Identification, significance and transmission of seed borne pathogens

S. B. MATHUR
Danish Government Institute of Seed Pathology for Developing Countries, Ryvangs Alle 78, DK-2900 Hellerup, Copenhagen, Denmark

M. P. HAWARE
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P.O., Andhra Pradesh 502 324, India

and

R. O. HAMPTON
USDA—ARS, Virology Laboratory,
Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA

Abstract

Several pathogens which cause important diseases in chickpea, faba bean, pea, and lentil are seed borne and seed transmitted. Examples are given to illustrate the importance of contaminated and infected seeds in the dissemination of diseases. Comments have been included on transmission by soil, infected plant debris, and vectors. Current laboratory seed health testing methods for detecting important fungi, viruses, and bacteria in pea, lentil, faba bean and chickpea are described.

Introduction

All four food legume crops of topical concern suffer from a number of diseases. Many of the economically important diseases are seed borne and seed transmitted; a check list is presented in the *Annotated List of Seed borne Diseases* (Richardson, 1979; 1981; 1983). We review here the significance of some of these important seed borne diseases on crop production, their transmission by seeds and other means, and laboratory testing methods for detecting pathogens in seeds of chickpea, faba bean, pea, and lentil.

Fungi

Chickpea

Chickpea is an important pulse crop in the Indian subcontinent, West Asia,

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Northern and Eastern Africa and Central and South America. Diseases are a major constraint to production. To date, 33 fungal, 1 bacterial and 7 viral diseases and phyllody (mycoplasma) have been reported on the crop from different parts of the world (Nene *et al.*, 1984). Some are of economic importance; these are Fusarium wilt (*Fusarium oxysporum* f.sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*), Ascochyta blight (*Ascochyta rabiei*) and Botrytis grey mould (*Botrytis cinerea*). Of the several diseases recorded, very few are reported as seed borne. Haware *et al.* (1978) have described the seed borne nature of *F. oxysporum* f.sp. *ciceri*, and seed borne diseases caused by this species and by *A. rabiei, B. cinerea, Colletotrichum dematium* and *Alternaria alternata*; methods for their detection in seed have been described in a technical bulletin (Haware *et al.*, 1986). This bulletin is primarily intended for seed production, seed certification, and plant quarantine personnel.

Fusarium wilt has been reported from several countries. In early infection it kills plants and can result in a total loss in yield. In India, it is estimated to cause a 10% annual yield loss (Singh and Dahiya, 1973). The fungus is a vascular parasite and is soil- and seed-borne. Seed borne infection is present in seeds harvested from plants which wilt after pod formation. Seeds from the wilted plants are generally small, wrinkled and discoloured. Though such seed can be detected visually, a seemingly normal seed may also harbour the pathogen. Therefore, it is important to test the seed for the presence of the fungus. Haware *et al.* (1978) showed that the fungus was present in the hilum region of the seed in the form of chlamydospore-like structures. These structures were thickwalled, spherical, closely packed, and connected by hyphal cells. Chickpea cultivars differ in the extent of yield loss and seed infection (Haware and Nene, 1980). The most common method of spread of the disease seems to be through seed and soil.

For detection, 400 seeds are surface-sterilized by immersing them for 2 min in 2.5% sodium hypochlorite. Seeds are then plated onto modified Czapek-Dox agar (10 per plate) and incubated at 20 °C for 8 d in a diurnal cycle of 12 h of near-UV light followed by 12 h darkness. The white cottony mycelium of *F. oxysporum* f.sp. *ciceri* can be observed emerging from the seed (Haware *et al.*, 1986). A seedling symptom test should be employed if agar medium is not available. Surface-sterilized seeds are sown into soil or fine riverbed sand in pots. These pots are kept in a growth chamber or in a glasshouse at 25 °C in a diurnal cycle of 12 h light and 12 h darkness. The seedlings should be monitored for at least 40 d for wilt symptoms. The seedlings from infected seeds generally show wilting between 15 and 25 d after sowing. The fungus can be isolated from roots. The wilt count closely agrees with the number of colonies detected on selective medium (Haware *et al.*, 1978).

Ascochyta blight is one of the most important diseases of chickpea, particularly in Pakistan, West Asia, and Northern Africa. In Pakistan, about 70% of the crop was lost to the disease in 1979 and in 1980 (Nene, 1982). It also appeared in epiphytotic form in parts of Punjab and Haryana States of

India during 1980 and 1981. For the first time, in 1983, Kaiser and Muehlbauer (1984) reported trace to severe incidence of blight in germplasm evaluation trials at Pullman, USA. Seventy-seven accessions out of the 125 tested were affected; cool, wet weather during June and July favoured infection and spread of the disease. According to these authors, the pathogen was introduced into the USA on seed imported from Syria and/or India. Measures taken to prevent spread and survival of *Ascochyta rabiei* included burning of plant debris, deep ploughing, destruction of all seeds, and crop rotation. The disease was also recorded in 1984 in 23 of 30 fields (588 of 811 ha) in the Nez Perce and Clearwater Counties of Idaho, USA (Derie *et al.*, 1985). One 20-ha field was ploughed down because of severe infection.

The most common and effective method of dissemination of *A. rabiei* appears to be by means of seed. Infected seeds are small, wrinkled and have dark brown lesions of various shapes and sizes. Pycnidia are found in deep lesions on such seed. If pods are infected at maturity, a seemingly normal seed may show only slight discolouration on the surface. Apparently healthy seed may also harbour the pathogen. Pycnidiospores obtained from the seed surface and pycnidia from 14-month old seed stored at 3° ± 1 °C showed only 33% germination (Maden *et al.*, 1975).

For seed health testing, potato dextrose agar with 1 g Dicrysticin-S per litre of medium is suitable. Seeds must be surface disinfected by dipping them into 2.5% sodium hypochlorite for 2 min. *A. rabiei* is slow-growing and if surface contaminants on the seed are not killed, the pathoghen may not be detected. Petri plates, each containing 10 seeds, are incubated in diurnal cycles of 12 h near-UV light and 12 h darkness at 22 °C for 10 d. The colonies of the fungus grow slowly on seed and are creamy white with black centers.

In the seedling symptom test, seedling emergence is not necessarily affected by seed infection. Indeed, the test does not give a reliable estimate of seed infection because, in many seedlings, the emerging shoots escape fungal contact and thereby no infection of *A. rabiei* is detected.

Faba bean

The crop is attacked by foliar, seedling, and root diseases. Some of the important fungal diseases reported to be seed borne are chocolate spot (*Botrytis fabae*), blight (*Ascochyta fabae*), and rust (*Uromyces vicia-fabae*).

Spread of *A. fabae* is mainly through seed. The fungus caused significant yield losses in both field and broad bean crops in New Zealand (Gaunt *et al.*, 1978): a reduction of 44% was recorded due to the severity of disease developing from seed with 12% infection compared to a genotype with only 0.2% seed infection. The significance of seed borne inoculum of Ascochyta was further emphasized by Gaunt and Liew (1981). In Canada, Wallen and Galway (1977) demonstrated that weed plants and buried host-material are of minor importance as sources of primary inoculum in the establishment of

the disease. However, in 1962, it was concluded by Geard in Australia that infected crop debris was an important means of carry-over.

Using *Ascochyta fabae* – infected seed (2–15% incidence), Hewett (1973) obtained seedlings with leaf lesions at Cambridge, UK. The fungus spread up to 10 m from individual infected plants in an average season and usually infected the new crop of seed. The amount of such infection from a single seed lot varied widely when samples were grown at different locations, presumably because of differences in local weather. Seed lots with about 1% infected seeds seem suitable for crop production but little or no *A. fabae* can be tolerated for seed intended for multiplication. Infection in commercial seed grown in UK has been greatly reduced by selection of clean seed. Health standards adopted in the Field Bean Seed Scheme may have eliminated *A. fabae* from one cultivar.

The use of healthy seed, as assayed by standard agar tests, has given significant increases in yields of field beans (Gaunt and Liew, 1981). According to these authors, use of seed lots with small proportions of infection represents the most economic and most efficient form of control. Before then, in 1950, Beaumont had concluded that the only satisfactory prevention measure was to use clean seed. Kharbanda and Bernier (1979) recommended soaking seed in benomyl-thiram mixture for 8 h to eradicate seed borne Ascochyta.

A seed health testing method to detect *A. fabae* was described by Hewett in 1966. For routine samples, 200 seeds (selected by a random halving method) were pre-treated by immersion for 10 min in sodium hypochlorite solution (approximately 1% available chlorine) and briefly drained. They were then placed onto potato dextrose agar in Petri dishes; the dishes, each containing 10 seeds, were incubated in tins (darkness) at 22 °C. After 5–7 d *A. fabae* produces a distinctive colony, approximately 25 mm in diameter. At ×25 to ×50 magnification the hyphae have a convoluted appearance. The under-side of the colony is mid-brown centrally, with shades of orange or green, paling towards the margin. A few pycnidia are usually present, ~~particularly where the seed meets the agar surface. Pycnidial formation is greatly stimulated by exposure to continuous fluorescent illumination.~~

Since 1981, The Working Group on Leguminosae, appointed by the Plant Disease Committee of the International Seed Testing Association, has been trying to standardize the agar plating method using seed samples of different origin and different incubation conditions. The work is now almost complete and the details of an internationally accepted seed health testing procedure will soon be published.

Several epiphytotics are reported to be caused by two species of *Botrytis; B. cinerea*, and *B. fabae*. Recent reports indicate, however, that *B. fabae* is the major cause of damage (Hanounik and Hawtin, 1982). *Botrytis fabae*, the cause of chocolate spot, has been isolated from seed (Leach, 1960; McKenzie and Morrall, 1975; Sode and Jørgensen, 1974). The fungus was not recovered from 16 of the 30 commercial seed lots tested by Harrison

(1978) in Scotland. Seed infection of *B. fabae* in the remaining 14 lots ranged from 1.0 to 8.8%. However, when three of the lots (with initial values of 8.8, 2.2 and 2.2% infection) were re-tested after storing for 9 months in paper sacks in the laboratory, *B. fabae* was not detected, which suggests that the fungus does not survive for long on dry seeds. Experimental work on seed transmission by Harrison (1978) has shown that seed borne *B. fabae*, although common in commercial seed stocks, may be unimportant in initiating an attack of chocolate spot, and that it may be possible to eliminate the fungus from seed by storing them for one year. No correlation was found between degree of seed infection and occurrence of *B. fabae* in Danish fields (Sode and Jørgensen, 1974).

B. fabae was isolated by Harrison (1978) using the agar plate method. One-hundred randomly-selected seeds were surface-sterilized by immersion in 10% Chloros (ICI Ltd; 1% W/V available chlorine) for 10 min, rinsed thoroughly in autoclaved water, and placed on 2% malt extract agar (MEA) in 10 Petri dishes at room temperature (20° ± 2 °C) and under normal laboratory lighting. Seeds infected with Botrytis were counted after 14 d.

Pea

Ascochyta Complex. Three species are involved: *Ascochyta pisi* causes leaf and pod spot; *Mycosphaerella pinodes*, the perfect stage of *Ascochyta pinodes*, results in blight; and *Ascochyta pinodella* (now called *Phoma medicaginis* var. *pinodella*) causes foot rot. All are widespread wherever pea crops are grown, especially in damp climates. Small amounts of seed borne inoculum can be of great epidemiological significance. Mixed infections often occur. According to Lawyer (1984), even a slight overall infection results in significant losses in both production and quality. Moderate or severe infection with *M. pinodes*, the most damaging of the three pathogens, can reduce yield by 50—75%. In Canada, Wallen (1965) found that under certain experimental conditions *A. pisi* caused a yield reduction of 11%, *P. medicaginis* var. *pinodella* 25% and *M. pinodes* 45%. Of the seed infected by *A. pisi*, 25% produced diseased seedlings, the remainder either did not germinate (because severe infection had killed the embryo) or emerge as seedlings with slight, suppressed infections. Secondary spread of the disease may occur from infected seedlings. Larger proportions of seeds and seedlings infected by *M. pinodes* and *P. medicaginis* var. *pinodella* are killed, often before plants mature to set seed. Wallen (1965) concluded that the relative infrequency of these two species in seed is an indication of their destructive nature. They survive well in soil through chlamydospores and pycnidia, and also perithecia in the case of *M. pinodes*. Using seed produced from dry areas is the best way to avoid seed borne infection from *Ascochyta* spp.

Different researchers have tried to detect seed borne infection by using various media and incubation conditions in order to develop a routine testing procedure (Anselme, 1962; Matthews, 1964; de Tempe, 1968). A review of

these methods indicates that plating of seeds, after surface disinfection, onto agar media (potato dextrose agar (PDA) or malt extract agar) followed by incubation for about 7 d in darkness or near ultra violet light at 20°—22 °C, gives more infection counts. Identification and distinction between the three species is convenient on agar. Plating seed onto PDA has been found very satisfactory at the Institute of Seed Pathology, as compared to the blotter method (Guilli, Hansen and Mathur, unpublished). Colonies of *A. pisi* are pale, with light-brown pycnidia scattered uniformly within the colony. Pycnidia of *M. pinodes* are darker (dark brown) and arranged in concentric rings, while the pycnidia of *P. medicaginis* var. *pinodella* are almost black, slightly larger and not arranged in rings. Whenever dark-coloured pycnidia are observed around infected seed, slide preparations must be examined under stronger magnification. Pycnidiospores of *P. medicaginis* var. *pinodella* are smaller and usually without septation.

The three most important diseases of field pea in South Australia are blight (*M. pinodes*), foot rot (*P. medicaginis* var. *pinodella*), and charcoal rot (*Macrophomina phaseolina*). All three diseases have seriously reduced economic returns; they can be seed borne or can be transmitted through the soil or in pea stubble. Ali *et al.* (1982) demonstrated that these pathogens, as well as *F. oxysporum* f.sp. *pisi*, can be detected easily by the agar plate method. Seeds, surface disinfected with sodium hypochlorite (3.5% available chlorine) for 5 min, are plated onto malt extract agar and incubated at 25 °C under a 12 h diurnal cycle of darkness and light (near ultra violet) for 7 d. Colony characters of the four species are shown in Ali *et al.* (1982).

Fusarium oxysporum f.sp. *pisi* causes wilt. In the USA, average annual losses of 2% were reported for the period 1951—1960 (USDA, 1965). Fusarium wilt has been destructive in the north central states (Chupp and Sherf, 1960) but has generally been controlled through development of resistant cultivars. A number of races of the wilt fungus are known to attack pea. Basically, the pathogen is a soil-inhabiting fungus; it survives in soil as chlamydospores which can be viable for longer than 10 years (Haglund, 1984). *F. oxysporum* f.sp. *pisi* is disseminated through the movement of contaminated soil or plant parts by water, wind, and/or people. Long-range dissemination occurs through transport of contaminated and/or infected seed. With three years of consecutive sowings of suspected seeds previously treated with Phygon and mercuric chloride, Kerling (1952) obtained considerable incidence of Fusarium wilt in The Netherlands. She concluded that the wilt infection was present in the testa of pea seed; an estimated 2% seed transmission occurred (which is more frequent than that recorded in the USA). In India, Lambhate and Bhide (1976) obtained 7 pathogenic isolates of the *F. oxysporum* f.sp. *pisi* race 1 from seed showing defective germination.

Other important pathogens in pea are *Erysiphe pisi, Peronospora viciae* and *Sclerotinia sclerotiorum*. The powdery mildew fungus, *E. pisi*, is prevalent worldwide and destructive in warm, moist climates (Uppal *et al.*, 1935).

Powdery mildew-infected plants developed from mercuric chloride disinfected seeds sown in sterilized soil. This evidence suggested that *E. pisi* is borne internally in the seed and that the pathogen can be detected by growing-on tests.

The downy mildew fungus, *Peronospora viciae*, is prevalent in cool, humid climates. Oospores were abundant in flowers (Safeeulla and Shaw, 1964). The oospores that adhere to and are embedded in the seed coat are not detected during normal handling and dissemination of the seeds. These observations suggest that dissemination of infected seeds assures dissemination of the pathogen and that oospores may be of far greater importance in the initiation of primary infections than was previously supposed. In 1951, Ciccarone (1952) had obtained downy mildew-infected pea plants raised in Italy from seed imported from Holland. No seed health testing method has been established for this pathogen.

Sclerotinia sclerotiorum causes stem rot and is widespread in cool moist conditions. It was recorded in Scotland on pea by Gray and Findlater (1960), infection probably being seed borne. Mycelium from infected seed develops rapidly in the blotter test, forming sclerotia (Noble, unpublished).

Lentil

In 1981, a list of diseases which attack lentil was published by Khare. About 20 fungi were mentioned as seed borne. Among these, species of *Fusarium, Rhizoctonia, Aspergillus, Sclerotium,* and *Botrytis* are reported to cause seed rot and seedling damage at the pre- and post-emergence stages.

Vascular wilt, caused by *Fusarium oxysporum* f.sp. *lentis,* is considered an important disease in the province of Madhya Pradesh, India, causing losses of more than 50% in some of the fields. Losses are large if wilt appears at the advanced stages of crop growth. Infected plants usually die and are barren; if infected at blooming, plants form only a few seeds (Khare, 1980). The fungus has been isolated from all plant parts, which indicates systemic infection from root to seed through stem, branches, pedicel, and placenta. According to Khare (1980), vascular wilt is basically a soil-borne disease, although seed transmission is extremely important in the transfer of the fungus from infected to uninfested areas.

Ascochyta blight (*Ascochyta lentis*) is a serious disease of lentil in several locations (Mamluk, 1983). The disease was reported from Canada for the first time in 1978, where 98% of the discoloured seeds and 48% of the normal seeds in the sample tested yielded the fungus. Lentil have been grown commercially in western Canada since 1970 (Morral and Sheppard, 1981). Surveys of seed samples clearly demonstrated that the disease was already widespread in Saskatchewan and Manitoba. A field survey in Saskatchewan in the summer of 1979 revealed very low levels of Ascochyta blight in southern and west-central areas. The disease was more prevalent in more

humid regions north of Saskatoon. Reductions in seed quality due to the disease are probably more important than losses in yield per se.

A. lentis was isolated from seeds of several imported accessions in the USDA lentil germplasm collection at Pullman, USA (Kaiser and Hannan, 1982). Seed infection alone frequently reduced shoot and root growth by 24—54% and seed yields by >25% in an environment that did not favour disease spread.

Morral and Sheppard (1981) used the following technique to isolate *A. lentis* from lentil seed: 200 seeds were taken from each sample, surface disinfected for 10 min in 0.6% sodium hypochlorite, plated onto V8 agar and incubated on the laboratory bench for at least 7 d. Colonies of the fungus were counted to derive percentage seed infection.

Certain fungi may be transmitted with seed as separate contaminants, e.g. the sclerotia of *Sclerotinia sclerotiorum* and plant debris infected with *Uromyces fabae* and *Peronospora lentis.* These fungi can be detected during inspection of dry seed and, particularly the last two, by examining seed washings (Washing Test).

Viruses

Although seed infection and pathogen association with crop seeds have been historically regarded as significant, seed borne viruses have appropriately caught the attention of plant scientists and seedsmen only in recent decades. Pea seed borne mosaic virus, for instance, was a newly described curiosity only 20 years ago (Inouye, 1967). Likewise, recognition of destructive new strains of cucumber mosaic virus, seed borne in several food legumes, has occurred only in the last decade (Davis and Hampton, 1986). Seed borne viruses that were formerly non-detectable, or detectable only with great difficulty, can now be readily detected by methods with unprecedented logistical power and sensitivity, e.g. enzyme-linked immunosorbent assay (ELISA), dot-ELISA, solid-phase radioimmunoassay, and nucleic acid blot hybridization.

Because of the growing cognizance of seed borne virus inoculum in plant disease epidemics, the possibility is being investigated of very small seed-transmission rates by viruses not generally recognized to be seed borne in economic plant hosts. Meticulous efforts are required to detect seed transmission of such viruses (Mikel *et al.*, 1984); yet such sparse inoculum, subsequently spread by insect vectors, is sufficient to disseminate, introduce and establish viruses into new geographic regions.

Thus, the number of known seed borne viruses will probably continue to increase for several distinct reasons, including: (a) discovery through epidemiological investigations of previously unrecognized viral inoculum sources, (b) discovery of seed borne viruses not previously detected because of the characteristically small seed-transmission frequencies, (c) discovery of viruses that are seed borne but symptomless in certain hosts, and (d)

discovery of seed borne viruses, by virtue of increasingly suitable and sensitive detection technology.

Seed borne viruses of food legumes

Viruses known to be seed borne in pea, lentil, and faba bean are presented with selected relations and control strategies, in Table 1. No viruses are known to be seed borne in chickpea. World-wide crop losses due to these seed borne viruses are very difficult to estimate. Losses within some semi-tropical agricultural regions, such as in northern India for example, are significant almost every year. Losses in temperate climate agricultural regions vary from year to year, depending upon three primary factors: (a) seed health of the principal seed lots sown, (b) severity to the crop of interactions between seed borne virus strain and plant host germplasm, and (c) seasonal weather factors which influence population densities and the migratory and feeding behavior of insect vectors.

The world area devoted to production of grain and vegetable pea is approximately 9 million ha (Makasheva, 1983), of which only about 0.7 million ha are harvested for food (dry seed, canned, or frozen). Nonetheless, pea seed borne mosaic virus (PSbMV) (Hampton and Mink, 1975), aphid-transmissible from seed-infected host plants, is perhaps the most significant seed borne virus of the four food legumes addressed at this Conference. A recently described strain of PSbMV (Hampton, 1982) is the only virus known to be seed-transmissible in lentil. The demonstrated seed-transmissibility of PSbMV in lentil and at least occasional seed-transmissibility in faba bean would seem to forecast its significance in these crops, as well as in pea.

PSbMV consists of at least two major strains, the pea strain (Hampton *et al.*, 1981) and the lentil strain, with numerous substrain variations (Hampton *et al.*, 1981). Interactions of strains with combinations of crop-plant genes result in a wide range of plant responses to PSbMV infection (Hampton and Marx, 1981; Hampton *et al.*, 1981), from symptomless infection to rapid necrotic collapse of whole plants. Particularly in the "symptomless to mild-symptom" portion of this range, the virus had escaped detection, identification, and control until 1967—77. Prior to and during that time, PSbMV became widely distributed throughout the world (Hampton, 1986) by means of infected seed lots.

Pea early browing virus, also seed-transmissible in pea and formerly of localized significance, is now being effectively controlled through de-circulation of infected seed lots and control of its nematode vector (*Trichodorus* spp.).

Faba bean is susceptible to 30 or more viruses, four of which are seed-transmissible: broad bean true mosaic virus (BBTMV) (Quantz, 1953), broad bean stain virus (BBSV) (Cockbain *et al.*, 1976), bean yellow mosaic virus (BYMV) (Quantz, 1954), and pea seed borne mosaic virus (Inouye, 1967). All except BYMV tend to have narrow leguminous-plant host ranges,

Table 1. Viruses that are seed borne in pea, lentil, and faba bean (none are known in chickpea).

Virus	Characteristics		Seed-borne in	Principal geographic areas	Priority control strategies
	Virus group	Vectors			
Pea seed borne mosaic virus	Potyvirus	Aphid spp.	Pea, Lentil, Faba bean	World-wide in pea	*Pisum* gene *sbm* *Lens* gene *sbv*
Bean yellow mosaic virus	Potyvirus	Aphid spp.	Faba bean	World-wide	Virus-free seed; *V. faba* resistance
Broad bean true mosaic virus	Comovirus	Probably beetles: *Cerotoma, Diabrotica*	Faba bean	Europe, NW Africa	Virus-free seed; Crop isolation
Broad bean stain virus	Comovirus	Weevils: *Apion, Sitona*; probably beetles.	Faba bean	Europe, NW Africa	Virus-free seed; Crop isolation
Pea early browning virus	Tobravirus	Nematodes; *Trichodorus* spp.	Pea	Europe	Virus-free seed; Avoid vector-infested soil

which somewhat limits their introduction and establishment into new agro-ecosystems. As has been true for PSbMV in pea, the rapid, long-range dissemination of BBTMV and BBSV in faba bean appears directly attributable to international shipments of infected seed lots.

PSbMV is being controlled in pea by extensive incorporation of the *Pisum* gene *sbm* into new cultivars, which confers either PSbMV-immunity or resistance, depending upon other complementary genes. Likewise, although the virus now occurs principally in *Lens culinaris* germplasm (Goodell and Hampton, 1984), control of PSbMV in this crop can be accomplished when necessary by the incorporation of *Lens* gene *sbv* into new lentil cultivars. Seed borne PSbMV appears not to occur generally in world faba bean crops, and has not been detected in commercial USA faba bean seed lots. No gene in *Vicia faba* is known to confer resistance to PSbMV, and may not be needed at this time.

Even after crop-plant genes have been incorporated into major crop cultivars, there are two control measures that will remain important to overall crop protection: the gradual improvement of crop seed-stocks, world-wide, through seed health testing (Neergaard, 1979), and a gradual up-grading of the seed health status of crop germplasm resources (Hampton, 1983), such that germplasm-borne pathogenes do not threaten breeding progenies derived from germplasm parents.

Virus detection in seeds

Virus detection has been revolutionized in the last decade by new testing methods (Banttari and Goodwin, 1983; Bryant *et al.*, 1983; Clark and Adams, 1977; Diaco *et al.*, 1985; Harrison *et al.*, 1983; Jaegle and Van Regenmortel, 1985) that are extremely sensitive for detecting small concentrations of virus, and which facilitate rapid, automatic and computerized processing of hundreds of plant-extract samples. These methods can be controlled to effectively minimize both "false positives" and "false negatives" in assay results. Moreover, specific parameters of the testing system can be computerized, modeled, and optimized (Clark and Barbara, 1987) to ensure the most efficient combinations of assay components.

Immunosorbent assays, because they have been extensively proven, are emphasized here. Typically, for such assays, sampled plants are homogenized in a special buffer, diluted optimally, and introduced into microplate test wells, following well-coating with specific anti-viral globulin. Subsequent treatments result in reactions that are either visible or can be quantified directly by photometry. Corresponding plant samples that contain known virus and others that are free of that virus are used to calibrate detecting instruments. Conclusions from assay results are usually based on appropriate statistical analyses; in many cases, however, more conservative decisions are made than would be allowed by formal statistics.

Supplementary tests are sometimes necessary in cases of questionable data

(e.g. reactions which exceed those of healthy-plant control samples, but which are much smaller than those for infected-plant control samples). Such anomalies may result from unrepresentatively small virus concentration in the sampled plant tissue. When occasional results remain unresolved by supplementary tests, the questionable status of the sample is recorded for further investigation. Partial serological relations (viral serotypes) can cause such results.

It is now clear that direct-seed assays, in which soaked seeds are homogenized and tested for virus presence by extremely sensitive immunosorbent assays, can yield erroneous data (e.g. detect seed borne viruses which would not be transmitted to resulting seedlings). Recent results with PSbMV in pea seeds (Maury *et al.*, 1987), however, indicate that removal of the pea seed testa, containing virus that would not be seed-transmitted, can remedy false positives. Accurate determinations of seed-transmitted viruses for specific seed lots, require either precursory, determinant assays of seed parts or grow-on tests with appropriate assays of seedling plants.

Bacteria

Bacterial blight of pea is the only important seed-transmitted disease in the four hosts of topical concern. The bacterium, *Pseudomonas syringae* pv. *pisi*, is carried by the seed in the form of a dry bacterial film on the seed surface, and also in the seed coat. The disease can cause significant crop losses when environmental conditions favour its development (wet soil and continuous cool and wet weather). Losses of up to 25% have been reported from the USA (Chupp and Sherf, 1960). When pea seedlings are infected, the entire crop may be lost. At later stages, defoliation, blasting of blossoms and pods, and unsightly pods can lessen yield and quality, and so reduce the value of the crop. Both internal and external seed infection can persist for at least three years on seed, although the degree of infection declines during each year the seed is stored (Lawyer, 1984). Seed lots are seldom held longer than three years before planting. The organism can be carried from one seed lot to another, and from seed to seed with certain fungicides. Farm machinery is also responsible for spreading infection wherever pea are planted. Overwintering and dissemination of the bacterium were dealt with extensively in a classical paper by Skovic (1927). The disease was found in New York State in 1979 after not being observed there for more than 25 years (Hunter and Cigna, 1981). The disease was associated only with plants grown from one seed lot. Planting clean seed and using cultivars resistant to *P. syringae* pv. *pisi* are the primary means of controlling bacterial blight.

Problems associated with detection of bacteria in seeds, using conventional procedures were discussed by J. D. Taylor during the First International Workshop on Seed Bacteriology held in 1982 at Angers in France. The dilution plating method for detection of *P. syringae* pv. *pisi* was also demonstrated. The method consists of three stages: extraction, isolation, and

identification. The bacteria are extracted by grinding seed and placing the flour into sterile tapwater where it is allowed to stand for 1—2 h. Isolation is carried out using dilution plating onto Petri dishes containing King's B medium, where *P. syringae* pv. *pisi* produces a greenish diffusable pigment which fluoresces blue under UV light. Some isolates are non-fluorescent and are more readily detected on 5% sucrose nutrient agar, where domed mucoid colonies are formed. The identity is confirmed by serological or host inoculation tests.

Care must be taken to distinguish the bacterial blight organism from the brown spot bacterium, *P. syringae* pv. *syringae* (syn. *P. syringae*). The latter, less important on pea than *P. syringae* pv. *pisi*, is an omnivorous pathogen and the two may be present on seed together. They cannot be distinguished from each other by biochemical tests. The two pathovars can, however, be differentiated by phage and serological tests as well as by pathogenicity tests conducted on pea and common bean; *P. syringae* pv. *pisi* does not infect common bean (*Phaseolus vulgaris*).

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