

Variability and Interaction Between Alfalfa Cultivars and Isolates of *Phytophthora megasperma*

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The author thanks F. E. Sabo for able technical assistance and for editing the manuscript.

Accepted for publication 27 September 1984.

ABSTRACT

Faris, M. A. 1985. Variability and interaction between alfalfa cultivars and isolates of *Phytophthora megasperma*. *Phytopathology* 75:390-394.

Six isolates of *Phytophthora megasperma* f. sp. *medicaginis* (*Pmm*) were tested individually for levels of virulence on 15 alfalfa cultivars having varying degrees of resistance to *Phytophthora* root rot (PRR). Stability parameters for the disease severity index were calculated by following Eberhart and Russell's regression technique. These included: regression coefficient (b), deviation from regression (S_D^2), and the coefficient of determination (r^2). Cultivar \times isolate interaction was caused by differences between the cultivars' fitted regression lines and by differences in the R_1 and R_2 response parameters. Only three cultivars possessed stable resistance to PRR. Some others had a high level of resistance to the mildly virulent isolates, but they were severely infected by the highly virulent isolates. Two

cultivars were susceptible to all isolates. Regardless of their virulence, all isolates had approximately equal response parameters; they inflicted the greatest damage on cultivars with the least resistance. However, it was clear that there were two levels of virulence among the isolates. The importance of interactions affecting infection by *Pmm* is illustrated in this work and the application of the genotype \times environment interaction analysis to their study is demonstrated. A low disease severity index (performance) and sensitivity to different levels of virulence (stability) must be considered when selecting the best alfalfa genotypes. Therefore, a sample of *Pmm* chosen from within the area of adaptation should be used to screen alfalfa genotypes for resistance to PRR.

Additional key words: virulence stability.

Phytophthora root rot (PRR) caused by *Phytophthora megasperma* Drechs. f. sp. *medicaginis* (*Pmm*) is a serious disease of alfalfa (*Medicago sativa* L.). It has been reported on alfalfa in the United States (5,6), Canada (2,3), Japan (24), and Australia (16,27). Disease resistance offers the most effective way of controlling PRR. Although many alfalfa cultivars and germ plasms with varying levels of resistance to PRR have been developed and released (1,14,17,21,26), there are few studies of the inheritance of

disease reaction to *Pmm* in alfalfa (18,19,23). Two different genetic mechanisms that condition the reaction to *Pmm* in cultivated alfalfa have been identified. Lu et al (23) reported that susceptibility appeared to be conditioned by one tetrasomic gene, *Pm*, with susceptibility incompletely dominant. Nulliplex plants were highly resistant and simplex plants were moderately resistant. Irwin et al (19) confirmed these findings and also identified another genetic mechanism in which resistance appeared to be conditioned by two incompletely dominant complementary genes *Pm1* and *Pm2*. Erwin (7) reported variation in virulence of some isolates and a loss in pathogenicity after several years of culturing. Gray et al (13) reported similar results.

Faris et al (9) observed a wide range in virulence among 26 isolates of *Pmm* (P) tested on three resistant and three susceptible alfalfa cultivars (C). There were significant isolate \times cultivar type

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(resistant or susceptible) ($P \times C$) interactions that revealed isolates with different virulence on the two cultivar types. Selection to incorporate PRR resistance into new alfalfa cultivars may be hampered by this $P \times C$ interaction, because the relative ranking of alfalfa plants may vary when they are tested against different isolates. The effect of such an interaction is similar in some respects to the genotype \times environment ($G \times E$) interactions that complicate the selection of superior crop plants to improve yield. In such situations, the relative ranking of genotypes will change from one environment to another (10). Various statistical techniques have been developed to study the $G \times E$ interactions, the component parts of genotypic effects, and the stability of response across environments (4,10,20,25). These statistical techniques have been used mostly in yield studies of various crops. However, Faris et al (8) used these techniques to study the stability of midge resistance of sorghum cultivars. More recently, Hobbs and Mohan (15) demonstrated the application of these techniques to study the symbiotic interaction of *Pisum sativum* L. and *Rhizobium leguminosarum*.

The objective of this work was to examine the effects of six isolates of *Pmm* on alfalfa cultivars and their interactions in PRR. Eberhart and Russell's (4) stability analysis was used to determine $G \times E$ interactions and examine them in greater detail to demonstrate the use of stability parameters in evaluating resistance.

MATERIALS AND METHODS

Six isolates of *P. megasperma* f. sp. *medicaginis* were used in this study (Table 1): DW12A and DW12B were the least virulent; DW31 and DW41 were moderately virulent; and Moore-6 and Quebec-5 were highly virulent on alfalfa (9). The isolates were classified into two morphological groups (9): those with small oogonia measuring 20–42 μm in diameter; and those with large oogonia measuring 43–60 μm in diameter. Isolates in group 2 were differentiated further into homothallic (2I) and heterothallic-like mating strains with limited fruiting capacity (2II). The six isolates were individually tested for quantitative virulence on 15 alfalfa cultivars having varying degrees of resistance to PRR (Table 2). Seeds of these cultivars were pregerminated for 24 hr and planted in sterilized soil in 10-cm-diameter plastic pots. Seedlings of each cultivar were thinned to 20 per pot with four replicate pots per cultivar. The pots were kept in the greenhouse for 2 wk and then transferred to controlled-environment cabinets maintained on a 16-hr light cycle at 25 C and an 8-hr dark cycle at 20 C. The plants in each pot were counted prior to inoculation, 3 wk after seeding.

The isolates of *Pmm* were first grown on pea agar medium (22) in 9-cm-diameter sterile petri plates and incubated for 5–7 days in the dark at 27 ± 2 C. Plugs (6-mm diameter) of mycelium were cut from the margins of these colonies and transferred to 125-ml Erlenmeyer flasks containing 50 ml of pea broth. There were 60 flasks per isolate, each seeded with eight mycelial plugs and incubated for 12–14 days in the dark at 27 ± 2 C. The resulting mycelial mats were filtered and comminuted in a Waring blender with distilled water for two 10-sec periods. The inoculum suspensions were diluted to 3,000 ml, and 50 ml of inoculum was poured on the soil surface of

TABLE 1. Geographic source and morphological groupings of the isolates of *Phytophthora megasperma*

| Isolate | Geographic source (township and county) | Morphological group ^a |
|----------|---|----------------------------------|
| DW12A | Matilda, Dundas, Ontario | 2II |
| DW12B | Matilda, Dundas, Ontario | 2I |
| DW31 | Winchester, Dundas, Ontario | 2I |
| EP41 | Gainsborough, N. Niagara, Ontario | 2I |
| Moore-6 | Seneca, Haldimand, Ontario | 1 |
| Quebec-5 | St. Blais, St-Jean Iberville, Quebec | 1 |

^a2II = large oogonia (43–60 μm in diameter), heterothallic-like mating strains with limited fruiting capacity; 2I = large oogonia (43–60 μm in diameter), homothallic; and 1 = small oogonia (20–42 μm in diameter), homothallic.

each pot. The soil in each pot was saturated with water once daily for 21 days following inoculation.

Twenty-one days after inoculation, each plant was individually scored for disease severity on the basis of a six-class scale (12): 1 = no symptoms, many fine roots present on main roots; 2 = no obvious root lesion, but most fine roots destroyed, leaving small black spots at the point of attachment; 3 = distinct localized lesion(s) on the taproot, or one or two secondary roots with tip rot, or both; 4 = part of the taproot rotted; 5 = nearly all of the taproot rotted, but plant still alive; and 6 = plants dead, including loss postinoculation. Disease severity index (DSI) was the average of disease severity scores of individual plants (about 20 plants per pot).

The experimental design was a randomized block with four replications. Variances of DSI data were homogeneous according to Bartlett's test. A combined analysis of variance was carried out on the DSI values to obtain sum of squares due to cultivars (C) and isolates (P) and cultivar \times isolate interactions ($C \times P$). Therefore, the performance of the k th replicate of the i th cultivar inoculated by the j th isolate is represented by the following linear model:

$$Y_{ijk} = \mu + d_i + \epsilon_j + g_{ij} + e_{ijk}$$

in which Y_{ijk} = DSI of cultivar i inoculated with isolate j on replicate k ; μ = grand mean over all replicates, cultivars, and

TABLE 2. Sources of alfalfa cultivars and their reactions to *Phytophthora megasperma*

| Cultivar | Source ^a | Reaction ^b |
|----------|-------------------------------|-----------------------|
| Agate | USDA and Minnesota AES | R |
| Answer | North American Plant Breeders | R |
| Apollo | North American Plant Breeders | R |
| Armor | North American Plant Breeders | R |
| Epic | Land O'Lakes, Inc. | R |
| Hi-Phy | Farmers Forage Research | MR |
| Iroquois | Cornell University | S |
| Lab 75 | Waterman-Loomis Co. | LR |
| Oncida | Cornell University | R |
| Peak | Land O'Lakes, Inc. | R |
| Saranac | Cornell University | S |
| Trident | North American Plant Breeders | R |
| WL-312 | Waterman-Loomis Co. | R |
| WL-313 | Waterman-Loomis Co. | LR |
| 120 | Dekalb Co. | R |

^aSource = Reports of the National Certified Alfalfa Variety Review Board (1963–1983).

^bR = resistant, MR = moderately resistant, LR = low resistance, and S = susceptible.

TABLE 3. Analysis of variance^a for disease severity index of alfalfa cultivars (C) inoculated with isolates (P) of *Phytophthora megasperma*

| Source of variation | $C \times P^b$ | | $P \times C^c$ | |
|-----------------------|----------------|---------------------------|-----------------------|--------------|
| | d.f. | Mean squares ^d | Source of variation | Mean squares |
| Blocks | 3 | 10.991 | Blocks | 3 10.991 |
| C | 14 | 2.171** | P | 5 50.810** |
| P | 5 | 50.810** | C | 14 2.171** |
| $C \times P$ | 70 | 1.346* | $P \times C$ | 70 1.346* |
| $C + (C \times P)$ | 75 | 4.643** | $P + (P \times C)$ | 84 1.483** |
| C (linear) | 1 | 251.051** | P (linear) | 1 30.391** |
| $C \times P$ (linear) | 14 | 2.810** | $P \times C$ (linear) | 5 1.012 |
| Pooled deviations | 60 | 0.914 | Pooled deviations | 78 1.143 |
| Pooled error | 252 | 0.943 | Pooled error | 252 0.943 |

^aThe variance is partitioned following Eberhart and Russell's (4) analysis of genotype \times environment interaction.

^bVariation of cultivars' disease severity indexes over environments (pathogen isolates).

^cVariation of isolates' disease severity indexes over environments (cultivars).

^dMean squares followed by one asterisk are significantly different at $P = 0.05$ and those followed by two asterisks at $P = 0.01$.

isolates; d_i = additive contribution of the cultivar i when $i = 1, \dots, c$; ϵ_j = additive contribution of the isolate j when $j = 1, \dots, p$; g_{ij} = cultivar \times isolate interaction of the cultivar i with isolate j ; e_{ijk} = residual variation contributed by replicate k when $k = 1, \dots, r$ of the cultivar i with the isolate j .

Stability parameters for the DSI data were calculated by following Eberhart and Russell's (4) regression technique as modified by Faris et al (8) to study pest-resistance stability. The model used in the regression is:

$$Y_{ij} = \mu_i + b_i I_j + d_{ij}$$

in which Y_{ij} = mean DSI of the i th cultivar inoculated with the j th isolate ($i = 1, 2, \dots, c$; $j = 1, 2, \dots, p$); μ_i = mean DSI of the i th cultivar over all isolates; b_i = stability parameter estimated by the

regression coefficient of the i th cultivar on the isolate virulence index (I_j); I_j = the isolate virulence index calculated as the mean DSI of the j th isolate minus the grand mean; d_{ij} = deviation from regression of the i th cultivar with the j th isolate. For each alfalfa cultivar, the mean DSI values of each isolate on that cultivar was regressed against the isolate virulence indexes. The isolate virulence index for each isolate was calculated by subtracting the grand mean from the mean DSI of that isolate over all cultivars. A cultivar with stable resistance is defined as one with a low mean DSI, a regression coefficient equal to zero ($b = 0$), and a deviation from regression as small as possible ($S_d^2 = 0$). Other response parameters were calculated; namely, the R_1 and R_2 of Langer et al (20). R_1 is calculated as the difference between the minimum and maximum DSI of a cultivar over all isolates, and R_2 is the difference between the DSI of a cultivar inoculated with the two isolates having the greatest and least average DSI values over all cultivars. Another indicator of response stability (in addition to S_d^2) was calculated, namely, the coefficient of determination (r^2) proposed by Pinthus (25).

A similar analysis of the $P \times C$ interaction to estimate response and stability of the different isolates was done based on the same models as shown above with cultivars and isolates reversed. In this case, a responsive, stable isolate causes a high mean DSI on alfalfa plants, produces a regression coefficient equal to one ($b = 1$), and a deviation from regression as small as possible ($S_d^2 = 0$).

RESULTS AND DISCUSSION

The DSI values of the cultivars inoculated with *Pmm* differed significantly (Table 3) when their mean squares were tested against the pooled error mean square. The most resistant cultivars were Answer, Peak, Trident, and Epic; the most susceptible cultivars were Hi-Phy and Lab 75 (Table 4). There was a highly significant difference in DSI values among the isolates of *Pmm*. The most virulent isolates were Moore-6 and Quebec-5 belonging to morphological group 1 (Table 1) which confirms our previous finding that isolates having small oogonia were highly virulent (9). The results of the combined regression analysis of variance for DSI (Table 3) showed that $C \times P$ interactions, C (linear), and $C \times P$ (linear) mean squares were all significant. This analysis indicated that the $C \times P$ interactions were a linear function of the additive effects due to differences in the level of virulence of the isolates. The $C \times P$ interaction (linear) sum of squares was caused by differences between the cultivars' fitted regression lines and indicated that each cultivar had its own characteristic linear response to the different isolates. The pooled deviations were not significantly larger than the pooled error. Therefore, departure from linearity did not exist. Thus, most of the $C \times P$ interactions could be predicted from the linear regressions of the cultivars on the isolate's virulence index (I_j).

The hypothesis that any regression coefficient does not differ from zero was tested by the appropriate t -test in which $\pm t = (b - \beta_0) / S_b$. By far, the most resistant of the cultivars tested were Trident, Answer, Epic, and Peak (Table 4) because their regression coefficients did not differ from zero. Their resistance is also stable because deviations from regression were estimated to be

TABLE 4. Mean disease severity index and estimate of response and stability parameters of 15 alfalfa cultivars (C) inoculated with six isolates (P) of *Phytophthora megasperma* (C \times P) and of six isolates of *P. megasperma* across 15 alfalfa cultivars (P \times C)

| | Mean | b^a | Response parameters ^b | | Stability parameters | |
|--|------|---------|----------------------------------|-------|----------------------|-------|
| | | | R_1 | R_2 | S_d^2 | r^2 |
| C \times P^c | | | | | | |
| Cultivars | | | | | | |
| Lab 75 | 3.44 | 0.962** | 2.27 | 1.95 | 0.029 | 0.94 |
| Hi-Phy | 3.20 | 0.952** | 2.27 | 1.06 | 0.525 | 0.83 |
| Saranac | 3.08 | 1.508** | 3.19 | 2.25 | 0.317 | 0.95 |
| WL-312 | 3.07 | 1.486** | 3.60 | 3.60 | 0.981 | 0.88 |
| Oneida | 2.95 | 0.532* | 1.51 | 1.43 | 0.158 | 0.75 |
| WL-313 | 2.75 | 1.329** | 2.66 | 2.46 | 0.077 | 0.96 |
| Iroquois | 2.72 | 1.672** | 3.44 | 3.00 | 0.090 | 0.99 |
| Armor | 2.69 | 1.434** | 3.20 | 3.05 | 1.863 | 0.81 |
| Agate | 2.64 | 0.831** | 1.92 | 1.46 | 0.244 | 0.86 |
| I20 | 2.56 | 1.068** | 1.40 | 2.28 | 0.000 | 0.96 |
| Apollo | 2.54 | 0.892 | 2.53 | 1.98 | 1.766 | 0.63 |
| Trident | 2.52 | 0.347 | 1.31 | 0.67 | 0.377 | 0.45 |
| Epic | 2.52 | 0.591 | 2.03 | 0.37 | 1.189 | 0.51 |
| Peak | 2.49 | 0.832 | 2.46 | 1.61 | 1.308 | 0.66 |
| Answer | 2.46 | 0.562 | 2.36 | 1.74 | 1.449 | 0.44 |
| P \times C^d | | | | | | |
| Isolates | | | | | | |
| Moore-6 | 3.99 | 1.34 | 1.77 | 0.55 | 0.754 | 0.44 |
| Quebec-5 | 3.92 | 1.75 | 2.57 | 2.32 | 0.669 | 0.44 |
| DW12A | 2.37 | 0.90 | 1.97 | 0.84 | 0.657 | 0.24 |
| DW12B | 2.19 | 0.65 | 1.67 | 1.67 | 1.283 | 0.16 |
| DW31 | 2.11 | 0.69 | 1.59 | 0.13 | 0.891 | 0.14 |
| EP41 | 2.06 | 0.68 | 1.99 | 0.34 | 1.188 | 0.11 |

^aRegression coefficients followed by one asterisk are significantly different from $b = 1$ at $P = 0.05$ and those followed by two asterisks at $P = 0.01$.

^b R_1 = the difference between the minimum and maximum disease severity index of a cultivar over all isolates; and R_2 = the difference between the disease severity index of a cultivar inoculated with the two isolates having the greatest and least average disease severity index values over all cultivars.

^cCultivars' disease severity indexes over pathogen isolates.

^dIsolates' disease severity indexes over cultivars.

TABLE 5. Correlation coefficients between mean disease severity index of alfalfa cultivars and response and stability parameters. Correlations for cultivars across isolates of *Phytophthora megasperma* are above the diagonal; correlations for the isolates across cultivars are below the diagonally aligned hyphens

| Parameter ^a | Mean | Regression | | | | |
|------------------------|----------------------|------------|---------|---------|---------|---------|
| | (\bar{x}) | (b) | R_1 | R_2 | S_d^2 | r^2 |
| Mean (\bar{x}) | — | 0.336 | 0.257 | 0.220 | -0.422 | 0.572* |
| Regression (b) | 0.943** ^b | — | 0.830** | 0.852** | -0.104 | 0.787** |
| R_1 | 0.527 | 0.755 | — | 0.756 | 0.297 | 0.398 |
| R_2 | 0.431 | 0.576 | 0.661 | — | 0.049 | 0.629* |
| S_d^2 | -0.618 | -0.697 | -0.448 | -0.109 | — | -0.567* |
| r^2 | 0.984** | 0.947** | 0.542 | 0.465 | -0.711 | — |

^a R_1 = the difference between the minimum and maximum disease severity index of a cultivar over all isolates; and R_2 = the difference between the disease severity index of a cultivar inoculated with the two isolates having the greatest and least average disease severity index over all cultivars.

^bCorrelation coefficients followed by one asterisk are significantly different at $P = 0.05$ and those followed by two asterisks at $P = 0.01$.

zero. Regressions of cultivar means against the isolate virulence index were compared (Fig. 1). Lab 75, Hy-Phy, Saranac, WL-313, WL-312, and Iroquois were sensitive to differences in the virulence level of the isolates; they showed more severe PRR symptoms when inoculated with the highly virulent isolates than with weakly virulent isolates. In contrast, cultivars Peak, Epic, Answer, and Trident generally showed low DSI when the mildly virulent isolates were used and also maintained a relatively high level of resistance against the highly virulent isolates. The apparent susceptibility of resistant cultivars such as WL-312 and Oneida could be attributed to the use of Canadian isolates which may have some specific virulence different from those used to develop these cultivars.

Regression values for some cultivars were plotted against their DSI mean (Fig. 2) to give a summary of cultivar responses. The apparent positive correlation between DSI means and regression coefficients was not statistically significant (Table 5). Therefore, according to the model for response and stability (8), only cultivars Peak, Epic, Answer, and Trident could be considered to have stable resistance to *Pmm*, since they showed a low mean DSI, $b = 0$, and $S_d^2 = 0$. Cultivars Saranac, WL-313, WL-312, and Iroquois all had a high level of resistance to the mildly virulent isolates, but they were severely infected by the highly virulent isolates (Fig. 1). Lab 75 and Hi-Phy had high DSI values with all isolates. The cultivar response is also indicated by R_1 and R_2 response parameters (Table 4). The three estimates of response (b , R_1 , and R_2) were highly correlated with one another (Table 5). Because they are calculated more easily, the R_1 and R_2 values might be most useful in selecting cultivars for response to *Pmm*.

The linear regression lines are measures of the relation of fungal virulence and cultivar reaction. Therefore, it is proposed that cultivars showing no response (ie, resistant) to isolates with varied degrees of virulence would have b values either equal to or close to zero. Subsequently, the results showed that the only cultivars to fit this criteria would be Answer, Epic, Peak, and Trident (Table 4). The first stability parameter (S_d^2) measures the deviations from regression lines. It is proposed that the cultivar with the smallest amount of variability around the regression line should be considered the most stable for its resistance to *Pmm*. However, neither pooled deviations (Table 3) nor any of the cultivars' deviations from regression were significantly different from zero

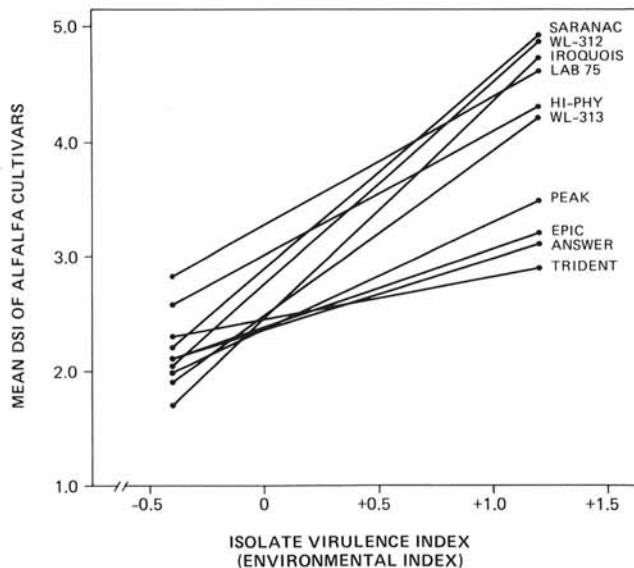


Fig. 1. The response of 10 alfalfa cultivars to isolates of *Phytophthora megasperma* with differential levels of virulence. Isolate virulence index was calculated by subtracting the grand mean from the mean disease severity index (DSI) of the j th isolate. The mean DSI values of alfalfa cultivars were based on the average of each cultivar-isolate combination. The mean DSI values of each cultivar were regressed against the isolate virulence indexes. Isolate virulence index for each isolate was calculated by subtracting the grand mean from the mean DSI of that isolate over all cultivars.

(Table 4). Hence, this parameter cannot be used with that set of data to differentiate among cultivars for stability of resistance.

Another stability parameter that was calculated is the coefficient of determination (r^2). It is worth noting that S_d^2 should decrease whenever r^2 increases (Table 4). As shown in Table 5, the S_d^2 and r^2 values were significantly, negatively correlated. Cultivars Answer, Epic, Peak, and Trident, which had the lowest responses to the isolates of *Pmm*, yielded the lowest r^2 values, i.e., 0.44, 0.51, 0.66, and 0.45, respectively. Also, r^2 was significantly correlated with the response mean \bar{x} ($r = 0.572^*$) which indicated that as DSI

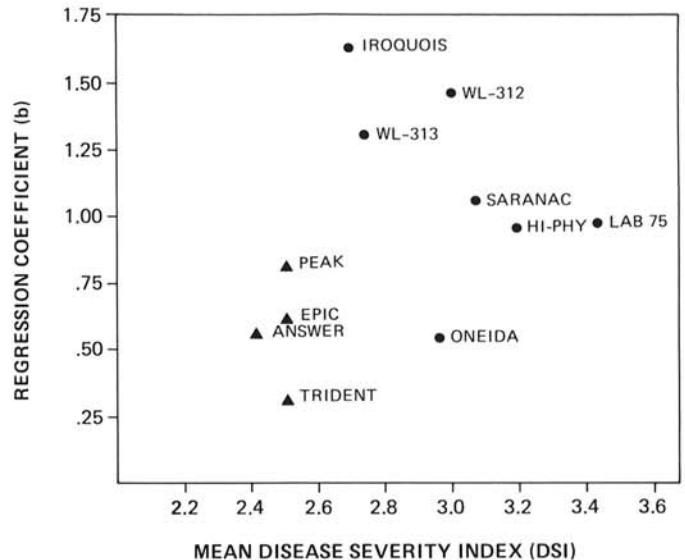


Fig. 2. The relation of mean disease severity index (DSI) and the response of alfalfa cultivars as measured by their regression coefficients. For all cultivars, S_d^2 was not significant. The regression coefficient did not differ significantly from zero for cultivars indicated by \blacktriangle . Mean DSI was calculated for each cultivar when inoculated with all the isolates. The mean DSI values of each cultivar were regressed against the isolate virulence indexes. Isolate virulence index for each isolate was calculated for any given cultivar, by subtracting the grand mean from the mean DSI of that isolate over all cultivars.

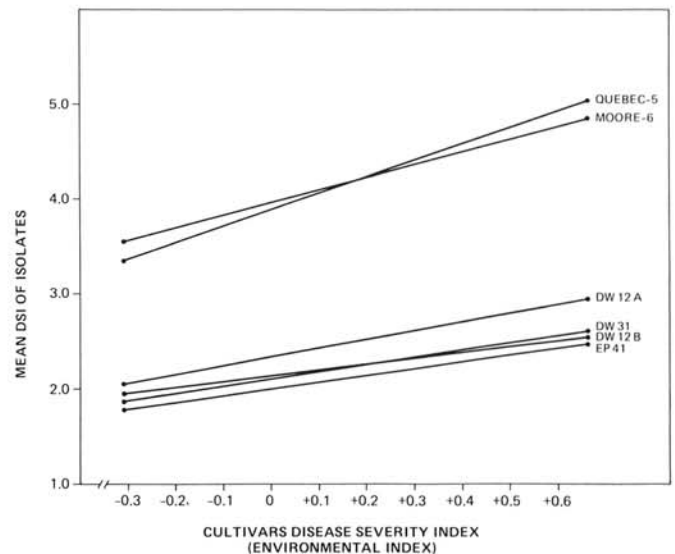


Fig. 3. The response of six isolates of *Phytophthora megasperma* expressed as mean disease severity index (DSI) on 15 alfalfa cultivars. Cultivar DSI was calculated by subtracting the grand mean from the mean DSI of the i th cultivar over all isolates. Mean DSI values of isolates were based on the average of each isolate-cultivar combination.

increased (ie, susceptibility to *Pmm*), r^2 also tended to increase and hence, S_d^2 decreased. In other words, decreasing DSI (higher resistance) seems to be associated with lower stability (higher S_d^2 and lower r^2). This is understandable for PRR resistance in alfalfa. Generally, within any cultivar the proportion of resistant plants ranges from 30–70% (19,23). Therefore, S_d^2 values will be lower and r^2 values will be higher on susceptible cultivars. On the other hand, the resistant cultivars with their mixture of resistant and susceptible plants will be expected to produce higher S_d^2 and lower r^2 values.

The results of the combined regression analysis of variance for DSI showed that $P \times C$ interactions and P (linear) mean squares were significant (Table 3). However, the $P \times C$ (linear) and the pooled deviations were not significant when tested against the pooled error. This revealed that the fitted regression lines of the six isolates had the same slope (ie, response) and that there was no difference in their mode of action on the alfalfa cultivars. There was no departure from linearity, therefore, $P \times C$ interactions could be predicted from the linear regressions of the isolates on the cultivar's index. The most virulent isolates in this case should have a high DSI mean and regression coefficient of $b = 1$. This is different from the criteria used when cultivars were considered. It implies that when virulence is stable it is expressed on all cultivars. For all six isolates, $b = 1$ (Table 4), which indicates that all isolates gave the same response, i.e., they inflicted the greatest damage on plants with the least resistance. However, it is apparent from Fig. 3 that there are two levels of virulence, with Moore-6 and Quebec-5 being more virulent than EP41, DW31, DW12A, and DW12B. The R_1 and R_2 response parameters were not significantly correlated (Table 5), but there appears to be a trend towards a positive relationship.

The isolates did not vary in their stability parameter (S_d^2) since they were all estimated at zero, which was expected because the pooled deviation mean squares were not significant (Table 3). The two most virulent strains have higher r^2 values than the other isolates (Table 4). The S_d^2 and r^2 values were not significantly correlated, but appeared to be negatively related. Therefore, it seems that S_d^2 would be more appropriate in assessing stability because r^2 values were significantly correlated with \bar{x} and b .

The importance of interactions affecting infection by *Pmm* is emphasized in this work and the application of the $G \times E$ analysis to their study is demonstrated. By this method, it is apparent that selection of alfalfa plants should be done by using highly virulent isolates. This is a fact that is recognized by all plant breeders. However, those isolates should be tested for virulence not only on susceptible cultivars (11) but on resistant ones, too. Also, it is important that increasing the level of resistance to *Pmm* in alfalfa cultivars resulted in increased stability of their response to isolates of varying virulence. The analysis revealed that isolates of the pathogen are stable in their performance, whether highly or moderately virulent. Variation in disease response depends upon the alfalfa plant itself. Since the $C \times P$ (linear) alone is significant, all the cultivar \times isolate interactions could be predicted from the linear regressions on the isolates' index. The regression coefficient (b) is a convenient measure of the relative sensitivity of the cultivars to different isolates of *Pmm*. There are two aspects that must be considered jointly in deciding which is the best alfalfa cultivar, namely, a low DSI (performance) and sensitivity to different levels of virulence (stability). The regression analysis has separated the alfalfa cultivars which are more resistant and stable. It is essential that breeding material be assessed at the outset for their relative mean performance, stability to different isolates of *Pmm*, and to be reassessed at appropriate stages throughout the breeding program. Our own results suggest that a relatively small, controlled experiment involving a few isolates will provide reliable information on both performance and stability. In practice, therefore, a sample of *Pmm* chosen from within the range normally available in the area of adaptation should be used to screen alfalfa genotypes. This approach is an addition to the plant breeders'

techniques and, if used correctly, should facilitate a better decision-making process in a particular breeding program.

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