Comparison of Seed Transmission and Survival of Xanthomonas axonopodis pv. phaseoli and Xanthomonas fuscans subsp. fuscans in Common Bean Seeds

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Xanthomonas axonopodis pv. phaseoli (Smith) Vauterin (Xap) and Xanthomonas fuscans subsp. fuscans Schaad et al. (Xff) cause indistinguishable symptoms known as common bacterial blight of bean (Phaseolus vulgaris L.). Seedborne inoculum is important in the survival and dissemination of these pathogens, and they can be transmitted from seed to growing plants, initiating damaging epidemics (4). Several reports have indicated that Xff strains are generally more pathogenic than Xap strains (3). However, there are no published studies comparing frequencies of seed transmission between the two species. Pathogen incidence on seeds is closely correlated with disease incidence in the seed production field (5), and there is usually a positive correlation between seed symptoms and the population of bacteria per seed. Therefore, the ability of the bacteria to survive on stored seed is an important epidemiological factor; if the two pathogens differ in this characteristic, it could represent a meaningful difference in disease risk associated with seedborne inoculum. In order to compare the seed transmission frequency and storage survival of the two bacteria, five strains of each species from different geographic origins were used to inoculate seeds of a susceptible snap bean genotype (cv. ‘Derby,’ Harris Moran Seed Co., Modesto, CA). Bacterial strains (courtesy of R. Gilbertson, V. Grimault, E. Vavricka, and A. Vidaver) originated from the USA (Michigan, Nebraska, or Wisconsin), Australia, Brazil, France, the Netherlands, or the United Kingdom. Strains were stored on silica beads (Microbank, Austin, TX) by shaking beads in a cell suspension, removing excess fluid, and freezing at -80°C. Bacterial suspensions were prepared from nutrient agar cultures grown at ambient temperatures (21°C to 24°C) and adjusted to the concentration of 10^6 CFU/ml in phosphate buffered saline (PBS). Seeds were immersed in the suspension for 5 min with vacuum and air-dried for 2 h in a biosafety cabinet. Control seeds were treated in the same manner but immersed in PBS only. Seed contamination levels were confirmed by culturing on nutrient agar medium. For each strain, three replicate samples of 25 seeds were planted in Cone-tainers (3.8 by 21 cm), one seed per Cone-tainer, in a greenhouse (24°C to 28°C) with supplemental high-pressure sodium lighting set for a 14 h photoperiod. An equal number of noninoculated seeds also was planted. Emergence was assessed 4-5 days after planting, and seedlings were left to grow another week to allow symptom development. Symptoms of common bacterial blight such as blight spots, brown necrosis on cotyledons, failure of leaf development and leaf lesions were observed and recorded. Infection was confirmed by culturing surface-disinfested tissue from both symptomatic and symptomless seedlings on MT semi-selective medium (1). The experiment was conducted twice. Both species reduced seedling emergence compared to the noninoculated control treatment; emergence for the control seeds was 86.7%, whereas it ranged from 65.3% to 70.7% for Xap-inoculated seeds and from 52.0% to 62.7% for Xff-inoculated
Fig. 1. Greenhouse emergence, disease incidence, and incidence of symptomless infection after inoculation of *Phaseolus vulgaris* (cv. 'Derby') snap bean seeds with five strains of *Xanthomonas axonopodis* pv. *phaseoli* (blue bars) and five strains of *Xanthomonas fuscans* subsp. *fuscans* (red bars). Individual strains are arranged in the same order as in the legend. Emergence of noninoculated control seeds is shown by the black bar. There were no symptoms or pathogen detection in seedlings from noninoculated seeds. Values are based on two experiments, each with three replications of 25 seeds for each strain. Emergence = 100 × number of seedlings emerged / number of seeds planted; disease incidence = 100 × number of seedlings with visual symptoms / number of seedlings emerged; symptomless infection = 100 × number of symptomless seedlings from which pathogen was isolated / number of seedlings emerged. Symptomless infection did not differ significantly among strains or species. Mean separation was conducted by Tukey's test (*P* = 0.05).
seeds (Fig. 1). Xff reduced emergence more than Xap ($P < 0.001$) and the incidence of common bacterial blight symptoms was higher in seedlings from Xff-inoculated seeds than in those from seeds inoculated with Xap ($P < 0.001$). Disease incidence ranged from 49.9% to 61.0% for seedlings from Xap-inoculated seeds and from 42.5% to 82.0% for Xff (Fig. 1). Both pathogens were re-isolated from all symptomatic seedlings and from a small percentage (mean 1.7 to 2.1%) of symptomless seedlings; symptomless infection did not differ significantly among strains or species (Fig. 1). Xff demonstrated a higher frequency of seed-seedling transmission (disease incidence + symptomless infection) than Xap ($P < 0.001$). Seedling infection with Xff was 70.4% across strains, compared to 57.0% for Xap. Four of the five Xff strains had higher disease incidence and transmission frequency than all five Xap strains, while one Xff strain from Nebraska had the lowest values for both variables. Survival (population size) of both species on inoculated, stored seeds was monitored monthly by dilution plating (2) during six months of controlled-environment storage. Over six months, populations in seeds declined from approximately $10^7$ cfu/seed to approximately $10^3$ cfu/seed, and survival did not differ significantly between the two Xanthomonas species. These results indicate that Xff presents a higher level of seed transmission risk in infected seeds of cv. ‘Derby,’ but the two common blight pathogens had similar survival characteristics in seed of this cultivar over the time period studied. Although the results represent a limited number of strains of each species, the higher disease incidence and seed transmission frequency of Xff compared to Xap reinforce the need for seed health tests that can differentiate the two species.

**Literature Cited**