AOSA-SCST SEED

PATHOLOGY SYMPOSIUM

Sheraton-Cavalier Motor Inn
Saskatoon, Saskatchewan
June 20, 1979

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INTRODUCTORY REMARKS

A.B. Ednie[1], *Chairman*

Seed pathology testing in North America has had a difficult time finding an important position in our seed laboratories' testing programs. General seed pathology testing, like vigour testing, has never been able to obtain full status in our testing programs perhaps because of the uncertainty of the significance of the analytical results was not seen to warrant the resource expenditures required to produce these test results.

Unlike germination and purity testing where results can be immediately translated into dollars and enforcement action, seed pathology only comes to the fore during isolated epidemic situations or when seed infection is known to be the sole or main cause of subsequent field infestations. North America has been blessed with a situation where most of its seed crops have been grown in dry non-maritime climates thereby producing relatively disease free seed. This, plus the confidence in the presence and rapid developments in pest control products, lead to a mainly "ho-hum" attitude towards the importance of seed-borne disease organisms.

Recent events in other countries in which North America has been developing markets for its seed and also events at home should cause us to once again review our capabilities to provide a seed pathology testing service to our seed industry. On the international scene our trading partners are having to institute protective quarantine measures to keep out unwanted pests. To achieve this goal they will require objective proof that the seed being imported into their phytosanitary zones do not contain certain diseases. They may even require a laboratory result quantitating the level of non-quarantine pathogens as a third measure of seed quality. This means that the industry will be requiring laboratory certificates indicating freedom from prohibited pathogens and the frequency of occurance of non-quarantine organisms that affect seed quality. Domestically we are seeing that the buyer as well as the seller are becoming more concerned about the sanitary condition of the seed in commerce as they become aware of the

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potential dangers seed-borne pathogens to crop production, i.e., bacterial blight of beans, black rot and blackleg of crucifers. This increase in interest is exemplified by the recent formation of a Seed Pathology Committee in the American Phytopathological Society and the formation of an Inter-American Working Group of Seed Pathologists. There is also an increasing number of papers in the scientific literature which are relating the incidence of seed-borne microorganisms to seed quality and performance. This is especially true with the literature dealing with soybeans. Last but not least is the erosion of our confidence in pesticides and the increasing concern about their long term effects on ourselves and the environment.

This symposium has been built around this background of an increasing interest and need for seed pathology testing service by the whole of the seed industry (producer, seller, buyer, and regulatory official) in North America. Our goal today is to bring to the seed technologist or the laboratory administrator some information on the scope of the expertise and physical resources necessary to mount a seed pathology testing program.

I have asked our speakers, who are well known in the field of seed pathology, to take into consideration that their audience is unfamiliar with this subject. The goal today is to bring to the seed technologist attention a new and challenging outlet for your talents and not to inhibit your interest in this field of testing by the over use of in house "technocratise".

The papers which follow then deal with the story and background of seed pathology from a pathologist's point of view. This is followed by a paper dealing with how the seed is a world in itself for our smaller co-inhabitors of the spaceship earth. Several papers on techniques follow with a concluding paper by a representative of the Seed Trade who will perhaps indicate the impact of seed pathology on the seedsman.

SEED PATHOLOGY— CONCEPTS AND METHODS OF CONTROL

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Abstract

Seed transmission, important in introducing, selectively perpetuating, and randomly distributing plant pathogens, is affected by the physical environment and by seed and soil microflora. Transmission may be prevented by seed-field culture and inspection, seed indexing and separatory procedures, chemical treatment, and thermotherapy. Aerated-steam treatment, followed by polyethylene glycol "invigoration" is a promising new method of eradicating seed-borne pathogens.

Additional key words: **transport; accompanying, external, and internal transmission; fungi; bacteria; viruses.**

Introduction

When plants emerged from the Paleozoic seas about 330 million years ago, they were accompanied by parasitic fungi and bacteria. When angiosperms became the dominant flora about 130 million years ago and seeds became the primary means of plant reproduction, parasitic fungi evolved mechanisms for seed transmission. Because of the extremely long period during which seed transmission of plant pathogens evolved, the host-parasite relationships are both complex and effective. Man may have begun disseminating plant propagules during his hunting, food gathering stage of development, perhaps 50,000 years ago. He came to depend on seeds for carrying his cereals from place to place and season to season as he developed agriculture 8,000-9,000 years ago in the eastern Mediterranean. The earliest evidence of plant pathogens on man's crops is from Jarmo 8,000 years ago. Man's knowledge of seed transmission of plant pathogens developed slowly from the sixteenth to the nineteenth century, and fairly rapidly since about 1880.

Seed transmission is the method par excellence by which plant pathogens are introduced into new areas, survive periods when the host is absent, are selected and spread as host-specific strains, and are distributed as random infection foci in plant populations.

Terminology

To state that a pathogen is seed-borne merely indicates that the pathogen and the seed are associated; it provides no information on the likelihood that a diseased seedling will be produced when the seed is planted, or how

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the association comes about. It is necessary to distinguish between *transport* of a plant pathogen from place to place or season to season and its successful *transmission* to the progeny. For example, powdery mildews of zinnia (*Erysiphe cichoracearum*), or pea and parsnip (*E. polygoni*) infect the seeds and are transported, but transmission to the seedlings has not been demonstrated. Only four of the many conflicting reports of seed-borne *Verticillium albo-atrum* or *V. dahliae* actually demonstrated transmission. The curly top virus is abundant in the perisperm of sugar-beet seed from infected plants, but is not transmitted to seedlings produced by them. There are a number of environmental factors, to be discussed later, that also affect the success of seed transmission.

Unfortunately, the numerous lists of seed-borne pathogens are based largely on the presence of pathogens on, in, or accompanying the seeds. They thus largely report seed transport, with transmission commonly assumed. Since the significant feature is transmission, these lists frequently are misleading. It should be stated in such lists whenever transmission has actually been demonstrated.

A pathogen is carried by seeds in one or more of three different ways:

1. *Accompanying*—It may independently accompany the seed without being attached to it. The pathogen may be in the form of sclerotia (ergot, *Claviceps purpurea*), seeds (dodder, *Cuscuta* spp.), infected bits of plant tissue (bacterial canker of tomato, *Corynebacterium michiganense*), smut balls (*Tilletia caries* on wheat), or as infested soil (club root of turnip, *Plasmodiophora brassicae*).
2. *External*—It may be carried passively on the surface of the seed as fungus spores (snapdragon rust, *Puccinia antirrhini*), vegetative cells (bacterial fasciation of sweet pea, *Corynebacterium fascians*), nematode larvae (stem and bulb nematode on alfalfa, *Ditylenchus dipsaci*), or a virus (tobacco mosaic virus on tomato).
3. *Internal*—It is carried, imbedded in plant parts essential for the production of the seedling, as fungus fruiting structures (late blight of celery, *Septoria apiicola*), fungus mycelium (alternaria blight of zinnia, *A. zinniae*), nematode larvae (*Anguina tritici* in wheat), or virus (lettuce mosaic in lettuce, bean common mosaic in bean). It is important to differentiate between infection of the embryo, in which the seed → progeny (SP) transfer has already occurred, and infection of parts other that the embryo, in which the SP transfer has yet to be made. This is especially true of viruses, because embryo invasion usually assures seedling infection, whereas infection of endosperm often does not produce an infected seedling.

Significance of Seed Transmission

In addition to the obvious efficient transfer of the pathogen spatially (geographic spread) and temporally (carryover from season to season),

there are several other unique but usually unrecognized significant features of seed transmission:

1. *Selection of host-specific strains of the pathogen*—Growth of a host in the seed field increases the inoculum of a pathogen able to attack it, and the probability of its infecting or contaminating the seed by that strain. Strains of *Rhizoctonia solani* virulent to pepper or tomato thus increase in the seed field, infect fruit in contact with the soil, invade the seed, and are thus carried to a new planting. Since the name *R. solani* includes a large group of biotypes differing in many significant respects, this mechanism assures the continued association of virulent strains with the host.
2. *Random infection foci in a field*—Planting infected or infested seed mixed with healthy seed distributes the pathogen randomly through the area. Such primary infection foci usually are more effective in starting an epidemic than are those from wind-blown spores or insect vectors, perhaps because infection occurs earlier. For example, planting lettuce seed having less than 0.003% infection with lettuce mosaic virus gives good commercial disease control in California, even though aphids may spread the virus into the planting from surrounding weedy fields. Similarly, halo blight of bean (caused by *Pseudomonas phaseolicola*) may produce an epidemic in Wisconsin from as few as 12 infected seeds per acre (0.02%).
3. *Prolonged transmission capacity*—Stored seeds remain viable for a longer time than vegetative propagules and have a longer potential transmission period. The seed and the pathogen remain viable for several years, although some pathogens may die before seed germination is greatly decreased.
4. *Long-distance dissemination*—Because of the long survival of pathogens in seeds, they are readily spread from continent to continent with maximal chance of successful infection of the host because they already are associated with it.
5. *Significance in certification and quarantine*—Quarantine regulations for seeds are less strict than are those for plants, plant parts, or soil, although they are at least as effective in pathogen dissemination. Unfortunately, these regulations realistically reflect the difficulties of detecting seed transmission. For reasons explained below, point-of-origin inspection may be unable to detect a pathogen although present in the field, examination of harvested seed is difficult and uncertain for low-incidence infections, and indexing methods are sometimes unreliable (virus-infected embryos may be symptomless on germination). However, certification based on sound biological facts may be very effective in reducing disease losses, as shown for lettuce mosaic on lettuce, and *Ascochyta* spp. on cannery peas in California.

6. *Relation to pathogen life history*—Seed transmission of a pathogen to a new area is more important than to one where it already occurs. Thus, the introduction of snapdragon rust (*Puccinia antirrhini*) to Australia in 1952 was more important than seed transmission within the U.S. Seed transmission of pathogens that are able to survive for long periods in soil (*Fusarium oxysporum* f. sp. *pisi* on pea) or even for short periods (*Alternaria zinniae* on zinnia), is more dangerous than transmission of pathogens that do not carry over in soil (*Puccinia antirrhini* on snapdragon). The wider the host range of a pathogen, the more dangerous is its introduction to a new area and the more difficult its eradication.

All nematode-transmitted viruses are seed-borne; because of the limited mobility of nematodes, those viruses that they transmit would not be successful if they were not seed-borne. On the other hand, no viruses spread by fungi are known to be seed-borne, perhaps because those microorganisms are successfully spread with wind-blown dust. Plants grown from seed infected with some viruses may produce seed with an increased percentage of virus transmission, and are more uniformly invaded than plants with current-season infection. Because of the greater uniformity of tissue invasion by the virus, the plants are better virus sources for insect vectors, and thus enhance spread. Because seedlings from virus-infected embryos tend to be symptomless, they must be indexed by inoculating into healthy seedlings.

7. *Relative importance in seed- and crop-production fields*—Some diseases are very important in seed fields, but are not seed borne and present no risk to the seed buyer. The virus and mycoplasm diseases, pea enation mosaic on sweet pea, aster yellows on delphinium, tomato spotted wilt in Compositae, and curly top of petunia are present to greater or lesser extent in California seed fields every year, but are not seed transmitted. *Pythium* and *Phytophthora* spp. cause root rot in seed crops in the field but are not seed transmitted.

Some diseases are important in seed fields and are seed transmitted, but are unimportant in crop fields. Garden stock (*Matthiola incana*) has a cold requirement for flower induction, and is grown for cut flowers during winter months. Fusarium wilt (caused by *F. oxysporum* f. sp. *matthiolae*) does not cause important loss of this cut-flower crop at the prevailing low soil temperatures, and thus cut-flower fields are uninjured. Since it takes nearly a year to produce seed on this plant, the seed matures in summer when soil temperatures are favorable to *Fusarium*, and severe losses may occur. Although seed transmitted, this disease generally is unimportant to the seed user. *Heterosporium tropaeoli* causes severe losses in California nasturtium seed fields, but for undetermined reasons is absent or unimportant elsewhere, although commonly seed transmitted. Control of these two diseases therefore has emphasized

reduction of losses in seed fields rather than producing seed free of these pathogens.

Some pathogens may be present in seed fields and infect or infest the seed produced, but rarely or never produce disease symptoms. *Alternaria zinniae* rarely produces disease in California seed fields because of the dry climate. Seedlings produced by infected seed are girdled and die, and the fungus sporulates on them. The fungus apparently survives in the soil until fall. The mature flowers absorb and retain moisture from the heavy autumn night fogs and dew, and are attacked by various molds, including *A. zinniae*. It grows down the petal into the fruit coat and seed. *Corynebacterium fascians* produces no fasciation or witches broom symptoms in California sweet pea or nasturtium seed fields because the soil does not remain moist enough near the plant crown for the bacteria to produce the growth substances that induce dormant buds to grow. However, the bacteria multiply sufficiently in the soil, or perhaps in the rhizosphere, that they get on sweet pea with dust during threshing, and on the surface of nasturtium seed in contact with soil. Although both of these pathogens produce no diseases in California seed fields, the seed produced is infected or infested. Point-of-origin inspection and certification for diseases of the types discussed in this section obviously is impossible.

Frequency of Seed Transmission

Commercial seed lots have highly variable percentages of infected seeds, ranging from a fraction of a percent to 100%, but well-cleaned seed usually has less than 5% seed transport. Because of the many complex events in the plant → seed (PS) transfer, in handling seed from harvest to planting (SS), and in the seed → plant (SP) transfer, transmission may occur in only one seed among thousands. However, this rarity is offset by the rate of increase of pathogens. For example, late blight (*Septoria apiicola*) infection on ten celery seeds in a seed bed may produce 15 million spores before time of transplanting. The greater the potential rate of increase of a pathogen, the less the amount of infection needed to produce an epidemic, and the more important the transmission.

Some representative percentages of infected seeds in commercial lots are: avocado sun blotch (virus), occasional seed; tomato bacterial canker (*Corynebacterium michiganense*), less than 1%; verticillium wilt (*V. albo-atrum*) in spinach, 9%; septoria late blight of celery, 40%; bean common mosaic (virus), over 50%; alternaria blight of zinnia, nearly 100%.

Factors Affecting Seed Transmission

Physical Environment

Infection of roots of safflower seedlings from bits of plant tissue carrying rust (*Puccinia carthami*) teliospores may be prevented at a soil temperature

of 30 °C. Because of the semi-arid conditions in the interior valleys of California, *Ascochyta*-infected pea seed and *Alternaria*-infected zinnia seed planted there will produce a seed crop free of these pathogens. Much of the U.S. seed industry is located in the western states, largely because the semi-arid conditions there reduce seed transmission of pathogens. However, there are occasional exceptions: bean seed infected with halo blight (caused by *Pseudomonas phaseolicola*), rarely produces diseased plants in California, but the bacteria rarely may spread to the seed surface during threshing; *P. lachrymans*, cause of angular leaf spot of cucumber, does not occur in California seed fields harvested only once, and is not then seed transmitted. Plants repeatedly picked over for processing while the plants are wet with dew may, however, develop the disease. Bean common mosaic (virus) is carried in seed produced on infected plants at 20 °C, but is not in seeds produced at 16.5 °C. China aster seed infested with *Fusarium oxysporum* f. sp. *callistephi* sown in steamed soil held at 15.6 °C will produce no evident wilt disease, even though some plants may be infected. Sown in soil at 25-27 °C, however, the seeds will produce conspicuously diseased plants that may produce infested seed.

Seed and Soil Microflora

Soil microflora may be antagonistic to a pathogen in or on the seed when it is sown, and the development of disease thus be suppressed. When infected or infested seed is sown in nearly sterile soil, a larger number of diseased seedlings will be produced than when sown in nontreated field soil. Some comparative data on the percentage of disease produced by such seed, sown respectively in nearly sterile and in nontreated soil, are: flax seed with *Polyspora lini*, 15.0 and 1.7%; flax with *Colletotrichum linicolum*, 66.3 and 17.0%; wheat seed with *Helminthosporium sativum*, 38.6 and 14.8%; oats with *H. victoriae*, 97.9 and 68.3%; *Senecio vulgaris* seed with *Verticillium albo-atrum*, 39.2 and 0%. For many years hundreds of tons of pea seed of susceptible varieties infested with *Fusarium oxysporum* f. sp. *pisi* have been planted for freezing, canning, and seed production in California, but wilt has appeared only in two small coastal areas. Due to the microflora of the suppressive soils, use of resistant varieties has not been necessary, as it is in Washington and Wisconsin. Antagonistic microflora on the seed also inhibit infection by seed-borne pathogens, as shown by Brazilian varieties of oats. These cultivars were considered to be resistant to *Helminthosporium victoriae* inoculated on the seed. However, hot-water treatment of the seed destroyed this resistance, and it was found that two seed-borne antagonistic fungi, *Chaetomium cochlioides* and *C. globosum*, were the cause of the "resistance".

Type of Seed Germination

Hypogeal, but not epigeal germination of seed restricts successful transmission of most rusts, powdery mildews, and downy mildews that in-

fect aerial parts. Infection by seed-borne pathogens that infect roots and stems are favored by both epigeal and hypogeal germination.

Control Methods

Preventive Methods in Seed Fields

1. *Selection of seed-production areas*—Seed should be produced in areas where the pathogen is unable to establish or maintain itself. The physical environment markedly restricts incidence of many diseases and prevents infection of the seed produced. Thus, pea seed free of *Ascochyta* spp. may be produced in south-central California because of the semi-arid conditions, and pea seed free of *Fusarium oxysporum* f. sp. *pisi* may be produced in California because of suppressive soils. Bean seed free of anthracrose (caused by *Colletotrichum lindemuthianum*), and cucurbit seed free of *C. lagenarium* and *Pseudomonas lachrymans* may be produced in areas that are rain-free during the growing season. Seed fields should be located as far as possible from crop-production fields or homeyard plantings of the crop. High value seed crops may justify soil treatment to reduce possibility of transmission of soil-borne pathogens.
2. *Culture practices*—Seed planted in seed fields should be free of pathogens; such seed can be obtained: (a) from areas free of the pathogen or where it is unable to infect; (b) by seed treatment to eradicate the pathogen; (c) from a carefully maintained nuclear block. Widely spaced direct seeding, instead of transplanting from seed beds, reduces the chance of spread; coupled with careful early roguing of diseased plants that appear, this will reduce seed infection. Fungicidal spraying will reduce some diseases (e.g., snapdragon rust) in the seed field and decrease the amount of inoculum on the seed.

   Avoidance of overhead irrigation is of great importance to avoid disseminating water-borne pathogens and making the environment favorable for infection. Furrow irrigation is preferable for seed crops. Harvesting of seed should be done before the autumn fogs and dews that favor seed infection. Hand removal of the senescing corollas of large-flowered petunias is often done to prevent rotting of the developing capsules by the gray mold, *Botrytis cinerea*. Use of plastic or chemically treated canvas ground sheets for collecting seed heads in the field lessens fabric decay and prevents pathogens such as *Rhizoctonia solani* from growing through them from the soil into flowers piled on them. Eradication of weed hosts of the pathogen in the field and adjacent fields will minimize pathogen spread.

   Host resistance to a pathogen will reduce the amount of inoculum produced and the possibility of seed transmission. Selection and breeding for varieties with non-transmission of seed-borne viruses, such as bean common mosaic, lettuce mosaic, squash mosaic, and soybean mosaic, is a promising but little-explored method.

3. *Point-of-origin inspection*—This method is useful in rejecting seed from fields with high incidence of a seed-transmitted pathogen. However, apparent absence of a disease may not insure freedom from the pathogen in the seed because (a) the pathogen may be present without producing symptoms in the field, as already explained, and (b) pathogen-free seed may be contaminated by the pathogen during threshing, cleaning, or other handling operations. Bacterial pathogens may be distributed from seed to seed in dried flakes, and air-borne fungus spores are scattered through a seed lot. Furthermore, seed lots frequently are intentionally mingled to raise the germination percentage of weak seed, to fill an order that exceeds the available supply of one lot, or to produce a "formula mix" of color range in flower seed.

Preventive and Control Methods for Harvested Seed.

1. *Seed indexing*—For reasons given above, the detection of seed-borne pathogens is best done after seed has been cleaned and packaged for sale. The best method is to plant the seed in soil, as this approximates actual conditions. The soil used should be: (a) non-treated field soil, to determine the actual field hazard; (b) treated at 100 °C/30 minutes with steam to indicate the maximum potential transmission; (c) treated at 60 °C/30 minutes with aerated steam to give some indication of the extent to which soil antagonists will inhibit transmission.

   A simple test developed by R.G. Grogan will indicate the presence of bacteria in a seed lot, but gives no indication of the percentage of infection. Newspaper is put over the hole in a six-inch clay pot, and an inch of sterile vermiculite placed on it. A layer of bean seed is placed on this, and the pot filled with vermiculite. The pot is filled with water overnight, and a hole punched in the paper the next day to drain the pot. The pot is kept watered so that the beans germinate and grow up through moist vermiculite. If any seeds are infected with *Pseudomonas phaseolicola*, several seedlings will show symptoms in the primary leaves.

   Special direct methods have been devised for microscopic examination of seed on blotters; these have been discussed by Neergaard (1977). These methods are subject to great variability in comparative tests in different laboratories, and must take into account special features of the pathogen. Pea seed infected with *Ascochyta pisi* can be detected by treating seed with sodium hypochlorite and planting on blotters. However, pea seed produced in the Pacific Northwest, contaminated only with surface dust containing chlamydospores of *A. pinodella*, will if similarly treated with sodium hypochlorite, give an erroneous negative reading on blotters.

   Indirect methods have also been developed for indexing seeds. Microscopic examination of the embryos of barley and wheat for smut

(*Ustilago nuda* and *U. tritici*, respectively) is an accurate and useful technique. Seed-borne bacteria may be detected by serological methods, by use of bacteriophages, and by inoculation of ground seeds on plates of selective media or on healthy seedlings. Seed-borne fungi may be detected by plating on selective media, and by immunofluorescence techniques. Viruses are more difficult to detect because, as already indicated, the seedlings produced may be symptomless, and must be indexed by inoculation on healthy seedlings.

2. *Separatory procedures*—Commercial cleaning of seed to free it of debris, weed seeds, sclerotia, and nongerminable or light seeds will reduce transmission of many pathogens (e.g., *Sclerotinia sclerotiorum* on bean, squash mosaic virus in squash seed, wheat cockle (*Anguina tritici* in wheat), but may spread pathogens such as bacteria or sticky-spored fungi. Ingenious types of screens, variable-pitch vibrating tables with controlled vacuum or air flow, specific gravity and spiral separators, and equipment to wash, scour, and polish seeds are commonly used. Specialized devices are available to remove dodder seed and to float ergots (*Claviceps purpurea*) from seed lots.
3. *Chemical seed treatments*—Protective treatments (e.g., thiram, maneb, captan, chloranil, dichlone) aimed at preventing attack of the seed by soil microorganisms are of little or no value in preventing seed transmission of pathogens. Eradicative chemical treatments (e.g., thiabendazole, carboxin, benomyl, hexachlorobenzene) are generally used to kill pathogens on the surface of the seed, between the glumes and the seed, or in shallow infections of the fruit or seed coats.
4. *Thermotherapy*—Heat treatment is effective against all classes of seed-borne pathogens, but usually is used against pathogens so situated as to be protected from chemicals. There are three types of such treatment—hot water, aerated steam, and hot dry air. Water has twice the thermal capacity of saturated aerated steam, and five times that of dry air. Temperature or time of treatment therefore increases from 49-57 °C/30 minutes for water, through 54-60 °C/30 minutes for aerated steam, to a range of 54 °C/5 hours to 95-100 °C/12 hours for dry air. Since temperature and time are more or less mutually compensating, it is desirable to standardize on a 30-minute period. The timing must be precise, and thermometers accurate in the treatment range must be used.

Hot water treatment has long been used and is very effective. For seeds, such as nasturtium and oats, that have air space between the fruit coat or glumes and seed coat, a presoak in cool water is desirable to displace the air. Seeds treated in hot water absorb much water, undergo severe leaching of soluble materials, require drying before storage or planting, and therefore sustain more injury than those treated with aerated steam. Seed coats of beans and peas may rupture during treatment, and those of garden stock, alyssum, and flax may exude mucilagi-

nous material that sticks the seeds together and makes handling difficult.

Treatment with aerated steam came into use after 1960 because of the disadvantages of hot water. Diluting steam with air lowers its temperature to any desired level. The aerated steam must move by pressure flow downward through the seed mass being treated in a double-walled container. The flow rate must be great enough to insure prompt (2 minutes) uniform heating of the seed mass. This is not possible in steam chambers, trays, or cloth or plastic mesh bags. Timing should begin when the output and input temperatures are approximately equal. At the end of the treatment period the steam is shut off and the seed temperature is lowered rapidly to about 32 °C by evaporative cooling from the continuing air flow. The seed will then retain only slightly more water than before treatment. Aerated steam treatments may sometimes by made more effective by holding the seed in moisture-saturated air at room temperature for 1 to 3 days prior to treatment. Bacteria and fungi apparently are made more thermally sensitive by this hydration. Hard seeds of legumes are not hydrated by this procedure, and it may be necessary to scarify lots having such seeds before hydration and treatment.

Any seed treatment decreases germination, particularly with seed of low vitality or more than one year old. Injury from thermotherapy is physiological, perhaps from damage to enzyme systems, and may be repaired if metabolic processes can proceed without cell enlargement or radicle emergence. Following heat treatment, if seed is held in polythylene glycol 6000 (PEG), such repair is achieved. Tomato seed with 99% germination fell to 54% following aerated steam treatment (60 °C/30 minutes—a very drastic treatment) and returned to 94% following PEG treatment. The figures for cabbage were 80, 15 and 31%, and for zinnia were 90, 39, and 65%. It has recently been found that some viruses may be inactivated by heat treatment of PEG-treated seed. Lettuce mosaic in lettuce seed was inactivated by holding seed moistened with PEG at 40 °C for 6-10 days in sealed petri dishes. Cherry leaf roll virus in *Nicotiana rustica* seed was inactivated by similar treatment for 14 days. Combining aerated steam and PEG treatments thus presents the possibility of using temperatures sufficiently high to insure killing the pathogen, with minimal seed injury.

Hot dry air is less effective than either of the above methods because microorganisms are less sensitive to dry than to moist heat. Hot air has therefore been relatively little used for seed treatment. Snapdragon rust urediospores are killed in one hour at 46 °C, or two hours at 43 °C. The sugar-beet nematode (*Heterodera schachtii*) may be killed in soil on sugar beet seed by hot air at 65-70 °C/5-10 minutes.

Following any thermotherapy treatment the seed should be treated with a mild protective fungicide. Since seed germination is somewhat retarded and weakened, this protection from soil microorganisms is beneficial.

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THE SEED: A MICROCOSM OF MICROBES

James B. Sinclair[1]

Abstract

Although seeds are considered to be small entities in man's perception of his surrounding environment, they are themselves a planet in which a microbial world exists. This microcosm is exemplified by the soybean seed and its associated microbes. The understanding of the relationships involved now has been realized as is its impact on seed quality and performance.

Additional index words: **seed pathology, soybean seed, *Glycine max*, seedborne microorganisms.**

Introduction

Every population of seeds has the potential of carrying a variety of microorganisms, including fungi (molds) and bacteria, many of which can cause disease in seedlings and plants or affect the embryo of the seed. The study of microorganisms associated with seeds is a very interesting one, since it is as varied as the kinds of seeds they attack.

There are some microorganisms, considered to be efficient, that live and reproduce on the outside of seeds without causing any visible harmful effect. Still others on the outside of the seed will become harmful when environmental conditions are favorable for their growth and reproduction. Many storage fungi fit into this latter category, including species of *Aspergillus*, *Fusarium*, *Penicillium*, etc. Still other fungi make their way into the nonliving outer tissues of seeds, such as bracts, pericarps and seedcoats. These survive in these tissues during conditions adverse for their growth and reproduction, ready to attack the germinating embryo when conditions are more favorable. Many examples of this type of seedborne microorganism can be given.

Another means of infection is the direct colonization of the embryo and/or ovule of the seed. The obligate and efficient fungal, bacterial and viral pathogens can be found in this group. The smut fungi and species of *Colletotrichum*, *Fusarium*, *Gloeotinia*, *Phytophthora*, *Plasmopora* and *Sclerospora*, among others, use this mechanism for survival in seeds. These microorganisms and some viruses may not kill the embryo, but delay its germination resulting in weak emerging seedlings and generally reduce seedling vigor. Thus, some of the most important effects that seedborne microorganisms and viruses have on seeds are to reduce storage life, germination, emergence and vigor, and eventually the adult plant yields.

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Individual seeds in each seed population may vary among themselves in the type and number of microorganisms and/or viruses that they carry. In studying microorganisms and viruses associated with seeds, a seed must be considered as a potential microcosm (i.e. a little world), of different microbes which may or may not interact with one another (6). We are only beginning to understand this microbe world associated with seeds.

Symptoms on Seeds

Seeds infected or contaminated with microorganisms often show characteristic symptoms. Neergaard (3) wrote a book on "seed pathology" and illustrates many of the symptoms associated with seeds infected by microorganisms and viruses. Commercially, seedborne microorganisms reduce the grade of grain at the elevator by causing reduced size, distortions, shrivelled kernels, discoloration, spotted seeds and stromatized seeds. United States growers often are "docked" because of poor quality grain based on these visible factors. The U.S. Department of Agriculture has set standards for judging grain quality based on visual symptoms (8).

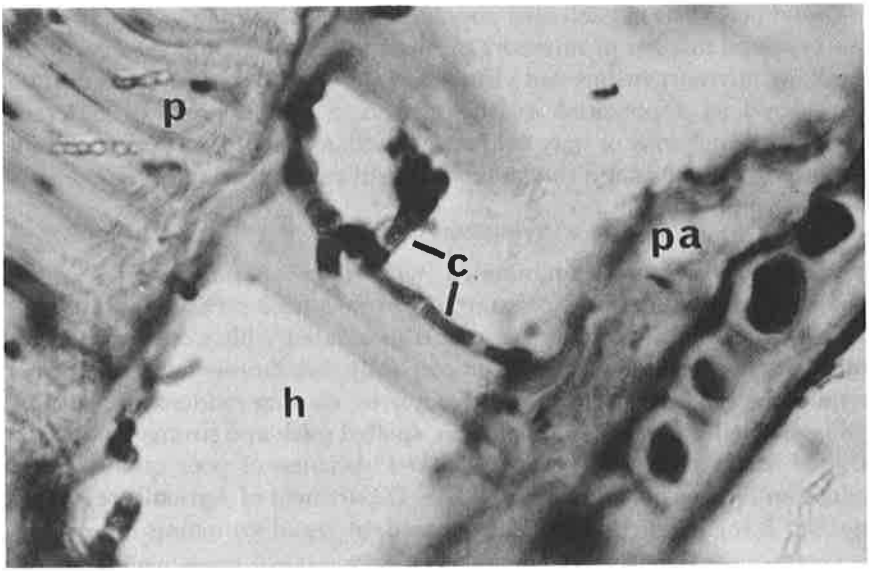
Soybean seeds with a heavy infection of *Phomopsis sojae* (causal fungus of pod and stem blight) appear discolored, fissured, flattened and may be all or partially covered with a greyish-white mycelium (1, 2). Soybean seeds infected with the anthracnose fungus, *Colletotrichum dematium* var. *truncata*, have irregular, greyish discolorations composed of minute black specks (4, 5). *Cercospora kikuchii* causes purple seed stain on soybean (1). Soybean seeds infected with *Bacillus subtilis*, however, do not have distinctive symptoms until seeds are incubated under high humidity and temperature (Fig. 2) (7).

It is well known that small grains infected with ergot fungi and other smut pathogens are not acceptable for processing. Such seed lots have to be cleaned before use. There are quarantines and embargoes on seeds known to be contaminated with bacteria, such as the black rot bacterium of crucifers. However, we are just beginning to understand some of the other effects due to contaminated seeds. We find that oil and flour derived from soybean seeds infected with *Phomopsis sojae* is of lower quality than that from seeds without symptoms of infection (Table 1) (2).

Seeds may be infected with a fungus and not show symptoms. When this is the case, fungicide seed treatment is more effective on seeds without symptoms than those with symptoms (Table 2).

Colonization of the Seed Coat of Soybeans

The seed coat of soybean as well as other large-seeded legumes, is made of three layers: the top or palisade layer, which is overlaid by a heavy waxy coating (cuticle); the middle or hour-glass cell layer (a spongy, honey comb-like layer); and the lower or parenchyma cell layer (made up of aluerone cells rich in protein) (Fig. 1). The hourglass cell layer and the parenchyma



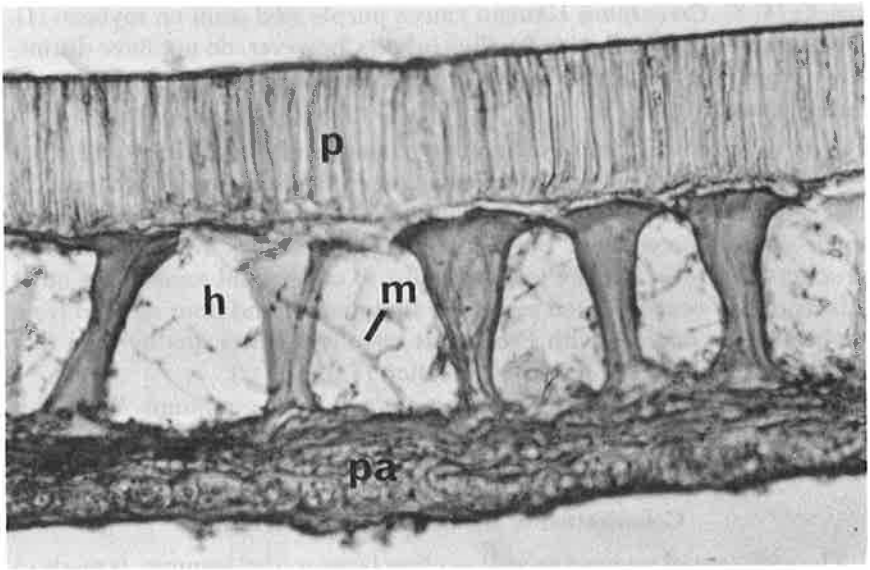


Figure 1. Cross-sections of soybean seed coats showing the presence of two fungi in the hourglass cell layer: at top, the fungus mycelium of *Phomopsis sojae* (m); at bottom, the fungus mycelium of *Colletotrichum dematium* var. *truncata* (c); p = palisade cell layer, h = hourglass cell layer, pa = parenchyma cell layer; m = mycelium of pod and stem blight fungus, and c = mycelium of anthracnose fungus.

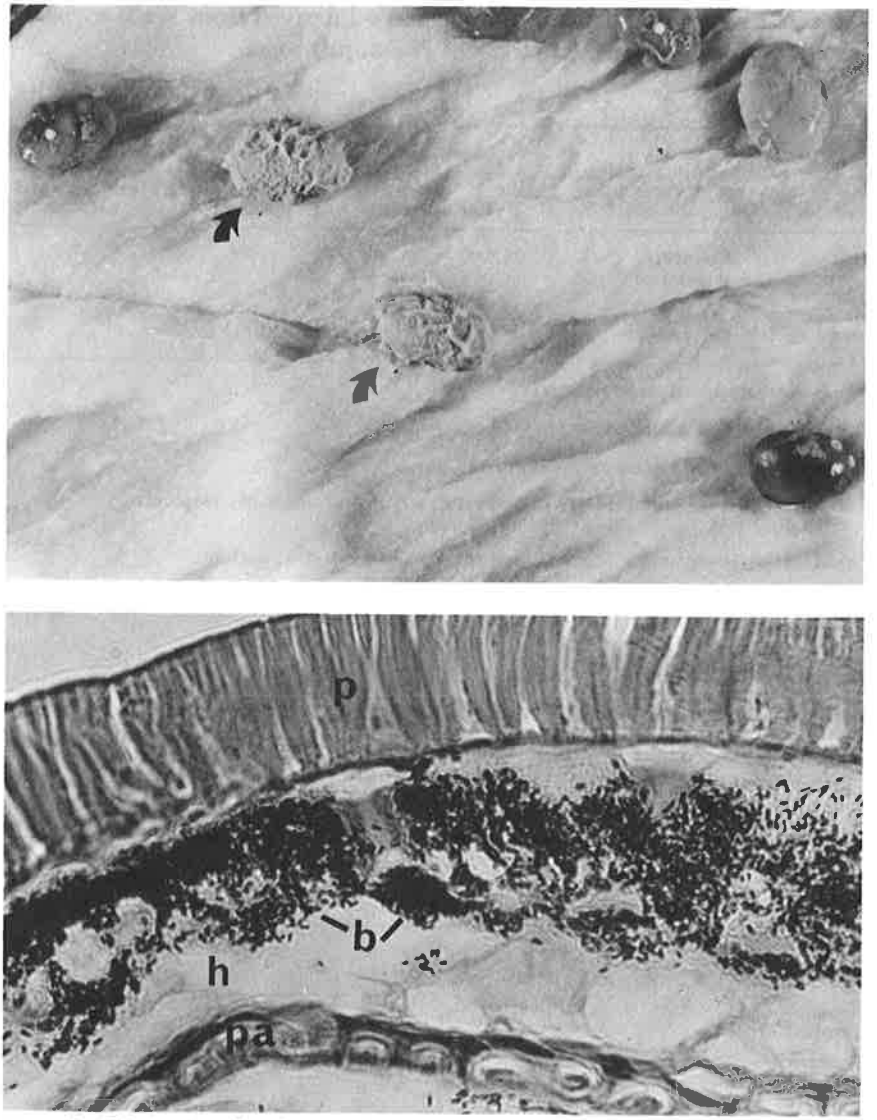


Figure 2. Colonization of soybean seeds by *Bacillus subtilis;* at top, dead soybean seeds on moist cellulose pads (28C) showing colony growth of *B. subtilis* covering individual seeds (arrows); at bottom, cross-section of a soybean seed coat showing the presence of the bacterium (b) in the hour-glass cell layer (h); p = palisade cell layer, h = hourglass cell layer, pa = parenchyma cell layer and b = stained bacterial cells.

layer may provide a nutritional base for fungi and bacteria. In studies in the Department of Plant Pathology, University of Illinois, several fungi and a bacterium have been observed in the hourglass cell layer. These include the fungi that cause anthracnose (*C. dematium* var. *truncata*) and pod and stem blight (*Phomopsis sojae*) (Fig. 1) and purple seed stain (*C kikuchii*);

Table 1. Description of soybean flour and oil derived from Wells soybean seeds with or without symptoms of *Phomopsis sojae*.

Seed lot	Sensory evaluation of flour for "off" odor[a]	Composition[b]		Oil	
		Oil (%)	Protein (%)	Peroxide value[c] (meq/kg)	Dark color and "off" odor
With symptoms	Moderately pronounced*[d]	25.6**[d]	43.3*	125**	+[e]
Without symptoms	Slightly perceptible	23.0	42.4	52	-

[a] Mean category based on 15 panelists.

[b] Mean based on 20 determinations.

[c] Mean based on four determinations.

[d] ** and * indicate statistically higher value at $P = 0.01$ and 0.05, respectively, using FLSD test.

[e] Qualitative observation: +, with dark color and off odor; -, without.

Table 2. Percentage[a] seed germination and percent recovery of *Phomopsis sojae* from Wells soybean seeds with and without symptoms of *P. sojae*, nontreatment or treated with thiram fungicide.

	Treated[b]		Nontreated		Control with seed treatment[c]
	Germination	% Recovery	Germination	% Recovery	
With symptoms	20	34**	12	84**	62
Without symptoms	91**	4	86**[d]	30	86*[d]

[a] Based on four replications of 100 seeds each.

[b] Seed treatment consisted of application of Arasan 50 Red at 125 g/100 kg of seeds before planting.

[c] Phomopsis control (C) =

$$\frac{\left(\begin{array}{c}\text{Phomopsis recovery without}\\ \text{seed treatment}\end{array}\right) - \left(\begin{array}{c}\text{Phomopsis recovery with}\\ \text{seed treatment}\end{array}\right)}{\text{Phomopsis recovery without seed treatment}} \times 100$$

[d] ** and * indicate statistically higher value at $P = 0.01$ and 0.05, respectively, using FLSD test.

and the bacterium, *B. subtilis*, that causes seed decay (Fig. 2) (1, 4, 5, 7). At the University of Puerto Rico, *P. sojae* has been found associated with seeds of common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*) and pigeon pea (*Cajanus cajan*).

The fungi and bacteria lie dormant in the soybean seed coat until conditions are favorable for their growth. Often these are the same conditions that favor seed germination. However, temperature and moisture that are not optimum for seed germination can still be adequate for the growth of microorganisms. Under these conditions, fungi and bacteria kill the seeds before they can complete the germination process or emerge from the soil.

Seedborne microorganisms not only result in lowered stands because of poor germination, but also reduce the market value of the crop for food and feed. Also, the seeds will carry the pathogens into the field to spread the infection.

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METHODS FOR ROUTINE DETECTION OF SEEDBORNE FUNGAL PATHOGENS[1]

J. W. Sheppard[2]

Abstract

Seeds may carry disease on their surfaces or within their tissues and may act as vectors introducing plant diseases to uninfested areas. Fungi are the most commonly isolated pathogens of seeds. Various methods for the detection of seed borne fungal pathogens including incubation tests, non-cultural techniques and virulence tests are discussed.

Additional Index Words: **agar plate test, blotter test, virulence testing, smut test.**

Introduction

Neergaard (3) has described seeds as both the victims and vehicles of disease. As vehicles of disease they may carry on their surfaces plant diseases into previously uninfested areas. As victims, they are subject to attack by a number of disease causing agents. Most plant diseases are caused by bacteria, viruses, nematodes or fungi.

More than 8,000 species of fungi have been identified as causing plant disease. Fungi occur in seeds more commonly than bacteria or viruses. The fungi which are found on seeds may be saprophytic or pathogenic. The saprophytes are non-host specific species which live and obtain their nutrients from non-living organic material, the seed coat for example. Pathogenic fungi however, are relatively host specific and obtain their nutrients from living organic material. Both may be found on the surface of the seed, in cracks or inside the seed coat, but the pathogens may also be found within the cotyledons or other tissues of the embryo (2).

Fungi are composed of a thread-like vegetative body called mycelium. Most fungi reproduce by means of spores which have a function similar to the seeds of higher plants. Like seed, the spores are of various sizes, shapes and colours, however, they are 300 to 600 times smaller than tobacco seeds. Some fungi do not form spores but reproduce by means of sclerotia which are really hardened clumps of fungal threads. Most seed-borne fungal pathogens are detected through the use of incubation tests. Seeds, usually 400, but often as many as 1000 are placed on a substrate which permits the fungi to grow out of the seed and develop to a stage where they can be identified by cultural and morphological characteristics. In most cases

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the presence of seed-borne fungi is expressed as percent infection. The two chief methods used in the routine detection of seed-borne fungal pathogens are the agar plate and the blotter test.

Agar Plate Test

Agar, a carbohydrate obtained from certain species of seaweeds, is widely used as a base for culture media for fungi and other microorganisms. Agar was first used by the Chinese over 1000 years ago but it was not until 1883 that Hesse, a student of the famous 19th century microbiologist Robert Koch recognized its value as a culture media after hearing of its use as a solidifying agent in jams and jellies from a friend of his wife in the Dutch East Indies. The most useful property of agar is the great difference between its melting point and the temperature at which it solidifies (1). Agar melts only when heated to 100 C and remains liquid until cooled to about 42 C. This property of agar permits infected plant material or seeds to be mixed with the medium while it is in a liquid state with little danger of killing any introduced fungal spores or mycelium. Agar itself provides few nutrients for the growth of fungal pathogens hence a number of "natural products" or extracts are added to the agar base in an attempt to reproduce the natural environment of the organism to be studied. Among the media widely used in the isolation of fungi from seeds are malt extract, potato dextrose and various vegetable or fruit decoctions such as V-8 juice or prune extract. These extracts may be supplemented to some degree by other sugars, salts, antibiotics and other agents. The agar is prepared for use by mixing the agar powder with an appropriate quantity of water and if necessary the required nutrients or vegetable extract. The mixture is then sterilized in an autoclave for 15-20 minutes. After sterilization the agar is cooled to about 50 C and at this point antibiotics may be added. The agar is dispensed into petri dishes and solidifies within 20 minutes after which the dishes are ready for use.

In most cases the seed must first be surface disinfected to eliminate contamination of the seed coat by saprophytic organisms which develop rapidly on the nutrient agar often inhibiting and completely obscuring the growth of the slower growing pathogens. Seeds are usually pretreated by soaking 5-10 minutes in a 2% hypochlorite solution such as javex or other common household bleach. The solution is poured off and the seed rinsed with sterile water prior to plating onto the agar surface. The efficiency of the surface disinfection procedure is increased by quickly passing the seed through a solution of 70% alcohol prior to the hypochlorite solution.

After surface disinfection, the seeds are placed one by one on the surface of the agar using forceps which have been dipped into 70% alcohol and passed through a flame. This "flaming" is repeated after each dish has been filled. The seeds are evenly spaced onto the agar surface. Usually 10 seeds per plate but this may vary according to the size of seed.

After plating, the dishes are placed in an incubator at 20-25 C for a period of 5-8 days. After incubation seed-borne pathogens present can be detected by colony and spore characteristics.

Blotter Test

The blotter test is actually a modification of a germination test as the seeds are placed on the surface of moistened filter paper discs or germination blotters. In the blotter test the blotters are saturated with sterile water and the excess water is poured off. Seeds are then sown onto the blotter singly with a pair of forceps or by use of a vacuum counter. As in the agar plate test the distance between seeds is dependent upon seed size, the prevalence and spreading capacity of the fungi that is being tested for and the duration of the test.

In the blotter test, the growth of seedlings often makes observation of fungi difficult and determination of percentage infection impossible. The use of a 0.2% solution of the sodium salt of 2,4-D prevents growth of seedlings of dicotyledonous plants with little effect on the growth of fungal pathogens. As an alternative to the use of 2,4-D for inhibition of seedling growth a freezing technique may be used. In this technique, seeds are sown onto moistened blotters and permitted to imbibe water for 24 hours. They are then placed at 15 C for 24 hours followed by a period of normal incubation. In most cases this is an environment 20-25 C with a 12 hour day/night NUV light cycle for a period of 5-7 days.

Virulence Test

In most cases the test for seed-borne pathogens ends with the recording of the incidence of the pathogen on the agar plates or blotters. Sometimes this is only the beginning. Some organisms must be further cultured, identified and re-inoculated into healthy host plants, and subsequently re-isolated before the test is completed. This procedure, known as Koch's postulates may be used to detect virulent and nonvirulent strains of a pathogen. Blackleg of rapeseed is a case in point, or, as is presently carried out in our laboratory, the detection of *Verticillium* spp. pathogenic to alfalfa from the inert material carried with alfalfa seed lots. In this procedure a 1 gm. seed sample is shaken in sterile water and the "wash water" diluted and spread onto the agar surface. The inert material and any other crop and weed seeds are also plated on agar. The plates are then incubated 3-7 days. Colonies of *Verticillium* are isolated from the agar surfaces and pieces of plant tissue are grown in pure culture until they can be identified as to species. Healthy 4 week old alfalfa seedlings are inoculated with a suspension of the fungus in water by placing the plants in a beaker of the suspension for ½ hour followed by the repotting of the plants. Symptoms of the disease can be observed 10-14 days later. Pieces of stem tissue from inoculated plants are then surface disinfected, sectioned and placed on agar and incubated

for 3-4 days after which *Verticillium* can be observed sporulating from the cut sections.

Non-Cultural Tests

Some fungal pathogens may also be detected in or on the seed without germination or incubation. Large fungal bodies such as sclerotia or ergot can be detected easily with the naked eye or low power magnification. Internal infection of loose smut (*Ustilago nuda*) in barley is present as hyphal strands within the embryo. The incidence of infection of seeds by this pathogen is determined by softening the seeds by soaking in sodium hydroxide solution overnight and isolating the embryo from the endosperm by washing the sample in a stream of warm water in a Grainger separator. The embryos are carried over the tip and are caught in a series of fine mesh sieves.

The sample is then further cleaned by repeated washing in a separatory funnel containing a solution of lactophenol and water. The embryos float and the chaff and endosperm tends to sink and are drawn off from the bottom. The recovered embryos are then placed into thick bottomed glass dishes and cleared by boiling in lactophenol 10-20 minutes after which they are arranged in rows in the dishes for examination under a steroscopic microscope to reveal the presence of the fungus in infected seeds.

Surface borne smuts can be detected by washing the spores from the seed surface by shaking a known quantity of seed in water to which a small amount of detergent has been added. The spores are collected by centrifuging and resuspending the resulting pellet in a small known quantity of fluid. Identification and enumeration of the spores is then made under a high power microscope.

In many ways the seed pathologist must combine the skills of the germination and purity analyst as well as those of the cultivar verification specialist. Our "seeds" are the minute spores of the fungi which adhere to the surfaces of the seed as well as the fungi within the seed. In our role as a germination analyst we separate the contaminants—the bacteria and other fungi from the pathogens being sought after. Finally we must verify that the organism we have obtained from the seed is in fact the cause of the disease we are seeking.

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ROUTINE METHODS FOR DETECTING AND ENUMERATING SEEDBORNE BACTERIAL PLANT PATHOGENS

J. W. Guthrie[1]

Abstract

The methods and procedures for routine testing for seed-borne-bacterial plant pathogens are still in the developmental stage. This is due to the slow development of research into bacterial plant pathology in general. However, several methods have been developed to handle various critical seed-borne bacterial disease situations. Efforts are now being made both in the United States and in the ISTA Plant Disease Committee to correct this situation.

***Additional index words:*—seed pathology, bacterial diseases, testing techniques.**

There are relatively few common routine methods currently used for detecting or identifying bacterial plant pathogens. So far, to my knowledge, if there are 20 laboratories making such tests there may well be 20 methods each with variations of an original procedure. This doesn't lead to the exactness that many seed testing laboratories strive to obtain. However, I don't believe that there have been any great blunders or errors because of this, but better organization seems appropriate.

Let me digress for a minute to bring you up-to-date. It is my guess that 30 years ago and maybe even as close as 20 years ago, the number of plant pathologists who were adequately schooled to work with bacterial plant pathogens could be counted on two hands. This unfortunate situation has been rapidly corrected and now in many states and provinces there exist skilled professionals whose responsibilities include research on bacterial plant pathogens. The progress has been excellent. Plant pathology departments of major universities now offer a special course in diseases incited by bacterial plant pathogens. However, I must throw a little more cold water on the situation by indicating that in many instances there is still no one experienced in the detection and enumeration of seed-borne bacterial plant pathogens. Perhaps this isn't as serious a deficiency as it first appears. We might have to retread some plant pathologists to think like a seed pathologist.

A few of the ways that have been used to approach this seed-borne problem include (a) detection of infected seed by external characteristics visible on the seed coat, (b) diagnosis of the problem by plant symptoms, (c) isola-

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tion of the bacterial pathogen to confirm and identify the disease by the organism itself and (d) to combine any of the above. Before I go into details of these procedures, there are two major obstacles to reckon with in developing a successful routine identification method. The first one is to obtain a source of seed with a constant and reliable level of seed-borne bacteria. Artificially contaminated seed is often so variable that even a simple seed treatment will eliminate the contaminating organism and no valid results obtained. Seed sources that are naturally infected with some bacterial pathogens are rather difficult to locate, and if research does not have an adequate seed supply, new methods for determining infected seed are not easily tested under laboratory, field or storage conditions.

The second obstacle which is common to many seed testing stations is adequate sample size (3). Where and how much seed is needed? Seed-borne bacterial plant pathogens can reside in extremely small numbers on or in seed. In bean seed, for example, in order to be statistically confident that a proper sample size was used for detection of the halo blight pathogen at the one percent level, about one-half of the sample must be tested. Can you imagine what this means? A seed source of 100,000 pounds shows that you must test 50,000 pounds. This is hardly practical and borders on insanity. A standard quantity of bean seed for such tests has been reduced to 5 to 10 pounds with the hope of better than average sample acquisition.

Over the years, however, various methods have been developed to detect seed infected by bacterial plant pathogens. Some of these methods are simple while others are of a more complicated nature.

White seeded beans infected with *Pseudomonas phaseolicola* (Halo blight) may fluoresce if viewed under ultraviolet light. However, broken seeds and seed coats also fluoresce. If all seed or seed parts that fluoresce are collected and tested by a standard seed soak procedure the odds on finding diseased seed are greatly facilitated. This is simply a procedure of concentrating fluorescent seed parts, some of which may be caused by infected seed. This procedure will not work with darkened skinned bean cultivators (4, 10).

Some cucumber seed at germination will, if infected, show symtoms of this on the cotyledons within 8-10 days according to a report from Israel. This is their routine test for cucumber seed infected with *Pseudomonas lachrymans*(9).

Taylor from England developed a test for detecting *P. phaseolicola* on bean that involves grinding the seed sample to a flour consistency, suspending in sterile water, allowing it to settle and testing the supernatant for the presence of the halo blight organism by a simple serial dilution onto a special agar media (8). This media (Kings B) allows the pseudomonads to fluoresce if present. The bacterial colonies that do fluoresce are removed and tested by other methods to determine the identity of the organism.

There are various methods for isolating pathogenic bacteria from seed.

They include the flour extraction procedure of Taylor's, or to simply incubate the seed in sterile water from 4 to 36 hours. This liquid can be either tested directly by an immunofluorescence serology (6) or the liquid used to inoculate test plants, or trasnferred to a special agar to make the final identification of the bacteria using biological or serological tests or a combination of these. Once a suspected bacterial colony is found, many different tests can be used. One test is serological which can be a simple micropercipitin, agar gel diffusion, or immunofluorescence (1, 3).

Some bacterial plant pathogens offer additional identifying procedures. *Xanthomonas* species are tested for their ability to convert starch so these organisms are streaked onto starch agar. A positive test, after applying a solution of iodine-potassium iodide (IKI) is a clear area on the agar (5). This is also used for identifying *X. campestris* (cabbage pathogen). *X. phaseoli* var. *fuscans* (a bean pathogen) will, after a few days growth on nutrient agar, turn the agar brown. A paper from Nebraska tells of a special agar for the isolation of a *Corynebacterium nebraskense* from infected corn tissue (7). Seed-borne plant pathogens from soybeans are readily isolated by using either moist cellulose pads or corn meal agar at 30 °C.

The use of bacterial phage was reported as good for the identification of *Corynebacterium michiganense* from tomato seed. Others have found that there was too much variation using the phage for this purpose (2).

Many plant pathogenic bacteria are gram negative whereas others are gram positive. This allows a unique separation of important bacterial pathogens. Species of the genus *Pseudomonas*, *Xanthomonas*, *Erwinia* and others are all gram negative whereas an important gram positive pathogen group are species of the genus *Corynebacterium*.

Sometimes the presence of bacterial slime on seed can be valuable as a determinant for identification. All that is necessary is to carefully touch a sterile needle to this residue and then inoculate the plant and wait for symptoms to develop. In addition, leaves from diseased peas can be placed just above a fresh pea pod and by passing a sterile needle through the leaf lesion into the pod can often result in a typical bacterial lesion.

In the U.S.A. there is an effort to collect, evaluate and disseminate recent developments in seed-borne bacterial plant pathogens under the auspices of regional research committes. One such committee (NCR-100) concerns itself with black rot of *Brassica* spp. as incited by *Xanthomonas campestris*. This group just met in Orlando, Florida to compare notes and determine future needs and directions. A second committee (NCR-135) is titled "Detection and Control of Plant Pathogenic Bacteria on Seed and Plant Propagative Materials". Some of the best researchers available are involved with this group. It is all encompassing in scope and the latest information is made available via their annual committee report.

The International Seed Testing Association (ISTA) Committee on Plant Disease (PDC), formed an eighth subcommittee or working group on seed-

borne bacterial plant pathogens in 1975 when Dr. Paul Neergaard of Denmark was chairman. The PDC is now under the leadership of Dr. Claude Anselme of France and is assisted by Dr. Johan Jorgensen from Denmark who are working diligently to try to develop certain standard procedures to be published in Seed Science and Technology, so that the more complex tests, i.e. immunofluorescence procedures can be made available to all who need it. There will also be a working sheet for each important seed-borne bacterial plant pathogen.

I hope that greater help will be forthcoming to assist all seed testing laboratories in this relatively difficult area of seed-borne diseases.

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METHODS OF DETECTING SEEDBORNE PLANT VIRUSES[1]

T. W. Carroll[2]

Abstract

A few seed transmitted viruses are carried on the surface of seed and in seed parts outside the embryo; however, most are carried in the embryo. The methods that can be used to detect seedborne viruses include both biological methods (grow-on test and direct seed test) and serological tests (agar double diffusion, radial diffusion, latex flocculation, ELISA, SSEM). These methods are described in some detail and all partially meet the criteria of simple, rapid, specific, sensitive and inexpensive that are distinctive for the "Ideal Method".

Additional index words: **seed transmitted viruses, serological tests, ELISA, SSEM, grow-on test, double agar diffusion test, radial diffusion test, latex flocculation.**

Introduction

Before I discuss the detection of seedborne plant viruses, I believe some statistics on plant viruses, in general, are in order. About 100 plant viruses are fairly well known. Perhaps another 100 or more are lesser known. An additional 500 or so transmissible plant pathogens are thought to be viruses, but their nature is not yet established beyond reasonable doubt. Of these viruses and pathogens, over 80 different viruses or virus-like agents have been recorded as being seed transmitted.

A few seed transmitted viruses are carried on the surface of seed, such as tobacco mosaic virus (TMV) on tomato seed. A limited number of seed transmitted viruses are carried in parts of the seed outside of the embryo. A good example of this is TMV in the testa or seedcoat of tomato. Most seed transmitted viruses, however, are carried in the embryo. Bean common mosaic virus (BCMV) and barley stripe mosaic virus (BSMV) are notable examples of embryo infecting viruses.

The economic importance of seedborne viruses is largely determined by the epidemiology of those viruses. For some viruses the primary reservoir and means of spread is the seed of the crops affected. In this case, control may be greatly simplified since the production and use of virus-free seed should provide satisfactory control of the viruses. Unfortunately, for many

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other seedborne viruses, seed of the crop is not the sole source of the viruses. Weeds common in the crop growing areas also serve as sources of inoculum. The epidemiology of some seedborne viruses is further complicated by the fact that insects and nematodes also spread the viruses. Control of these latter viruses requires more difficult measures.

It is apparent that what is needed for the detection of seedborne plant viruses is an "Ideal Method". This method would be simple, rapid, specific, sensitive, and inexpensive. The method would be simple so that no elaborate analytical procedures would be required. Furthermore, it should be simple in order to test a number of different samples simultaneously. In other words, the method could be standardized for routine testing. The "Ideal Method" should also be simple because the interpretation of results would be unequivocal.

The "Ideal Method" should also be rapid to insure that the results would be obtainable in a matter of hours or even minutes. This would mean that large numbers of samples could be run in a short time.

The "Ideal Method" should be specific in that it would detect only the intended virus or viruses. Thus, the method would serve to both detect and identify viruses.

The "Ideal Method" should be sensitive because low concentrations of virus would be readily detected ($< \mu g/ml$ or about 1 to 10 billion virus particles per ml). The virus could be detected in a single seed or it could be detected in one infected seed per 1,000 healthy seeds.

The "Ideal Method" should be inexpensive because no sophisticated equipment would be required and no special facilities would be needed. Furthermore, no highly trained personnel would be necessary. In short, the cost per unit tested would be cheap.

Unfortunately, not one of the present methods for detecting seedborne viruses includes all of the attributes of the "Ideal Method". Some methods are simple and rapid, while others are complex and time consuming.

My talk today will focus on embryo transmitted viruses. It will pertain only to the plant viruses infecting the true seeds, or the ripened ovules of crop plants. In the short time allotted to the subject, I will give an overview of some of the currently used methods for detecting seedborne plant viruses. I will also mention the main attributes of these methods.

For ease of discussion, related or similar detection methods have been grouped within a broad category.

Biological Methods

The biological methods include two important tests. The first test is known as the growing-on test. On a world-wide basis, this test probably constitutes the single most important method. Often times, particularly in the developing countries, it is the only test used.

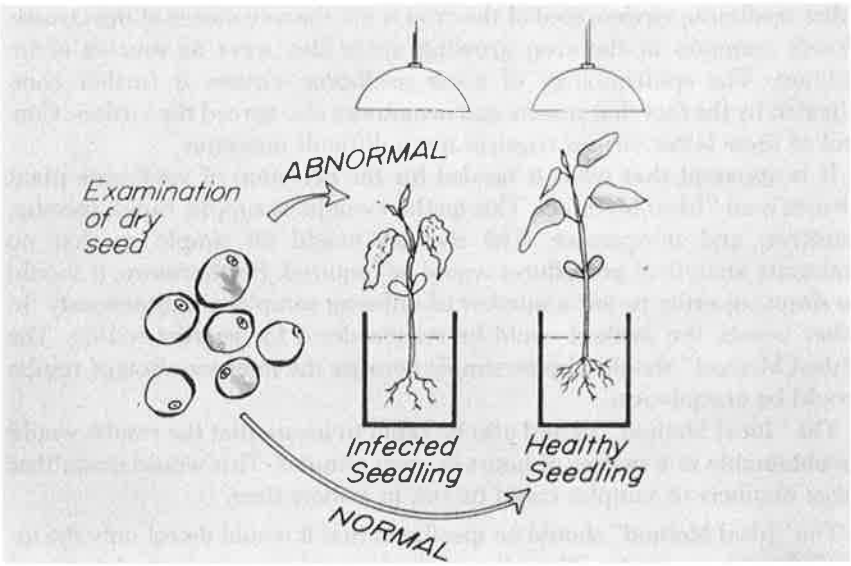


Figure 1. Schematic representation of the growing-on test.

Growing-on test: The growing-on test as illustrated in Fig. 1, begins with an examination of dry seed. Any abnormal seed is noted, and usually that seed is tested separately from the normal seed. Next, the seed to be tested is planted in various substrates so progeny plantlets or seedlings can be observed for characteristic symptoms of virus diseases. The progeny should be maintained in a suitable environment. The temperature and light regimes should be appropriate for the virus disease or diseases in question. In addition, the progeny plants should be kept free of pests such as insects and mites.

The growing-on test is fairly simple and relatively inexpensive. It offers the most direct evidence of seed transmission of a plant virus. Generally it detects all moderate and severe strains of a virus, and it may detect some mild strains too. Single seeds can be tested and the incidence of virus determined within the seed lot. This test has been used for many seedborne viruses and is presently used in the Salinas Valley of California for screening commercial lettuce seed lots for lettuce mosaic virus.

Frequently, an infectivity assay is used in combination with the growing-on test. Progeny plants from the seed tested are triturated (ground) in an appropriate medium and the resulting triturate (sap) inoculated onto special test plants. These test plants are capable of specifically responding locally or systemically to the virus tested. In other words, this infectivity assay enhances the sensitivity, and in some cases, the selectivity of the growing-on test. With the proper test plants, single seed infections can be detected. Virus concentrations of 1 to 10 μg/ml have been discovered.

Direct seed test: The second type of biological test is the direct seed test and it is diagrammatically represented in Fig. 2. It is similar to the growing-on test and it includes an examination of dry seed and an infectivity assay. Once again, any abnormal seed is recorded and processed separately from the normal seed. All seeds are usually soaked in an aqueous medium and then triturated by some means (Wiley mill, etc.). The slurries produced are then applied to indicator or test plants. The slurries containing virus produce symptoms typical for a given virus on the indicator plants. Single seeds or composite seed samples may be processed. The strong points of the test are its relative simplicity, specificity and selectivity. The incidence of plant virus in a population of seed may be enumerated by this test. This test will soon be used routinely for the detection of pea seed-borne mosaic virus in pea seed. The seed indexing program will be conducted by the Palouse Seed Co. under the supervision of the Washington and Idaho State Departments of Agriculture. *Chenopodium amaranticolor* (purple pigweed) will be the indicator plant. One infected seed can be detected within a mixture of one infected and 9 virus-free seeds.

Serological Tests

Serological tests comprise the next group of detection methods. These tests involve physicochemical reactions between blood sera (usually rabbit) and plant viruses. The blood sera used have been taken from animals that have been injected with virus preparations. These animals do not

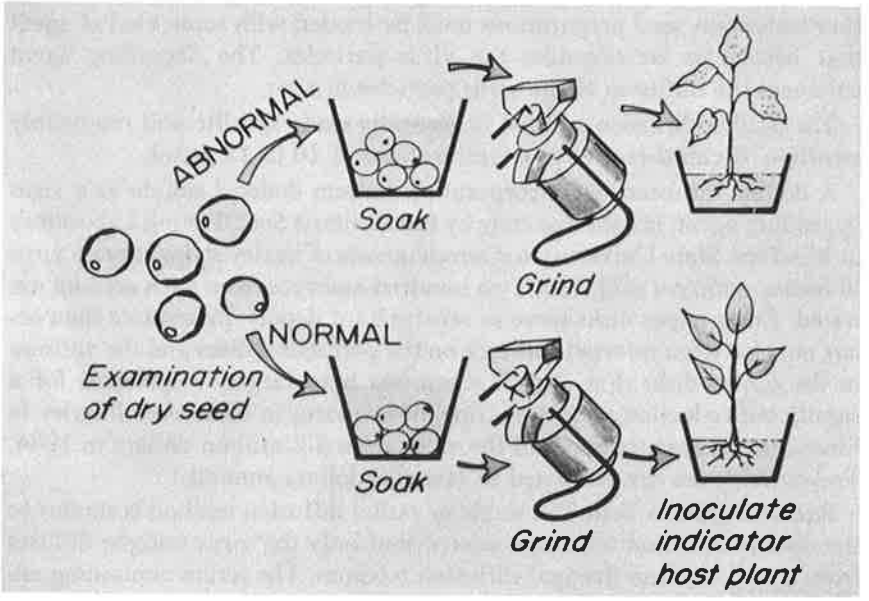


Figure 2. Schematic representation of the direct seed test.

become infected by the plant viruses, but produce antibodies to the viruses via their immunological protective system. For a given virus, a sample of blood is removed from an animal after sufficient time has elapsed following injection of the virus, and the blood serum carrying the antibody molecules specific for that virus is then separated from the blood. Most of the tests to be described below are based on the antibodies in the serum precipitating the plant viruses located in the juice of triturated seeds. The analyst then examines the test material for these precipitates. The serological tests usually detect all strains of a virus. Once again, for all of these tests, a dry examination of seed preceeds the actual serological test.

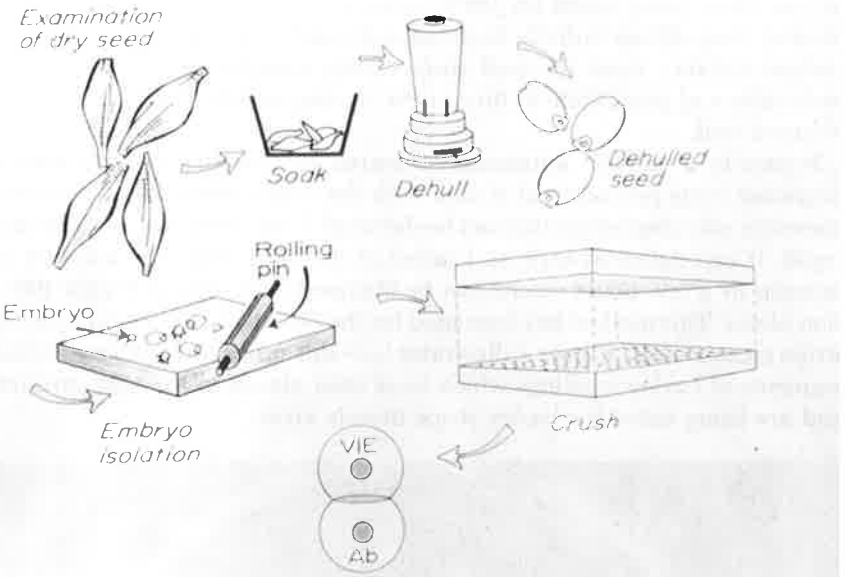
Double Diffusion Test: The double diffusion technique has been used more extensively than any other serological method. It can assay a single seed or parts of a seed. Usually the seed to be tested is soaked in tap water. Then the whole seed or one of its parts (embryo, for example) is triturated and the resulting triturate is transferred to a well cut in a diffusion medium. Commonly, the diffusion medium is an agar gel. Thereafter an antiserum specific for a suspect virus in the seed is placed in a separate well. In time the virus particles (antigen) and the antibody molecules diffuse toward one another. Since this diffusion is in two different directions, it is called double diffusion. When the two seroreactants reach a point in the gel where the relative concentration of each is serologically equivalent, antigen and antibody molecules complex, precipitate, and become immobilized. The precipitate appears as a white band, at some point between the two wells.

To detect viruses that have an elongated structure by the double diffustion technique, seed preparations must be treated with some kind of agent that breaks up or degrades the virus particles. The degrading agent enhances the diffusion of the virus particles in agar

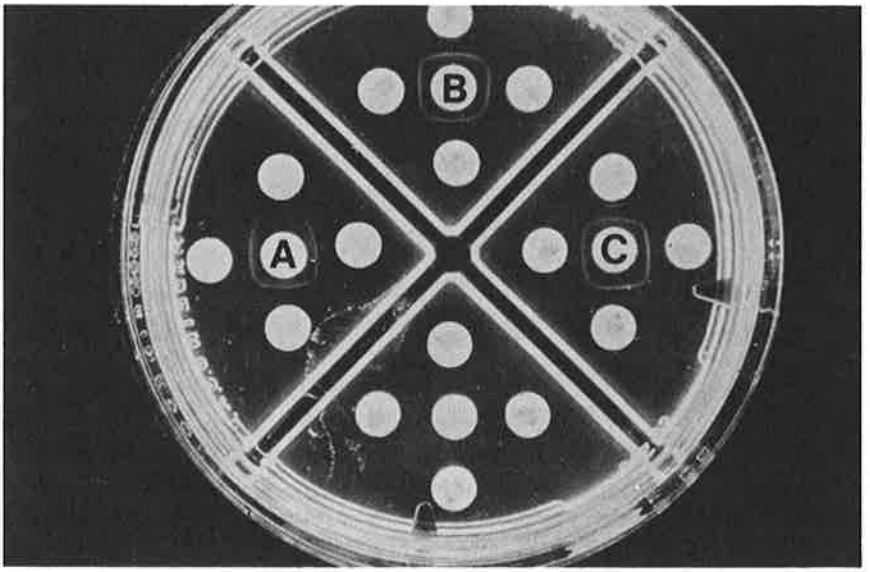
The double diffusion method is generally quite specific and reasonably sensitive. It can detect virus concentrations of 10 to 25 μg/ml.

A double diffusion test incorporating sodium dodecyl sulfate as a virus degrading agent, is used routinely by the Montana Seed Testing Laboratory at Montana State University for serodiagnosis of barley stripe mosaic virus in barley embryos (Fig. 3A). Two hundred embryos from each seed lot are tested. Filter paper disks serve as seroreactant depots. Precipitate lines occur only between infected embryos on the peripheral disks and the antisera in the center disks (Fig. 3b). The test has been largely responsible for a significant reduction of barley stripe mosaic virus in commercial barley in Montana. Losses attributed to the virus were 3.2 million dollars in 1964. Today the losses are estimated at 100,000 dollars annually.

Radial Diffusion Test: The single or radial diffusion method is similar to the double diffusion technique except that only the virus antigen diffuses from a well out into the agar diffusion medium. The serum containing antibodies to a specific virus has been included in the agar in this test. Therefore the procedure is to charge the wells with the seed or seedling



A. Schematic showing the preparation and serological analysis of barley embryo extracts. Antigen from a virus infected embryo (VIE) formed a precipitate (lens shaped area) with antibody (Ab) molecules.



B. Quadrant petri dish with white precipitates due to positive virus-antibody reactions. Precipitate lines occur only between infected embryos on the peripheral disks and the center antisera disks A, B and C. (Reproduced by permission of the American Phytopathological Society from Carroll, 1980).

Figure 3. The agar double diffusion test.

preparations being tested for the presence of virus. If virus molecules are present, they diffuse radially from the wells and when they have reached a certain distance from the well surface they complex with the antibody molecules and precipitate in this region. A ring or halo results around the charged well.

Normally a subunit antiserum (prepared by injecting rabbits with a degraded virus preparation) is used with the radial diffusion method and therefore only degraded virus can be detected. This method is sensitive and rapid. It can detect as little as 1 μg/ml of degraded virus. In a matter of minutes or a few hours results can be obtained with pre-made agar diffusion plates. This method has been used for the detection of seedborne barley stripe mosaic virus. Figure 4 illustrates how this procedure works with leaf segments of barley seedlings which have been placed in the agar medium and are being tested for barley stripe mosaic virus.

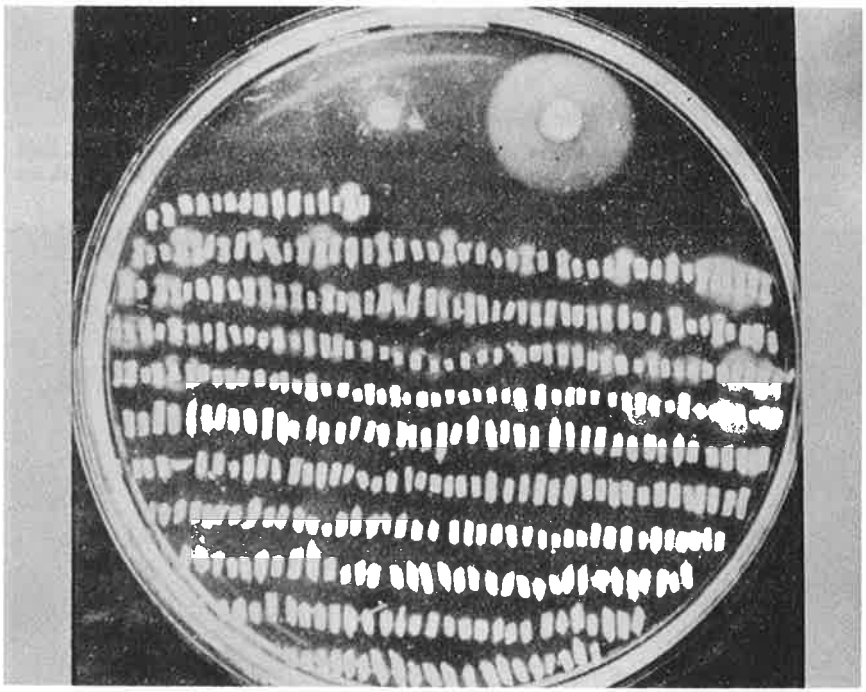
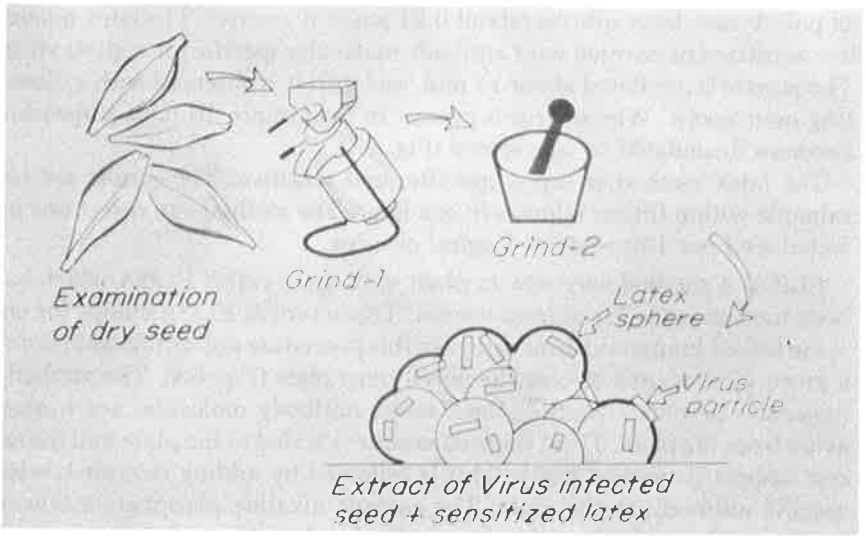


Figure 4. Petri dish with radial diffusion test of barley leaf segments. A positive test is indicated by the presence of a white precipitate at the ends of the segments in the agar medium. (Reproduced by permission of the American Phytopathological Society from Slack and Shepherd, 1975).

Latex Flocculation Test: Latex flocculation is another serological method and is represented diagrammatically in Fig. 5A. Dry seeds or seedlings have been tested by this method. For seeds, lots of one hundred seeds are ground in a Wiley mill. Then about 0.1 g of the ground seed material is placed in 2



A. Schematic of latex flocculation method.

B. Two 100 μl pipettes containing suspensions of antibody-sensitized latex. Virus presence in the upper pipette caused the suspension to become flocculated or aggregated. Absence of virus in the lower pipette was indicated by the dispersed appearance of the latex suspension. (Reproduced by permission of Zeitschr. Pflkrankh. Pflschutz. from Lundsgaard, 1976).

Figure 5. The latex flocculation test.

ml of buffer and further ground in a mortar with a pestle. About 20 μl of this seed extract are then placed in a 100 μl pipette to which 10 μl of tagged latex had been previously added. The tagged latex consists of a suspension

of polystyrene latex spheres (about 0.81 μm in diameter). The latex spheres are sensitized or covered with antibody molecules specific for a given virus. The pipette is oscillated about 15 min. and then it is observed with a dissecting microscope. Where virus is present in the sample the latex suspension becomes flocculated or aggregated (Fig. 5B).

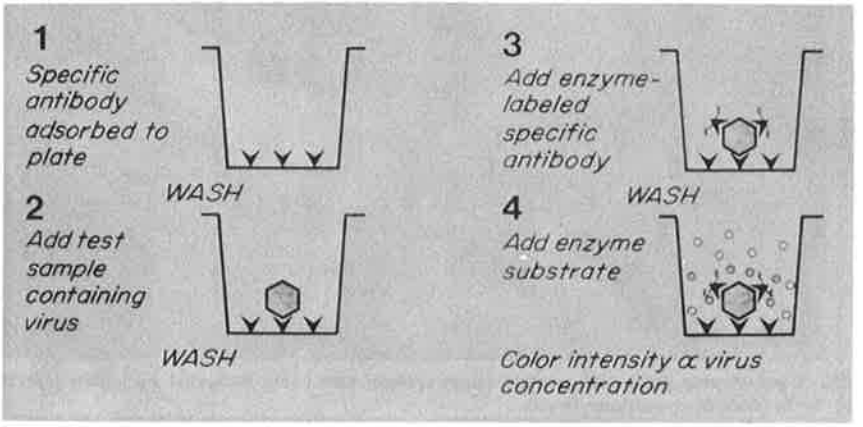
The latex method is rapid, specific, and sensitive. The results are obtainable within fifteen minutes to one hour. The method can detect one infected seed per 100 seeds or 1 μg/ml of virus.

ELISA: A method very new to plant virology is called ELISA which has been used to assay for animal viruses. The acronym ELISA stands for enzyme linked immunosorbent assay. In this procedure antiserum specific for a given virus is used to coat the polystyrene plate (Fig. 6A). The antibody molecules become adsorbed. Next, excess antibody molecules are washed away from the plate. Then, the seed sample is added to the plate and the excess sample is washed away. This is followed by adding enzyme-labeled specific antibody to the plate. The enzyme alkaline phosphatase is conjugated to the antibody molecules specific for the virus under examination. The excess labeled antibody molecules are removed from the plate by washing. Finally, enzyme substrate is added to the plate. The substrate is para-nitrophenyl phosphate. The enzyme hydrolyzes the substrate. Hydrolyzed substrate is determined by measuring the extinction at 405 nm wavelength spectrophotometrically or by visual observation. For this assay dry-milled or water-soaked seed is extracted with buffer (Fig. 6B). The seed preparations are then ground further with another buffer and a surfactant. Afterwards, the seed extracts are then applied to polystryrene plates following the standard ELISA procedure. Virus infected seed preparations would produce a yellow color (Fig. 6C).

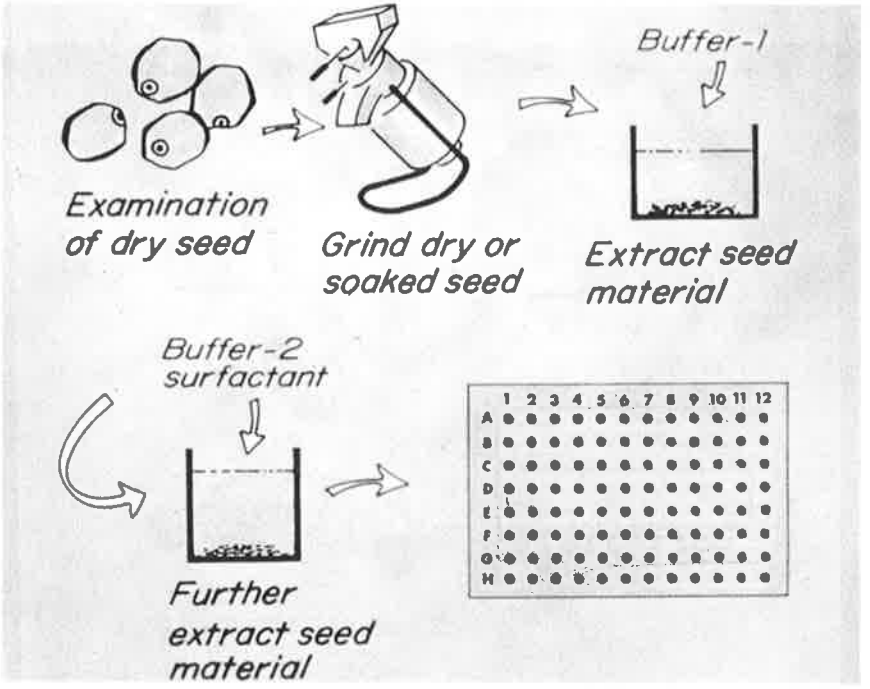
High sensitivity is the main attribute of the ELISA method. Some workers claim to detect 0.1 μg/ml of virus. The method has been used experimentally to detect tobacco ringspot and soybean mosaic viruses in soybean seed. Low levels of infection (1-4%) could be detected in extracts from seed lots.

SSEM: The final technique I will discuss is call SSEM. This acronym stands for serologically specific electron microscopy. This method and a few others comprise the category of methods known as immunoelectron microscopy. Essential steps in the SSEM procedure are as follows: (1) The copper specimen support or grid is covered with a parlodion film. (2) The filmed grid is floated on antiserum diluted 1:100 to 1:5000 in tris buffer. (3) After 30 minutes the grid is washed repeatedly to remove unadsorbed serum proteins. (4) The grid is floated on an extract of virus-infected tissue from 10 min. to 24 hours. (5) The grid is washed repeatedly to remove cellular debris and salts. (6) The grid is then stained with 5% uranyl acetate for 1.5 minutes or longer. (7) The grid is washed with distilled water or 95% ethanol, and finally (8) The grid is blotted dry and examined in the electron microscope. The procedure is shown in Fig. 7A. For seed

diagnosis, dry seed is ground by mortar and pestle or Wiley mill or soaked seed is homogenized (Fig. 7B). The seed preparation is applied to an electron microscope grid containing an antiserum specific for the virus in question.

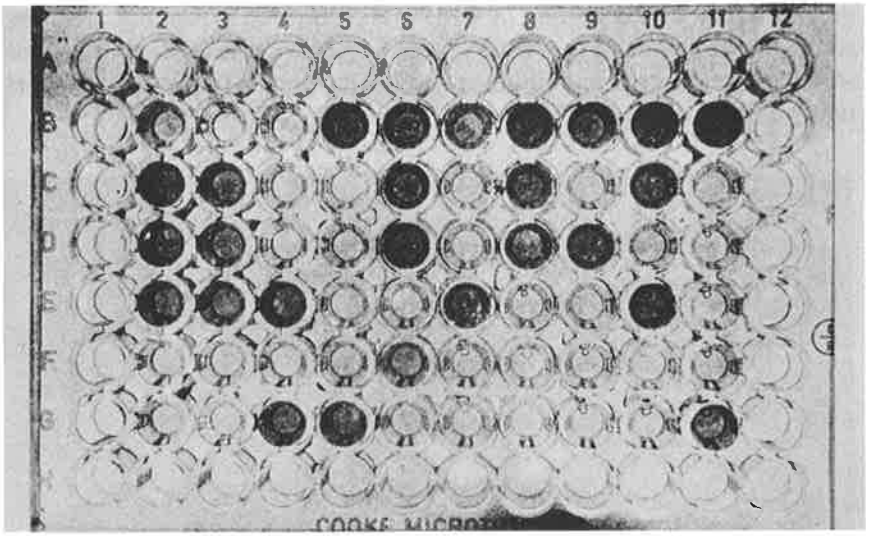


A. Principle of the ELISA method.



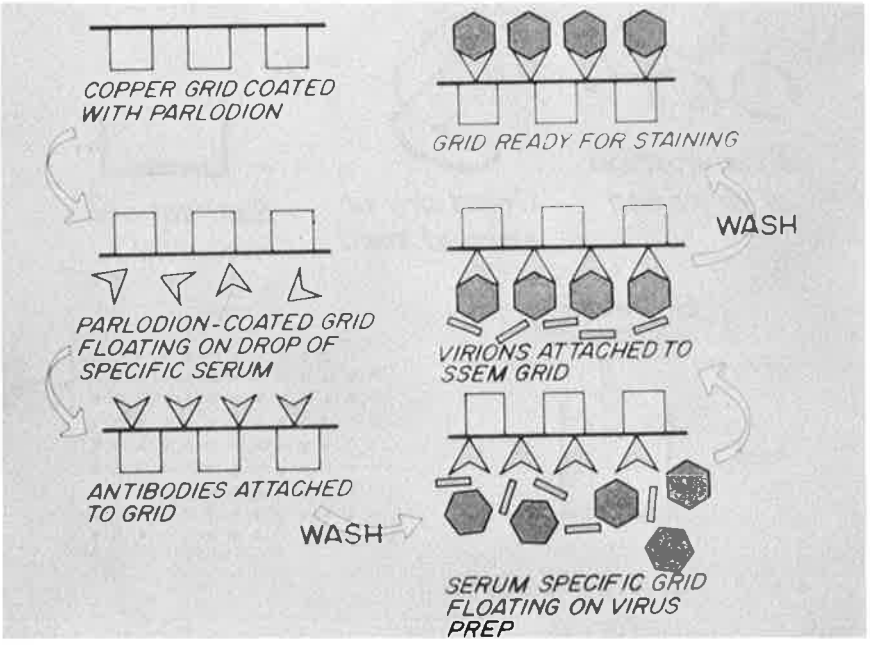
B. Schematic of ELISA test procedure.

Figure 6. The enzyme linked immunosorbent assay (ELISA) method.



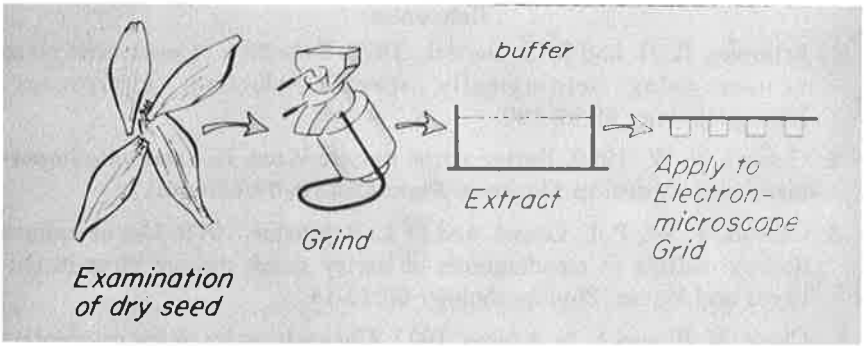
6C. A polystyrene plate with positive (virus present) tests being indicated by yellow colored wells (dark in appearance here).

Figure 6 (Continued)

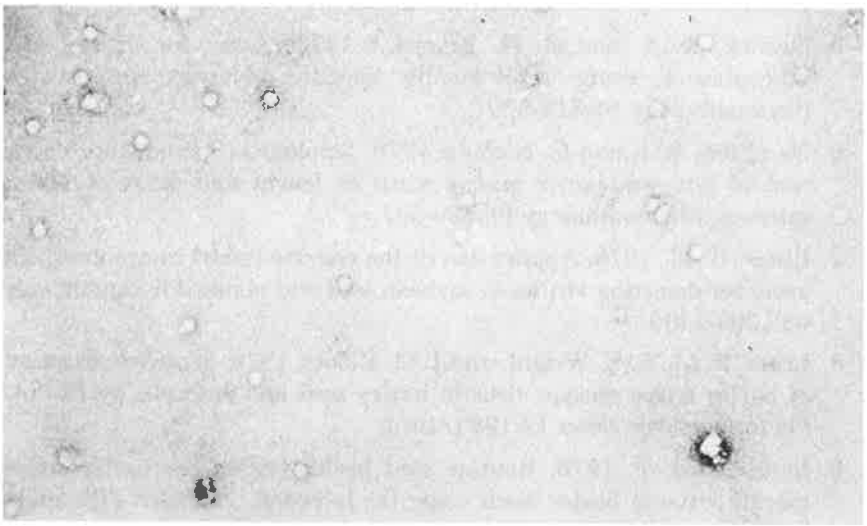


A. Principle of the SSEM method.

Figure 7. The serologically specific electron microscopy (SSEM) method.



7B. Schematic of the SSEM method.



7C. Electron micrograph showing presence of the spherical particles of tobacco ringspot virus. (Reproduced by permission of the American Phytopathological Society from Brlansky and Derrick, 1979).

Figure 7 (Continued)

Samples from infected seed lots reveal the presence of virus particles characteristic for the suspect virus. When tobacco ringspot (Fig. 7C) and soybean mosaic viruses were tested, each virus could be detected in a singly-infected soybean seed. The SSEM method was sensitive enough to detect the two soybean infecting viruses in a mixture of one infected seed per 1,000 healthy seeds. The method is simple for trained personnel, and it is rapid, selective, and sensitive. It requires the use of a transmission-electron microscope.

The references which follow give additional details of the methods described in this presentation.

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EPIDEMIOLOGICAL ASPECTS OF SEED DISEASE CONTROL[1]

D. C. McGee[2]

Abstract

An increased understanding of the disease cycle of seed-borne plant pathogens can lead to more effective and efficient disease control schemes through improvement and integration of seed disease control practices.

Additional index words: **seed pathology.**

Introduction

Diseases directly affecting seed are important limiting factors in seed production resulting in loss of seed yield, reduction in seed quality, and carryover of pathogens to subsequent crops. Seed diseases are controlled by methods such as seed health testing, seed treatment, and application of fungicides to the growing crop. These control practices often have been developed by an empirical means, however, with little consideration for the disease epidemiology. Application rates for fungicide seed treatments, for example, usually are determined by relating treatment rates to subsequent disease development in a series of field tests carried out in different locations under a variety of environmental conditions. If repeated often enough, this type of experiment may provide reasonably reliable information on application rates, but very little knowledge is obtained about the disease epidemiology. As a result, failures in control practices cannot be explained, and more fungicides tend to be used than are necessary. By obtaining an understanding of the epidemiology of seed diseases, control practices can be much improved.

The Disease Cycle

The life cycle of a plant pathogenic organism can be viewed as consisting of four basic phases, survival, transmission, infection, and disease development (Fig. 1). The survival phase occurs during the period when a susceptible host is not available. Pathogens can survive in various ways; in the soil, on crop residues, on alternative hosts, or in association with seeds. The next phase, transmission to a susceptible host plant, can be effected by environmental factors such as wind, rain, and insects or by planting infected seed. After contact is made between the pathogen and host, the process of

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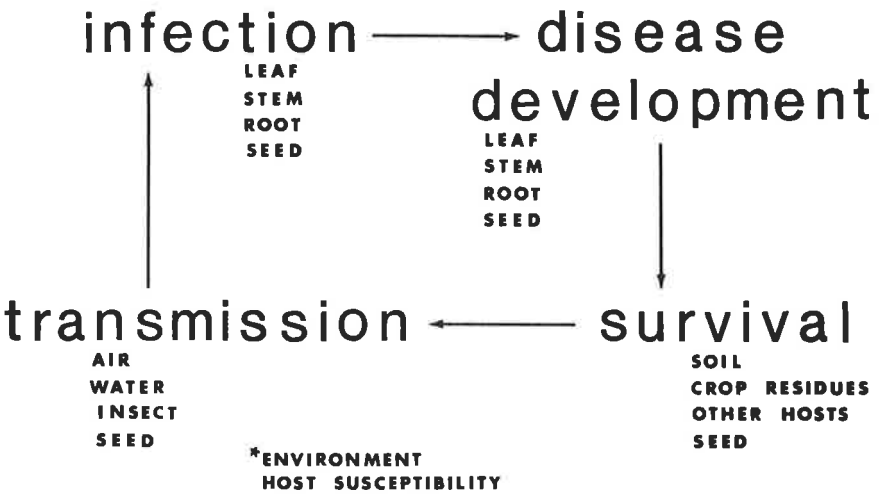

Figure 1. A simplified plant disease cycle.

infection may occur, during which time the relationship between the two is established. Any part of the plant can be infected, including seeds. Further progression of this relationship represents the final, disease development, phase of the cycle. All phases of the disease cycle can be influenced by environmental factors. If for example, free water is required for infection to take place, then rainfall probably will be required for this phase of the cycle to occur. If a pathogen is killed by low temperature, then the amount of inoculum that survives might be influenced by the severity of the winter. The life cycle also is influenced by genetic factors such as the susceptibility of the host plant to prevailing races of the pathogen. The amount of disease that finally develops is the net effect of all these factors. Seeds, clearly, can be involved in all phases of the cycle. The epidemiology of seed diseases therefore can be defined as the study of relationships between the seed-related aspects of the disease cycle and the various factors that influence the cycle.

The Role of Seed in the Disease Cycle

Although large numbers of microorganisms are associated with seeds, few of them are of significance with respect to pathogenicity. The majority are non-pathogens, for which there is no evidence that they cause disease as a result of their presence on seed. Examples are *Alternaria* spp. on grass seeds and *Chaetomium* spp. on soybean seeds. Some of these fungi may, in fact, have the capacity to colonize seeds at the expense of pathogenic fungi. Another group includes pathogens of a crop that are seed-borne, but the seed-borne phase of the disease has little significance with respect to disease development. An example is *Leptosphaeria maculans*, the cause of blackleg

of oilseed rape. Although seed-borne inoculum is important as a means of introducing the pathogen into a new area, seed-borne inoculum is insignificant compared with that carried on crop residues in areas where this disease is established. A third group consists of pathogens for which infected seed is the main source of inoculum, and, when seed infection is controlled, the disease is effectively controlled. Examples are lettuce mosaic virus and *Gloeotinia temulenta*, the cause of blind seed of rye-grass. Finally there is a group of pathogens that directly affect the seed, resulting in loss in viability or other seed quality characteristics. *Phomopsis* spp., the cause of pod and stem blight of soybeans, is an example of this group. The significance of seed-borne organisms obviously must be determined before attempts are made to control them.

Controlling Seed Diseases

Methods of controlling seed diseases have the objective of interfering with the disease cycle in a way that will reduce subsequent disease development. Seed treatment affects the cycle at the survival and transmission phases (Fig. 1) by reducing seed-borne or soil-borne inoculum. Seed health testing can be employed when enough is known about the disease cycle to establish tolerance levels for seed-borne pathogens. Cultural practices, such as crop rotation, destruction of residues, or alteration of harvest date, can affect any phase of the cycle. Fungicide application to the growing crop usually affects the infection and disease development phases. The use of foliar fungicides to control pod and stem blight (*Phomopsis* spp.) of soybeans is a good example of a control practice developed with little consideration for the disease epidemiology. The main effect of this disease is a reduction of seed viability. Foliar applications of benomyl at the mid pod growth stage and 14 days later can control pod and stem blight under some circumstances, but treatment results often are unsatisfactory. Present knowledge of the epidemiology of the disease indicates that the fungus infects the pod, remains in an inactive phase for an undetermined period of time, and then infects the seed. It also is known that, to be effective, the fungicide must be applied before seed infection occurs. Because the time when the fungus moves from pod to seed is not clear, the fungicide may be applied either too early or too late. Another reason for the apparent "ineffectiveness" of the treatment is that it often is applied when disease pressure is slight, and no improvement in yield or seed quality is noted by the grower. This is a problem particularly in states such as Iowa, where pod and stem blight often is not severe. With a better understanding of the epidemiology of pod and stem blight disease, the timing of application of the fungicide could be greatly improved, and the need for application of the fungicide might be determined by using a predictive scheme. Clearly, by studying the epidemiology of seed diseases, improvement and integration of control practices can be achieved, resulting in more effective disease control.

IMPORTANCE OF SEED PATHOLOGY IN SEED TRADE QUALITY CONTROL PROGRAMS

John E. Cross[1]

Abstract

Seedsmen attempt to reduce seed-borne pathogen levels by planting disease-free stock seed and careful inspection of both seed fields and seed lots. When seed-borne pathogens are detected the seed lot may be treated, destroyed or specially labeled. Seedsmen cannot guarantee absolute freedom from seed-borne pathogens.

Additional index words: **seed pathology, quality control product liability.**

Introduction

It is a great pleasure to represent the seed trade at this symposium. I speak as a member of the A.S.T.A. phytosanitary subcommittee, but the examples which I will use are drawn from my experience with Asgrow.

Many of my comments represent the A.S.T.A. position on proposals made by the European and Mediterranean Plant Protection Organization (EPPO). This group had developed proposed quarantine lists for seed-borne pathogens in Europe, North Africa, and the Middle East.

Concerns of the Seed Industry

Disease-free stock seed. The fundamental strategy in producing high quality commercial seed is to plant high quality stock seed. The basic philosophy is the same whether we are talking about genetic quality or pathological quality. Seed companies go to considerable expense to produce disease-free stock seed. For example, we produce lettuce stock seed in insect-proof greenhouses to avoid lettuce mosaic virus spread by aphids. In halo blight of garden beans we avoid sprinkler irrigation, treat our stock seed with streptomycin sulfate, and are developing a test procedure to index stock seed.

Field inspection. Although this is an integral part of Quality Control it is fraught with danger. Too frequently infection is not detected, or there are symptomless carriers. Nevertheless, gross infection can be detected and the seed crop destroyed.

There is an interesting liability question when a seed-borne pathogen is detected in a seed production field, and the crop is destroyed. Was the pathogen on seed stock, in the grower's field originally, or introduced from an outside source. In general, seed production contracts do not address this important point.

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Seed Evaluation. As you well know the data is only as reliable as the sample. It appears that seed-borne pathogens frequently are more concentrated in certain parts of the seed field and so it is difficult to obtain a representative sample. The pathogen may be more prevalent in certain portions of the lot e.g., certain size grades. With halo blight of beans we are experimentally testing gravity cull samples, since the likelihood of finding the pathogen is much greater in shriveled seed than in normal seed. As an industry we have difficulty with the concept of "zero tolerance' if this means absolute freedom from a pathogen in a commercial seed lot. In lettuce mosaic we claim zero in 30,000—which means that 30,000 seeds were tested and no infection was found. However, the seed lot may well contain one infected seed in 100,000. The epidemiologists need to tell us how much infection is tolerable. Under which environmental conditions will epidemics develop? What are we to do with seed lots which contain one infected seed in 10,000? There is very little information in the literature about tolerances for seed-borne pathogens.

A recent paper by Anselme and Baker (1) describes 61 important seed-borne bacteria, fungi, viruses, and nematodes. It outlines their economic importance, distribution, and available testing methods.

Table 1 outlines the testing methods which are available for these various classes of causal agents. It is interesting to note, that official methods do not exist for major bacterial pathogens such as *Pseudomonas phaseolicola* and *Xanthomonas campestris*, which are of great concern to both seedsmen and customers.

Table 1. Availability of test methods for the various classes of seed-borne organisms.

	No Method Available	Only a Laboratory Method Available	ISTA Method Available
Bacteria	4	3	0
Fungi	12	7	24
Viruses	4	2	0
Nematodes	0	0	3

There is also a requirement to have a precise identification of the pathogen. The customer needs to know not only the level of contamination, but also the virulence of the pathogen. Serological techniques may identify the pathogen, but may not differentiate between virulent and avirulent strains. Specific races of many seed-borne pathogens have been identified, and host plants differ in resistance to these races. In certain instances the seedsman must identify the actual race which is present on or in the seed. This is a time consuming and expensive task.

Product Liability. This is an area of increasing concern to both seedsmen and customers. The concerns include:

1. Economic loss from contaminated seed in the current crop. For example, the halo blight pathogen may produce lesions on bean pods which have to be removed before the pods can be processed.
2. Economic loss in adjoining plants or fields. For example, the black rot pathogen (*Xanthomonas campestris*) can readily spread from cabbage in a transplant bed or greenhouse and contaminate millions of adjoining plants of other crucifer species. Ten dollars worth of seed can contaminate hundreds of thousands of dollars worth of transplants.
3. Contaminating subsequent crops. Certain seed-borne pathogens can survive on crop debris, weed hosts, etc. and provide a source of inoculum in succeeding years.

Action by Seedsmen

Seed treatment. When seed-borne pathogens are detected it is logical to attempt to control them with pesticides or hot water treatments. This is seldom completely effective. The pesticide may control surface contaminants but be ineffective against internal infection. Many effective pesticides are not registered in certain countries e.g., mercury, streptomycin. Heat sterilization is effective in certain instances, but may have an adverse effect on the vigor of the seed.

Labeling. The seedsmen has a real dilemma when the only available seed of a variety is infected, and seed treatment is not possible. The only reasonable approach seems to be to inform the customer of the facts and let him decide whether or not to plant the seed. One approach to labeling is illustrated in the tag which we used on garden bean seed produced in Idaho in 1977.

Tag: Caution: Halo Blight (*Pseudomonas phaseolicola*) was found in Idaho, where this seed lot was produced. We did not detect Halo Blight in the seed field which produced this lot. As an added precaution we have treated the seed with streptomycin. Nevertheless, we cannot assume that this seed is free of the Halo Blight organism.

Note: In addition to all other disclaimers contained herein Asgrow specifically disclaims all express and implied warranties related to freedom from Halo Blight.

The important point here is that we did everything practical to control *Pseudomonas phaseolicola*, but still could not guarantee complete freedom from the pathogen.

Discard lot. If the seed is not merchantable because of the presence of a seed-borne pathogen, then of course it must be discarded.

Conclusion

Although this brief presentation has stressed concerns and problems, the future is still bright. The seed industry recognizes the legitimate concerns of both customers and control officials. We are devoting significant resources to developing improved production techniques to produce disease-free seed, and are working closely with public institutions on techniques for evaluating seed lots. We solicit your assistance and guidance as we sail through these relatively uncharted waters.

Literature Cited

1. Anselme, C. and Baker 1979. Paper 79-020 presented at the F.I.S. meeting in Switzerland, May 1979.