

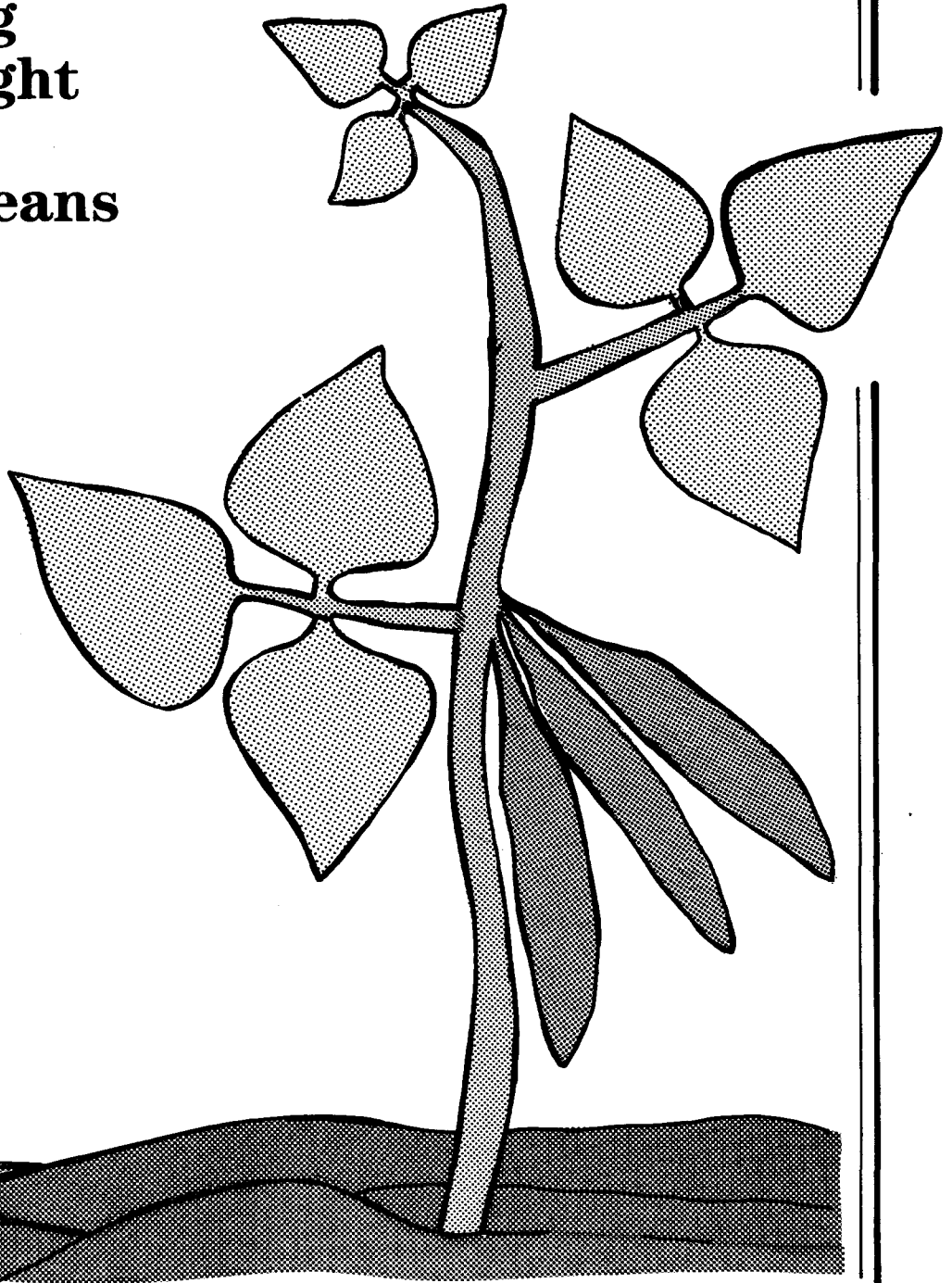


The Dome Test

For Detecting Bacterial Blight Pathogens in Dry Edible Beans

James R. Venette
Plant Pathologist

H. Arthur Lamey
Extension Plant Pathologist



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NDSU EXTENSION SERVICE
North Dakota State University, Fargo, ND 58105

Bacterial pathogens can spread rapidly in a bean crop and can cause serious losses by reducing yield and quality of harvested seeds. Bacterial bean blights are controlled primarily by planting seed that contains few pathogens. Seed produced in the humid Midwest has a greater potential for being contaminated than seed produced in arid western states.

Scientists at the North Dakota Agricultural Experiment Station have developed a rapid and simple test for detecting bacterial pathogens in bean seeds. The seed test allows quality assessment so that seeds with few pathogens can be chosen for planting. The test involves extracting bacteria from seeds, introducing the bacteria into seedlings, then growing the seedlings in plastic domes which create conditions favorable for disease development. The amount of disease in the seedlings is related to numbers of pathogens in the seed. A judgment on the relative risk from planting tested seeds can be made.

What makes bacterial blights different from other bean diseases?

Four major and persistent bean diseases: rust, white mold, root rot, and bacterial blights, are found in North Dakota. Rust is controlled with resistant varieties and relatively inexpensive fungicides. Rust epidemics usually develop slowly and can be monitored. White mold can be controlled with expensive fungicides and with plant resistance coupled with some cultural techniques. Plants are most susceptible to white mold during the blossom period, but spread from one infected plant to another is limited. Root rot pathogens generally build slowly in soils so careful field monitoring and record keeping can help growers identify their least infested soil. Resistance to some of the root pathogens is present in certain varieties, and fungicide seed treatments can reduce early season damage. Cultivation can encourage root growth which reduces root rot damage.

There is no satisfactory control for bacterial blights once they are established in a field. Varieties with resistance to certain bacterial blights have been developed, but most commercially grown varieties are susceptible to one or more of the blight pathogens. Incorporation of resistance genes into commercially acceptable varieties is a lengthy process made difficult by variation in the pathogen population and by different genes controlling numerous aspects of resistance. Copper fungicides might reduce the impact of bacterial blights, but copper sprays have not proven effective in North Dakota. Cultural techniques do little to limit the diseases.

The primary (and almost only) control of bacterial diseases is to keep the pathogens out of the fields. The pathogens do not survive well in soil or rotted debris. These bacteria are predominately restricted to edible beans (*Phaseolus*). Secondary hosts such as weeds and wild plants are minor sources of pathogens in North Dakota. The major method for introducing bacterial pathogens into a field is by contaminated seed.

How much loss will bacterial pathogens cause?

There is no satisfactory answer because it is difficult to determine what constitutes a loss. Bacterial pathogens can cause reduction in yield. They can cause discolored seed which increases dockage. They can cause shrunken seed with reduced weight. Off-type beans are more difficult to market and generally receive lower prices. Contaminated seed beans germinate poorly and can serve as a source of infection for other beans.

Disease-caused yield loss estimates ranging from no apparent loss to total destruction of a field have been made, but supportive information detailing circumstances of the losses is lacking. It is difficult to estimate losses when there are no disease-free plots or fields for comparison.

Some important generalizations can be made: 1) Bacterial disease losses are related to the weather, especially rainfall. Losses are greatest in growing regions with large amounts of rainfall. Contaminated seed grown for several generations in desert environments (supported by surface irrigation) can produce plants with no apparent disease and seed with little contamination. 2) Losses are greatest when the blights affect plants early. Seedlings and rapidly growing plants are most susceptible. In North Dakota, rainfall is mostly in the spring which promotes rapid plant growth and spreads the bacteria. 3) Entire fields can be infected from a few contaminated seeds. An acre of beans with perhaps 70,000 to 100,000 plants can be infected from a dozen or possibly fewer infected plants (providing the weather is conducive for spread). 4) Losses cannot be eliminated by seed treatments because seed treatments cannot eliminate bacteria from seeds. The antibiotic streptomycin is commonly applied in a seed treatment slurry and can reduce bacterial numbers on the outside of bean seed coats, but has little effect on bacteria within the seed. The antibiotic seed treatment is good protection against secondary infection of the seed.

What is meant by bacterial blights of beans?

Beans are susceptible to two "blight" pathogens, one causing halo blight and one causing common blight. A similar bacterial disease is brown spot. Because all of these diseases have many characteristics in common (i.e. they are seedborne, spread by splashing rain, similar host range, etc.) they are sometimes collectively called the blights. Each blight has unique characteristics that help in diagnosing a particular problem (see NDSU Extension Circular PP-576, Dry Edible Bean Diseases).

How does anyone know if bean seed is contaminated with bacterial blight pathogens?

A generalization might be made: clean seed comes from healthy plants. Early growers were urged to plant small plots of beans in different areas and from different sources. They collected the harvest from the healthiest plots and used this for planting the next year's production. While the plan was probably successful in the short term, the process was not efficient and sometimes forced a choice of a "least bad" plot. Growers were also urged to purchase clean seed or quality seed from whatever sources they could find if their own seed was of poor quality.

Seed certification programs were a major advance in assuring growers that the seed they purchased was true to type and from healthy plants. Plant health was determined by trained professionals who inspected each field and made a judgment on the plants' health. Standards varied slightly from state to state. Some states allowed a few plants to be infected with bacterial blight (a tolerance level) while other states demanded that seed come from fields in which absolutely no bacterial blight was detected, generally based on two field inspections.

Depending on weather and other factors such as insect damage, blight symptoms might not be obvious in the field and might be overlooked by even the most diligent inspector. Sometimes seed from apparently healthy plants was sown and the crop was devastated by bacterial blight. It became obvious that some sort of seed test was needed to reinforce field inspection. One of the earlier tests was the "grow out." The plan was simple; grow a sample of the seed one year and if it performed well, it was probably suitable for large plantings the next year. This plan had many difficulties and generally is no longer used in commercial seed production. Variations of the plan are used in quarantine procedures.

Harvested beans from heavily infected fields can appear very different from beans taken from healthy fields. Seedsmen wondered if direct evaluation of seeds prior to planting might be useful in identifying seeds practically free of bacterial pathogens. When seeds from infected plants were hand picked (to remove discolored, shrunken, obviously or even apparently infected seeds) and then grown, bacterial blight was still serious. The conclusion was that clean seed could not be identified visually and that some sort of laboratory test was needed.

Over time, a number of tests to detect seedborne bacterial pathogens in beans have been proposed. The tests vary in their complexity, speed, specificity and sensitivity. There is no general agreement on one test that is best and there is no procedure specified by official seed testing agencies.

If no test is best, then why is the dome test used to evaluate North Dakota beans?

Part of the argument surrounding use of various tests is that the purpose of a test might not be clear. In some cases, procedures might be designed to detect certain specific bacterial pathogens. These tests produce yes-no results; the pathogen is there or it is not. If the test is extremely sensitive, a high percentage may test "yes" and few seeds would be available for growers. If the test is not very sensitive, only the most severely infected seeds would be eliminated and a grower would have increased chance of loss from bacterial blight.

An associated concern is the specificity of a test. It would be desirable to have a test that detected only plant pathogenic bacteria. If a test is not very specific, it may detect plant pathogens along with other nonpathogenic bacteria. Lack of specificity would make more samples of beans appear as if they have pathogens when actually they may not. If a test is too specific, it may detect only a fraction of the pathogen population and seed lots would be declared clean when they actually are contaminated. Complicating specificity is the fact that the pathogens are variable in a number of characteristics and can acquire new genetic information from cohabiting bacteria.

Ideally, we would like an extremely sensitive test that is quantitative; that is, it would show how many of each of the blight-causing bacteria are present in a bean sample. This is difficult, and few of the seed tests are quantitative. Part of the rationale is that standards have (or approach) a "zero balance" for blight bacteria. If any are found, the lot is disqualified. Another part of the rationale is that no one can relate numbers of bacteria in a seed test with performance of that seed in the field. It is known that infected seed planted in an environment conducive for blight development and spread stands a greater chance for significant loss. Quantitation is directly related to the assignment of risk in the use of a tested seed lot.

The dome test is sensitive. NDSU researchers were able to detect about 600 bacteria per milliliter (about one-fifth teaspoon) of soaked-bean suspension. That suspension can contain a number of other bacteria sometimes exceeding a total of 100 million bacteria per milliliter. This would be equivalent to detecting one bacterium from among 167,000 others.

The dome test detects pathogens. The test itself is based on the susceptible response of bean seedlings. The susceptible response is the development of small water-congested spots on bean leaves that mark the areas where bacteria are growing and destroying leaf tissue. These spots are called lesions. The fact that plant pathogenic organisms are causing the bean seedling lesions has been corroborated by other tests.

The dome test is quantitative. The number of lesions that develop on seedlings is proportional to the number of bacteria in the soaked-bean suspension.

How is the dome test used to determine if one lot of seed has more bacterial contamination than another lot of seed?

The purpose of the dome test is not to determine which pathogens are present; rather, it is to evaluate the potential risk from the use of that seed. The comparison is made on the general level of contamination rather than on quantitation of any specific pathogen. In the dome test, the unit that is evaluated for presence of blight bacteria is the primary leaf on the seedlings. When pathogen numbers are low, no or few lesions develop on the primary leaves. When pathogen numbers are high, the lesions can be so

numerous that they make the entire leaf appear water-congested. In the test about 30 seeds are planted, which means that a potential of 60 primary leaves will be evaluated for each dome test.

Are the lesions actually counted?

As the dome test was being developed, numbers of lesions on each leaf were counted. In some cases, 100 or more lesions developed on a single leaf. It soon became apparent that counting lesions on each leaf would be too slow and tedious to be practical. To speed counting, diagrams of leaves with different numbers of lesions were prepared. Each leaf was then compared to the diagram, and the closest lesion count assigned to that leaf. Eight diagrams were prepared and these were numbered 0-7. A leaf equivalent to the 0-numbered diagram would not have any lesions. A leaf that appeared as the diagram numbered 1 would have one lesion, a leaf appearing as diagram 2 would have two to four lesions, etc. A calculation value was assigned for purposes of averaging. The complete relationship is as follows.

Diagram Value	Lesion Range	Calculation Value
0	0	0
1	1	1
2	2-4	3
3	4-9	6
4	9-18	12
5	18-37	25
6	37-75	50
7	75-150	100

Once all the leaves in a dome had been evaluated, the calculation values were averaged. The average calculation value was compared to the lesion range and translated into an equivalent diagram value which is reported as the rating of the dome. In written reports to growers, the average value and the equivalent diagram value (the dome rating) are reported.

Let's follow an example using only four leaves for simplicity. The first leaf looked like diagram #4, the second leaf looked like diagram #5, the third leaf looked like diagram #6, and the last leaf looked most like diagram #2. To determine the dome rating, we add the calculation values of each of the diagrams — in this case $12 + 25 + 50 + 3 = 90$. The total number of lesions (90) would be divided by the number of leaves (4) to derive the average number of lesions per leaf — in this case 22.5. If we go to the lesion range, we find this value falls in the range represented by the diagram leaf #5 and the lot is assigned the same rating value of 5.

On the written report to growers, there sometimes appears an emergence count or a note that emergence was poor. Some of this seed may have a standard germination test result (the one that appears on the tag) of 90+ percent. This discrepancy can be confusing.

As part of the dome assay, pregerminated seeds are infiltrated with liquid from soaked beans. The pregerminated seed are from the lot being tested, which eliminates some external sources of bacteria. Generally 30 pregerminated seeds are planted $\frac{3}{4}$ inches deep in sterilized growth medium (vermiculite). A few days later, the seeds emerge from the growth medium and begin growing. Usually, not all of the seeds emerge. Studies have shown that pregerminated seeds with high numbers of bacteria rot in the medium. At this point, there is no satisfactory method to factor this information into the dome results, except that emergence is considered. If emergence is less than 60 percent, the seed is viewed as potentially poor quality and a retest is in order.

Standard germination tests record the amount of seed that germinate in wet blotters under controlled environmental conditions. The standard germination percentage should not be confused with the dome test emergence percentage.

How does the grower know that seed is safe, healthy, or fit to plant?

Since the bacterial pathogens spread so easily, probably no seed lot can be considered completely safe, but some lots are safer than others. The dome rating gives a comparative evaluation. One comparison is internal; that is, seeds that rated 5 in a dome test would have many more pathogenic bacteria than seeds that rated 3. One could surmise that planting seed rated 5 would be riskier than planting seed rated 3. Another comparison is with seed from other

areas, especially those thought to be the best available. When a number of dome tests were made on seed from western growing regions, the ratings ranged from 2-5 with the greatest numbers of samples being 3-4. When seeds (both bin run and seed-bean seeds) from North Dakota were tested, the ratings ranged from 3-6 with the greatest number being 4-5. The bacterial contamination levels of western-grown seed were used to establish the "acceptable" level of contamination for North Dakota certified seed. Because no seed lot can be declared safe, probably none could be absolutely condemned as unfit to plant because of bacterial contamination. Seeds with a dome rating of 6 or 7 would be likely to have poor field emergence and would be most likely to sustain heavy losses even in weather only moderately conducive to blight spread.

Can the dome rating be related to yield loss?

Not yet. Some experts suggest that a test like the dome test has little value unless the results (the dome rating) can be correlated with losses in the field. Such a correlation would establish a threshold value for bacterial disease. Threshold values are used extensively for assessing insect damage and losses from some pathogenic fungi like those causing smut on cereals. Establishing the correlation would be easier if the weather were consistent from year to year. It is not. Further, yield loss is generally based on comparable healthy beans growing in test plots with diseased beans. Entirely satisfactory methods of keeping the pathogens from infesting the healthy plots have not been found. Many years of data will allow a "statistical" answer to the question.

Is the quality of North Dakota grown seed related to the weather?

When the growing season is dry, more seed lots pass the dome test.

How stable or reliable is the dome rating?

In repeated trials, the dome rating from multiple tests of a single lot of seed seldom varied by more than 1 unit. The dome rating also improves if the multiple tests are made over time. Studies have shown that the number of bacteria in stored seed declines over time.

How well does the dome rating represent the bacterial contamination in a particular lot of seed?

The dome test is made on about a pound of seed, although more is requested from growers to allow for retesting. This small sample may represent nearly any amount of seed. How well the sample represents the lot depends on how well the sample is taken. Since the bacterial diseases may not be randomly or uniformly spread through a field, all portions of the field should be represented. There are cases where seed fields were rejected from certification based on field inspection, but the harvest was tested by the dome test and passed. One explanation is that the sample was predominately from a portion of the field with less bacterial disease.

Practical factors largely overshadow statistical considerations in determining sample size. Consider this: a 20-acre seed bean field yields 2,000 pounds per acre for a total yield of 40,000 pounds of navy beans at 2,400 seeds per pound, giving a count of 96 million beans. Of the 96 million beans, about 2,400 will be tested or 0.0025 percent of the beans. Assume the beans are planted the next year at a seeding rate of about 40 pounds per acre (or a plant population of 96,000), and further assume that 12 infected plants per acre can initiate an epidemic. To make calculations, we must also assume that the dozen infected plants came from infected seed and that none of the other seeds had any pathogens. Calculations show that 0.0125 percent of the seed planted per acre was infected. Since 0.0025 percent of the beans would be sampled, this would be equivalent of checking 2.4 plants per acre to find one of the twelve from among 96,000.

From the calculations, it would appear that the dome test might seldom detect pathogens. In practice, it does detect them. The power of the test comes from the fact that the pathogens are widely dispersed on and in the bean plants even though they may not be causing obvious disease symptoms.

The dome test cannot show how many beans are infected or how many infective bacteria are present in each bean. Results of many years testing indicate that under good growing conditions, the amount of bacterial contamination in a seed lot is reasonably uniform. Limited experience with drouthy beans suggests that the infection is much less uniform. Repeated tests on seeds from drouth-stressed beans may produce more erratic dome test ratings.

A seed lot may contain much more bacterial contamination than indicated by the dome test. In the dome test, seeds are rigorously cleaned of foreign material and culls. The seeds are washed to remove dust and disinfested in sodium hypochlorite to kill bacteria on the outside of the seed coats. The only bacteria that are detected are those from internal portions of the seeds. If a lot of seed contains infested trash, small seed that should have been removed in cleaning, or has not been properly treated with an antibiotic seed treatment, the level of contamination in that lot may be high.

Does an antibiotic seed treatment improve the dome rating and reduce the risk from planting contaminated seed?

Antibiotic seed treatment (streptomycin) probably has its greatest activity against external (surface) bacteria. The dome rating estimates internal seed contamination. For the risk assessment that is associated with the dome rating, it is assumed that the seeds are probably treated. The antibiotic should not improve the dome rating but it will reduce the risk of disease in the field.

Do different pathogens cause different amounts of disease?

Quantification of disease intensity or disease severity is difficult, but halo blight is a cooler season disease and is more likely a problem in cool wet weather, especially early in the growing season. Common blight is much more prevalent in warm weather and is more prevalent mid to late season. Brown spot seems to affect plants early to mid season. Brown spot lesions can be invaded by an *Alternaria* fungal pathogen and the *Alternaria* can cause serious plant damage.

Without supplemental tests, identification of the pathogens cannot be made in the dome procedure. As technology improves it should be possible to test a great number of seeds from a particular lot determining both type and amount of bacterial contamination.

Who is responsible for the running of the dome test on beans?

In December 1988, the NDSU Extension Service assumed responsibility for testing bean seeds. The extension service is administering the Seed Health Testing Laboratory as an adjunct to the Plant Diagnostic Laboratory. Any dry edible beans can be tested. If the sample is furnished by a grower who wishes to plant bin run seeds, the report will be returned as an advisory on the potential risk of blight from using the beans as seed. The seed health testing laboratory is acting as an agent for the North Dakota State Seed Department, which is responsible for certification requirements. If the sample is provided by a certified seed grower, the results of the tests are sent to the state seed department, which determines if the seed is suitable quality for certification.

What does a person need to do to have a sample of beans dome tested?

Collect a representative sample of about 4 pounds of beans. (Note: a 3-pound coffee can holds about 5 pounds of beans.) Clean the beans, hand-picking if necessary to remove insects, rocks, dirt-balls, plant debris, weeds (especially common cocklebur), broken seeds, or other non-bean contaminants. If the seeds are mailed, pack them to reduce cracking. Send them to:

Seed Health Testing Laboratory
North Dakota State University
P.O. Box 5012
Fargo, ND 58105

There is a fee associated with the test. The current (1989) charge is \$40 for each sample tested. You may wish to phone (701-237-7854) prior to sending beans to learn of schedules, delays, or fee changes. Ordinarily the test takes two to three weeks from receipt of the sample until results are available. Results are not sent until fees are collected, so including payment with the sample card can speed responses.

The dome test does not include supplemental tests such as standard germination tests. Contact the state seed department regarding those tests.

