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Screening techniques and sources of resistance to root rots and wilts in cool season food legumes

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Abstract

Soilborne, fungal pathogens of cool season food legumes, including seed and seedling blights, rot rots, and wilts are described. Seed and seedling diseases are caused primarily by *Pythium* and *Rhizoctonia* spp. The most important fungi causing root rots include *Aphanomyces euteiches, Fusarium solani, Pythium* spp., *Sclerotium rolfsii*, and *Macrophomina phaseolina*. Wilt is caused primarily by various host-specific forms of *Fusarium oxysporum*. This paper discusses these diseases and screening procedures that emphasize standardization of inoculum levels, maintenance of virulent pathogen cultures, inoculum growth media, environmental conditions, and host plant age. Sources of resistance to these diseases are discussed.

Seedling diseases

Any environmental or physiological factors which delay emergence or result in uneven stands, such as: a) poor seed vigor; b) cold wet soil; c) poor seedbed preparation; d) herbicide injury; or e) crusting of soil after planting can predispose developing plants to seedling disease. Worldwide, seedling diseases of peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), faba beans (*Vicia faba* L.), and lentils (*Lens culinaris* Medik.) are caused primarily by *Pythium* spp. and *Rhizoctonia solani* Kuehn.

Pythium seed and seedling rot

Pythium ultimum Trow and sporangial forms resembling *P. ultimum* are often described as seed and seedling pathogens of both chickpeas and peas. Other species, such as *P. splendens* Braun and *P. irregulare* Buisman (Harman, 1984; Van der Plaats-Niterink, 1981), have also been reported as pathogenic to peas. In studies conducted at Prosser, Washington, Plant Introduction (PI) accessions resistant to *P. ultimum* were also resistant to other *Pythium* species (Kraft, unpublished data). In addition, zoospore inoculum, produced by such species as *P. irregulare*, was equal in pathogenicity to mycelial inoculum produced by *P. ultimum*.

Stasz & Harman (1980) reported that resistance in peas to Pythium seed and seedling rot was due to differences in numbers of infections occurring at a given inoculum level. They also reported that aging seeds prior to planting increased exudation, decreased vigor, and increased disease severity. Woyke (1987) reported that large seed was more vigorous and resistant to seedling attack by *Pythium* than small seed. Pea seed with the dominant *A* gene for anthocyanin production was more resistant than lines possessing the recessive *a* gene for lack of anthocyanin. Resistance is due to the presence of delphinidin, an anthocyanin (anthocyanin-aglycone) pigment in the testae, which is fungistatic to a number of seed and root pathogens. However, peas

with an *A* gene can be susceptible in the seedling stage, despite the presence of delphinidin, if they exude sufficient amounts of reducing sugars (Kraft, 1977). Similar to peas, kabuli chickpea seeds, lacking pigmentation in the testae, are extremely susceptible to *Pythium* attack, as compared to pigmented, desi types (Kaiser & Hannan, 1983).

Methods for screening peas for resistance to *Pythium* include infesting soil with: cornmeal-sand inoculum (Perry, 1973); inoculum produced on medium-saturated vermiculite (Kraft & Roberts, 1969); oospore inoculum (Stasz & Harman, 1980); or soaking seed in a hyphal suspension prior to planting (Ohn *et al.*, 1978). Soil infested with inoculum of *P. ultimum*, grown on vermiculite saturated with a basal medium, is used at Prosser. Infested soil is air-dried to induce the formation of thick-walled sporangia and oospores of *P. ultimum*. This soil is then mixed with sufficient quantities of non-infested soil so that test lines are exposed to a population of 200 to 500 propagules of *P. ultimum* g^{-1} of air-dry soil (Mircetich and Kraft, 1973).

Several PI accessions, including 257593, 140165, 166159 and 140295 with the dominant *A* gene for pigmented testae, were found resistant and were used in breeding for resistance at Prosser (Kraft & Roberts, 1970). However, more advanced pea breeding lines with the recessive *a* gene have been developed, which are resistant to *Pythium* seed and seedling attack. Early generation lines (F_3-F_6) are planted in the field with and without seed treatment fungicides to screen for resistance to *Pythium* pre- and post-emergence damping-off.

Rhizoctonia seed and seedling rot

The imperfect stage classification of *Rhizoctonia solani* is based on the anastomosis grouping concept (AG). The pathogen responsible for seed and seedling disease is classified in AG4 (Anderson, 1982). *Rhizoctonia solani* (AG4) can attack pea seedlings whenever environmental conditions are favorable. For seedling infection to occur, the sclerotium or hyphal fragment must germinate or resume growth. The resulting hyphae may grow through soil for several millimeters to infect the epicotyl, seed, or hypocotyl of a seedling host. The pathogen prefers well aerated areas at the soil surface and is most aggressive under warm, moist (24 to 30°C) conditions.

Screening tests for resistance to *R. solani* have utilized the following procedures: a) mycelial discs from potato-dextrose agar (PDA), V8 juice agar, or synthetic media (McCoy & Kraft, 1984); b) maize kernel inoculum (Shehata *et al.*, 1981, 1984) placed at the base of seedling stems; and c) sclerotia infested soil (McCoy & Kraft, 1984). McCoy & Kraft (1984) reported that resistance to *R. solani* was positively correlated with epicotyl thickness when test lines were grown in soil infested with 20 sclerotia g^{-1} of soil. Shehata *et al.* (1983) reported that pea lines resistant to Fusarium root rot were susceptible when grown in soil infested with both *R. solani* and *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyd. & Hans. The type of nutrient medium used to produce primary inoculum of *R. solani* can affect overall disease severity ratings. For example, mycelial discs of *R. solani* incubated on PDA or V8 juice agar caused lower disease ratings than mycelial-disc inoculum from dextrose-asparagine agar. However, the overall relationship among test lines remained similar (McCoy & Kraft, 1984). Table 1 lists those pea lines reported resistant to *R. solani* AG4.

Table 1. Pea lines resistant to *Rhizoctonia solani* AG4

Pea line #	Reference
Dark Skin Perfection	McCoy and Kraft, 1984 Shehata *et al.*, 1981
B77-634-4	McCoy and Kraft, 1984 Shehata *et al.*, 1981
Pi 189171	McCoy and Kraft, 1984 Shehata *et al.*, 1981
PI 197990	McCoy and Kraft, 1984 Shehata *et al.*, 1981
74SN3	Shehata *et al.*, 1981
PI 257593	Shehata *et al.*, 1981

In India, *R. solani* can sometimes cause heavy damage to seedling and adult lentil plants depending upon the time of infection. In both cases, affected plants exhibit yellowing of leaves progressing from lower to upper plant parts. The roots of the affected plants become reddish brown with a clear constriction at the collar region or below (Shukla *et al.*, 1972; Kannaiyan & Nene, 1973; Shatla *et al.*, 1974). The main root below the constriction remains healthy in the early stages of infection but may become infected at a later date. In such cases, the affected seedlings/plants are easily pulled out of the soil, but some break at the constriction point. When infection occurs in the late

seedling stage, the plants can survive for some time but exhibit a progressive yellowing of leaves from the base upwards.

To screen lentil lines for resistance to *R. solani* in the greenhouse, the fungus is grown on PDA and the mycelial mat mixed thoroughly with the planting medium (Kannaiyan & Nene, 1973). Lentil seedlings are grown in sterilized soil for 10 days after which the top 2.5 cm soil is removed and a uniform amount of fungus inoculum placed near the collar region of each seedling. Then the soil is replaced. Mortality counts are taken every 5 days. None of the 158 lentil lines/accessions tested through a pot-screening method in the greenhouse were found resistant; however, line UPL 172 showed the lowest mortality of 30% followed by 40% in line UPL 288 (Kannaiyan, 1974).

Collar rot of lentil, caused by *Sclerotium rolfsii* Sacc., is omnipresent and can cause heavy losses at the early stages of crop growth. Infected plants droop and ultimately exhibit damping-off symptoms. In the final stages of the disease cycle, white strands of the fungus and sclerotia are formed around the collar region. The disease is more severe under sufficient soil moisture conditions and ambient temperatures of around 28 to 30°C (Aycock, 1966; Mathur & Deshpande, 1968; Khare, 1980).

In the greenhouse, screening for collar rot resistance is conducted either in trays or large pots (Kannaiyan & Nene, 1976; Khare, 1980). Pot screening is conducted similarly to the method described for wet root rot (Kannaiyan & Nene, 1973). In another method, *S. rolfsii* is multiplied on soil-maize medium (Kannaiyan & Nene, 1976); or sand-oatmeal (Claudius & Mehrotra, 1973) for 12 days. Screening trials are conducted in 30-cm-diameter pots using 200 g inoculum in 5 kg^{-1} of soil. Infested pots are covered with moist jute bags for 48 h and then planted with 50 seeds per pot. Another technique involves inoculating each plant with two mature 21-day-old sclerotia at the hypocotyl region and covering with moistened soil (Mohammad & Kumar, 1986). After a 14 to 21 day incubation, test lines are rated for percentage damping-off.

Of several hundred lentil lines and accessions tested for resistance to collar rot in the greenhouse, 12 (JL 678, JL 719, JL 727, JL 828, LP 18, LP 288, LP 338, LP 379, Pant 370 P 23, Pant 638, Pusa 1, and Pusa 3) were found resistant with less than 10% mortality (Kannaiyan, 1974; Mohammad & Kumar, 1986), respectively.

Root diseases

Fusarium root rot

Fusarium root rot of peas is caused by *F. solani* f. sp. *pisi* (Kraft *et al.*, 1981). This is a serious disease of peas in all USA pea-producing areas. This pathogen is now also recognized as a serious root pathogen of chickpeas, especially in warm growing conditions (Bhatti & Kraft, 1992a). Fusarium root rot is distinct from Fusarium wilt and usually occurs in conjunction with other diseases of peas and chickpeas (Kraft *et al.*, 1981; Bhatti & Kraft, 1992a,b). This pathogen can reduce pea yields from a constant 10% to as high as 50%. *Fusarium solani* f. sp. *pisi* usually invades the cotyledonary attachment area of both peas and chickpeas. Initial symptoms on primary and secondary roots consist of reddish-brown streaks that later coalesce. The external root color becomes dark reddish-brown to black, especially at the soil line and in the cotyledonary attachment area. Above ground symptoms of severely infected chickpea and pea plants include stunting, graying, yellowing, and necrosis of lower foliage. Chlamydospores are the naturally occurring survival structure in field soil.

Whalley (1984) developed a rapid test for screening peas for resistance to *F. solani* f. sp. *pisi in vitro*. Test seeds are surface disinfested and germinated on moist filter paper until the plumules are 30 mm long. Resultant seedlings are then transferred to test tubes and suspended in 0.1% water agar containing 1×10^6 conidia ml^{-1}. Peas are incubated for 14 days in a growth chamber set at 24°C. Lockwood (1960) screened PI accessions in pure culture by pipetting a 1×10^6 ml^{-1} conidial suspension onto seed planted in autoclaved quartz sand. Plants were dug and read 23 days later and scored on a 0 to 9 scale. An infested soil technique was developed at Prosser where seeds are planted in soil infested with 20,000 to 40,000 colony forming units (cfu) g^{-1} soil in a controlled environment (24°C day, 15°C night, 6480 lux maximum illumination with a 16 h day) (Kraft, 1975). Plants are harvested 14 to 21 days after emergence and each plant is scored on a 0 to 5 scale with 5 indicating a completely rotted root. For segregating material, plants with more healthy root systems (i.e., less epicotyl and root necrosis than the susceptible control) are transplanted into an autoclaved potting mixture. Transplants that survive are grown to seed set.

Good seed vigor is an important consideration in comparing one line to another. A line with poor seed vigor may be susceptible to Fusarium root rot when in fact it is resistant (Kraft, 1986), which is similar to an earlier report on Pythium seed rot of peas (Stasz & Harman, 1980). Recently, we have tested a technique first developed by Dr. Simon Menzies, Plant Pathologist, DSIR, Auckland, New Zealand (retired), to screen peas for resistance to Fusarium root rot. Untreated seeds of a test line are soaked overnight at room temperature, in a conidial suspension of *F. solani* f. sp. *pisi* adjusted to 1×10^6 spores ml^{-1}. Inoculated seed are then planted into coarse-grade perlite in plastic flats and incubated in a growth chamber at a constant 24°C with a 16 h photoperiod at 6480 lux. The perlite is kept moist throughout the 14 day incubation period by watering with micropore filtered water (0.45 μ). Resultant plants are scored for disease severity using the 0 to 5 scale and segregating plants are saved for seed production as mentioned previously.

Fusarium root rot of chickpea

There are few reports describing *F. solani* f. sp. *pisi* as an important pathogen of chickpea (Kraft, 1969; Grewal *et al.*, 1974; Westerlund *et al.*, 1974). However, *F. solani* is often cited as a serious pathogen of chickpeas (Viswakarma & Chaudhary, 1981; Nain & Agnihotri, 1984; Mani & Sethi, 1985; Mario & Carolina, 1987; Nene, 1987), but it is unknown if this pathogen is actually *F. solani* f. sp. *pisi*.

Several chickpea lines from ICRISAT and ICARDA were evaluated for resistance to *F. solani* f. sp. *pisi* (Bhatti & Kraft, 1992b) using the technique described earlier (Kraft, 1975). Only four lines of 39 tested exhibited some resistance when the incubation temperature varied from 22 ± 3°C. However, when the incubation temperature was 25 ± 3°C no lines tested were resistant.

Progress has been made and will continue in developing peas with quantifiable resistance to Fusarium root rot and with acceptable horticultural attributes. Table 2 lists a number of PI accessions and breeding lines of peas which are resistant/tolerant to *F. solani* f. sp. *pisi*.

Table 2. Pea lines resistant/tolerant to *Fusarium. solani* f. sp. *pisi*

Pea Line	Reference
PI 140165	Kraft, 1975
PI 164417	King *et al.*, 1960
PI 164837	King *et al.*, 1960
PI 164971	King *et al.*, 1960
PI 165577	King *et al.*, 1960
PI 165965	King *et al.*, 1960
PI 166082	King *et al.*, 1960
PI 166084	King *et al.*, 1960
PI 169606	King *et al.*, 1960
PI 171816	King *et al.*, 1960
PI 173057	King *et al.*, 1960
PI 174921	Kraft, 1975
PI 174922	Kraft, 1975
PI 179969	Kraft, 1975
PI 196013	Kraft, 1975
PI 196021	Kraft, 1975
PI 196022	Kraft, 1975
PI 242028	Kraft, 1975
PI 257593	Kraft, 1975
VR-410-2	Kraft and Giles, 1978
VR-1492-1	Kraft and Giles, 1978
RR-1178	Kraft, 1984
WR-1167	Kraft, 1984
792022	Kraft, 1981

Dry root rot of Chickpea

Because disease development is highly influenced by temperature and soil moisture, screening for resistance to dry root rot of chickpeas caused by *Rhizoctonia bataticola* (Tabenhaus) E. J. Butler [syn. *Macrophomina phaseolina* (Tassi) Goidanich] in the field is not practical. High temperatures and dry soil conditions at flowering and podding stage can dramatically increase dry root rot severity. However, Fusarium wilt screening plots at ICRISAT center became infested with the dry root rot pathogen, and were used in eliminating chickpea breeding lines susceptible to dry root rot. There are no reported cases of uniform and effective dry root rot disease screening plots being developed for chickpeas. Pot culture techniques for greenhouse screening and a paper towel technique for laboratory screening were developed (Nene *et al.*, 1981). Further improvement of pure culture screening techniques is needed to correspond more closely with results obtained in the field.

Dry root rot of lentil, caused by *R. bataticola*, is becoming an increasingly important lentil disease in India under both dry and humid climates. The affected plant exhibits sudden drooping of top leaves and drying without showing any yellowing. The roots turn ashy to

ashy-brown and desiccate. Black sclerotial bodies of variable size and shape develop on the surface as well as within the root. The pathogen is also responsible for pre- and post-emergence seed and seedling rots which reduce plant stands.

Evaluation of lentils for resistance to dry root rot is usually conducted in the laboratory (Kannaiyan & Nene, 1976) using the paper towel method (Deshkar *et al.*, 1973). The inoculum is raised in Richard's liquid medium (Deshkar *et al.*, 1973) or PDA (Van Rheenen *et al.*, 1989) for 6 days. The resultant mycelial mat, separated by filtration, is macerated in a sterilized blender for 30 seconds with sterilized water to get a suspension (10:100, v/v). The mycelial suspension is transferred to an autoclaved enamel tray. Paper towels (30 × 30 cm) are first autoclaved at 15 psi for 15 minutes at 110°C, and then immersed in the fungal suspension and removed. Seeds are placed at constant intervals on the towel which is then folded and kept for 3 days in a moist chamber at 25–28°C. The numbers of diseased seeds and seedlings are recorded and diseased seedlings discarded, while the healthy ones are transferred to pots containing soil infested with *R. bataticola* for further screening. The optimum temperature for disease development appears to be 30–35°C (Singh & Nema, 1987; Van Rheenen *et al.*, 1989). Unfortunately, using the techniques described above, no sources of resistance to dry root rot of lentils, caused by *R. bataticola*, have been identified.

Common root rot of peas

Common root rot of peas, caused by *Aphanomyces euteiches* Drechs., occurs in most pea growing areas of North America, Europe, Australia, New Zealand, and Japan (Pfender, 1984). This disease can infect peas at any age and plant symptoms can appear as early as 10 days after emergence, if the inoculum level is high and soil moisture levels are conducive for disease development. Straw-colored lesions spread through the root cortical tissues, which become soft and darken as secondary organisms invade the colonized tissues. Microscopic examination of infected cortical tissue will reveal typical oospores of the pathogen. The oospores are readily recognized and are sufficiently distinct to permit quick identification. They are large (25 to 30 μ) and contain a unique, large oil globule.

Oospores can persist in soil for years. Upon germination, oospores form hyphae or sporangia. Sporangia, which are undifferentiated from hyphae, produce asexual primary zoospores which quickly encyst. Soon secondary zoospores emerge from these cysts and swim to a susceptible host where they encyst, germinate, and infect.

All published reports on screening peas for resistance to *A. euteiches* in pure culture have utilized zoospores as the primary inoculum source. Zoospore inoculum has been produced on corn kernel broth (Haglund & King, 1961), on maltose-peptone broth (Carmen & Lockwood, 1960), and on pea seed broth (Kraft, 1988). At Prosser, 10 g of pea seed are placed in 200 ml of glass distilled water and autoclaved for 0.5 h. An agar disc of *A. euteiches*, from a colony margin, grown for 5 days on 2% cornmeal agar is aseptically placed in each flask. Inoculated flasks are incubated for 5 to 7 days in the dark at room temperature. Resultant mycelial mats are washed three times in sterile tap water and drained. Mats are then placed in a mineral salts solution (Carmen & Lockwood, 1960) and aerated overnight with filtered air. Seven mats are placed in 250 ml of mineral salts solution. The mats are harvested by swirling several times in the salt solution and removed. The resultant zoospore suspension is then counted with a hemacytometer. Zoospore counts usually range from 300,000 to 800,000 ml^{-1}. Zoospore numbers are adjusted to 200,000 per ml^{-1}.

Five-day-old seedlings, germinated in coarse grade perlite, are removed and dipped in the zoospore suspension for 1 minute and transplanted back into the perlite. Inoculated plants are compared to an uninoculated control of the same line after a 14 day incubation period under greenhouse conditions (25 to 30°C daytime temperatures). Any test line which produces at least 70% of the fresh weight of that line's uninoculated control is considered resistant.

Marx *et al.* (1972) reported that tolerance to Aphanomyces root rot was associated with dominant, undesirable alleles at three unlinked marker loci (*le*-long internodes; *A*-anthocyanin pigment production; and *Pl*-pigmented hilum). Substitution of recessive alleles which express horticulturally desirable traits at each of these loci resulted in a reduction in tolerance. However, Kraft (1988) reported that resistance to *A. euteiches* was recovered in breeding lines with desirable horticultural traits. Resistance did not break down. Resistance was expressed as less disease and fewer oospores produced in resistant plant roots than in susceptible roots. Lewis & Gritton (1988) reported that resistance to *A. euteiches* appears to be quantitatively inherited with low inheritability. A recurrent selection program where disease pressure is intense was used to increase resistance to common root rot in horticul-

Table 3. Pea lines resistant/tolerant to *Aphanomyces euteiches*

Pea Line	Reference
PI 166159	Lockwood, 1960
PI 167250	Lockwood, 1960
PI 169604	Lockwood, 1960
PI 175227 (sel)	Marx *et al.*, 1972
PI 176721	Lockwood, 1960
PI 180693	Lockwood, 1960
PI 180702	Lockwood, 1960
PI 180868	Lockwood, 1960
PI 184129	Lockwood, 1960
Minn 108	Davis *et al.*, 1976
Minn 494A-1	Haglund and King, 1961
792022	Kraft, 1981
75-786	Kraft and Tuck, 1986
84-1638	Kraft and Tuck, 1986
84-1930	Kraft and Tuck, 1986
86-2236	Kraft, 1989
90-2079	Kraft, 1992
90-2131	Kraft, 1992
90-2322	Kraft, 1992
Wis 8901-RR	Gritton, 1990
Wis 8902-RR	Gritton, 1990
Wis 8903-RR	Gritton, 1990
Wis 8904-RR	Gritton, 1990
Wis 8905-RR	Gritton, 1990

turally acceptable types. The recent release of several public germplasm lines with resistance to *A. euteiches* greatly improves the prospect of developing commercial cultivars with improved resistance/tolerance to this important disease (Davis *et al.*, 1976; Kraft, 1986, 1989, 1992; Gritton 1990). Table 3 lists PI accessions and breeding lines resistant to *A. euteiches.*

Fusarium wilt

Pea wilt

Wilt of peas, caused by *Fusarium oxysporum* Schl. f. sp. *pisi* (Van Hall) Snyd. & Hans. race 1, was first described in 1924 (Linford, 1928). Resistance to this disease was determined to be inherited by a single dominant gene (Wade, 1929; Walker, 1931). Race 2 was found when race 1 resistant cultivars were developed and again attributed to a separate, dominant gene (Hare *et al.*, 1949). Races 3 and 4 found in the Netherlands and Canada, respectively, are thought to be more virulent strains of race 2 (Hubbeling, 1974). In addition, the genetic basis for resistance in the host to races 3 and 4 was not defined. In 1970, race 5 was detected and described from northwestern Washington state. All cultivars known to be resistant to races 1 and 2 were susceptible (Haglund & Kraft, 1970). Resistance to race 5 was first found in USDA PI accessions and was also attributable to a single dominant gene. In 1979, race 6 was also described from northwestern Washington state (Haglund & Kraft, 1979). This new race was pathogenic on cultivars and PI accessions resistant to races 1, 2, and 5, and resistance was again reported to be governed by a single, dominant gene.

Techniques to screen peas for resistance to wilt include: 1) pruning roots while submersed in a conidial suspension (Wells *et al.*, 1949; Haglund, 1989); 2) pouring mycelial fragments and conidia into a trough adjacent to seedling roots growing in sand (Armstrong & Armstrong, 1974); and 3) pouring conidia and hyphal fragments into holes punched into potting soil with a pointed rod to wound roots (Doling, 1963). In Washington state, resistance to race 1 wilt is determined under field conditions at Pullman, Washington, where a race 1 field nursery was established by Dr. F. J. Muehlbauer, USDA/ARS. As described by Wade (1929), elimination of race 1 susceptible cultivars is complete when inoculum levels are high. Resistance to races 2, 5, and 6 is determined in the greenhouse under pure culture conditions. Tests to screen for race 2 resistance are conducted when ambient greenhouse temperatures range from 20 to 24°C (Wells *et al.*, 1949). Tests for race 5 and 6 resistance are conducted in the winter months when ambient greenhouse temperatures range from 15 to 21°C.

All cultures of races 2, 5, and 6 used as primary inoculum are derived from single spores on 2% water agar (Toussoun & Nelson, 1976) and increased on fresh PDA under fluorescent light with a 12 h photoperiod. Only colonies appearing to be representative of the wild type are maintained in soil tubes (2 ml conidial suspension placed in 10 g of autoclaved soil mix in a test tube) (Toussoun & Nelson, 1976). Primary inoculum of a test isolate is produced by dispersing a small amount of infested soil on a PCNB plate (Nash & Snyder, 1962) and a resulting colony is selected, which is representative of the wild type for each race. A small agar plug from the colony margin of a 5-day-old culture is placed in 50 ml of liquid Kerr's medium (Kerr, 1963). Inoculated flasks are incubated for 5 days on a rotary shaker at 120 cycles per minute with constant fluorescent light on a laboratory bench. Spore concentrations

for each isolate are determined with a hemacytometer. Usually, three separate isolates of each race are combined so that the fungal spore concentration is 1×10^6 conidia ml^{-1}.

Seeds of each test line are surface disinfested with a 0.53% NaOCl solution before planting in coarse, autoclaved perlite. All seedlings are inoculated in the third to fourth node by carefully removing each plant, dipping and pruning one-half of the root system of each plant with a razor blade while immersed in a conidial suspension. Inoculated seedlings are transplanted back into the perlite and incubated on a greenhouse bench until wilt symptoms are evident and/or known susceptible controls are severely wilted or dead. Wilt symptoms consist of stunting, yellowing, dying of lower leaves, curling of leaf margins, and usually death of the plant (Kraft & Haglund, 1978). The pathogen should be readily isolated from the above ground stem of any susceptible, inoculated plant when whole plant symptoms are evident. Suggested pea lines to use as differentials to distinguish races 1, 2, 5, and 6 are shown in Table 4.

Lentil wilt

The disease is a serious threat to lentil production in many parts of the world and is caused by *Fusarium oxysporum* f. sp. *lentis* (Vasudeva + Srinivasan) Gordon. This pathogen is responsible for severe grain losses (Fleischmann, 1937; Khare, 1980; Bayaa *et al.*, 1986). The disease appears either in the early stage of crop growth (seedling wilt) or during reproductive growth (adult plant wilt) (Khare, 1981). Moderately high soil temperatures (20 to 25°C) which favor fungal growth, and sunlight, which enhances transpiration, seem to be the key factors determining symptom expression. In Syria, wilt usually appears in April/May (Erskine *et al.*, 1990). However, seedling wilt was very widespread in the wilt sick plot at Tel Hadya, Syria, in November 1991, on 1-month-old seedlings of a susceptible cultivar, probably because of abnormally high temperatures in October (Bayaa & Erskine, unpublished).

Typical symptoms of wilt are first seen as sudden drooping of leaflets starting at the plant top and progressing downward. Leaflets close and do not shed prematurely, turning dull green. Finally the whole plant wilts. Wilting may be unilateral and confined to individual branches. When wilt appears at flowering no seeds are produced. When wilt occurs in mid-late pod filling, yield is drastically reduced and resultant seeds are often shrivelled. Vascular discoloration of infected plants sometimes occurs.

Lentil wilt screening procedures

To screen under field conditions, the wilt nursery is infested by repeated cultivation of a susceptible cultivar and the incorporation of wilted plant material (Bayaa & Erskine, unpublished; Khare *et al.*, 1990). In order to increase disease pressure, lentil wilt inoculum can be increased on sterilized lentil seeds and uniformly spread in the nursery before sowing a susceptible cultivar. Screening is initiated when a uniform and high level of wilt damage is observed. Information on appropriate inoculum levels of *F. oxysporum* f. sp. *lentis* for field evaluation is lacking. This could be determined by estimating the number of propagules g^{-1} of soil using plate dilution with selective medium for *Fusarium* (Nash & Snyder, 1962; Komada, 1975). Test lines should be interplanted with a susceptible check, repeated every two to four test rows. Disease incidence should be recorded regularly during the season (Khare *et al.*, 1990). The wilt-sick plot should be monitored for possible interactions with other soil microorganisms.

Laboratory and greenhouse pure culture screening

Pure cultures of the lentil pathogen are maintained in autoclaved soil as mentioned previously (Toussoun & Nelson, 1976). Primary inoculum of *F. oxysporum* f. sp. *lentis* can be increased on any suitable medium (Kerr, 1963) and then mixed with the planting soil according to procedures previously described (Kannaiyan & Nene, 1976). Various growth media to increase inoculum are reported including PDA, lentil extract, dextrose agar (LD), and Richard's solution as the most commonly used media (Kannaiyan & Nene, 1978; Khare, 1980; Bayaa & Erskine, 1990).

The water culture technique, originally described by Wensley & McKeen (1962) and Roberts & Kraft (1971), was modified by Omar *et al.* (1988), and used to screen lentil lines for resistance to wilt. The roots of 10-day-old seedlings, grown previously in sterilized sand, were dipped in a spore suspension of 10^5 spores ml^{-1}. The seedling reaction was rated on a 0 to 7 scale described by Dixon & Doodson (1971) approximately 7 to 10 days later. Using the water culture technique of Roberts & Kraft (1971), the optimum spore concentration to differentiate between susceptible and resistant lines in 7 days was found to be 1.5×10^5 to 2×10^5 or

Table 4. Suggested pea lines to differentiate races 1, 2, 5, and 6 of the Fusarium wilt fungus

	Wilt Reaction[a]				Reference[b]
Pea Line	*R 1*	*R 2*	*R 5*	*R 6*	
M410	S	S	S	S	Brotherton Seed Co.
Vantage	R	S	S	S	Brotherton Seed Co.
Mini	S	R	S	S	Asgrow Seed Co.
Mini 93	R	R	S	S	Asgrow Seed Co.
Sundance II	R	S	R	S	Pure Line Seed Co.
Grant	R	S	S	R	Brotherton Seed Co.
WSU 23	R	R	R	S	W. A. Haglund
SWU 28	R	S	R	R	W. A. Haglund
74SN5	R	R	R	R	J. M. Kraft

[a] R = resistant; S = susceptible.

[b] Brotherthon Seed Co., Inc., Moses Lake, WA 98837; Asgrow Seed Co., Twin Falls, ID 83303; Pure Line Seeds, Inc., Moscow, ID 83843; Dr. W. A. Haglund, NW Washington Research & Extension Unit, Washington State University, Mt. Vernon, WA 98273.

6.5×10^5 spores ml^{-1} (Bayaa & Erskine, unpublished; Kamboj *et al.*, 1990).

Seeds may also be sown in autoclaved soil and inoculum, grown in a liquid medium for 14 days, applied 14 days after sowing (Bayaa & Erskine, 1990). At ICARDA, a spore concentration of 2.5×10^6 microconidia ml^{-1} was used to inoculate sterilized field soil in the greenhouse to screen for wilt. The resultant inoculum concentration was 5.0×10^4 microconidia g^{-1} soil (Bayaa & Erskine, unpublished). Other reports have described the use of oatmeal-sand inoculum mixed with planting soil. However, the authors believe that it is impossible to quantify inoculum produced on oatmeal-sand and incorporated into soil. The ability to standardize inoculum levels is extremely important to interpret results from one evaluation test to the next.

Wilt severity readings are recorded either at 56 days after planting for seedling wilt or during pod development for adult plant wilt (Bayaa & Erskine, unpublished). The observations on seedling/plant mortality are converted to percent wilt for use on a 1 to 9 scale where: 1 = 1% or less plants wilted; 3 = 1 to 10% plants wilted; and 9 = 51% or more plants wilted. Observations on the intensity of wilting are also recorded to assess symptom development on test lines (Bayaa & Erskine, 1990).

The effects of various environmental factors on pathogen growth and disease expression have been studied (Dhingra *et al.*, 1974; Khare, 1980; Saxena & Khare, 1988; Erskine *et al.*, 1990). Lentil suffered more damage (48%) in sandy loam soil than in clay soil (22%). The mortality of lentil plants increased with soil pH up to 7.5 beyond which it declined. The optimum soil temperature for disease development was found to be 20 to 25°C. The optimum soil moisture level for disease expression is 25% in the soil used at ICARDA.

Kannaiyan & Nene (1976) reported that 32 out of 158 lines were found immune under glasshouse conditions. However, none of them was immune, resistant or tolerant under field conditions, which indicated that their inoculation procedure was not adequate, or different races or strains were prevalent at their test site. Khare (1980) identified 25 out of 440 lines as resistant. Of these, JL 80, JL 500, JL 674, Pusa-3, and Pant 234 were highly promising. At ICARDA, sources of wilt resistance have been identified. These are distributed in the Lentil International Fusarium Wilt Nursery (LIFWN) for testing under field conditions at different locations. Their resistance was confirmed in Egypt (Hamdi *et al.*, 1991). Mihov *et al.*, (1987) reported four cultivars with resistance under field conditions in Bulgaria. Seven lines out of 100 were found resistant to wilt in Bangladesh (Hossain *et al.*, 1985). Five breeding lines (JL 599, JL 632, JL 674 JL 1005, and L 406), which combined immunity to rust and resistance to lentil wilt, have been reported (Nene *et al.*, 1975;

Pandya *et al.*, 1980). Resistance to wilt was also found among wild relatives of lentil (Bayaa *et al.*, 1991).

In reporting resistance, it is very important to mention the crop growth stage of the screening. Most lentil lines, exhibiting resistance at the seedling stage, lose their resistance at the adult stage (ICARDA, 1990).

Chickpea wilt

Fusarium wilt, caused by *Fusarium oxysporum* Schlecht emd.:Fr. f. sp. *ciceris* (Padwick) Matuo & K. Sato, is an important soilborne disease of chickpea. This disease has been reported from most all the chickpea growing regions in the world (Nene & Reddy, 1987). Although no precise information on yield losses from this disease is available, a rough estimate of about 10% yield loss was reported from India (Singh & Dahiya, 1973) and Spain (Trapero-Casas & Jiménez-Díaz, 1985), and up to 40% in Tunisia (Bouslama, 1980). The disease can appear at any stage of plant growth. The chickpea wilt pathogen can penetrate young seedling roots directly. Penetration occurs mainly through the cotyledons, or close to the cotyledons, and to a lesser extent in the zone of elongation and maturation (Jiménez-Díaz *et al.*, 1989a). Symptoms can develop in a highly susceptible cultivar within 25 days after sowing, but can also occur up to podding stage. Early wilting causes more loss than late wilting, but seeds from late-wilted plants are lighter, rougher, and duller than those from healthy plants (Haware & Nene, 1980).

The pathogen is both soilborne and seed transmitted. Seedborne inocula can be eradicated by seed dressing with commercial rates of Benlate T (30% benomyl + 30% thiram) (Haware *et al.*, 1978). *Fusarium oxysporum* f. sp. *ciceris* is pathogenic to *Cicer* spp. and can invade roots of other grain legumes (Haware & Nene, 1982a), melon, potato, sugarbeet, vetch, white lupine, *Amaranthus retroflexus*, and *Chenopodium album* (Trapero-Casas & Jiménez-Díaz, 1985; Cabrera de la Colina *et al.*, 1987) without causing external symptoms. Crop rotations to control chickpea wilt are not feasible because *F. oxysporum* f. sp. *ciceris* can reproduce on symptomless carriers and chlamydospores can survive for at least 6 yr in the soil. The most practical and economical control of Fusarium wilt of chickpea is resistant cultivars (Nene & Reddy, 1987).

The existence of pathogenic races in populations of *F. oxysporum* f. sp. *ciceris* is well established. Haware & Nene (1982b) first identified races 1, 2, 3, and 4 in India based on the differential reactions of ten chickpea lines. Later, three additional races, namely races 0, 5, and 6, were identified in Spain (Jiménez-Díaz *et al.*, 1989b). Race 0 is the least virulent of the seven races described. It is not pathogenic to desi line JG 62, which is susceptible to all other races. All race 0 isolates tested so far induce a progressive foliar yellowing as compared to the severe leaf chlorosis, flaccidity, and early wilt induced by races 1–6. Race 0 is widespread in southern Spain and seems to occur in Tunisia (Nene & Sheila, 1986). Italian isolates of *F. oxysporum* f. sp. *ciceris* have been identified as belonging to race 0, although the disease reaction of differential lines correspond to race 1 (Frisullo *et al.*, 1989). Recently, Phillips (1988) described a race 6 in California based on the disease reaction of the ten differential lines of Haware & Nene (1982b) in naturally infested field plots. No difference can be established as yet between race 6 from Spain and California until cross inoculation studies are made under standardized conditions. Furthermore, the possibility that differential host-interactions between more than one race in the field cannot be ruled out. The race status of *F. oxysporum* f. sp. *ciceris* in California seems to be more complex than that reported by Phillips (1988). Buddenhagen & Workneh (1988) concluded that wilt isolates from three central coast counties in California could be classified into at least two pathogenicity groups. Jiménez-Díaz (unpublished) carried out cross inoculation studies of differential lines with races 0 and 5 from Spain and with four isolates of *F. oxysproum* f. sp. *ciceris* provided by Buddenhagen and Workneh. Three of the isolates were similar to races 0, 1, or 5, respectively, and the fourth one resembled race 6.

Screening techniques

Efficient field, greenhouse, and laboratory procedures to evaluate chickpea lines for resistance to Fusarium wilt have been developed and standardized at ICRISAT (Nene *et al.*, 1981). Haware & Nene (1980, 1982b) reported on screening under controlled conditions using a pot-culture inoculation method in artificially infested soil. Stock isolates of *F. oxysporum* f. sp. *ciceris* should not be maintained by repeated transfers on growth media because loss of virulence through mutation can occur (Toussoun & Nelson, 1976; Burnett, 1984). We recommend that virulent isolates be stored as dormant cultures in either sterile soil (Toussoun & Nelson, 1976), silica gel (Windels *et al.*, 1988), or sterile filter paper (Correll *et al.*, 1986).

Recent work at ICRISAT (1989) indicates that a threshold of 483 propagules of race 1 g^{-1} (ppg) of soil is required for a 100% wilt incidence of JG 62. The late wilting cultivar K850 had a resistant reaction with that inoculum density, but wilt incidence increased with an increased inoculum level of 3283 ppg of soil. No wilt occurred with the resistant cultivar WR 315 at any inoculum level tested. Plants inoculated by the pot-culture method are grown in the greenhouse or in a growth chamber adjusted to a 14 h photoperiod of fluorescent light at 252 μE m^{-2} s^{-1} (Jiménez-Díaz *et al.*, 1989b, 1991). Chauhan (1963) reported that the optimum temperature for infection was 25°C, whereas disease developed with low severity at 35°C and no disease occurred at 15°C. However, Bhatti & Kraft (1992a) found that in soil artificially infested with 500 and 1000 ppg of *F. oxysporum* f. sp. *ciceris* wilt was severe at 25 and 30°C, but moderately severe at 15 and 20°C. No disease developed at 10°C even with an inoculum density of 5000 ppg.

Screening of a large number of germplasm accessions is more efficiently accomplished in the field. Experience at ICRISAT indicates that the development of a wilt-sick plot for field screening is relatively easy (Nene & Haware, 1980). Initially, a test site is selected where wilted chickpea plants are frequent and the soil is a slightly alkaline vertisol. Next, a highly susceptible cultivar is grown at this site for 2 to 3 seasons and infected plant debris is incorporated into the soil. Since 1980, wilt-sick plots have been used for resistance screening at many research centers including ICRISAT Center, Bihar, and Ludhiana (India), El Bajio and Culiacan (Mexico), Faisalabad (Pakistan), Beja (Tunisia), Santaella (Spain), and the Central Valley of California, USA.

Screening for resistance in a wilt-sick plot may present difficulties such as: a) the occurrence of other soilborne pathogens of chickpea; b) uneven distribution of the pathogen(s); c) the existence of more than one race of *F. oxysporum* f. sp. *ciceris*. Additionally, the yearly use of a wilt-sick plot for screening of different germplasm may lead to an uneven shift in the population and/or race distribution of the pathogen in soil. Screening chickpeas for resistance to Fusarium wilt in Santaella, Spain illustrates some of the points raised above. This wilt-sick plot was established in a naturally infested field in 1981 and since then has been continuously used for wilt resistance screening of germplasm from ICARDA, ICRISAT, and the regional breeding program. In 1983, a uniform wilt reaction occurred in highly susceptible lines, but cultivar JG 62 had a resistant reaction indicating that race 0 was prevalent in the plot. Results from field screening in 1989 indicated that a race shift may have occurred in the plot and at least two pathogenicity groups are now present in the plot (Jiménez-Díaz *et al.*, 1991). Furthermore, large differences occurred in the disease reaction of selected lines in the field. These same lines exhibited resistant to moderately resistant reactions when they were inoculated with isolates representative of the two pathogenicity groups and with races 0 and 5 of the pathogen in the greenhouse.

Development of wilt resistant sources and cultivars

Good progress has been made in identifying sources of resistance and development of wilt resistant, high-yielding cultivars (Singh, 1987; Singh & Reddy, 1991). At ICRISAT over 150 wilt resistant desi and kabuli germplasm lines are available (Haware *et al.*, 1989), and additional sources of resistance to wilt and root rots have been identified (Reddy *et al.*, 1990a,b). Multilocation testing has shown that a few lines have broad-based resistance, such as ICC–2862, ICC–9023, ICC–9033, ICC–10803, ICC–11550, and ICC–11551, or broad based and stable resistance, such as ICC–267, ICC–858, and ICC–8933 (Nene *et al.*, 1989).

At Santaella, Spain, a total of 2702 kabuli lines have been screened for resistance to Fusarium wilt in collaboration with ICARDA, including 713 FLIP lines in 1987, 991 ILC lines in 1989, and 196 FLIP and 802 ILC lines in 1990. The most promising resistant lines identified in 1987 were screened again in 1989, and these together with resistant lines identified in 1989 were also screened in 1990. Four lines [FLIP 84–43 C (ILC–5411), FLIP 85–20 C, FLIP 85–29 C, and FLIP 85–30 C] had a resistant reaction, and six additional lines had a resistant to moderately resistant reaction, in replicated tests in 1987 and 1989. These lines also tolerate cold and Ascochyta blight (Jiménez-Díaz *et al.*, 1991). In the 1990 replicated test, lines FLIP 85–20 C, FLIP 85–30 C, and ILC 219 were resistant, while the other lines showed a moderately resistant reaction. In addition, FLIP 87–26 C, FLIP 87–78 C, and FLIP 87–82 C, and ILC–267, ILC–1278, and ILC–1300 were resistant among those screened in 1990 (Jiménez-Díaz *et al.*, unpublished).

Fifteen accessions of 11 wild *Cicer* spp. and nine accessions of *C. pinnatifidum* were screened in pot culture inoculations for resistance to races 0 and 5 of *F. oxysporum* f. sp. *ciceris* in collaboration with the USDA-ARS Western Regional Plant Introduction

Station, Pullman, Washington (Kaiser & Jiménez-Díaz, unpublished). *Cicer bijugum*, *C. cuneatum*, and *C. judaicum* were resistant to both races, and *C. canariense* and *C. chorassanicum* were resistant to race 0 but susceptible to race 5. All accessions of *C. pinnatifidum* were susceptible to race 5 but PI 458555, PI 458556, and PI 510654 were resistant to race 0.

Resistance to Fusarium wilt of chickpeas has been successfully incorporated into high yielding desi and kabuli backgrounds, including ICCV 2, 3, 4, and 5 (Kumar *et al.*, 1985) and ICCV 6 (Ghanekar *et al.*, 1990) from ICRISAT; "Surutato 77", "Sonora 80", "Gavilan", "Kino", and "Tubutama" from Mexico (Morales, 1986); "UC 15" and "UC 27" from California (Buddenhagen *et al.*, 1988); and "Andoum 1" (Halila & Harrabi, 1990) from Tunisia. However, in most cases, wilt resistant kabuli cultivars are susceptible to Ascochyta blight and cultivars resistant to Ascochyta blight are susceptible to wilt (Halila & Harrabi, 1990; Jiménez-Díaz & Trapero-Casas, 1990).

Concluding remarks

Significant progress has been made in developing resistance to *Pythium* seed and seedling diseases in peas. Kabuli chickpeas are extremely sensitive to *Pythium* seed rot and preemergence damping-off, whereas desi chickpeas are resistant. Most likely desi chickpeas are resistant to *Pythium* due to thickened testae which reduce nutrient leakage during germination and the presence of fungistatic, anthocyanin compounds. More effort is needed to develop kabuli chickpeas with *Pythium* resistance. Resistance to Rhizoctonia seed and seedling rot in peas and lentils has been identified but much more work is needed to develop commercial cultivars with significant *Rhizoctonia* resistance. Techniques have been developed to screen lentils for resistance to collar rot caused by *Sclerotium rolfsii* and sources of resistance have been identified. However, the best sources of lentils with resistance to collar rot still exhibited a 10% mortality rate. Good resistance to Fusarium root rot of peas has been reported. This resistance is available in lines with acceptable horticultural type and significant progress is being made to develop commercial pea cultivars with resistance to this pathogen. However, the threat of new races of *F. oxysporum* f. sp. *pisi* developing exists in areas where short rotations are common. Resistance to Fusarium root rot of chickpea is not stable at high temperatures (30°C) and additional research is needed to identify resistant, chickpea germplasm. In addition, the *formae speciales* of *F. solani*, attacking chickpeas in the Middle East and North Africa needs to be determined.

Significant progress has been made in developing resistance to Aphanomyces root rot of peas with acceptable horticultural type. However, as is the case with most root diseases, this resistance is not complete and will break down with high inoculum levels and/or climatic conditions favorable to disease development. Good single gene resistance exists to the four races of the pea wilt pathogen. Apparently, resistance to all four economically important races of Fusarium wilt in peas is governed by single, independent, dominant genes. Screening techniques are described and are reproducible from one location to another around the world. Resistance to lentil wilt, caused by *F. oxysporum* f. sp. *lentis*, has been reported at ICARDA, ICRISAT, Bangladesh, and Bulgaria. Unfortunately, lentil breeding lines or cultivars exhibiting wilt resistance in the seedling stage are often not resistant in the adult stage. More work is needed to find mature plant resistance to lentil wilt and in developing accurate screening procedures for this pathogen. Significant progress has been made in finding resistance to chickpea wilt, caused by *F. oxysporum* f. sp. *ciceris*, and this resistance has been incorporated into high yielding kabuli and desi cultivars. Currently, seven races (0, 1, 2, 3, 4, 5, and 6) of *F. oxysporum* f. sp. *ciceris* are recognized. More work needs to be conducted on determining the genes responsible for resistance to these races and to find linkages that can been used as markers in a breeding program. Lastly, more effort is needed to combine Fusarium wilt resistance with Ascochyta blight resistance in chickpeas.

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