Seed Contamination in Transmission of Halo Blight in Beans

TABLE 1. EFFECT OF METHOD OF INOCULATION ON INFECTION AND SEED TRANSMISSION OF HALO BLIGHT IN TWO VARIETIES OF BEAN PRODUCED WITH FURROW IRRIGATION AT DAVIS, CALIFORNIA

Method of inoculation	Red Kidney			Gallatin 50		
	Number plants*			Number plants*		
	With stem lesions & systemic symptoms	Killed	Seed trans-mission†	With stem lesions & systemic symptoms	Killed	Seed trans-mission†
Seed contaminated with dust	2	0	2/3	1	0	3/3
Cotyledon	257	24	3/3	273	98	3/3
Primary leaf	12	2	3/3	5	3	3/3
Trifoliate leaf	7	0	2/3	0	0	3/3

* Total number of plants from three replicated plots of 110 plants each.
† Positive seed transmission in 100-seed samples mechanically harvested from three replicated plots.

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This paper presents evidence that seed transmission of halo blight can result from infestation as well as infection, and that infestation probably is more important, especially under furrow irrigation in low rainfall areas where secondary spread during the growing season is rare or nil.

HALO BLIGHT OF BEAN (*Phaseolus vulgaris* L.) caused by *Pseudomonas phaseolicola* (Burk.) Dows. has been controlled successfully for many years in the midwestern states and other areas with summer rainfall by using seed produced in the semiarid West where, under normal low rainfall conditions, the absence of splash dispersal precludes or greatly diminishes spread of the causal organism. The rainfall during the growing season in some western areas of bean seed production is occasionally greater than normal—resulting in spread of halo blight and contamination of seed. Severe epidemics have occurred in Wisconsin and other parts of the country in recent years, indicating that some stocks of western-grown seed were contaminated with the halo blight organism.

Halo blight has occurred very rarely in California where the average total rainfall during the growing season (June through September) is very low—less than 0.5 inch. During the past two years, however, the incidence of the disease has increased and, in several instances when seed produced in Idaho was planted in sprinkler-irrigated fields in California, spread occurred and considerable damage was caused. When the same seed stock was planted in furrow-irrigated fields, however, the incidence of disease was very low: only a few plants were found with lesions on the primary leaves or water-soaked lesions at the cotyledonary node, and there was no obvious secondary spread during the growing season. In beans intended for processing this low incidence is of no consequence. However, since some of these furrow-irrigated fields were intended for seed production, tests were made to determine whether the resulting seed was contaminated. Two of three seed lots tested (from a known contaminated seed stock) grown under furrow irrigation in the San Joaquin Valley in 1964 showed positive seed transmission.

Although the halo blight organism can move through the xylem and parenchyma tissues of the bean plant and does produce systemic chlorosis of the upper trifoliate leaves, it may not be present in all tissues that show symptoms. It has been reported that *Pseudomonas phaseolicola* can enter the seed by way of the raphe or the micropyle, and thus can occur in or under the seed coat.

Typical leaf and pod infections occurred in the sprinkler-irrigated plots and were especially abundant close to the sprinkler outlets. This confirms previous observations that sprinkler irrigation provides conditions favorable for spread and infection of wet-weather pathogens that (under furrow irrigation in arid climates) ordinarily spread very little, if at all. Seed was mechanically harvested from these plots and transmission of halo blight was demonstrated consistently when several 100-seed samples were tested. Thus, it is obvious that seed produced under sprinkler irrigation from contaminated seed is quite likely to be infected or contaminated.

The number of plants that developed stem cankers and systemic symptoms, and that were killed by halo blight, was much greater when plants were inoculated in the cotyledonary axis (table 1). Also, following a 0.53-inch rain that occurred on August 8 (a very unusual occurrence at Davis during the summer months), a few lesions developed on pods in close proximity to the cankered stems. The other methods of inoculation resulted in considerable localized infection on inoculated leaves that usually did not cause systemic symtoms, and there was no evidence of leaf or pod spotting resulting from secondary spread. Nevertheless, mechanically harvested seed from plants in practically all the plots was contaminated as indicated by tests of 100-seed samples from each replicate.

Because results from repeated tests had shown that inoculation of halo-blight-free seed with dry pulverized inoculum consistently resulted in seed transmission, an attempt was made to determine whether transmission in seed from these plots was attributable to infection or infestation. One hundred lesion-free pods of Gallatin 50 and Red Kidney were hand-picked from cotyledonary-inoculated, furrow-irrigated plants with obvious stem lesions and systemic symptoms. The pods were allowed to air dry in the laboratory and were surface sterilized by immersion in a 1-in-10 solution of NaOCl bleach for 15 minutes. Seed from each pod was hulled out and planted separately in individual pots of vermiculite. In 466 seeds of Gallatin 50 and 383 seeds of Red Kidney, no transmission occurred, but two 100-seed samples of mechanically harvested seed from these same plots, tested at the same time, showed positive. These results indicated that infection of seed by systemic movement of bacteria did not occur in the samples tested, but it is conceivable that under other conditions seed infection may occur more frequently.

Additional tests were made to determine the amount and consistency of transmission in seeds from pods with visible lesions. Pods with lesions were hand-harvested from sprinkler-irrigated plots. They were allowed to dry and were surface sterilized as before. Each pod was split open and individual seeds were picked out with sterilized forceps and tested separately for seed transmission by planting in vermiculite with five halo-blight-free seeds. Seed from 97 pods were tested and 81% of the pods showed positive transmission from one or more seeds. Fifty-one per cent of the seeds that came from directly underneath or adjacent to a lesion transmitted, while only 18% of the seed removed by the distance of at least one seed from a lesion transmitted. These data indicate that penetration of pods by the bacteria is restricted, and that some seed directly underneath or adjacent to lesions may not be infested or infected. Examination of the inner pod walls underneath several lesions revealed that the bacteria often had not penetrated to the seed cavity thus providing a probable explanation for the lack of transmission from many of the seeds in close proximity to lesions.

Since it appeared that surface contamination of seed is an important source of seed-borne inoculum, a number of tests were made in an attempt to find an effective seed treatment for elimination of this inoculum. Most of the tests were made on seed artificially contaminated with dust because we assumed that this inoculum was surface-borne, whereas naturally contaminated seed might also have had internal infection. Results from these tests (unpublished and Treatment No. 2 in table 2) were quite variable and seed transmission generally was not completely eliminated.

Internal contamination

Since the dust inoculum was not eliminated by any of the treatments, it apparently was not entirely surface-borne. Examination of a number of seeds with a dissecting microscope showed that bits of the dust often were present in the hilum area which has many small vacuoles resembling a sponge. Also in some seeds, but not all, the micropyle was completely open. In adition to this, many seed coats were cracked and split so that the cotyledons were exposed to the exterior. Therefore, tests were made to determine whether surface sterilization would eliminate seed transmission by dust contamination if these openings were eliminated prior to contaminating the seed. Melted vaspar (mixture of equal parts of paraffin and vaseline) was used to cover the hilum, raphe, and micropyle. Intact seeds so covered absorbed water very slowly; thus, all seeds that had wrinkled seed coats after soaking in water or bleach for 15 minutes were eliminated because upon examination they were found to have rifts and splits in the coats. Whereas surface sterilization of dust-contaminated seed reduced, but did not eliminate, seed transmission, it was eliminated when seeds were treated with vaspar and selected to eliminate damaged seed coats prior to contamination. It appears, therefore, that contaminating inoculum can be internal as well as external. Thus, failure to eliminate seed transmission by surface sterilization does not necessarily indicate that the contaminating inoculum came from internal infection that occurred during the growing season.

TABLE 2. EFFECT ON HALO BLIGHT SEED TRANSMISSION OF COVERING THE HILUM RAPHE AND MICROPYLE BEFORE SURFACE STERILIZATION OF SEED ARTIFICIALLY CONTAMINATED WITH DUST INOCULUM

Treatment	Seed transmission (100-seed samples)
1. Dust contaminated and rinsed with water	2/2
2. Dust contaminated and surface sterilized by immersion in 1 in 10 NaOCl bleach solution for 15 minutes	2/5
3. Hilum, raphe, and micropyle covered with vaspar; dust contaminated and rinsed with water	2/2
4. Hilum, raphe, and micropyle covered with vaspar; dust contaminated and surface sterilized by immersion in 1 in 10 NaOCl bleach solution for 15 minutes	0/5
5. Uncontaminated seed (control)	0/6

Discussion

The method that has been used successfully for many years for control of wet-weather pathogens is the use of pathogen-free seed produced in the semiarid West. Even though some sources of western-grown bean seed have been contaminated or infected with halo blight in recent years, there is no reason to abandon a method of control that has proved so effective.

The results of this study show that infestation of bean seed with the halo blight organism can result in seed transmission. Thus, the production of halo-blight-free seed is difficult because seed can become infested from seed-borne infected plants or other sources of inoculum, even though secondary spread did not occur during the growing season. Infestation apparently occurs during threshing, but also could occur during cleaning or other operations after the seed is harvested. Inasmuch as *P. phaseolicola* can survive in infected dust for at least 13 months, contaminated threshing machines and seed-cleaning equipment could be sources of inoculum for seed infestation from one season to the next and possibly longer, as has been found with angular leaf spot of cotton. Therefore, even under the most favorable growing conditions, a seed crop is likely to become contaminated should contaminated or infected seed be planted. Whether two or more seed generations under California conditions are required for elimination of seed transmission has not been determined with certainty, but two seed stocks were apparently disease-free after increase for two years from a known contaminated seed stock. The long history of freedom from the disease in California-produced seed also shows that clean seed can be produced consistently.

Summary

Apparently disease-free seed was obtained by hand picking lesion-free pods from systemically infected plants. Thus, the hand picking of pods from apparently healthy plants in fields where there had been little or no secondary spread should be a sure way of obtaining at least a small nucleus foundation stock of clean seed for increase.

Our results—showing that surface sterilization of artificially infested seed is not completely effective, apparently because the inoculum is not completely external—indicate that chemical treatments should not be relied upon to eliminate either infestation or infection.

Most foreign quarantine regulations and state seed certification programs depend heavily on field inspection for certification of seed lots for freedom from halo blight contamination—a method which may be effective for detecting halo blight in rainy areas or under sprinkler irrigation where secondary spread has occurred abundantly, but is practically worthless when beans are grown under furrow irrigation in a dry climate as in California. For example, several fields observed during 1965 that were planted with contaminated seed showed that, when grown with sprinkler irrigation, infection was usually readily apparent; but with furrow irrigation, detection of disease was extremely difficult. In some cases infection was detected when plants were small (in the first trifoliate leaf stage) but not after the plants were full grown. By this time most of the seed-borne infected plants had died or were stunted and overgrown by normal plants in the row. A more meaningful criterion for certification would be to test for seed transmission after seed processing, including seed treatment, has been completed.

The apparent absence of the disease in fields planted with contaminated seed under arid conditions certainly does not assure freedom from seed transmission of halo blight. Thus, seed producers should not rely on field inspection, or on one-season reproduction under arid conditions, for elimination of halo blight inoculum from seed stocks.

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