Techniques for Evaluating Sugarbeet for Resistance to Cercospora beticola in the Field[1]

E. G. Ruppel and J. O. Gaskill[2]

Received for publication December 18, 1970

Evaluations of sugarbeet (*Beta vulgaris* L.) breeding material and varieties in the field for resistance to leaf spot, incited by *Cercospora beticola* Sacc., can best be done under a moderately severe and uniform disease epiphytotic. Artificial inoculation and environmental conditions favoring the pathogen are necessary for consistent development of such an epiphytotic.

Since 1956, we have successfully employed essentially the same techniques to create epiphytotics of leaf spot in our nurseries near Fort Collins, Colorado. Frequent inquiries concerning techniques prompted us to relate the following details of our methods. The techniques may be applicable in research on similar leaf diseases of other crops.

Source of Inoculum

Each year, buffer rows of sugarbeet in the evaluation nursery are inoculated in addition to the material to be evaluated. To minimize the possibility of strain selection within the fungus, four lines of sugarbeet are used as buffer rows. Currently, these include moderately leaf-spot-resistant GW 674-56C and SP 5481-0, highly resistant SP 5822-0, and highly susceptible R & G Pioneer.

Approximately 6 to 8 weeks after inoculation, when the epiphytotic is at its peak and the fungus is sporulating profusely, infected leaves are harvested from the buffer rows. These leaves are dried in an open shed and stored in burlap bags in an unheated, well-ventilated building until the following year. Presently, 2.6 kg of dried leaves of GW 674-56C, 1.9 kg each of SP 5481-0 and SP 5822-0, and 1.3 kg of R & G Pioneer are used to make 50 gal of inoculum.

Inoculum Preparation

Aliquots of dried leaves are wetted and rubbed together by hand in a galvanized tub containing 13 gal of water. Periodically, the water is squeezed from the leaves and they are removed to make room for additional dry leaves. The rubbing is con-

[1]Joint contribution of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, the Colorado State University Experiment Station, and the Beet Sugar Development Foundation. Publication approved by the Director, Colorado State University Experiment Station, as Scientific Series No. 1581.

[2]Research Plant Pathologists, Crops Research Laboratory, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Fort Collins, Colorado 80521.

tinued until the above amounts of leaves have been processed. After most of the wetted leaves have been removed, the suspension is brought up to 12 gal with water and passed through a 60-mesh brass dairy screen to remove leaf debris. This constitutes the stock spore suspension. For inoculum, the suspension is diluted to 50 gal with water.

In practice, the number of 50-gal batches of inoculum needed is predetermined, and that number of tubs is used for the wetting and rubbing process. Rubbing of leaves can be done in any tub without regard to the source beet line. Final batches of stock spore suspensions must be thoroughly mixed to assure uniformity. Inoculum is used immediately after preparation.

Inoculum Application

The inoculum is placed in a 55-gal drum mounted on a Farmall-A tractor.[3] Spraying is done at 100 psi with the tractor driven at full throttle in second gear (ca. 3.5 mph). The spray boom, mounted at the rear, is equipped with nozzles to cover four rows of beets with three nozzles per row (Figure 1). The nozzles are positioned to spray the center (5X nozzle) and both sides (10X nozzles) of each row. Approximately 50 gal of inoculum are applied per acre.

Figure 1.—Application of *Cercospora beticola* inoculum in leaf spot nursery.

Inoculations with *Cercospora* at Fort Collins usually are conducted in early July, approximately one month after thinning. At this time, the beets are about 2 months old, and daily mean temperatures are high enough to promote infection by the fungus.

Post-inoculation Irrigation

To assure adequate humidity and moisture for spore germ-

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

ination and penetration, the foliage of inoculated beets is kept continually wet for two to three days immediately after inoculation by intermittent overhead sprinkling from 8:00 A.M. until sundown. Sprinkling in this manner is resumed about 2 weeks later, and usually is performed on 2 or 3 nonconsecutive days each week until the end of August. The plants are furrow irrigated as needed.

Disease Cycles and Evaluations

The first leaf spots usually appear approximately 12 days after inoculation. Maximum primary leaf spot development is attained about 9 to 12 days later. These leaf spots then serve as the source of inoculum for additional disease cycles which are promoted by the sprinkling regime described above.

The most reliable disease ratings usually are made approximately 5 to 8 weeks after inoculation. It is important that these ratings be made when the epiphytotic is at or very near its peak, not after maximum symptoms in susceptible lines have begun to decline. In some years, disease ratings also are made on symptoms of primary infection, about 3 weeks after inoculation. These ratings, though not as reliable as later ratings, are made as a precaution against the loss of foliage due to hail or other unpredictable causes.

Our ratings are based on a scale of 0 to 10, with 0 = no apparent infection and 10 = complete defoliation. In practice, we have not encountered a 0 or 10 rating. The range of ratings actually used (i.e. 1 to 9, inclusive) has proved to be quite practical as a means of recording varying degrees of resistance or susceptibility among plots and among individual plants within plots (1,2)[4]. A contrast of resistant and susceptible varieties is shown in Figure 2. The consistency and reliability of our method

Figure 2.—*Cercospora* resistant and susceptible sugarbeet varieties in leaf spot nursery.

[4] Numbers in parentheses refer to Literature Cited.

is exemplified by data presented in Table 1. The repeating lines of sugarbeet (US 201, SP 5822-0, R & G Pioneer, 52-334) were similarly ranked according to leaf spot resistance in both years.

Table 1.—Evaluations of leaf spot resistance in several lines of sugarbeet in 1969 and 1970 at Fort Collins, Colorado.

Line	Mean leaf spot ratings[1]	
	1969[2]	1970[3]
US 201	1.5 a	1.3 a
FC (504 × 502/2) × SP 6322-0	—	1.8 a
SP 5822-0	1.8 a	3.0 b
GWI-29	3.3 b	—
52-305 CMS	3.5 b	—
US H9B	—	5.7 c
52-305 CMS × 52-407, F_1	5.3 c	—
R & G Pioneer	5.3 c	7.0 d
52-305 CMS × 52-407, F_1	5.8 c	—
52-334	7.0 d	7.3 d
General mean	4.2	4.4
F (Lines)	45.59	74.82
F (required at 1% level)	3.65	5.64
C.V. (%)	13.9	12.1

[1] Rating based on a scale of 0 to 10, with 0 = no apparent infection and 10 = complete defoliation; means followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.
[2] Means of four replications.
[3] Means of three replications.

We have found that the position of the sun or the amount of available light can affect the appearance of the infected plants. Therefore, we attempt to rate whole experiments, or at least complete replications, under similar lighting conditions. On sunny days we prefer to conduct evaluations with the sun at our back.

Wide variations in plot size and shape and in number of replications are dictated by the requirements of different experiments, and by seed supplies and available space (1,2). Two-row plots are preferable to 1-row plots for varietal or line evaluations. The principal advantage in the use of 2-row plots is that ratings can be made without interference of leaves from plants in adjacent plots. The plot configuration most commonly used in our current work is 2 rows × 12 feet, with rows 22 inches apart.

Frequently it is desired to select individual plants for leaf spot resistance in segregating populations without use of individual-plant ratings. For this purpose, we use 6-ft bamboo canes to mark the location of plants that appear most resistant when the epiphytotic reaches a severe stage. These plants are re-examined at weekly intervals for a period of 2 or more weeks after marking, and canes are removed from plants that appear to have insufficient resistance. No additional plants are marked during these re-examinations.

Spore Quantity and Quality

The quantity of spores in our inoculum varies from year to year depending upon the number of spores on the source leaves when they are harvested. In 1969, for example, we applied about 2.5×10^5 spores per linear foot of row, whereas in 1970 over 1.7×10^6 spores were applied per foot. A satisfactory epiphytotic developed in both years. As expected, primary infection was more severe in 1970.

In 1970, samples were taken of stock spore suspension, inoculum at the midpoint of application, and inoculum at the end of application. Spore counts indicated a mean of approximately 99.3×10^4 spores/cc in the stock suspension and about 21 to 23×10^4 spores/cc in the inoculum samples. Viability tests indicated about 2% germination of spores in the stock suspension in contrast to 49% in the inoculum samples. Apparently, the concentrated suspension contained an intolerable amount of a substance(s) that inhibited spore germination.

Conclusion

The above techniques for creating an epiphytotic of leaf spot in the field, and for evaluating resistance of sugarbeet lines and individual plants, are characterized by their simplicity, reliability, and low cost. Their effectiveness in the development of breeding lines and hybrids with high resistance to leaf spot, in combination with other desirable characters, has been proven by experience at Fort Collins.

Summary

Techniques used in the field at Fort Collins, Colorado, for creating uniform epiphytotics of *Cercospora* leaf spot and for evaluating resistance of lines and individual plants may be summarized as follows:

1. Dried, *Cercospora*-infected leaves from sugarbeet lines varying widely in degree of resistance are used for inoculum preparation.
2. Source leaves are wetted and rubbed together by hand in water to free spores. The resultant spore suspensions are diluted with water and sprayed on beet foliage at 100 psi and a rate of approximately 50 gal/acre.
3. Foliage is kept wet for 2 to 3 days after inoculation by intermittent overhead sprinkling.
4. Sprinkling for 2 or 3 days a week is resumed soon after the symptoms of primary infection appear (ca. 2 weeks after inoculation).
5. The most reliable disease ratings usually are made when the epiphytotic is at its peak, about 5 to 8 weeks after inoculation. By this time disease intensity has advanced

substantially beyond that resulting from primary infection.

6. Ratings are based on a scale of 0 to 10, with 0 = no apparent infection and 10 = complete defoliation.
7. Since amount and quality of light affect the appearance of infected plants, whole experiments or complete replications are rated under similar lighting conditions.
8. Six-foot bamboo canes are used to stake individual resistant plants in segregating populations for selection purposes. Staking is performed when the epiphytotic reaches a severe stage. Subsequently, the staked plants are reexamined, and canes are removed from those individuals that appear to have insufficient resistance.

Number of spores applied per linear foot of row of beets has varied from 2.5×10^5 in 1969 to 1.7×10^6 in 1970. Satisfactory epiphytotics occurred in both years.

Spore counts in 1970 of stock suspension and diluted inoculum indicate 99.3×10^4 and 21 to 23 $\times 10^4$ spores/cc, respectively. Viability tests indicated only 2% spore germination in the stock suspension, whereas 49% of the spores germinated in the more dilute inoculum.

Literature Cited

(1) GASKILL, J. O., C. L. SCHNEIDER, A. M. MURPHY, and G. E. COE. 1967. Breeding for combined resistance to leaf spot and curly top in sugar beet. J. Am. Soc. Sugar Beet Technol. 14 (6): 518-537.

(2) SMITH, G. A. and J. O. GASKILL. 1970. Inheritance of resistance to *Cercospora* leaf spot in sugarbeet. J. Am. Soc. Sugar Beet Technol. 16 (2): 172-180.