

EFFECT OF PRIOR INOCULATION OF LEAVES OF DRY BEANS WITH THE
COMMON BLIGHT PATHOGEN ON THE DISEASE REACTION OF
SUBSEQUENTLY INOCULATED LEAVES AND PODS AND THE REACTION OF
DETACHED VERSUS ATTACHED PODS

H.M. Ariyaratne¹, D. P. Coyne¹, Anne K. Vidaver², and K. M. Eskridge³
Departments of Horticulture¹, Plant Pathology², and Biometry³, University of Nebraska,
Lincoln, NE 68583-0724

Low heritability estimates of the leaf and pod reaction to *Xanthomonas campestris* pv *phaseoli* (Smith) Dye (*Xcp*) in dry beans (*Phaseolus vulgaris*) were reported by several workers (Coyne et al., Proc. Amer. Soc. Hort. Sci. 86:373-379, 1965; Aggour & Coyne, J. Amer. Soc. Hort. Sci. 114(5):828-833, 1989; Arnaud-Santana et al., J. Amer. Soc. Hort. Sci. 119(1):116-121, 1994). Segregating bean progenies often differ in the number of days for flowering and pod development. Varying temperatures occurring at different times during pod development affect reaction of the pods to *Xcp* thus increasing the environment variance leading to lower heritability estimates for the disease reactions. Some workers used the same *Xcp* strain to detect disease reactions in different plant parts such as leaves, canopy and pods (Schuster et al., HortScience 18:901-903, 1983; Silva et al., Theor. Appl. Genet. 78: 619-624, 1989; Arnaud-Santana et al., J. Amer. Soc. Hort. Sci. 119(1):116-121, 1994) but did not determine if prior inoculation of a plant part affected the reaction of later inoculated plant parts (leaves and pods). Induced systemic resistance to pathogens were observed in tobacco inoculated with heat-killed bacteria (Lozano & Sequeira, Phytopathology 60:875-879, 1970) and cucumber with living bacteria (Caruso & Kuć, Physiological Plant Pathology 14:191-201, 1979). It is important to first determine if the reactions of detached pods are similar to attached pods, and also if prior inoculation of leaves with virulent or low virulent strains of *Xcp* would affect subsequent reactions of the leaves and pods. The objectives of this research were to study (a) the effect of initial inoculation of bean leaves with *Xcp* on subsequent inoculated leaf and pod reactions and (b) the *Xcp* reaction in attached bean pods versus detached pods.

Materials and Methods: A split-plot experimental design with two replicates was used, with bean lines as whole-plots, and bacterial inoculations and pod treatments as sub-plots. PC 50 (sus.), XAN 159 (res.), BAC 6 (res.) and HT 7719 (sus.) were grown in the growth chambers under controlled temperature ($24 \pm 2^\circ$ C day/ $21 \pm 2^\circ$ C night) and 12 HR day length. The two *Xcp* strains (10^7 cfu/ml) V3S8 (NE, high virulence) and Xps (NE, low virulence) were used for inoculating bean plants. Experiments were conducted twice.

The following inoculation treatments were used; (a) Inoculation of first trifoliolate, then subsequent inoculations of another trifoliolate (fully expanded behind growing point) and green pods at pod filling stage with a virulent strain (V3S8) and low virulent strain (Xps) separately, (b) Same as above except no inoculation of first trifoliolate, (c) Control: inoculation with buffer only. Two attached pods and two detached pods were inoculated for each treatment. Inoculated detached pods were placed in the sealed 5"X 7" plastic bags punched with two small holes and suspended from a stake supporting the same plant.

The multiple needle method (Andrus, *Phytopathology* 38:757-759, 1948) was used to inoculate leaves. The pods were punctured with a dissecting needle and then a Pipetteman was used for pod inoculations (Arnaud-Santana et al., *J. Amer. Soc. Hort. Sci.* 119(1):116-121, 1994). Leaf and pod disease reactions were recorded 7, 10 and 14 days after inoculations.

Results and Discussion: The ANOVA for disease reactions of the first trifoliolate bean leaves showed significant differences between lines. Differences in leaf reactions to the two strains were detected. V3S8 was more virulent on the first trifoliolate leaves than strain Xps.

The ANOVA for the reactions of fully developed leaves at the pod filling stage indicated significant interactions between lines and inoculations. The interaction occurred because the disease reactions of the two resistant lines were nearly uniform with both strains while the two susceptible lines varied in response to these strains. Strain V3S8 was more virulent than strain Xps. Comparison of reactions to either strain used in inoculations 1 and 2 in all lines showed no significant differences indicating that prior leaf inoculations with *Xcp* had no effect of disease reactions resulting from subsequent inoculations of later developed leaves.

The ANOVA for pod reactions to *Xcp* indicated that there was no significant interactions between lines and pod treatments, inoculations and pod treatments, or lines, inoculations and pod treatments. Detached pods showed similar disease reactions as attached pods. None or slight symptoms on pods were observed in the two resistant lines. A significant interaction occurred between lines and inoculation treatments. Strain V3S8 was more virulent than Xps on the two susceptible lines. The comparison of inoculations with either the virulent or low virulent strains in all lines showed that there were no significant difference between pod reactions of prior leaf inoculated plants and those not receiving a prior leaf inoculation.

No statement regarding induced resistance can be made based on these results because avirulent strains or dead bacterial cells were not used in the prior inoculation. However, the results are useful to breeders because they clearly demonstrate that separate prior inoculations with virulent or low virulent *Xcp* strains do not influence the reactions from subsequent inoculation of different plant parts with *Xcp*.

Conclusions: (1) Inoculation of detached pods with *Xcp* under controlled temperature conditions showed similar disease reactions as attached pods. This inoculation method can be used to reduce environment variance affecting disease reactions and to improve heritability estimates for more effective selection and for better detection of quantitative trait loci (QTL's) with molecular markers if desired. (2) There was no effect of prior inoculation of *Xcp* on the disease reactions of subsequently inoculated leaves and pods of the common bean lines tested. Different plant parts of the same plant of common bean can be used to assess disease reaction to *Xcp* at different or simultaneous times of inoculation without affecting the disease reaction in each plant part.