and Barnes (5). Their greenhouse 'sand tank' method was also tested twice but disease ratings of the small seedlings presented some difficulties. A greenhouse method (pot test) developed at Ottawa (3,4) will be described here in greater detail. The main objective of the present work was to determine a relationship between the percentage of resistant plants (%R) and the disease severity index (DSI) under both field and greenhouse conditions.

Materials and methods

Field test. Frosheiser and Barnes (5) described the method used in Minnesota field trials but additional details are provided for the Ottawa test. At the Central Experimental Farm a field which had previous outbreaks of Phytophthora root rot (PRR) was chosen. It has a clay-loam soil with poor drainage but no mineral deficiencies as determined by soil tests. Each spring (May) after seedbed preparation, scarified alfalfa seeds of various lines were planted by hand in 1.5 m rows, 0.6 m apart in a randomized complete block design with 4 replications. At least 120 seeds were sown in each row. A stand count (emergence) was made 2-3 weeks after seeding (this count was used to determine the number of dead or missing plants at a later date). When seedlings were 4-wk-old, they were inoculated by pouring a mycelial suspension of three virulent *P. megasperma* f.sp. *medicaginis* (Pmm) isolates (3,4) at their base at the rate of ca. 0.9 g wet mycelium per row (dry weight 0.16 g). The suspension was prepared from mycelial mats grown in liquid medium in flasks for two weeks as described earlier (3.4). Plots were kept wet by sprinkler irrigation (1-2 h per day) for the next two weeks. In the following week the soil was allowed to dry for necessary weeding and cultivating.

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Plots were, alternately, kept saturated for two weeks and allowed to dry for one week until plants were 16-17 wk-old when they were dug and rated for root rot according to Frosheiser and Barnes' description of six disease severity categories (5). The percentage of resistant plants: %R = number of plants in categories 1 and 2 times 100 divided by total number of plants, and the disease severity index: DSI = [Σ(number of plants × category value) / total number of plants1 were calculated. Dead or missing plants were included in the category 6, based on the previous stand count. Data were analysed by using available computer programs (11) on correlation and regression (12).

Greenhouse test. A uniform potting soil mixture containing 1:2:3:1 parts by volume of garden soil, peat, sand and perlite with additional 0.15% superphosphate and 0.08% lime was used in all experiments. The soil was distributed evenly in 10 cm plastic pots. Scarified alfalfa seeds were germinated on moist filter paper in 9 cm petri plates (24-48 h) and then 22 seedlings per pot were uniformly distributed over the moistened soil and lightly covered with finely screened (3 mm mesh) soil, which was then dampened with a mist of water. Pots were placed on greenhouse benches receiving a 14 h photoperiod with ca. 200 μmol m^{-2} s^{-1} at the plant surface. When the seedlings emerged, they were thinned to 20 per pot and allowed to grow for the next three weeks. These seedlings were inoculated by pouring a mycelial suspension of the same three isolates of Pmm (described previously) on the soil surface (ca. 0.9 g/pot). Pots were placed on 5 cm plastic saucers and from this time the soil was kept at field capacity (by adding small amounts of water to each pot until it just started to drain into the saucer) for the next 10 days. During the following 11 days, the saucers were removed and the pots were watered every other day to avoid overwatering. In preliminary l'looding trials with uninoculated seedlings, no abnormality was noted until 12 days, although it has been recently reported that anatomical and physiological changes in the tap roots can be detected even after four days of flooding (2,13). After the incubation period of 21 days, plants (6-wk-old) were rated for root rot using the 1-6 severity categories (5). The %R and DSI were calculated and analysed.

Results and discussion

Relationship between % resistant plants and disease severity. A high degree of correlation and consistent linear relationship between the percentage of resistant plants (%R) and disease severity index (DSI) were found in all groups of alfalfa lines, large (e.g. 66) or small (e.g. 8), tested in the field during 1983-1986 (Table 1). The correlation coefficients (r) were greater than -0.9, all significant at $P \leq 0.01$ (12) and the linear regression equations were very similar each year. Log (natural) transformation of either %R or DSI or both did not alter the results but actual (raw) values are presented in the tables 1-3. A strong relationship between %R and DSI (r = -0.78 to -0.97, significant at $P \leq 0.01$) and similar linear regression equations were found in the greenhouse tests also (Table 2). Examination of six individual cultivars (tested twice in the greenhouse) also led to the same conclusions. Results were similar in both field and greenhouse tests and all data pairs (%R and DSI) were plotted to illustrate their relationship (Fig. 1). It is noteworthy that the two measurements (%R and DSI) are not independent as they are based on the same alfalfa population. Therefore it was not surprising that the two were correlated. It is clear however that either %R or DSI can be used as a measurement for evaluating PRR resistance in alfalfa lines. Since the DSI has a narrow, discrete range (1-6) but %R

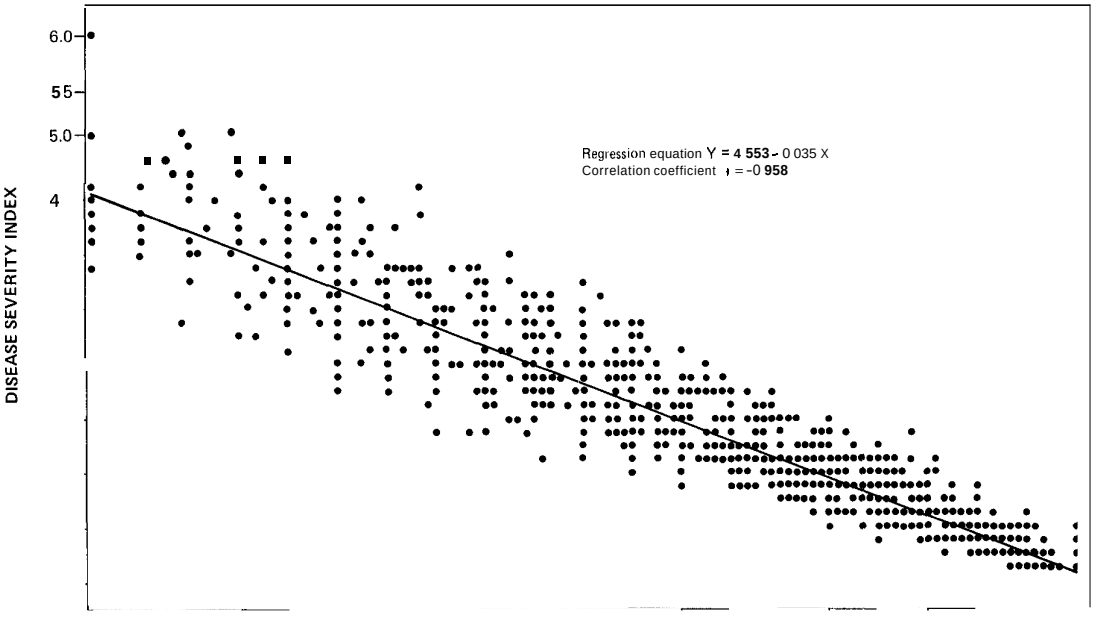

Figure 1. Relationship between percent resistant plants and disease severity index (of a total of **2394** pairs of data points from all tests combined, **515** are shown and the remaining **1879** coincided).

Table 1. Correlation coefficients (r) and regression equations ($Y = a + bX$) for the percent resistant plants (% R = X) and the disease severity index (DSI = Y) and their ranges in a number of alfalfa lines under field conditions during 1983-1986.

No. of Lines	r Values[a]	Regression Equations	Ranges of % R	Ranges of DSI
25 (1983)	-0.94	$Y = 5.10 - 0.038 X$	34 - 99	1.3 - 1.8
37 (1984)[b]	-0.96	$Y = 4.47 - 0.035 X$	14 - 100	1.2 - 4.2
29 (1984)	-0.95	$Y = 4.33 - 0.033 X$	24 - 100	1.3 - 3.8
8 (1984)	-0.97	$Y = 4.72 - 0.038 X$	14 - 90	1.2 - 4.2
66 (1985)	-0.94	$Y = 4.71 - 0.036 X$	9 - 100	1.0 - 5.1
61[c] (1985)	-0.93	$Y = 4.92 - 0.037 X$	0 - 100	1.0 - 3.7
30 (1986)	-0.96	$Y = 4.97 - 0.039 X$	27 - 97	1.2 - 4.0

[a] All r values significant at $P \leq 0.01$ (see ref. 12, p. 174)

[b] These 37 lines were divided into two groups: 29 unnamed and 8 named (Answer, Apollo, Iroquois, Peak, Saranac, Trident, Turbo and Vernal) for separate analysis.

[c] These 61 lines had 2 instead of the usual 4 replications, each containing more than 60 plants at the time of emergence.

Table 2. Correlation coefficients (r) and regression equations ($Y = a + bX$) for the percent resistant plants (% R = X) and the disease severity index (DSI = Y) and their ranges in a number of alfalfa lines under greenhouse conditions.

No. or Names of Lines X Reps[a]	r Values[c]	Regression Equations	Ranges of % R	Ranges of DSI
28 (1984) X 10	-0.96	$Y = 4.38 - 0.033 X$	7 - 100	1.1 - 4.9
66 (1985) X 10	-0.96	$Y = 4.35 - 0.032 X$	0 - 100	1.1 - 4.9
81 (1985) X 2	-0.96	$Y = 4.05 - 0.030 X$	0 - 100	1.0 - 4.5
30 (1986) X 10	-0.97	$Y = 4.82 - 0.038 X$	0 - 100	1.1 - 5.1
Answer (1st run)[b] X 20	-0.96	$Y = 5.60 - 0.047 X$	0 - 100	1.1 - 6.0
Answer (2nd run) X 20	-0.94	$Y = 3.78 - 0.026 X$	50 - 90	1.5 - 2.6
Apollo (1st run) X 20	-0.88	$Y = 4.28 - 0.030 X$	30 - 81	1.4 - 3.4
Apollo (2nd run) X 20	-0.94	$Y = 3.57 - 0.023 X$	60 - 100	1.3 - 2.3
Iroquois (1st run) X 20	-0.94	$Y = 4.63 - 0.034 X$	0 - 100	1.5 - 4.6
Iroquois (2nd run) X 20	-0.88	$Y = 4.23 - 0.031 X$	20 - 55	2.7 - 3.9
Saranac (1st run) X 20	-0.87	$Y = 4.79 - 0.037 X$	0 - 100	1.3 - 6.0
Saranac (2nd run) X 20	-0.78	$Y = 4.07 - 0.024 X$	25 - 65	2.5 - 3.9
Trident (1st run) X 20	-0.97	$Y = 4.50 - 0.034 X$	18 - 100	1.1 - 3.7
Trident (2nd run) X 20	-0.95	$Y = 3.84 - 0.028 X$	55 - 100	1.0 - 2.6
Vernal (1st run) X 20	-0.85	$Y = 4.84 - 0.036 X$	20 - 82	1.9 - 4.5
Vernal (2nd run) X 20	-0.96	$Y = 4.47 - 0.036 X$	5 - 55	2.6 - 4.4
Above 6 cvs (1st run) X 20	-0.92	$Y = 4.90 - 0.038 X$	0 - 100	1.0 - 6.0
Above 6 cvs (2nd run) X 20	-0.97	$Y = 4.35 - 0.033 X$	5 - 100	1.0 - 4.4

[a] A replication is a pot of alfalfa seedlings.

[b] Individual named cultivars were tested two times (runs) in the same greenhouse.

[c] All r values significant at $P \leq 0.01$ (see ref. 12, p. 174).

has a wide range (0-100), the use of %R would seem more appropriate for separating or grouping of alfalfa lines despite the fact that within individual lines the range may be large (Table 1 and 2). Furthermore, evaluation of lines by %R is simple, objective and time-saving as noted by other workers (8).

Correlation between field and greenhouse tests. Since the number of replications in the field and greenhouse varied, the mean %R and DSI values for each corresponding alfalfa lines were used for obtaining correlation coefficients (r) and regression equations (Table 3). In all, except the test with 61 lines with two replications, the r values were significant at $P \leq$

Table 3. Correlation coefficients (r) and regression equations (Y = a + bX) for field (Y) and greenhouse (X) tests using the mean[a] values of percent resistant plants (% R) and disease severity index (DSI) of corresponding alfalfa lines during 1984-1986.

No. of Lines	r values for		Regression Equations for	
	% R	DSI	% R	DSI
6 (1984)	0.95**[b]	0.95**	Y = -0.98 + 0.83 X	Y = 0.87 + 1.14 X
25 (1984)	0.58**	0.57**	Y = 16.49 + 0.58 X	Y = 1.26 + 0.56 X
66 (1985)	0.58**	0.44**	Y = 62.63 + 0.32 X	Y = 1.06 + 0.27 X
61 (1985)[c]	0.13ns	0.12ns	Y = 83.58 + 0.07 X	Y = 1.40 + 0.08 X
30 (1986)	0.77**	0.70**	Y = 46.51 + 0.51 X	Y = 0.70 + 0.47 X

[a] The mean values of % R and DSI of each line were derived from 4 reps. in the field and 20 reps. in the greenhouse.

[b] ** indicate significance at $P \leq 0.01$; ns = non-significant.

[c] These 61 lines had only 2 reps. in both field and greenhouse.

0.01 but they varied from 0.58 to 0.95 for %R, and from 0.44 to 0.95 for DSI. The regression equations for field (Y) on greenhouse (X) differed between years, indicating a lack of consistency in their relationship. It was generally observed that variability in greenhouse data was less than in field data. The greater variability in field tests was likely due to several (unknown or unavoidable) biotic and environmental factors affecting disease development. Results indicate that in order to increase accuracy and consistency in the evaluation of alfalfa lines, greenhouse tests should also be used in addition to field trials for determining resistance to Phytophthora root rot.

Conclusions

There is a strong linear correlation between the percentage of resistant alfalfa plants (%R) and Phytophthora root rot severity index (DSI) both under field and greenhouse conditions. To simplify the disease assessment procedure only %R values can be used for evaluating alfalfa lines, and field trials should be supplemented with greenhouse tests to confirm PRR resistance.

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Literature cited

1. Anonymous. 1980. Field crop recommendations. Ministry of Agriculture and Food, Ontario. Publ. 296. 64 pp.
2. Barta, A.L., and A.F. Schmitthenner. 1986. Interaction between flooding stress and Phytophthora root rot among alfalfa cultivars. Plant Dis. 70:310-313.
3. Faris, M.A. and F.E. Sabo. 1981. Progress report on pathogenicity differential studies of 74 ***Phyrophrhora megasperma*** Dreschsler isolates from alfalfa. Forage Notes 25(2):23-30.
4. Faris, M.A., F.E. Sabo and D.J.S. Barr. 1983. Studies on ***Phytophrhora megasperma*** isolates with different levels of pathogenicity on alfalfa cultivars. Can. J. Plant Pathol. 5:29-33.
5. Frosheiser, F.E. and D.K. Barnes. 1973. Field and greenhouse selection for Phytophthora root rot resistance in alfalfa. Crop Sci. 13:735-738.
6. Gray, F.A., R.B. Hines, M.H. Schonherst and J.D. Naik. 1973. A screening technique useful in selecting for resistance in alfalfa to ***Phyrophrhora megasperma.*** Phytopathology 63: 1185-1188.
7. Heisey, R.F. 1979. An investigation of techniques and methods useful in breeding alfalfa for resistance to Phytophthora root rot. M.Sc. Thesis. Cornell University, Ithaca, N.Y. 14850. U.S.A.
8. Hohrein, B.A., G.A. Bean and J.H. Graham. 1983. Greenhouse technique to evaluate alfalfa resistance to ***Phytophrhora megasperma*** f.sp. ***medicaginis.*** Plant Dis. 67: 1332-1333.
9. Irwin, J.A.G.. S.A. Miller and D.P. Maxwell. 1979. Alfalfa seedling resistance to ***Phyrophrhora megasperma.*** Phytopathology 69: 1051-1055.
10. Kuan, T.L. and D.C. Erwin. 1980. ***Forma specialis*** differentiation of ***Phytophthora megasperma*** isolates from soybean and alfalfa. Phytopathology 70:333-338.
11. SAS User's Guide. 1985. Statistics. SAS Institute Inc., Cary, North Carolina, U.S.A.
12. Snedecor, G.W. and N.G. Cochran. 1956. Statistical Methods (5th edition). Iowa State University Press, Ames, Iowa, U.S.A. 534 pp.
13. Zook, D.M., D.C. Erwin and L.H. Stolzy. 1986. Anatomical, morphological, and physiological responses of alfalfa to flooding. Plant and Soil 96:293-296.