

Factors Affecting Pea Seed and Seedling Rot in Soil

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Journal Series Article No. 7248, Michigan Agricultural Experiment Station, East Lansing.

Accepted for publication 20 August 1975.

ABSTRACT

Effects of temperature, soil moisture, cultivar, seed color, and seed treatments on incidence of pea seed and seedling rot in soil naturally infested with *Pythium ultimum* and artificially infested with *Fusarium solani* f. sp. *pisi*, were investigated in controlled environmental chambers and in field plots. Disease incidence was greater with the wrinkled-seeded cultivar Miragreen than with the smooth-seeded cultivar Alaska, and was greater with green than with yellow Miragreen seeds. Seed and seedling rot were greater with high, than with low, soil moisture. Soaking Miragreen seeds in water at 22 C for 48 hours prior to planting reduced incidence of seed and seedling rot below controls,

presumably due to removal of the bulk of seed exudates stimulatory to pathogens. However, soaking for 48 hours at 10 or 15 C did not decrease, but usually increased, seed and seedling rot over controls, possibly because of increased exudation due to low-temperature injury. Incidence of rot was greater when growth chamber temperatures were alternated on a diurnal cycle to simulate field conditions than when they were held constant, suggesting that alternating temperatures favored both *Fusarium* spp. and *Pythium* spp., which have differing optima for disease development.

Phytopathology 66:188-192.

Additional key words: *Pythium ultimum*, *Fusarium solani* f. sp. *pisi*, environmental effects.

The spermosphere (34) or spermatosphere (31) has been defined as the zone of soil surrounding a germinating seed in which microbial activity is stimulated by nutrient exudates. The cotyledons, radicle, hypocotyl, and epicotyl base of germinating pea (*Pisum sativum* L.) seedlings are located within or near the spermosphere (27), and are susceptible to infection by *Fusarium solani* (Mart.) Appel & Wr. emend Snyd. & Hans. f. sp. *pisi* and *Pythium ultimum* Trow (2, 3, 4). Infection by either pathogen causes tissue decay in, and even death of, the seedling (4, 10, 16, 18). *Fusarium solani* f. sp. *pisi* and *P. ultimum* are widespread in pea-growing regions (4, 8, 10), and were more destructive when both were present, than when only one was present (3, 16). *Pythium* and *Fusarium* populations were much greater in spermosphere than in nonspermosphere soil (30, 36), particularly when seeds with rapid imbibition rates such as peas were planted (30).

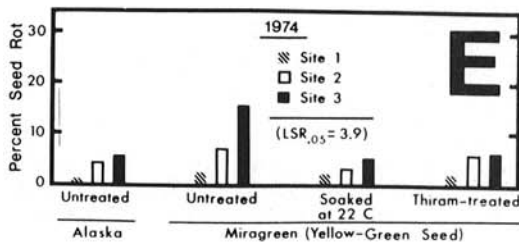
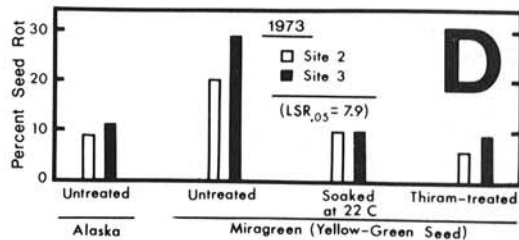
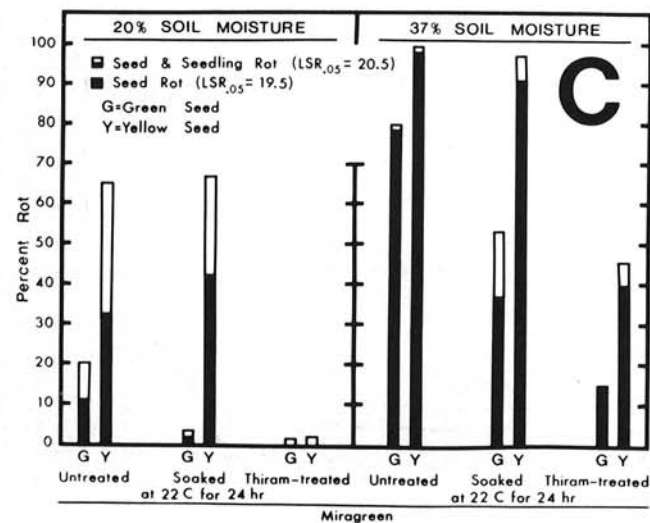
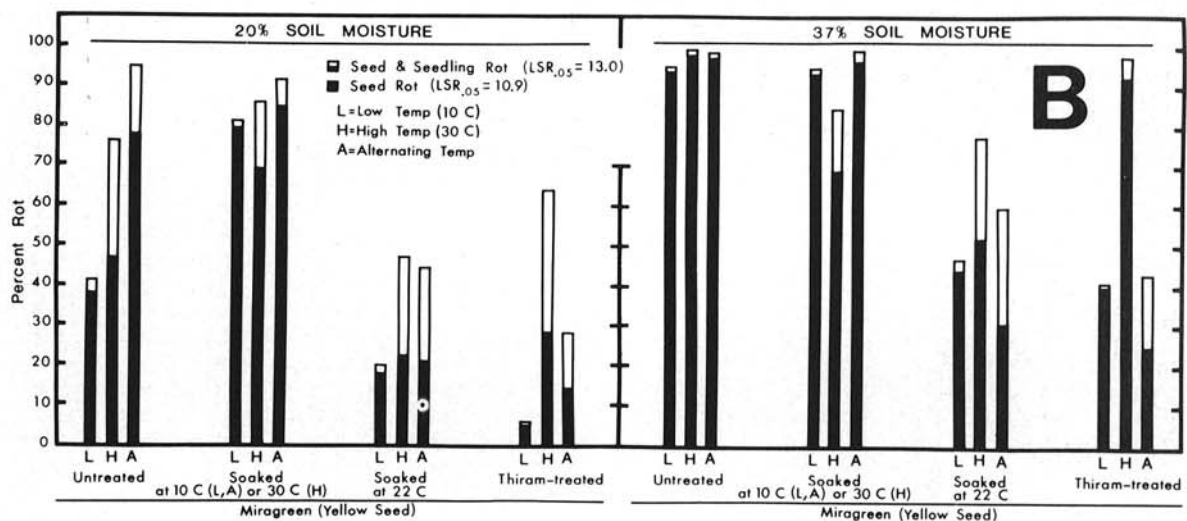
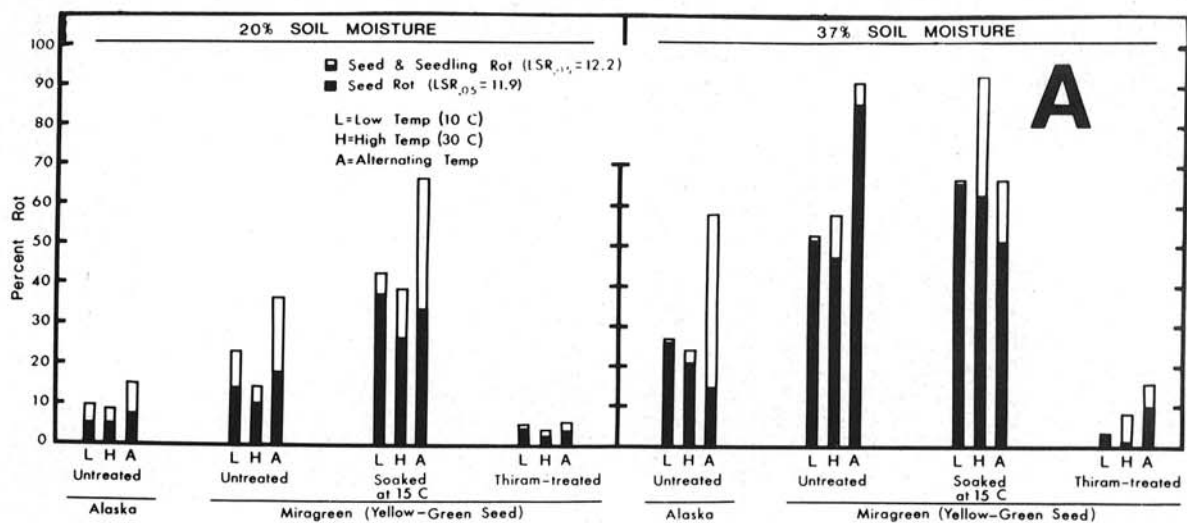
Improved techniques for measuring fungal stimulation in the spermosphere of beans (32) and peas (27) have made it possible to quantitatively study effects of environmental factors on the spermosphere. The magnitude of the spermosphere effect, as measured by

Fusarium solani chlamydospore germination, was greater at high than at low soil moisture (27, 32), increased with decreasing soil temperatures at 50% soil moisture, was greater for a wrinkled-seeded than a smooth-seeded pea cultivar, and was greatly reduced by soaking pea seeds prior to planting (27). The purpose of this study was to determine the relation between the magnitude of the spermosphere effect and incidence of pea seed and seedling rot. A preliminary report has been published (28).

MATERIALS AND METHODS.—*Selection and treatment of seeds.*—Wrinkled-seeded (cultivar Miragreen) and smooth-seeded (cultivar Alaska) pea seeds were obtained from Ferry-Morse Seed Co., Mountain View, Calif. Seeds with cracked testae or off-color spots were discarded. Remaining seeds had at least 98% viability. Miragreen seeds varied in color, and were separated into lots of yellow, yellow-green, and green.

Some seeds were coated with thiram (tetramethylthiuram disulfide) at a rate of 0.8 g thiram (active ingredient) per kilogram of seed. Other seeds were

Fig. 1-(A to E). Incidence of pea (*Pisum sativum* "Miragreen" and "Alaska") seed and seedling rot in soil naturally infested with *Pythium ultimum*, and artificially infested with *Fusarium solani* f. sp. *pisi*. Miragreen seeds were coated with thiram at a rate of 0.8 g thiram (active ingredient) per kilogram of seed, or were soaked in water at 10, 15, 22, or 30 C for 48 hours (unless otherwise specified) prior to planting; some seeds of both cultivars (Miragreen and Alaska) were planted without treatment. (A to C) Growth chamber experiments. Temperatures were maintained at 10, 22, or 30 C, or were alternated every 12 hours, starting with a low of 10 C and a high of 25 C, and increasing the alternating lows and highs by 1 C every 48 hours until the final range was from 15 to 30 C. A) Effect of soil moisture, temperature, cultivar, and seed treatment on incidence of rot in soil infested with 4×10^5 *F. solani* f. sp. *pisi* chlamydospores per gram. B) Effect of soil moisture, temperature, and seed treatment on incidence of rot in soil infested with chlamydospores (4×10^5 /g) and macroconidia (6×10^5 /g) of *F. solani* f. sp. *pisi*. C) Effect of soil moisture, seed treatment, and seed color on incidence of rot in soil at 22 C. D and E). Field trials. Effect of cultivar, seed treatment, and soil characteristics on incidence of seed rot. Soil moisture levels at sites 1 and 3 were consistently greater than at site 2. Site 1 was not infested with *F. solani* f. sp. *pisi*.



surface-disinfected for 30 minutes in 0.5% sodium hypochlorite containing 1 ml of Tween-20 (polyoxyethylene sorbitan monolaurate) per liter, followed by 24 or 48 hours of soaking in aerated or nonaerated water at 10, 15, 22, or 30 C. Some seeds were planted with no treatment.

Source, preparation, and infestation of soil.—Conover sandy loam was used in field trials, and for growth chamber experiments. Soil characteristics included: 43% moisture holding capacity, 2.7% organic matter, 54% sand, 29% silt, 17% clay, and pH 6.6. The soil was naturally infested with *Pythium ultimum*.

In growth chamber experiments, soil was passed through a 2-mm opening (9-mesh) sieve to remove large stones and break up large aggregates. The soil was then mixed in a concrete mixer while a suspension of *F. solani* f. sp. *pisi* chlamydo-spores, prepared as previously described (27), was added with an atomizer until the infestation level reached 4×10^5 spores/g dry weight of soil. In one experiment, to increase inoculum load, 6×10^5 *F. solani* f. sp. *pisi* macroconidia per gram dry weight of soil were added to soil previously infested with chlamydo-spores. In field trials, a suspension of *F. solani* f. sp. *pisi* chlamydo-spores was sprayed onto the soil surface and incorporated into the upper 7-10 cm prior to planting in 1973.

Control of soil moisture and temperature.—Screen-bottomed metal soil boxes (50 × 107 × 43-cm deep) were placed in an empty water tank in a growth chamber. Soil at 20% moisture (oven-dry weight basis) was compacted uniformly in each box to a depth of 10 or 30 cm. Pea seeds (four replicates of 25 each) were planted 10-15 mm below the surface of the infested soil for each treatment. Water was added to the water tank until the water level reached the base of the soil in the soil box. Water then moved upward by capillary action, quickly establishing and maintaining a soil moisture content in the upper 2.5 cm of soil of 20 or $37 \pm 2\%$, using soil depths of 30 or 10 cm, respectively.

Seeds were incubated in growth chambers for: (i) 21 days at 10 C, (ii) 10 days at 22 C, (iii) 10 days at 30 C, or (iv) 14 days under a 12-hour alternating diurnal temperature cycle, in which temperatures were first alternated between 10 and 25 C for 2 days, then high and low temperatures were increased by 1 C every 2 days so that final temperatures alternated between 15 and 30 C. The purpose of the alternating temperature cycle was to simulate the diurnal, gradually increasing temperature cycles which are common in field soils in spring when peas are planted, providing optimum conditions for disease development (10, 18, 21). A 12-hour photoperiod was alternated with a 12-hour dark period; when alternating temperatures were used, the higher temperature was synchronized with the light period and the lower temperature with the dark period. Seedlings were 5-10 cm in height at the end of the incubation periods.

Peas were planted at three sites on the Michigan State University farm on 18 April in 1973 and 1974. Seed treatments were replicated four times at each site, with 100 seeds per replicate. Soil temperatures in April and May of 1973 and 1974 ranged from 0-32 C, as determined by a Tempscribe recording thermograph with the probe positioned 2.0 cm below the soil surface. For determination of soil moisture content, soil cores (3 cm in

depth) were collected twice weekly throughout the season. Soil moisture levels at site 2 were consistently 4-5% less than at sites 1 and 3, due to higher elevation. Sites 2 and 3 were artificially infested with *F. solani* f. sp. *pisi*; site 1 remained uninfested. Incidence of seed rot in the field was determined 4-5 weeks after planting.

Assessment of seed and seedling rot.—Seeds which failed to extend a plumule above the soil surface were counted as rotted. Seedling rot included all dead and unhealthy seedlings showing signs of wilt, acute stunting, or decaying plumules, which were often engulfed in a mass of white mycelium. All experiments were repeated at least once, and were statistically analyzed using a split plot or a split-split plot analysis of variance. The least significant range (LSR_{0.05}) between means was determined using Tukey's test (33).

RESULTS.—*Effect of soil moisture.*—Seed and seedling rot in growth chamber experiments were usually greater at 37% than at 20% soil moisture (Fig. 1-A, B, C). In field plots, incidence of rot among untreated seeds was greater in the wetter soil (site 3) than in the drier soil (site 2) (Fig. 1-D, E) where soil was infested with *F. solani* f. sp. *pisi*.

Effect of cultivar.—Seed and seedling rot were greater for untreated Miragreen than for Alaska peas at both 20% and 37% soil moisture (Fig. 1-A). Similarly, in the field more untreated Miragreen seeds rotted than Alaska seeds at all planting sites (Fig. 1-D, E).

Effect of seed color.—Green Miragreen seeds had a much lower incidence of rot than yellow Miragreen seeds at either 20% or 37% soil moisture (Fig. 1-C).

Effect of temperature.—Seed and seedling rot in soil infested with 4×10^5 *F. solani* f. sp. *pisi* chlamydo-spores per gram were greater in most treatments when gradually increasing temperatures were alternated diurnally, than when temperatures remained constant at 10 or 30 C (Fig. 1-A). When the *F. solani* f. sp. *pisi* inoculum consisted of 4×10^5 chlamydo-spores and 6×10^5 macroconidia per gram dry weight of soil, incidence of seed and seedling rot in most treatments was greater at 30 C than at 10 C (Fig. 1-B).

Effect of soaking seeds.—Incidence of seed and seedling rot among seeds soaked in aerated deionized water for 48 hours at 22 C prior to planting was always much less than among untreated seeds (Fig. 1-B, D, E); however, incidence of seed and seedling rot among seeds soaked at 10, 15, or 30 C for 48 hours was usually as great or greater than among untreated seeds (Fig. 1-A, B). Miragreen seeds were also soaked in nonaerated tap water at 22 C for only 24 hours before planting in soil infested with *F. solani* f. sp. *pisi* and *P. ultimum*. Incidence of seed and seedling rot at 22 C among soaked yellow seeds was similar to untreated yellow seeds at both 20 and 37% soil moisture (Fig. 1-C). However, incidence of seed and seedling rot among soaked green seeds was lower than that of untreated seeds.

DISCUSSION.—Pea (17) and bean (25) seeds exude nutrients during germination, the greatest proportion of which are simple sugars such as glucose, maltose, sucrose, and fructose. Such sugars stimulate spore germination and germ tube growth of seed-rotting fungi (5, 25). Incidence of rot has been directly correlated with the quantity of carbohydrate exuded by soybeans (12), beans (24), and peas (20). Pea seeds germinate beneath the

soil surface and the cotyledons remain confined to spermosphere soil throughout seedling development. This is disadvantageous to the seedling if seed-rotting organisms within the spermosphere are stimulated to produce an active vegetative mycelium capable of infecting the seed or seedling axis, particularly when soil moisture and temperature are unfavorable for seed germination and seedling growth. The ability of *Pythium ultimum* sporangiospores to germinate within 1.5 hours after receiving the seed exudate stimulus (32), and the rapid growth rate of the mycelium at 12-30 C (18) produced a characteristic "balling" of soil around seeds (5, 9). "Balled" seeds may rot prior to emergence (4), produce seedlings which rot following emergence (3), or produce weakened seedlings (21).

Generally, environmental conditions which adversely affect seedling growth are most conducive to pre- and postemergence damping-off (18). Pre-emergence rotting of peas is most severe in cool, wet soil conditions in which seedling emergence would be delayed and pathogen spore germination would be greatest due to a large spermosphere effect (2, 9, 21, 27, 32). Peas are considered to be a cool temperature crop which must be planted early in the spring in temperate regions to produce maximum yields (21). Fungicide seed treatments have been widely used to minimize pre-emergence rotting in peas, though they have not always adequately controlled the disease (9, 11, 19).

The spermosphere effect (27) and incidence of seed and seedling rot (Fig. 1-B, D, E) were both considerably reduced by soaking Miragreen peas in water at 22 C for 48 hours before planting. However, seed germination and seedling emergence were impaired when seeds were soaked at high (30 C) or low (1-15 C) temperatures (15, 22, 26), or for more than 48 hours (14, 15). The high incidence of rot among Miragreen seeds soaked for 48 hours at 10, 15, or 30 C (Fig. 1-A, B) was possibly due to temperature injury.

The green color of pea and lima bean seeds occasionally fades as seeds mature, a process known as bleaching (23, 35, 37). Bleached peas appear yellow, and are sometimes referred to as "blonds" (35). Incidence of pea seed and seedling rot among untreated yellow Miragreen peas was greater than among green seeds (Fig. 1-C), presumably because yellow seeds exuded more carbohydrate and had a larger spermosphere effect than green seeds (29). Soaking green seeds for 24 hours before planting was effective in reducing incidence of rot (Fig. 1-C), apparently because carbohydrate exudation from green seeds had subsided to very low levels prior to planting (29). The spermosphere effect around yellow Miragreen seeds soaked for 24 hours was only slightly less than around unsoaked seeds (Short and Lacy, unpublished), indicating that considerable exudation occurred, even after 24 hours of soaking. Lima bean seed rot was greater and seedling vigor has been reported to be lower (23, 37) for bleached than for nonbleached seeds, although the mechanism(s) involved were not determined. The loss of green color in peas and lima beans, and the mechanism(s) by which bleaching increases susceptibility to seed decay, merit further study.

Temperature affects not only the amount of seed exudation (29), but also bacterial competition for exudates (1), the growth rate of the host (18), and the

growth rate of the pathogen (10, 18). Optimum temperature for seed and seedling rot caused by *Pythium ultimum* (12-25 C) is lower than for *F. solani* f. sp. *pisi* (24-33 C) (10, 18, 21). Thus, seed and seedling rot at 10 C (Fig. 1-A, B) may have been caused primarily by *P. ultimum*, while that at 30 C may have been due mainly to *F. solani* f. sp. *pisi*. The greater incidence of rot under alternating temperatures than at constant 10 or 30 C (Fig. 1-A, B) was likely due to temperatures favoring disease development by both pathogens at different times of the day (3, 16). This was supported by a much lower incidence of rot at constant 10 C than at either constant 30 C or at alternating temperatures, when the *F. solani* f. sp. *pisi* inoculum load was increased by adding macroconidia (Fig. 1-B).

Leach (18) tried to predict the fate of seeds, including peas, planted in *Pythium*-infested soil by calculating ratios of rates of emergence of the host in pasteurized soil to rates of growth of the fungus in potato-dextrose broth at temperatures from 4-35 C. These ratios have not always proven to be useful in predicting damping-off (6, 7), perhaps because the effect of soil moisture on the host and pathogen were not considered (4, 13, 18).

Due to recent progress in measuring the spermosphere effect (27, 32), a more direct approach to predicting pre-emergence rotting of seeds is possible. The radial extent and intensity of the spermosphere effect, as measured by *F. solani* f. sp. *pisi* chlamydospore germination at 10, 22, or 30 C (27), increased with various pea cultivar-soil moisture combinations in the following order: Alaska, 20% soil moisture; Miragreen, 20% soil moisture; Alaska, 50% soil moisture; Miragreen, 50% soil moisture. Incidence of pea seed and seedling rot in growth chambers (Fig. 1-A) at 10 C, 30 C, or under alternating temperatures, increased in precisely the same sequence, suggesting a direct relation between incidence of rot and the magnitude of the spermosphere effect at any particular temperature.

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