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**American Pomological Society U. P. Hedrick Award for a research paper by a student: (1990 First Place Award)**

# Comparison of Rating Methods for Bacterial Spot Resistance in Japanese-Type Plum[1]

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## Abstract

Rating methods were compared in field experiments to determine usefulness in breeding bacterial spot [*Xanthomonas campestris* pv. *pruni* (Smith) Dye] resistant plums (*Prunus salicina* Lindl. and hybrids). Methods compared were measurements of number, size and incidence of leaf spots, percentage affected leaf area and stem canker length. Measurements of spot size and spot number were equal in repeatability and overall correlation with other rating methods but the correlations between these methods were moderately low (r = 0.54 to 0.59) indicating that they may be under separate genetic control and that ranking of cultivars will change with the rating method. The measurement of percentage affected leaf area by use of photographic standards was least repeatable (t = 0.42) and stem canker length was most repeatable (t = 0.58). The moderately high broad sense heritabilities (0.42 to 0.58) obtained for all rating methods indicates improvement of bacterial spot resistance should be possible.


Bacterial spot [*Xanthomonas campestris* pv. *pruni* (Smith) Dye] in Japanese-type plums (*Prunus salicina* Lindl. and hybrids) can be expressed as leaf spots, fruit spots, or stem cankers (3). Incidence in one organ does not necessarily relate to incidence in another for the same genotype (4) and so ranking of cultivar susceptibility can alter depending on the character measured. We are measuring resistance to leaf spots and stem cankers to determine the genetic transmission of these traits. The present study was to determine the most useful method of rating artificially and naturally inoculated trees in the field.

## Methods

### Experiment 1—Rating of naturally infected plum seedlings.

Fifteen plum seedlings which each differed in susceptibility to bacterial spot were planted in a high density nursery system (6) and rated in September 1989 when 18 months old. The trees were naturally infected with the bacterial spot pathogen and leaf infection was rated twice by each of 9 rating methods. The methods are described in Table 1 and consist of 3 measures of the incidence of leaf spots (I1, I2, I3), 3 measures of the number of spots per leaf (N1, N2, N3), and 3 measures of spot size (L1, L2, L3). Seedlings were ranked from 1 to 15 by each of the 9 methods and Spearmans correlation was used to compare rankings by the different methods.

### Experiment 2—Rating of artifically inoculated, grafted plum clones.

Thirty-nine plum genotypes consisting of cultivars and breeding selections were propagated on seedling peach rootstock and planted at Gainesville, Florida in a high density nursery system at spacings of 30 cm by 90 cm. The experimental design was completely randomized with 4 ramets per genotype. Trees were summer budded in May 1989 and transplanted when dormant in January 1990. Trees were irrigated with overhead sprinklers and were not sprayed with any bacteri-

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cides. Each tree was inoculated by plunging and swirling the top 10 leaves on one branch into a suspension of $2.5 \times 10^8$ bacterial cells per ml of *X. campestris* pv. *pruni* strain F89-1 in early June and by injecting, with a 26-gauge needle and syringe, the same inoculum into 3 sites within the top 15 cm of one stem.

The rating methods consisted of counting the number of spots per leaf on the 2 leaves with highest incidence of disease 2 weeks after inoculation; estimating the percentage of diseased leaf area via standardized photographs (Fig. 1) at 6 weeks after inoculation; measuring the length of the largest spots running along the veins, or the diameter of the largest discrete spot at 4 weeks after inoculation; and measuring the length of stem cankers at the injection sites at 7 weeks after inoculation (Table 1). All data were analyzed after a log plus one transform and the variances were estimated using the SAS VARCOMP procedure (5).

## Results and Discussion

### Experiment 1—Rating of naturally infected plum seedlings

It is difficult to compare rating systems when there is no well defined standard because the ranking of methods can alter depending on which standard is chosen as a yardstick. For example in Table 2 the method L2 rates highly when compared with I1 ($r = 0.79$) but lower when compared to N1 ($r = 0.44$). To overcome this problem an overall measure of the value of each of the 9 rating methods was obtained by calculating the average correlation coefficient of a method with all other methods i.e. by calculating marginal means from the correlation matrix (Table 2). There were no significant differences among the methods by this criterion, but there was a trend of higher correlations for samples of most severely affected leaves than for random or systematic samples i.e. L1 and L2 compared to L3 and N3 compared to N1. It thus

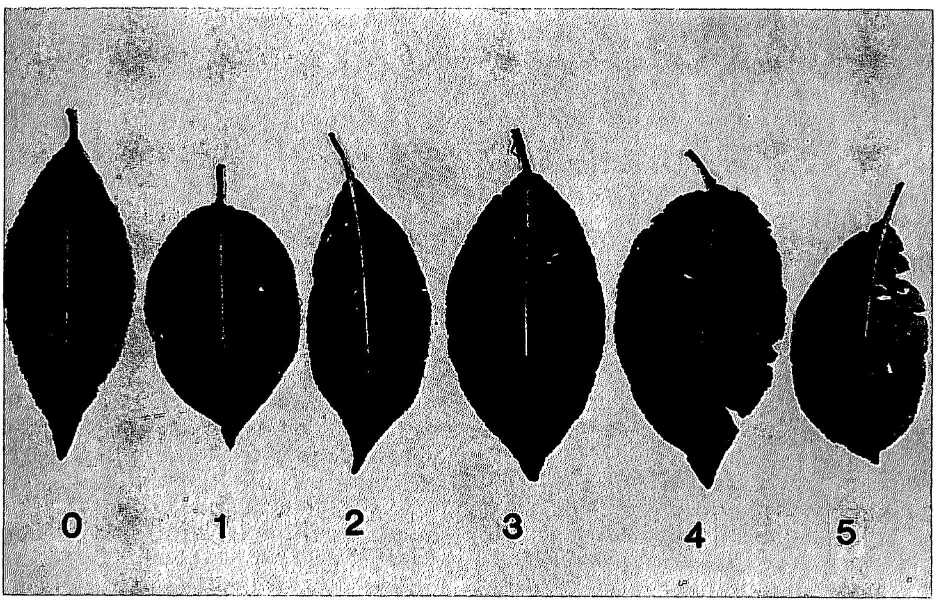


**Figure 1. Photograph standards for estimating percentage leaf area affected by bacterial spot, averaged on worst 2 leaves. (0 = no disease; 1 = up to 1% leaf area affected; 2 = 1-5%; 3 = 6-10%; 4 = 11-15%; 5 = over 15%).**

**Table 1. Explanation of methods used in rating plum resistance to bacterial spot.**

| Symbol | Meaning |
|---|---|
| **Experiment 1—Rating of naturally infected plum seedlings** | |
| I1 | Incidence of infected leaves on 0 to 5 scale (adapted from Werner et al. 1986). |
| I2 | Incidence of infected leaves measured by counting 20 leaves at 10 cm intervals. |
| I3 | Incidence of infected leaves measured by counting the first 20 leaves on 3 separate shoots. |
| N1 | Number of spots per leaf on the 15th leaf on 5 branches. |
| N2 | Number of spots per leaf on the leaf 14 cm from the tip on 5 branches. |
| N3 | Number of spots per leaf on the 3 most severely affected leaves. |
| L1 | Diameter (mm) of the 5 largest discrete spots. |
| L2 | Diameter (mm) of the 5 largest spots along veins. |
| L3 | Diameter (mm) of 5 randomly chosen spots. |
| **Experiment 2—Rating of artificially inoculated, grafted plum clones.** | |
| SN1 | Number of spots on worst leaf. |
| SN2 | Mean number of spots on worst 2 leaves. |
| PHOTO | % diseased leaf area estimated by photographs. |
| SL1 | Maximum spot length (mm) along vein. |
| SL2 | Mean of spot length (mm) of 2 largest spots along vein. |
| SD | Maximum spot diameter (mm) of discrete lesion. |
| CL1 | Basal stem canker length (mm). |
| CL2 | Mean of 2 stem canker lengths (mm). |
| CL3 | Mean of 3 stem canker lengths (mm). |

appears that rating of most severely rather than randomly sampled leaves is an acceptable method. Spot size correlations were generally higher than those for spot number but the differences were not significant and so there does not appear to be any advantage in measuring spot number rather than spot size on terms of the overall correlation coefficients.

**Experiment 2—Rating of artifically inoculated, grafted plum clones**

The value of a rating system depends on its repeatability, ability to detect differences and time to accomplish in the field. Genetic gain in a breeding program will be strongly affected by rating method via influence on heritability. The repeatability of the 9 methods was measured by the intraclass correlation (t) which specifies the proportion of variation between genotypes in relation to the total variation (7). Higher t values represent less variability between ramets within the one genotype and so greater repeatability. There was a trend of increasing t values with increasing numbers of observations i.e. SN2 > SN1, SL2 > SL1, and CL3 > CL2 > CL1 (Table 3). Stem cankers were generally easier to measure than leaf spots and therefore had higher repeatabilities. The repeatability for measuring leaf spot number (SN1 and SN2) was not significantly different from the repeatability for measuring leaf spot size (SL1 and SL2), and thus there is no advantage in one method over the other. The photo method had the lowest repeatability which may be related to: (1) inability of the observer to relate % leaf infection to the photograph standard, or (2) the probable existence of 2 or more types of resistance i.e. leaf spot size and leaf spot number.

All correlations between leaf rating systems were highly significant ($P < 0.01$) but accounted for only a small to medium proportion of the variation when the methods measuring spot size, spot number and the photograph system were compared (Table 4). The lowest coefficient of determination was $R^2 = 0.29$ for SN2 with SD and the highest was $R^2 = 0.67$ for PHOTO with SL2. This would indicate that the ranking of cultivars alters depending on whether the disease is measured by

Table 2. Spearmans correlations between the 9 methods for rating bacterial spot resistance of naturally infected plum seedlings (Experiment 1).

| Method[1] | I2 | I3 | N1 | N2 | N3 | L1 | L2 | L3 | Mean |
|---|---|---|---|---|---|---|---|---|---|
| I1 | 0.79 | 0.54 | 0.52 | 0.61 | 0.58 | 0.60 | 0.79 | 0.53 | 0.62 |
| I2 | | 0.61 | 0.63 | 0.66 | 0.60 | 0.65 | 0.72 | 0.55 | 0.65 |
| I3 | | | 0.67 | 0.59 | 0.41 | 0.56 | 0.56 | 0.44 | 0.55 |
| N1 | | | | 0.41 | 0.44 | 0.59 | 0.44 | 0.58 | 0.54 |
| N2 | | | | | 0.54 | 0.66 | 0.55 | 0.43 | 0.56 |
| N3 | | | | | | 0.76 | 0.61 | 0.67 | 0.58 |
| L1 | | | | | | | 0.72 | 0.64 | 0.65 |
| L2 | | | | | | | | 0.64 | 0.63 |
| L3 | | | | | | | | | 0.56 |

All correlations differ significantly from zero at $P < 0.05$.
[1]See Table 1 for explanation of method symbols.

spot number or spot size. This was confirmed with a Spearmans ranked correlation test which showed the correlation of genotypes ranked from 1 through to 39 by the different methods spot number (SN2) and spot size (SL2) was $r_s = 0.49 \pm 0.14$. The moderately low correlations ($r = 0.54$ to $0.59$) between ratings of spot size and spot number are probably because these traits are controlled by different genes.

The high correlations between SN1 and SN2 ($r = 0.99$); SL1 and SL2 ($r = 0.99$); and among the CL measurements ($r = 0.92$ to $0.98$) were expected as they are correlations between measurements of the same traits but with varying degrees of replication. By similar reasoning SD was more related to SL ($r = 0.74$ and $0.75$) than to SN ($r = 0.54$) because SD and SL are both measuring the trait of lesion expansion.

Table 3. Estimates of variance components and repeatability for the 9 methods used to rate bacterial spot resistance of artifically inoculated, grafted plum clones (experiment 2).

| Method[1] | $V_B$ | $V_w$ | t | s.e. |
|---|---|---|---|---|
| SN1 | 0.360 | 0.408 | 0.47 | 0.09 |
| SN2 | 0.387 | 0.399 | 0.49 | 0.08 |
| PHOTO | 0.038 | 0.051 | 0.42 | 0.09 |
| SL1 | 0.161 | 0.191 | 0.46 | 0.08 |
| SL2 | 0.163 | 0.158 | 0.51 | 0.08 |
| SD | 0.040 | 0.041 | 0.49 | 0.08 |
| CL1 | 0.112 | 0.133 | 0.46 | 0.08 |
| CL2 | 0.132 | 0.136 | 0.49 | 0.08 |
| CL3 | 0.124 | 0.089 | 0.58 | 0.08 |

[1]See Table 1 for explanation of symbols.
$V_B$ = between genotype variance.
$V_w$ = within genotype variance.
t = intraclass correlation (repeatability).

The intraclass correlation is also called the clonal repeatability and can be used as a measure of heritability in the broad sense (1). The between genotype variance will be an overestimate of total genetic variance to the extent that some environmental effects may be transmitted to clonal descendants (2). Moderately high broad sense heritability estimates (0.42 to 0.58) were obtained in this study using all rating methods which indicates that about 50% of the variability is due to genetic causes and is potentially useful for improvement of bacterial spot resistance in plums. Further studies are required to determine what proportion of this genetic variability is due to additive gene action and therefore useful in a phenotypic recurrent mass selection program. More rapid gain is expected for resistance to stem cankers than to leaf spots because of the higher broad sense heritability estimates.

In this study the severity of leaf infection after artificial inoculation was equally well measured by spot number and by spot size but a combination of the two, as obtained by percentage affected leaf area, was less repeatable. The moderately low correlation between measures of spot number and

**Table 4. Correlations between the 9 methods for rating bacterial spot resistance of artificially inoculated, grafted plum clones (experiment 2).**

| Method[1] | SN2 | PHOTO | SL1 | SL2 | SD | CL1 | CL2 | CL3 |
|---|---|---|---|---|---|---|---|---|
| SN1 | 0.99 | 0.63 | 0.59 | 0.58 | 0.54 | 0.49 | 0.58 | 0.53 |
| SN2 | | 0.63 | 0.58 | 0.57 | 0.54 | 0.48 | 0.58 | 0.53 |
| PHOTO | | | 0.81 | 0.82 | 0.68 | 0.57 | 0.58 | 0.60 |
| SL1 | | | | 0.99 | 0.74 | 0.56 | 0.58 | 0.59 |
| SL2 | | | | | 0.75 | 0.54 | 0.56 | 0.57 |
| SD | | | | | | 0.52 | 0.54 | 0.51 |
| CL1 | | | | | | | 0.95 | 0.92 |
| CL2 | | | | | | | | 0.98 |

All correlations differed significantly from zero at $P < 0.05$.
[1]See Table 1 for explanation of method symbols.

spot size indicates that each trait requires individual selection but also that selection of one trait should result in improvement of the other. Further studies are required to investigate the genotypic and environmental correlations of these traits. After natural progression of the disease the incidence of leaf spot infection was also a useful measure of disease resistance. Resistance to stem cankers was measured simply and with the highest repeatability by the average length of 3 artificially inoculated cankers.

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# Comparison of Micropropagated and Runner Propagated Strawberry

A recent report on the performance of 'Olympus' strawberries propagated through tissue culture or by runners showed that yield of micropropagated plants was not greater than plants from runners. Significant variability existed from the subclones from micropropagation with the highest yielding subclone. However after runner propagation for 4 years, selected subclones showed no difference in yield. The difference among subclones of 'Olympus' were not stable and were most likely transient response to the micropropagation environment and not due to genetic changes.

From: Moore et al. 1991. Field Performance of 'Olympus' Strawberry Subclones. HortScience 26(2): 192-194.