DETERMINANTS OF PEA SEED AND SEEDLING ROT

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ABSTRACT

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By

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The mechanisms by which biotic and abiotic factors interact to affect incidence of seed and seedling rot in pea (Pisum sativum L.) were investigated. Exudates from germinating seeds stimulated spore germination of pathogenic fungi in spermosphere soil. This phenomenon has been called the spermosphere effect. The effects of soil moisture, soil temperature, seed age, seed color, and seed treatments on seed exudation, spore germination, and incidence of disease were investigated.

I. A method was developed for directly observing germination of Fusarium solani f. sp. pisi chlamydospores in 1-mm increments of distance from the pea seed coat. The maximum distance from seeds at which spores germinated was greatest at 10 C and 50% soil moisture, and never exceeded 7 mm under any conditions tested. More spores germinated at 50% than at 20% soil moisture. More germination occurred near the emerging radicle than in other areas of the spermosphere. Spore germination decreased with increasing temperature at 50% soil moisture, but at 20% soil moisture a higher percentage of spores germinated at 22 than at 10 or 30 C. The

wrinkled-seeded pea cultivar Miragreen supported more spore germination in the spermosphere than did the smooth-seeded cultivar Alaska. When Miragreen seeds were soaked in aerated water for 48 hours prior to planting, spores germinated only in the millimeter of soil nearest the seed and the spore germination was only one-sixth as great as in the same zone near unsoaked seeds. It is suggested that spore germination is a function of the availability of nutrients exuded by the seed.

II. Exudates from surface-sterilized pea seeds were collected during 4 days of germination in sterile leaching systems of glass beads. Total carbohydrates were measured using a modified anthrone analysis. The amount of carbohydrate exuded from pea seeds was influenced by incubation temperature, cultivar, seed age, and also by factors associated with seed color and rate of water imbibition. The greater part of the carbohydrate was exuded during the first 18 hours of seed germination at 22 and 30 C, but significant exudation persisted for about 48 hours at 10 C. Total exudation over a 4 day period was greater at 10 C than at higher temperatures. Wrinkled-seeded Miragreen peas exuded more carbohydrates than the smooth-seeded Alaska cultivar, and yellow ("blond") Miragreen seeds exuded more carbohydrates than green Miragreen seeds under most experimental conditions. Eight-year-old Miragreen seeds exuded up to ten times more carbohydrates than one-year-old seeds, and seeds which required only 5 hours to complete swelling in water exuded more nutrients than seeds needing 8 or 12 hours to complete swelling.

III. Wrinkled-seeded Miragreen and smooth-seeded Alaska pea seeds were planted in soil artificially infested with Fusarium solani f. sp. pisi and naturally infested with Pythium ultimum. Incidence of seed and seedling rot was determined in growth chambers. 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. Every other day for 10 days the alternating lows and highs were each increased by 1 degree until the final range was from 15 to 30 C. Incidence of seed rot in the same soil in field plots was also determined. Miragreen seeds rotted more frequently than Alaska seeds. Incidence of rot was greater with yellow than with green Miragreen seeds. More seeds rotted at high than at low soil moisture. Seed and seedling rot were most severe when temperatures were alternated between the lower temperatures favorable for disease development by P. ultimum and the higher temperatures favorable for F. solani f. sp. pisi infection. Soaking Miragreen seeds in water at 22 C for 48 hours prior to planting reduced the incidence of seed and seedling rot; however, soaking seeds at 10-15 C for 48 hours usually increased seed and seedling rot, perhaps because of low temperature injury. However, in the absence of such putative seed injury, incidence of seed and seedling rot at any soil temperature tested was directly related to the magnitude of the spermosphere effect, which in turn was influenced by the amount of seed exudation and by soil moisture.

DETERMINANTS OF PEA SEED AND SEEDLING ROT

By

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To Karen

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GENERAL INTRODUCTION

In 1904 Hiltner (2) observed that microorganisms were more abundant in soil surrounding plant roots than in soil remote from roots, and called this zone of soil the rhizosphere. In 1947 Slykhuis (13) observed that microbial activity in soil surrounding germinating seeds was greater than in soil distant from seeds, and Verona (14) later referred to this zone of soil as the spermosphere. Amino acids, simple sugars, and presumably other substances are exuded from roots and seeds (8,10) providing a food supply readily utilized by many soil microorganisms. This loss of nutrients can be beneficial to the plant if symbionts such as mycorrhizal and rhizobial species are stimulated. However, seedling exudates may also stimulate plant pathogenic microorganisms, initiating a series of events which frequently result in a weakening or killing of the host.

Techniques used to measure root and seed exudates and resultant microbial activity have had serious limitations. For example, it has not been possible to directly measure exudation in soil, since sugars and amino acids are readily utilized by the indigenous soil microflora (4). Hence, exudates have been collected from microbe-free plants <u>in vitro</u> under conditions quite different from the conditions of the soil environment (5). Imprecise methods of

sampling soil in close proximity to roots and seeds have hindered attempts to define quantitatively and qualitatively the rhizosphere and spermosphere. Usually, roots have been carefully removed from soil, and soil firmly adhering to roots was defined as rhizosphere soil (3,9). Soil firmly adhering to seeds was called spermosphere soil (13). Seeds or roots with adhering soil were then vigorously agitated in sterile distilled water to suspend the soil particles and microorganisms in solution. Aliquotes of the soil suspension were serially diluted and plated out on agar media for determining microbial populations as a measure of microbial activity (3). With this method it was impossible to determine differences in magnitude of microbial activity at defined distances from seeds or roots.

A better understanding of the nature and extent of the rhizosphere and spermosphere would likely further our understanding of the mechanisms involved in seed and root rot diseases (1,4,6). For several reasons seeds seemed more suitable than roots for an initial attempt at refining techniques of measuring exudation and resultant pathogenic activity in soil. It seemed likely that the location of microbial activity around a single, stationary, hypogeously germinating seed could be measured more precisely than about an elongating, branching root system. In addition, exudation from seeds is transitory in contrast to the continual loss of nutrients from growing root tips (7,11,12); thus, it also seemed likely that the time course of exudation and the spacial distribution of exudates could be assessed more accurately with seeds than with a growing, fibrous root system.

Spores of many seed-rotting fungal pathogens germinate in response to seed exudates, particularly the sugar components (8,10, 11). The purposes of this investigation were: (i) to refine *in vitro* techniques of collecting and analyzing carbohydrate exuded during seed germination; (ii) to develop a technique for directly measuring pathogenic fungal spore germination in soil surrounding seeds; (iii) to determine how various seed characteristics, environmental factors, and cultural practices affect seed exudation and spore germination; and (iv) to relate seed exudation and the consequent germination of pathogenic spores to incidence of seedling mortality with the hope of ultimately using the information obtained to control seed and seedling diseases.

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PART I

GERMINATION OF *FUSARIUM SOLANI* F. SP. *PISI* CHLAMYDOSPORES IN THE SPERMOSPHERE OF PEA

Germination of Fusarium solani f. sp. pisi Chlamydospores in the Spermosphere of Pea

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ABSTRACT

A method was developed for directly determining amount of spore germination in pea spermospheres in 1-mm increments of distance from the seed coat. The greatest distance at which *Fusarium solani* f. sp. *pisi* chlamydospores germinated was established within 24 h at 22 or 30 C, and never exceeded 7 mm under any conditions tested. More spores germinated, and the spermosphere was larger at 50% than at 20% soil moisture. More germination occurred near the emerging radicle than in other areas of the spermosphere. Greatest distances from seeds at which spores germinated decreased with increasing temp at 50% soil moisture. At 20% soil moisture, a higher percentage of spores germinated at 22 than at 10 or 30 C. The wrinkle-seeded pea cultivar 'Miragreen' supported more spore germination in the spermosphere than did the smooth-seeded cultivar 'Alaska'. If Miragreen seeds were soaked in aerated water for 48 h prior to planting, spores germinated only in the millimeter of soil nearest the seed and percentage germination was one-sixth that of spores in the same zone near unsoaked seeds.

Phytopathology 64:558-562.

Additional key words: agar-embedded soil columns.

Spores of most plant pathogenic fungi do not germinate in soil unless provided with some external stimulus (10). Germinating seeds exude nutrients capable of stimulating microbial activity, including spore germination (17). The zone around the seed into which exudates diffuse and microbial activity increases, has been designated the spermosphere (24) or spermatosphere (22). *Fusarium solani* f. sp. *phaseoli* chlamydospores were reported to germinate as far as 10-12 mm from germinating bean seeds (23). Attempts to ascertain sporangial germination (23) and populations (21) of *Pythium ultimum* at varying distances from seed surfaces, were less successful.

Inadequate techniques of observing activities of microorganisms in soil has been a major obstacle in determining quantitative dimensions of the spermosphere. The objective of this investigation was to determine the amount of chlamydospore germination of *F. solani* (Mart.) App. & Wr. em. Snyd. & Hans. f. sp. *pisi* occurring in different regions, and at various distances from germinating seeds of *Pisum sativum* L. as influenced by: (i) cultivar; (ii) soil temp; (iii) soil moisture; and (iv) soaking of seeds prior to planting. A preliminary report has been published (19).

MATERIALS AND METHODS.—*Selection and preparation of seeds.*—Wrinkle-seeded ('Miragreen') and smooth-seeded ('Alaska') pea cultivars obtained from Ferry-Morse Seed Co., Mountain View, Calif. were used. Individual seeds were selected on the basis of uniformity in size and color (yellow in the case of Miragreen; green for Alaska) and freedom from spots or cracks on the seed coat. Prior to planting, seeds were surface disinfested for 10 min in 0.5% sodium hypochlorite containing 1 ml of Tween 20 (polyoxyethylene sorbitan monolaurate) per liter, followed by 5 min of rinsing in sterile distilled water. In one experiment, seeds were soaked for 48 h in aerated water prior to planting.

Source, preparation, and infestation of soil.—Conover loam soil from the Michigan State University farm, collected from an area free from recent pesticide application, was used in all experiments. Soil was stored at 15-20% moisture at 22-25 C in closed plastic containers. Prior to use, soil was air-dried and passed through a 30-mesh sieve. Water-holding capacity of this soil was 61%, organic matter content was 3.4%, and pH was 6.6. The soil contained 18% clay, 15% silt, and 67% sand.

Chlamydospores of *F. solani* f. sp. *pisi* were produced in shaken liquid culture as follows: Macroconidia were removed from potato-dextrose agar (PDA) plates in 25 ml of sterile distilled water, combined with 40 ml of potato-dextrose broth, and agitated on a reciprocal shaker for 48 h. The germinated conidia were washed and suspended in 40 ml of sterile soil extract [prepared by mixing 1 liter of water with 1 kg of Conover loam, allowing the mixture to stand for 48 h, and filtering the supernatant through a 2.2-μ Gelman membrane filter (1)]. Germinated conidia agitated in soil extract produced an abundance of chlamydospores free from mycelium within 7 days. Chlamydospores were washed, resuspended in distilled water, and agitated at low speeds for 1-2 h in a Sorvall Omni-Mixer to break up aggregates of chlamydospores. The chlamydospore suspension was adjusted to 2.5×10^7 chlamydospores/ml using a hemacytometer. Air-dried, sieved soil was placed in a mixing apparatus, and the spore suspension was applied with an atomizer until a final concn of 1.6×10^6 chlamydospores/g dry wt of soil and a 20% soil moisture were simultaneously attained.

Adjustment of soil moisture.—Infested soil was placed in 2.5-cm-diam Pyrex glass tubes with a fine screen fastened to the base. Uniform compaction in the upper 2.5 cm of soil was attained by dropping the tubes from a height of 15 cm until further

compaction ceased. One pea seed, with the hilum oriented downward, was centered in the upper 2.5 cm of each soil column prior to the addition and compaction of the uppermost 1.25 cm of soil. The lower ends of the columns were immersed to a 1-mm depth in a water bath, permitting the upward movement of water by capillary action. Moisture levels of 20% and 50% were established in the upper 2.5 cm of soil by using 33 and 8.1-cm soil columns, respectively. This system maintained a constant moisture level in the upper 2.5 cm of soil during all stages of seed germination.

Determination of spore germination.—Spores were incubated at 10, 22, or 30 C in soil columns for 24-96 h following seed placement. Columns were then dried with a stream of air by applying a vacuum to the lower ends, infiltrated with 2% molten water agar, cooled, and the agar hardened by immersing in ethanol for 12 h. Agar-embedded soil columns were extruded from the Pyrex tubes and a 2-mm-thick cross-section in the vicinity of the seed was removed with a razor blade. A small sharpened spatula was used to serially remove blocks of soil at millimeter increments from the seed surface in the area of radicle emergence and opposite the radicle area (Fig. 1-F). Soil cores above and below the seed were removed with a 4-mm-diam cork borer, and serially sectioned in millimeter increments with a razor blade.

Each block of soil was placed on a microscope slide, and a drop of 5 N HCl added to dissolve the agar. Soil smears were made with 0.1% aniline blue in lactic acid, similar to the technique of Nash et al. (13). The smears were examined for chlamydospore germination at a magnification of X430. Fifty chlamydospores were counted per slide. Treatments were replicated five times and experiments were repeated once with similar results. Statistical differences between treatments were determined using a two-way analysis of variance following angular transformation of data.

RESULTS.—Preliminary experiments were carried out to determine the optimum time after planting to sample chlamydospore germination in the spermosphere. Germination at 22 C was determined after 24, 42, 48, or 72 h, using both pea cultivars and moisture levels of 20% and 50% (Table 1). The greatest distance from the seed at which spores germinated in any treatment was established within 24 h. Percentage germination was greatest 42 h after planting at 50% soil moisture and 48 h after planting at 20% soil moisture. Extensive mycelial growth after 42 h at 50% moisture obscured additional germination; however, this did not occur at 20% soil moisture. During preparation of soil smears from the 50% moisture samples, many ungerminated spores appeared to become dislodged from entangled hyphae of germinated spores, possibly resulting in an underestimate of spore germination. Germ tube lysis further reduced apparent spore germination 72 h after planting at 22 C (Table 1). No lysis was observed up to 96 h after planting at 10 C, but at 30 C lysis was already evident 30 h after planting. In later experiments (Fig. 1), soil columns were incubated for 24 h at 30 C; for 42 and 48 h at 50% and 20% soil moisture, respectively, at 22 C; and for 96 h at 10 C.

Effect of different regions of the spermosphere.—Spore germination was always greater near the emerging radicle than in other regions of the spermosphere (Fig. 1-A, B, E, F). However, no differences in germination in the other areas (hilum, opposite hilum, and opposite radicle) were found; hence, only data from the radicle and opposite radicle regions were plotted. At 50% soil moisture and 22 C, spores germinated with greater frequency at a given distance from the seed near the radicle than in other regions of the spermosphere. At 20% soil moisture, spore germination near the emerging radicle of either cultivar was consistently greater than in other areas only in the millimeter of soil directly adjacent to the seed. Maximum spore germination observed was 70% in the millimeter of soil (50% moisture) adjacent to the radicle area of the cultivar Miragreen. Spore germination declined with increasing distance from the seed, and was never detected further than 7 mm from the seed (Fig. 1-C).

Effect of soil moisture.—Spores germinated 2-5 mm further from the seed when soil moisture was increased from 20% to 50%, regardless of cultivar or temp used (Fig. 1-A, B, C, D). Spore germination at a given distance from the seed was also considerably greater at 50% than at 20% soil moisture.

Effect of cultivar.—Spores germinated at greater distances and in higher frequencies at comparable distances from the Miragreen than the Alaska cultivar under all soil moisture and temp conditions (Fig. 1-A, B, C, D).

Effect of temperature.—The region of the spermosphere sampled was opposite the radicle (Fig. 1-F), since the amount of spore germination in this area was indicative of that in most other regions of the spermosphere. At 50% soil moisture, the maximum distance from the seed at which chlamydospores germinated decreased with increasing temp with both cultivars (Fig. 1-C, D). However, at 20% soil moisture, germination was greatest at 22 C and was least at 10 C.

Effect of soaking seeds.—Spore germination was confined within 1 mm of the surface of Miragreen pea seeds soaked in aerated water for 48 h before planting (Fig. 1-E). Germination within 1 mm of soaked seeds was one-sixth that of spores in the same zone near unsoaked seeds.

DISCUSSION.—The method of embedding and sectioning soil described enabled quantitative measurement of spore germination in the spermosphere with a precision heretofore not possible (14, 21, 22, 23). This technique could also be employed for determining spore germination in the rhizosphere. Undoubtedly, amount and rapidity of exudation are two very important considerations in selecting a host for study. If amount of exudation restricted spore germination to within 1 mm of the seed or root, this technique would not be as applicable. We have not been able to remove soil sections in increments of less than 0.5 mm.

The ideal time for assessing spore germination in

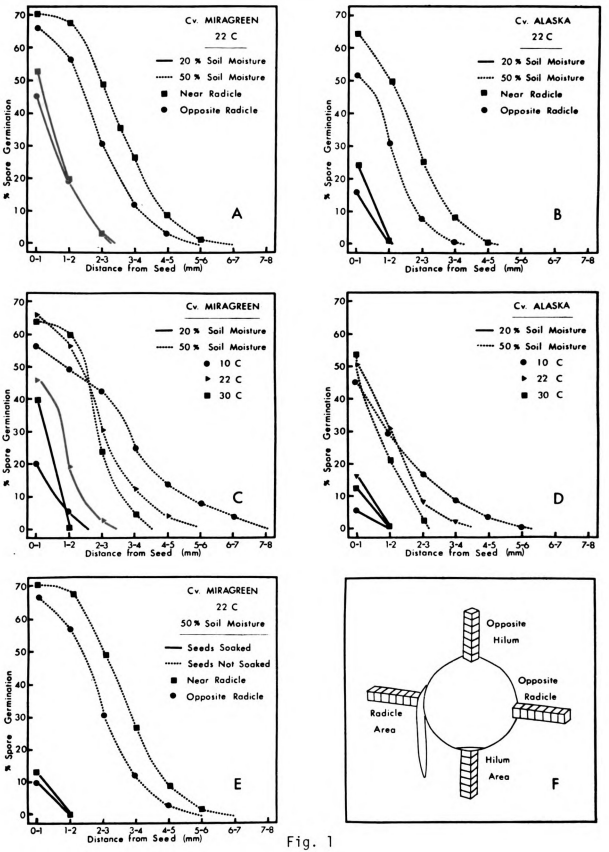
Cv. MIRAGREEN
22 C
20 % Soil Moisture
50 % Soil Moisture
Near Radicle
Opposite Radicle
% Spore Germination
Distance from Seed (mm)
A
Cv. ALASKA
22 C
20 % Soil Moisture
50 % Soil Moisture
Near Radicle
Opposite Radicle
% Spore Germination
Distance from Seed (mm)
B
Cv. MIRAGREEN
20 % Soil Moisture
50 % Soil Moisture
10 C
22 C
30 C
% Spore Germination
Distance from Seed (mm)
C
Cv. ALASKA
20 % Soil Moisture
50 % Soil Moisture
10 C
22 C
30 C
% Spore Germination
Distance from Seed (mm)
D
Cv. MIRAGREEN
22 C
50 % Soil Moisture
Seeds Soaked
Seeds Not Soaked
Near Radicle
Opposite Radicle
% Spore Germination
Distance from Seed (mm)
E
Opposite Hilum
Opposite Radicle
Radicle Area
Hilum Area
F

Fig. 1

TABLE 1. Apparent germination of *Fusarium solani* f. sp. *pisi* chlamydospores at 22 C in the spermospheres of two pea cultivars at various times after planting in soil at 20% and 50% moisture

Cultivar	Soil moisture (%)	Time after planting (h)	Chlamydospore germination (%) at incremental distances from radicle (mm)						
			0-1	1-2	2-3	3-4	4-5	5-6	6-7
Miragreen	50	24	61	53	38	23	13	2	0
		42	70	68	49	27	9	2	0
		48	50	34	23	8	3	0	
		72	...[a]	...	...	...	...	...	...
Alaska	50	24	45	33	21	12	0		
		42	65	50	25	8	1	0	
		48	61	38	13	2	0		
		72	45	13	1	0			
Miragreen	20	24	50	31	4	0			
		48	66	26	2	0			
		72	45	13	1	0			
Alaska	20	24	22	8	0				
		48	32	4	0				
		72	22	1	0				

[a]No data due to extensive germ tube lysis.

soil is when the last germ tube has appeared. Unfortunately, by that time some germ tubes have lysed, and at 22 C and 50% soil moisture extensive mycelial growth also had occurred and made counting of germinated spores difficult. Both chlamydospore germination and germ tube lysis were less at the lower soil moisture (Table 1), in agreement with Cook and Flentje (5). It was possible, however, to accurately ascertain the greatest distance from seeds at which spores germinated. Lysis, mycelial growth and delayed spore germination were most pronounced within 2 mm of the seed. Thus, the values in Fig. 1 probably underestimate the final extent of chlamydospore germination close to the seed, but become increasingly more accurate toward the periphery of the spermosphere.

Severity of pre-emergence damping-off in peas has been directly correlated with amount of carbohydrate exudation during seed germination (7, 12). Wrinkle-seeded cultivars exuded more sugars than the less-susceptible smooth-seeded cultivars (7). Pre-emergence damping-off and carbohydrate exudation were greater in wet than dry soils (6, 7, 8, 9). The data (Fig. 1-A, B) suggest that at constant temp, spermosphere size is directly related to carbohydrate exudation. The considerably larger spermosphere at 50% soil moisture is likely due to (i) an increase in exudation, and (ii) a facilitated diffusion of these sugars through soil water (23).

The greater spermosphere effect near the radicle than in other areas of the seed (Fig. 1-A, B) is also likely due to greater amounts of nutrient exudation. The micropyle is a major portal through which seeds imbibe water (11) and simultaneously exude sugars (18, 20). Exudation from soybean (4), bean (16), and broad bean (15) was greatest in the micropylar zone, where the radicle penetrated the seed coat.

Schroth et al. (18) reported a 50% increase in carbohydrate exudation from seeds of the pea cultivar Alaska as temp was increased from 15-30 C. Bacterial competition for these nutrients would also increase with temp in the range of 10-30 C (2). Thus, at 10 C, the slow rate of bacterial consumption of these exudates may have enabled their diffusion further from the seed than at warmer temp. Hence, spermosphere size appeared to be inversely related to temp at the 50% soil moisture level (Fig. 1-C, D). However, at 20% soil moisture, the spermosphere was larger at 22 C than at 10 C, conceivably due to an insufficient increase in microbial activity to consume the greater amount of exudates as quickly. But when temp was further increased to 30 C, the smaller spermosphere effect was likely due to increased microbial competition. Any direct effect of temp on spore germination seems unlikely, since chlamydospore germination on PDA was greater than 90% at 10, 22, or 30 C.

Size of the spermosphere in which pathogenic spores germinate may be directly related to disease severity. Pre-emergence rotting of peas is most severe in cool wet weather (3, 8), conditions which resulted in a large spermosphere (Fig. 1-C, D). The Miragreen

Fig. 1-(A to F). Germination of *Fusarium solani* f. sp. *pisi* chlamydospores in the spermospheres of pea cultivars 'Miragreen' and 'Alaska.' **A & B)** Effect of soil moisture in two regions of the spermosphere. **C & D)** Effect of temp and soil moisture. Region of sampling was opposite the radicle. **E)** Effect of soaking seeds for 48 h prior to planting. **F)** Regions of the spermosphere sampled.

cultivar had a larger spermosphere than the less susceptible Alaska cultivar (Fig. 1-C, D). Flentje and Saksena (7) obtained a 5-fold increase in emergence by soaking wrinkle-seeded peas for 20 h prior to planting in soil infested with *Pythium*.

Since little exudation occurred after imbibition of water was complete (approximately 8 h) (20), fully swollen seeds when planted should exude considerably less nutrients than unsoaked seeds. Soaking pea seeds for 48 h prior to planting drastically reduced the volume and intensity of the spermosphere effect (Fig. 1-E), and presumably inoculum potential. Knowledge of the effect of soil moisture, temp, type of cultivar, cultural practices, and other factors in altering spermosphere dimensions may be useful in controlling pre-emergence rotting of peas and other agricultural crops.

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PART II

CARBOHYDRATE EXUDATION FROM WRINKLED AND SMOOTH PEA SEEDS IN RELATION TO TEMPERATURE, AGE, AND SEED COLOR

INTRODUCTION

Nutrients exuded during seed germination diffuse into surrounding soil where they stimulate an increase in microbial activity known as the spermosphere effect (22,25). Recently, Fusarium solani chlamydospore germination was used as an index for determining the magnitude of the spermosphere effect in soil around bean (24) and pea (22) seeds. Unfortunately, it has not been possible to directly measure the magnitude of seed exudation in natural soil, since sugars and amino acids are quickly utilized by the indigenous soil microflora (1,2,10,12). However, exudation has been measured in vitro from seeds germinating submerged in water (11,16,18), on moist filter paper (19,20,23) and cheese cloth (9), and in sterile moist sand (20,21). The purpose of this investigation was to refine in vitro techniques of collecting seed exudates so that the relationship between seed exudation and the spermosphere effect could be determined.

Pea (Pisum sativum L. "Alaska" and "Miragreen") seeds seemed most appropriate for such a study for the following reasons: (i) the magnitude of the spermosphere effect using F. solani f.sp. pisi as an index has been determined for these cultivars (22); (ii) the dramatic decrease in the spermosphere effect produced by soaking Miragreen pea seeds prior to planting (22) merited a definitive

explanation; (iii) the effect of temperature on carbohydrate exudation from wrinkled-seeded pea cultivars has not been examined, and was inclusive with smooth-seeded Alaska peas (21); (iv) pea seed exudation is of sufficient magnitude to enable measuring carbohydrate exudation from individual seeds (10,18); and (v) the effect of maturation on seed exudation (23), particularly when accompanied by a loss of color (14), has not been investigated under sterile conditions.

MATERIALS AND METHODS

Source and Treatment of Seeds

Wrinkled-seeded (Miragreen) and smooth-seeded (Alaska) pea cultivars were purchased from Ferry-Morse Seed Co., Mountain View, Calif.; all seed was less than a year old when used, except in one experiment where 8-year-old seed was also planted. Seeds were selected for use on the basis of weight [220-240 mg (fresh weight)/ seed] and freedom from spotted or cracked seed coats. Miragreen seeds were separated into lots of yellow, yellow-green, and green seeds. All seeds were surface-sterilized for 30 minutes in 0.5% sodium hypochlorite containing 1 ml of Tween 20 (polyoxyethylene sorbitan monolaurate) per liter, followed by a 5 minute rinse in sterile distilled water. Seeds with defective seed coats began to swell during this pregerminative treatment, and were discarded. Swelling is indicative of imbibition of water, and thus was used as a visual criterion for determining the apparent amount of time required to complete imbibition in moist glass beads or in sterile distilled water.

Collection of Exudates

Two sterile leaching systems were used to collect exudates during the first 2-4 days of seed germination at 10, 22, and 30 C.

One system consisted of a separatory funnel connected with Tygon tubing to a Pyrex glass cylinder (25-mm-diameter X 90-mm-long)

with a rubber stopper at each end, containing 20 gm of 1-mm-diameter glass beads, and equipped with an air vent plugged with cotton, and a 7-mm-diameter drainage outlet (Fig. 1-A). Following sterilization of this apparatus, sterile distilled water was poured aseptically into the separatory funnel, a single surface-sterilized pea seed was placed aseptically within the glass bead matrix, and the glass cylinder was covered with aluminum foil to exclude light. Water was then percolated through the glass beads at a rate of 10 ml/hour using a peristaltic pump. A fraction collector was utilized for collecting the leaching water at hourly intervals in test tubes containing 95% ethanol to prevent possible utilization of exudates by contaminating microorganisms in the collection tubes.

The other leaching apparatus (Fig. 1-B) consisted of a modified petri dish (100-mm-diameter X 80-mm-deep) containing 300 gm of 1-mm-diameter glass beads, which was connected to a separatory funnel (containing sterile distilled water) above and a collection flask below. Surface-sterilized pea seeds were individually soaked for 8 hours in 5 ml of sterile distilled water, and grouped according to the apparent amount of time required to complete imbibition: 5, 6, or 8 hours. Seeds which had completed imbibition after 8 hours were placed in the leaching system for exudate collection. Seeds which had not completed imbibition after 8 hours were left in water until swelling was visually complete (about 12 hours).

In one experiment, surface-sterilized seeds were individually soaked in 5 ml of sterile distilled water for 24 or 48 hours,

and were grouped according to imbibition rate. Ten seeds of a comparable imbibition rate were aseptically placed within the glass bead bed in each petri dish (Fig. 1-B). Exudates were collected at the time of planting and 24 hours after planting.

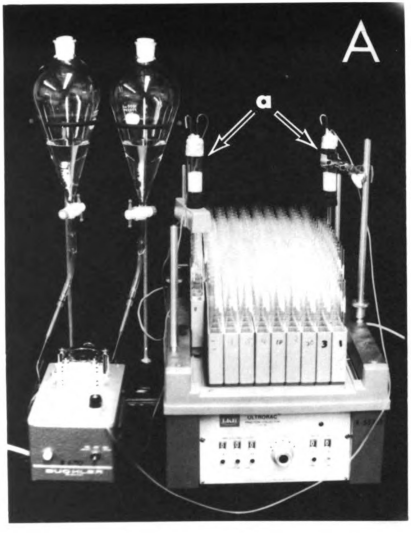
Analysis of Exudates

Leachings from both systems were tested for sterility on potato-dextrose agar and contaminated experiments were discarded. Cellular debris was removed from the leachings by filtration through a Millipore filter with a 0.22 μm pore diameter. Leachings from petri dish systems containing 10 seeds were condensed to dryness at 40 C with a Rotavapor high vacuum evaporator, and redissolved in 10-25 ml of distilled water. Leachings collected hourly from individual seeds germinating in glass cylinders were condensed to dryness at 40 C using a manifold to which were attached 40 pasteur pipettes arranged to direct a filtered air stream into test tubes containing leachings (Fig. 2); the exudates were redissolved in 1 ml of distilled water. Total water-soluble carbohydrate was determined by a modified anthrone analysis (17) in which 1 ml of exudate was mixed with 9 ml of anthrone reagent, placed in a boiling water bath for 10 minutes, and cooled to room temperature. Optical density at 600 nm was measured with a Bausch and Lomb Spectronic 20 spectrophotometer, and carbohydrate concentrations were determined using glucose as a standard. Experiments in which exudates were collected hourly from individual seeds were repeated 5 times; experiments with 10 seeds/petri dish were repeated once. Significant

differences between means were determined at the 5% probability level using Ducan's multiple range test (7).

The quantitative determination of carbohydrate exuded every hour from individual pea seeds germinating in sterile glass beads represents a refinement of previous methods (9,11,16,18,19,20,21,23) with the following advantages: (i) variability among seeds could be determined, eliminating the possibility that exudation means might be distorted by a few unusually leaky seeds (18); (ii) the probability of contamination was much less when a single surface-sterilized seed was transferred to a sterile environment than when many seeds were involved, and contaminated or non-viable replicates were easily discarded without nullifying the entire experiment; and (iii) seed exudation has usually been studied when seeds germinated submerged in water, whereas a glass bead environment more nearly represented a natural soil environment.

Fig. 1-(A & B). Sterile leaching systems used to collect carbohydrate exudates from surface-sterilized pea seeds during 4 days of germination in glass bead environments. (A) Using a peristaltic pump, water flowed at 10 ml/hour from the separatory funnel to the glass cylinder (a) where it percolated through the glass beads in which a single seed was germinating; leachings were collected every hour in test tubes on a fraction collector. (B) Exudates from 10 seeds germinating in glass beads in a modified petri dish were collected every 8 hours by twice flooding the glass beads, and allowing the leachings to drain into an Erlenmeyer flask.



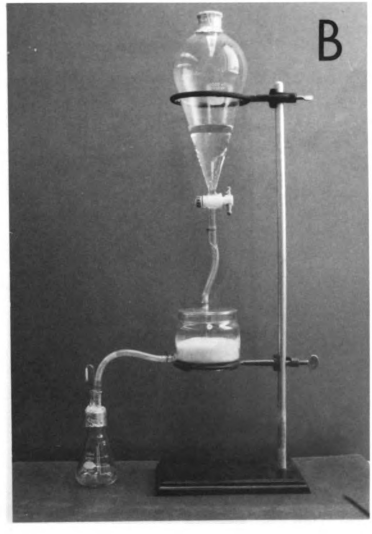


Fig. 1.

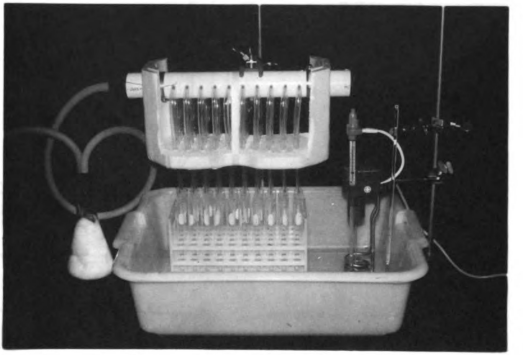


Fig. 2. Apparatus used to condense hourly leachings from individual pea seeds. Leachings were condensed to dryness at 40 C using a manifold with 40 pasteur pipettes to direct a cotton-filtered stream of air into 40 test tubes containing leachings.

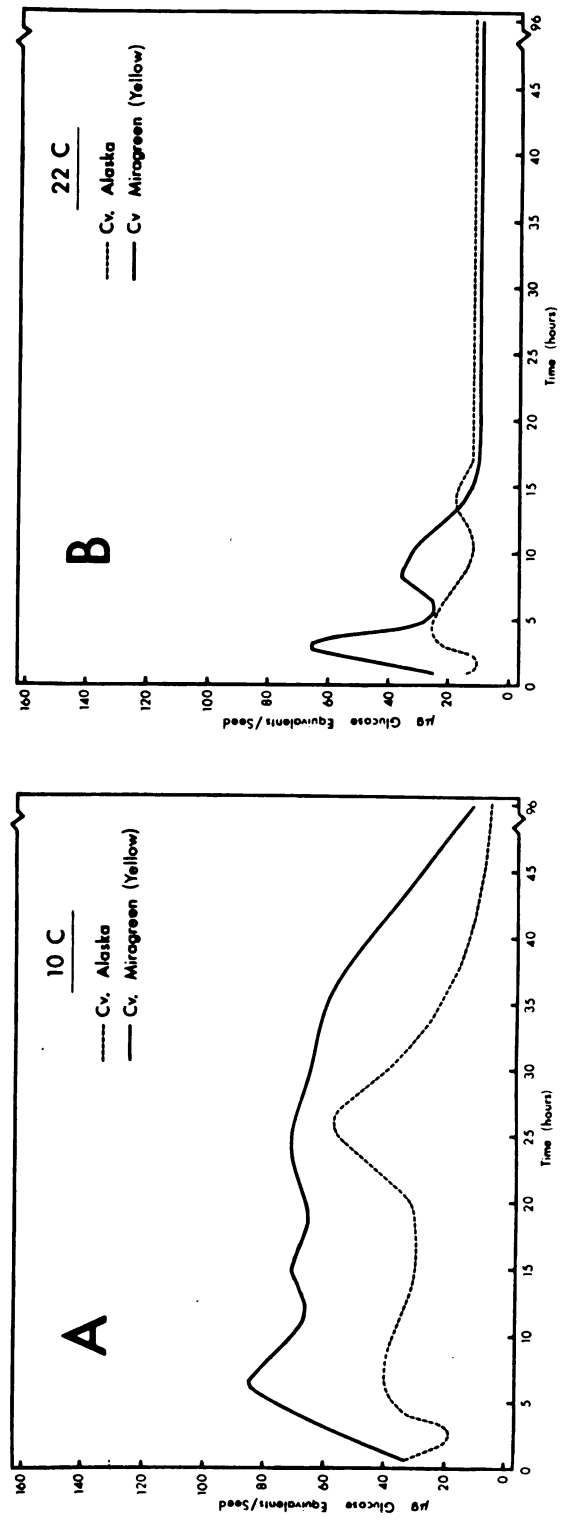


Fig. 3-(A to C). Amount of carbohydrate exuded from Miragreen and Alaska pea seeds at hourly intervals during the first 4 days of seed germination at three temperatures. Exudates were collected from 6 seeds of each cultivar germinating individually in a sterile, moist glass bead environment. (A) 10 C; (B) 22 C; (C) 30 C.

TABLE 1.--Effect of temperature and cultivar on water imbibition and carbohydrate exudation by pea seeds during 4 days of germination in a sterile, moist glass bead environment.

Temperature (°C)	Cultivar	Seed Color	Apparent Imbibition Time[a] (hours)	µg Carbohydrate[b] per Seed		
				Initial 18 hours	Subsequent 78 hours	Total
10	Alaska	Green	15 (11-17)	592 AB[c]	1050 A	1642 A
	Miragreen	Yellow	23 (15-32)	1222 C	2156 B	3378 B
22	Alaska	Green	13 (11-16)	295 A	911 A	1206 A
	Miragreen	Yellow	8 (6-14)	498 AB	755 A	1253 A
30	Alaska	Green	8 (8-10)	515 AB	653 A	1168 A
	Miragreen	Yellow	7 (6-12)	1085 BC	1038 A	2123 AB

[a]Mean and range of time required for seeds (15/treatment) in sterile glass beads to appear completely swollen.

[b]Mean carbohydrate exuded from 6 individual seeds germinating in glass cylinders containing sterile, moist glass beads. Carbohydrate was analyzed by the anthrone method and is expressed as glucose equivalents.

[c]Means in each column followed by the same letter do not differ significantly (P = 0.05) by Duncan's multiple range test.

TABLE 2.--Relation of cultivar, seed color, age, and imbibition rate to carbohydrate exudation. Surface-sterilized pea seeds were individually soaked for 8 hours in sterile distilled water, or until imbibition was completed, and were then placed within the glass beads of a petri dish leaching system. Carbohydrate exuded during the soaking period and during the remainder of 2 days in glass beads was collected three times each day for quantative analysis.

Cultivar	Seed Color	Seed Age (years)	Apparent Imbibition Time[a] (hours)	µg Carbohydrate[b] per Seed[c]		
				Day 1	Day 2	Total
Miragreen	Yellow	8	5	6450	669	7119
Miragreen	Yellow	<1	6	1228	140	1368
Miragreen	Yellow	<1	8	718	84	802
Miragreen	Yellow-green	<1	8	680	25	705
Miragreen	Green	8	6	3162	290	3452
Miragreen	Green	<1	8	296	12	308
Miragreen	Green	<1	12	174	11	185
Alaska	Green	<1	8	434	22	456

[a]Time required for seeds to appear completely swollen while soaking in sterile distilled water.

[b]Glucose equivalents as determined by anthrone analysis.

[c]Mean of 10 seeds/treatment.

TABLE 3.--Seed color and imbibition rate of Miragreen peas in relation to carbohydrate exudation during 24 or 48 hours soaking in sterile distilled water at 22 C, and during a subsequent 24 hours at 22 C in petri dishes containing sterile, moist glass beads.

Seed Color	Apparent Imbibition Time[a] (hours)	µg Carbohydrate[b] per Seed[c]		
		Soak Time		Incubation Time in Glass Beads
		24 Hours	48 Hours	24 Hours
Yellow	6	1230		112
Yellow	6		2680	27
Yellow	8	1340		130
Yellow	8		1450	54
Yellow-green	8	885		60
Yellow-green	8		1070	14
Green	8	340		15
Green	8		425	16
Green	12	200		20
Green	12		245	10

[a]Time required for seeds to appear completely swollen during first 12 hours of soak period.

[b]Glucose equivalents as determined by anthrone analysis.

[c]Mean of 10 seeds/treatment.

RESULTS

Effect of Temperature

Leachings were collected from individual surface-sterilized pea seeds at hourly intervals during the 96 hour incubation period at 10, 22, and 30 C, and each sample was analyzed for carbohydrate (17). The greater part of the carbohydrate was exuded by each cultivar during the first 18 hours of incubation at 22 and 30 C, but significant exudation persisted for about 48 hours at 10 C (Fig. 3-A,B,C).

When amounts of carbohydrate collected during the first 18 hours, the subsequent 78 hours, or the total 96 hours were compared at 10, 22, and 30 C (Table 1), Miragreen peas exuded significantly more carbohydrate when incubated at 10 C than at 22 or 30 C; however, the differences between 10 and 30 C were not statistically significant. Total exudation from Alaska peas at similar periods was not significantly affected by temperature.

Effect of Cultivar

The average amounts of carbohydrate exuded from Miragreen seeds during the first 18 hours of germination were significantly greater than from Alaska seeds at each temperature tested (Fig. 3-A,B,C). The average amounts of carbohydrate exuded from Miragreen seeds between the 18th and 48th hours of collection were

significantly greater than from Alaska seeds at 10 C, but not at 22 or 30 C. Differences in exudation between the two cultivars were not significantly different between the 48th and 96th hour of incubation at any temperature.

Effect of Aging

Eight-year-old Miragreen seeds exuded up to 10 times more carbohydrate during the first 2 days of germination at 22 C than seeds less than one year old (Table 2). Old seeds swelled more quickly than young seeds; for example, young green Miragreen seeds submerged in water always required 8 or more hours to complete swelling compared to 6 hours for 8-year-old seeds (Table 2).

Relation to Imbibition Rate

Seeds of each cultivar required more time to complete swelling at low (10 C) than at high (30 C) temperatures (Table 1). Seeds which imbibed water rapidly at 22 C exuded more carbohydrate than seeds of a comparable age and color which imbibed more slowly (Tables 2 and 3). In addition, seeds submerged in water swelled more rapidly and exuded more carbohydrate than seeds in moist glass beads; this was most apparent with Alasks seeds at 22 C (Tables 1 and 2).

Effect of Soaking Seeds

Soaking Miragreen peas in water at 22 C for 48 hours prior to planting in *Fusarium solani* f. sp. *pisi*- and *Pythium ultimum*-infested soil was effective in reducing incidence of seed and

seedling rot (p. 46). A very small percentage of F. solani f. sp. pisi chlamydospores germinated in soil around seeds which had been soaked prior to planting (22). Seeds which were submerged in water at 22 C for 48 hours exuded very small amounts of carbohydrate during a subsequent 24 hour period in glass beads (Table 3). Apparently, seeds planted after 48 hours of soaking no longer exuded significant amounts of pathogen-stimulating carbohydrate. However, differences in exudation and in incidence of rot (p. 46) were observed in seeds of different color.

Relation to Seed Color

Total carbohydrate exudation during 2 days of germination at 22 C was considerably greater from yellow than from green Miragreen seeds of a comparable age (Table 2), and exudation from yellow seeds subsided more slowly than from green seeds (Tables 2 and3). For example, after 24 hours of submergence in water at 22 C, yellow seeds exuded approximately 6 times more carbohydrate than green seeds during the following day in a glass bead environment (Table 3). Both the amount and the rate of exudation from yellow-green seeds were intermediate between green and yellow seeds (Tables 2 and 3).

DISCUSSION

The simultaneous uptake of water and exudation of cell solutes by germinating pea seeds apparently occurs over the entire surface of the seed coat; however, the micropyle provides an orifice through which these processes occur most efficiently (13,22). Sugars and amino acids are exuded primarily from the symplast of the cotyledons (11,23), though the seed coat and superficial residues may also contribute small amounts of solutes. Two mechanisms of exudation have been proposed. Larson (11) observed that submerged pea seeds imbibed water and exuded solutes more readily following removal of the seed coat; he proposed that rapid imbibition disrupts membrane organization resulting in the leakage of cell contents. Simon and Raja Harun (23) hypothesized that cell membranes lose their integrity as seeds dry at maturity, and thus solutes leak out of cells during imbibition while membrane integrity is being re-established. Electron micrographs of pea cotyledons revealed a fragmentation and gradual breakdown of membranes during maturation (3), and their subsequent re-development during germination (4).

Reports (8,11,23) that pea seed exudation subsides considerably following completion of imbibition were confirmed. Imbibition in moist glass beads was visually complete in 6-16 hours at

22 and 30 C, but required 11-32 hours at 10 C (Table 1). Carbohydrate was exuded in large amounts for approximately 24 hours longer at 10 C than at 22 or 30 C (Fig. 1-A,B,C). Larson's hypothesis would predict membrane damage and subsequent exudation to be less at 10 C than at 22 or 30 C because of a slower rate of imbibition. On the contrary, carbohydrate exudation at 10 C was considerably greater than at 22 or 30 C (Table 1). Moreover, imbibition at 10 C was more rapid for Alaska (15 hours) than for Miragreen (23 hours) peas, yet Alaska peas exuded less carbohydrate than Miragreen peas (Table 1), which is also inconsistent with Larson's hypothesis. Thus, our data support the hypothesis of Simon and Raja Harun (23).

Individual seeds of both cultivars consistently revealed two peak periods of exudation at all three temperatures (Fig. 3-A,B,C), although the second Miragreen peak at 10 C was obscured by the different times (hours 14-37) at which exudation from individual seeds was most intense. Seed metabolism was probably quite low during the first exudation periods since imbibition would have only begun; however, metabolic processes are known to be rapidly increasing during the times coinciding with the second peak exudation periods (14). Thus, the second peak may represent a time of increasing catabolic activity, perhaps essential to restoring membrane integrity. Indeed, exudation subsided more quickly in time (position) and rate (slope) as temperature was increased from 10 to 30 C (Fig. 3-A,B,C) as would be expected if metabolic activity were involved. Since the sugar content of pea seeds increases during the first week of germination (4), the small amounts of carbohydrate exuded during the

third and fourth day of germination (Fig. 3-A,B,C) were likely due to a restoration of membrane integrity rather than to a depletion of carbohydrate reserves.

Miragreen seeds exuded considerably more carbohydrate than Alaska seeds during the first 12-20 hours of seed germination at 10, 22, or 30 C (Fig. 3-A,B,C). Similarly, the magnitude of the spermosphere effect as measured by F. solani f. sp. pisi chlamydospore germination (22) was greater with Miragreen than with Alaska peas, indicating a causal relationship between nutrient exudation and spore germination in the spermosphere. Moreover, seed exudation subsided after 24-48 hours of soaking in water at 22 C (Table 3); consequently, when soaked seeds were planted in soil, the spermosphere effect was much smaller than when seeds were not soaked (22). Thus, the amount of carbohydrate exuded in vitro was directly related to the magnitude of the spermosphere effect in soil at 10, 22, or 30 C.

Temperature did not significantly affect total carbohydrate exudation from smooth-seeded Alaska peas with (Table 1) or without (21) seed coats; however, the pattern of exudation was affected by temperature (Fig. 3-A,B,C). In addition, the quantity and composition of carbohydrate exuded by Alaska peas lacking seed coats was much different than from seeds with intact testae (11). However, wrinkled-seeded peas exuded more electrolytes at 10 C than at 20 C (18). Similarly, the first 18 hours of in vitro carbohydrate exudation from wrinkled-seeded Miragreen peas was greater at 10 and

30 C than at 22 C (Table 1). The inverse relationship between temperature and spermosphere size in soil at 50% moisture (22) was probably due to exudate diffusion away from seeds being impeded more by bacterial utilization at 30 C than at 22 and 10 C (2).

Maturation of pea seeds during moist, hot, sunny weather causes many green peas to turn yellow (14) due to chlorophyll loss (5), a process known as bleaching or "blonding" (6,14,26). Green and yellow peas did not differ in quantity or composition of carbohydrates (14); however, leakage of sugars, amino acids, and carboxylic acids was much greater from yellow than from green seeds germinating in non-sterile water (14) or in sterile glass bead environments (Tables 2 and 3). Further, exudation from yellow and green seeds increased with maturation (23) and age (Table 2) (14). Loss of moisture as seeds mature (15) occurs concurrently with loss in membrane structure (3). Stressful maturation conditions, such as those in which "blonding" of peas occurs, may alter the normally slow organized breakdown of membrane systems (3), resulting in excessive exudation during seed germination (15).

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PART III

FACTORS AFFECTING PEA SEED AND SEEDLING ROT IN RELATION TO SEED EXUDATION AND THE SPERMOSPHERE EFFECT

INTRODUCTION

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 nutrients exuded from the seed. Improved techniques of delimiting the spermosphere of beans (32) and peas (28) have made it possible to study effects of environmental factors on the spermosphere. The magnitude of the spermosphere effect, as measured by Fusarium solani chlamydospore germination, was directly related to soil moisture (28,32), inversely related to soil temperature at 50% soil moisture, greater for a wrinkled-seeded than a smooth-seeded pea cultivar, and greatly reduced by soaking pea seeds prior to planting (28).

The cotyledons, radicle, hypocotyl, and epicotyl base of pea (Pisum sativum L.) seedlings are located within the spermosphere (28) and are susceptible to infection by Fusarium solani (Mart.) Appel & Wr. emend Snyd. & Hans, f. sp. pisi and Pythium ultimum Trow (2,4,5). Infection by either pathogen causes tissue decay and even death of the seedling (5,11,17,19). F. solani f. sp. pisi and P. ultimum are widespread in occurrence in pea-growing regions (4,5,9,11), and are most destructive when both are present (4,17). Pythium and Fusarium populations were much greater in spermosphere than in non-spermosphere soil (30,36), particularly

when seeds with rapid imbibition rates such as peas were planted (30). The purpose of this study was to determine the relationship between the magnitude of the spermosphere effect and incidence of pea seed and seedling rot. A preliminary report has been published (29).

MATERIALS AND METHODS

Selection and Treatment of Seeds

Wrinkled-seeded (Miragreen) and smooth-seeded (Alaska) pea cultivars obtained from Ferry-Morse Seed Co., Mountain View, Calif., were used. Seeds with cracked testae or off-color spots were discarded. Miragreen seeds varied in color, and were separated into lots of yellow, yellow-green, and green.

Seed treatments included coating seeds with a slurry of thiram [tetramethylthiuram disulfide, 2 oz (active ingredient)/ 100 lb seed], or surface-disinfecting seeds 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 non-aerated water at 10, 15, 22, or 30 C. Some seeds were planted with no treatment.

Source, Preparation, and Infestation of Soil

A sandy loam soil from the Michigan State University farm was used for field trials and 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 9-mesh (2 mm opening) 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 chlamydospores, prepared as previously described (28), was added with an atomizer until the infestation level reached 4.0 X 10^5 spores/gm dry weight of soil. In one experiment, approximately 6.0 X 10^5 F. solani f. sp. pisi macroconidia/gm dry weight of soil were also added. In field trials, a suspension of F. solani f. sp. pisi chlamydospores was sprayed onto the soil surface and incorporated into the upper 3-4 inches prior to planting in 1973.

Control of Soil Moisture and Temperature

Sieved soil at 20% moisture (oven-dry weight basis) was compacted uniformly into 50 X 107 cm, screen-bottomed metal soil boxes sitting in an empty water tank in a growth chamber. Pea seeds (4 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 moved upward by capillary action, quickly establishing and maintaining soil moisture in the upper 2.5 cm of soil at 20 and 37 ± 2% using soil depths of 30 and 10 cm, respectively.

Seeds were incubated in a growth chamber 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 degree every 2 days, so that final temperatures alternated between 15 and 30 C. The purpose of the alternating temperature cycle was to simulate diurnal, gradually increasing temperatures common in spring when peas are planted, to maximize the spermosphere effect (28), and to provide optimum conditions for disease development (11,19,22). A 12-hour photoperiod provided by incandescent and fluorescent lights was alternated with a 12-hour dark period; when alternating temperatures were used, the higher temperature was synchronized with the light period. Seedlings were 5-10 cm in height at the end of the incubation periods.

Peas were planted at 3 sites on the Michigan State University farm on April 18, 1973 and 1974. Seed treatments were replicated 4 times at each site with 100 seeds/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. Soil cores (3 cm in depth) were collected twice weekly throughout the season for determining soil moisture content. Soil moisture at site 2 was consistently 4-5% less than at sites 1 and 3 due to higher elevation. Site 1 was not artifically infested with F. solani f. sp. pisi, thus serving as a control. 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 statistically analyzed using a split plot or a split-split plot analysis of variance. The least significant range ($LSR_{.05}$) between means was determined using Tukey's test (33).

RESULTS

Effect of Soil Moisture

Seed and seedling rot in growth chamber experiments were always greater at 37% than at 20% soil moisture (Fig. 1-A,B,C). Incidence of seed rot in field plots was greater in the wetter soil (site 3) than in the drier soil (site 2) (Fig. 1-D,E).

Effect of Cultivar

Incidence of seed and seedling rot were greater for Miragreen than for Alaska peas at 20% or 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 planted in soil at either 20% or 37% moisture (Fig. 1-C).

Effect of Temperature

Seed and seedling rot in soil infested with 4.0×10^5 F. solani f. sp. pisi chlamydospores/gm 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 4.0×10^5 chlamydospores and 6.0×10^5 macroconidia/gm dry weight of soil,

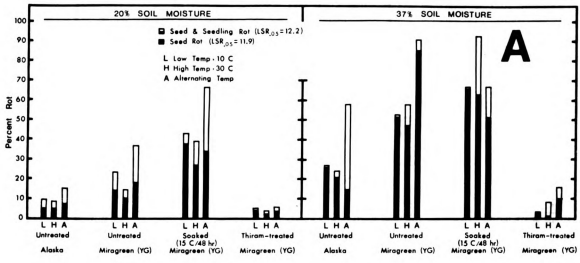
incidence of seed and seedling rot in most treatments was greater at 30 C than at 10 C (Fig. 1-B).

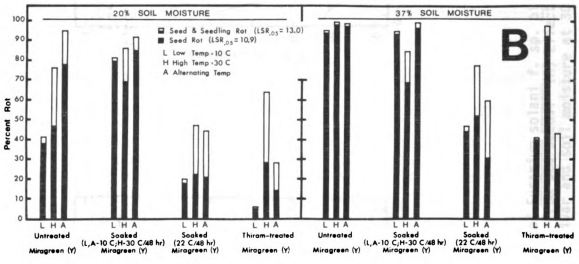
Effect of Soaking Seeds

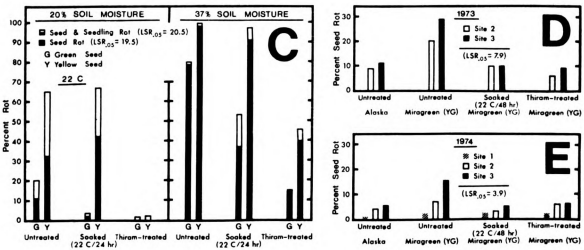
Incidence of seed and seedling rot among seeds soaked in aerated deionized water at 10, 15, or 30 C for 48 hours prior to planting was greater in most treatments than among untreated seeds (Fig. 1-A,B); however, incidence of rot among seeds soaked similarly at 22 C was always much less than among untreated seeds (Fig. 1-B,D,E). For example, soaking seeds for 48 hours at 22 C was as effective at minimizing seed rot in the field as treating seeds with thiram (Fig. 1-D,E).

Miragreen seeds were also soaked in non-aerated tap water at 22 C for 24 hours before planting in F. solani f. sp. pisi- and P. ultimum-infested soil. 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 levels (Fig. 1-C). However, incidence of seed and seedling rot among soaked green seeds was lower than that of untreated seeds and was comparable to that of thiram-treated green seeds at 20% soil moisture, but not at 37% soil moisture.

Fig. 1. Effect of soil moisture, temperature, cultivar, and seed treatment on incidence of pea seed and seedling rot in soil naturally infested with Pythium ultimum and artificially infested with Fusarium solani f. sp. pisi. Some seeds were planted with no treatment; others were coated with a slurry of thiram [2 oz thiram (active ingredient)/100 lb seed], or were soaked in water at 10, 15, 22, or 30 C for 24 or 48 hours prior to planting. (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 degree every other day until the final range was from 15 to 30 C. (A) Alaska and yellow-green (YG) Miragreen seeds were planted in soil infested with 4.0×10^5 F. solani f. sp. pisi chlamydospores/gm. (B) Yellow (Y) Miragreen seeds were planted in soil infested with an F. solani f. sp. pisi inoculum of 4.0×10^5 chlamydospores and 6.0×10^5 macrooconidia/gm. (C) Yellow (Y) and green (G) Miragreen seeds were planted in soil at 22 C. (D & E). Field trials. Alaska and yellow-green Miragreen seeds were planted in mid-April 1973 and 1974. Soil moisture levels at sites 1 and 3 were consistently greater than at site 2. Site 3 was not infested with F. solani f. sp. pisi.


20% SOIL MOISTURE
37% SOIL MOISTURE
Seed & Seedling Rot (LSR.05 = 12.2)
Seed Rot (LSR.05 = 11.9)
L Low Temp - 10 C
H High Temp - 30 C
A Alternating Temp
A
Percent Rot
L H A
Untreated
Alaska
Untreated
Miragreen (YG)
Soaked (15 C/48 hr)
Miragreen (YG)
Thiram-treated
Miragreen (YG)


20% SOIL MOISTURE
37% SOIL MOISTURE
Seed & Seedling Rot (LSR.05 = 13.0)
Seed Rot (LSR.05 = 10.9)
L Low Temp - 10 C
H High Temp - 30 C
A Alternating Temp
B
Percent Rot
Untreated
Miragreen (Y)
Soaked (L,A-10 C,H-30 C/48 hr)
Miragreen (Y)
Soaked (22 C/48 hr)
Miragreen (Y)
Thiram-treated
Miragreen (Y)


20% SOIL MOISTURE
37% SOIL MOISTURE
Seed & Seedling Rot (LSR.05 = 20.5)
Seed Rot (LSR.05 = 19.5)
G Green Seed
Y Yellow Seed
22 C
C
Percent Rot
G Y
Untreated
Soaked (22 C/24 hr)
Thiram-treated
1973
Site 2
Site 3
(LSR.05 = 7.9)
D
Percent Seed Rot
Untreated
Alaska
Untreated
Miragreen (YG)
Soaked (22 C/48 hr)
Miragreen (YG)
Thiram-treated
Miragreen (YG)
1974
Site 1
Site 2
Site 3
(LSR.05 = 3.9)
E

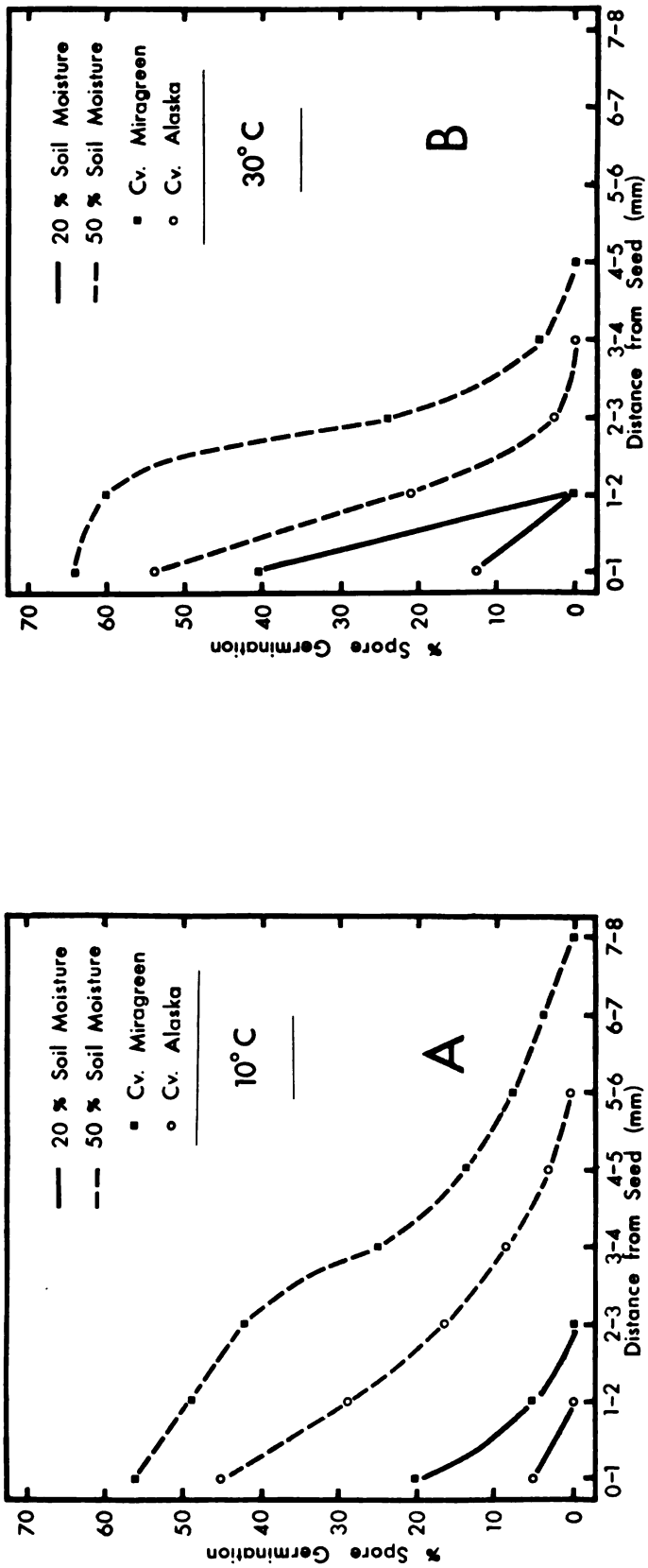


Fig. 2-(A & B). Germination of Fusarium solani f. sp. pisi chlamydospores in the spermasphere at incremental distances from pea seeds. (A) Effect of cultivar and soil moisture at 10 C. (B) Effect of cultivar and soil moisture at 30 C.

DISCUSSION

Pea seed cotyledons germinate beneath the soil surface and remain confined to the spermosphere soil throughout seedling development. This is disadvantageous to the seedling if seed-rotting organisms within the spermosphere have been 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 1/2 hours after receiving the seed exudate stimulus (32), and the rapid growth rate of the mycelium at 12-30 C(19) produces a prolific growth of Pythium mycelium in the spermosphere responsible for the characteristic "balling" of soil around seeds (6,10). "Balled" seeds may rot prior to emergence (5), produce a seedling which rots following emergence (4), or result in a weakened seedling (22).

Pea (18) and bean (26) seeds exude nutrients, the greatest proportion of which are simple sugars such as glucose, mannose, sucrose, and fructose. Such sugars are capable of stimulating spore germination and germ tube growth of seed-rotting fungi (6, 26). Incidence of rot has been directly correlated with the quantity of carbohydrate exuded by soybeans (13), beans (25), and peas (21). However, amount of nutrient exuded in vitro may not

always be indicative of the extent of pathogen activity in the spermosphere. For example, Miragreen seeds exuded significantly more carbohydrate at 10 C than at 22 C (p. 23), but F. solani f. sp. pisi chlamydospore germination in soil at 20% moisture was considerably greater at 22 C than at 10 C (28). It is also conceivable that seeds, like roots (3), could exude inhibitors of spore germination and mycelial growth which could affect the activity of the pathogen in the spermosphere.

Leach (19) tried to predict the fate of seeds, including peas, planted in Pythium-infested soil by calculating the ratio of the rate of emergence of the host in pasteurized soil to the rate of growth of the fungus in potato-dextrose broth at temperature increments from 4-35 C. Ratios below unity, indicating that the growth rate of the pathogen exceeded that of the host, were associated with severe pre-emergence infection; as the ratio increased beyond unity, the disease declined accordingly. However, these ratios have not always proven to be useful in predicting damping-off (7,8), perhaps because the effect of soil moisture on the host and pathogen were not considered (19). For example, raising the soil moisture level increased pea seed exudation, producing a higher incidence of pre-emergence rot (14).

A more direct approach to predicting pre-emergence rotting of seeds is possible due to recent progress in measuring the spermosphere effect (28,32). 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 (28), increased with various pea cultivar-soil moisture combinations in the following order (Fig. 2-A,B): Cv. Alaska, 20% soil moisture; Cv. Miragreen, 20% soil moisture; Cv. Alaska, 50% soil moisture; Cv. 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, indicating a direct relationship between incidence of rot and the magnitude of the spermosphere effect at any particular temperature.

A simple relationship between incidence of rot and the spermosphere effect was neither expected nor found when temperature was included as a variable, because temperature affects not only amount of seed exudation (p. 23), but also bacterial competition for exudates (1), growth rates of host and pathogen (19), and rate of disease development (11,19). 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) (11,19,22). 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 mainly due to F. solani f. sp. pisi. The greater incidence of rot under alternating temperatures than at 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 (4,17). When the F. solani f. sp. pisi inoculum was increased 2.5 fold (Fig. 1-B), incidence of rot was much less at 10 C than at 30 C or when temperatures ranged from 10-30 C.

Generally, environmental conditions which adversely affect seedling growth are most conducive to pre-and post-emergence damping-off (19). 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 (2,10,22,28,32). Peas are a cool temperature crop which must be planted early in spring in temperate regions to produce maximum yields (22). Fungicide seed treatments have been widely used to minimize pre-emergence rotting in peas, though they have not always adequately controlled the disease (10,12,20). Since Pythium and Fusarium populations are much higher in spermosphere than in non-spermosphere soil (30,36), minimizing the spermosphere effect might suppress an increase of these pathogens, as well as disease incidence.

Seed germination and seedling emergence were impaired when seeds were soaked in water at high (30 C) or low (1-15 C) temperatures (16,23,27), or for more than 48 hours (15,16). Thus, the high incidence of rot among Miragreen seeds soaked for 48 hours at 10, 15, or 30 C (Fig. 1-A,B) was probably due to temperature injury. However, the spermosphere effect (28) 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. Thus, soaking pea seeds under optimum conditions may be a useful cultural practice by which gardeners can minimize pea seed and seedling rot.

The green color of pea and lima bean seeds occasionally fades as seeds mature, a process known as bleaching (24,35,37). 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 than green seeds (p. 24). As a result, the spermosphere effect would have been greater with yellow than with green seeds. The spermosphere effect around yellow Miragreen seeds soaked in water at 22 C for 24 hours was only slightly less than around unsoaked seeds (unpublished data), indicating that considerable exudation occurred even after 24 hours of soaking; consequently, incidence of rot among yellow seeds was not reduced by 24 hours of soaking; (Fig. 1-C). However, soaking green seeds for 24 hours before planting was effective in reducing incidence of rot, apparently because carbohydrate exudation from green seeds had subsided to very low levels prior to planting (p. 25). Lima bean seed rot and seedling vigor have also been reported (24,37) to be considerably greater with bleached than with non-bleached seeds, though 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.

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CONCLUSIONS

CONCLUSIONS

Success and efficiency in minimizing plant disease losses are dependent on our understanding of the mechanisms by which biotic and abiotic factors interact to prevent pathogens from locating, penetrating, and invading host tissues. Plant disease control measures are often directed at inhibition of the pathogen after it is in contact with the host. However, an equally important goal is to prevent potential pathogens from even encountering susceptible hosts. Above-ground plant structures are difficult to maintain free of pathogens due to wind, rain, and insect dispersal of pathogen propagules. However, contact between host and pathogen in soil is largely dependent on growth on the host, or the pathogen, or both. Fortunately, most fungal spores do not germinate and grow through soil without an external source of nutrients. Germinating seeds exude sugars, amino acids, and probably other substances which diffuse into surrounding soil and stimulate spore germination and vegetative growth of fungi, including seed pathogens. The increase in microbial activity in soil around germinating seeds has been called the spermosphere effect.

Pea seeds exuded sufficient quantities of pathogen-stimulating carbohydrates to enable refinement of previous methods of measuring *in vitro* carbohydrate exudation and *Fusarium solani*

chlamydospore germination in the spermosphere. In addition, seed and seedling decay are frequently very severe for crops such as peas which exude large amounts of carbohydrates during germination, and then remain within the spermosphere soil where pathogen activity has been stimulated. Thus, garden peas and Fusarium solani f. sp. pisi were a particularly appropriate host and pathogen, respectively, for studying the relationship between carbohydrate exudation, the spermosphere effect, and incidence of seed and seedling rot.

The total amount of carbohydrate exudation and the magnitude of the spermosphere effect were directly related to the incidence of pea seed and seedling rot at 10 C, 30 C, or when temperatures were alternated. More specifically, carbohydrate exudation, the spermosphere effect, and incidence of rot were greater for wrinkled-seeded Miragreen than for smooth-seeded Alaska peas, greater at high than at low moisture conditions, and much less for seeds soaked at 22 C for 24-48 hours than for seeds not soaked prior to planting. Carbohydrate exudation and incidence of seed and seedling rot were greater with yellow than with green seeds; and exudation was greater from old than from young seeds. Temperature affected not only carbohydrate exudation, but probably microbial competition for exudates, growth rates of host and pathogen, and rate of disease development as well. However, when temperatures were alternated to maximize carbohydrate exudation, the spermosphere effect, and rate of disease development, incidence of seed and seedling rot were greatest.

Research efforts and recommendations to growers for minimizing pea seed and seedling rot should be based on three principles: (i) minimizing carbohydrate exudation, (ii) minimizing the spermosphere effect, and (iii) optimizing conditions for seed germination and seedling growth. Peas are a cool temperature crop which must be planted early in spring in temperate regions to ensure high yields; thus, temperature can be "controlled" only to the extent to which peas can be planted in areas where temperatures are conducive both to high yields and to minimum rot. Similarly, rainfall is usually beyond human control and not entirely predictable; however, pea seed and seedling rot can be minimized by planting seeds in well-drained fields, and at times other than immediately preceding an extended rainy period. Unfortunately, the latter variable is rarely under the growers' control.

For centuries man has carefully selected and treated seeds to improve emergence and yields. For example, smooth-seeded pea cultivars are generally less susceptible to seed rot than wrinkled-seeded cultivars. But, planting smooth-seeded rather than wrinkled-seeded peas is no real solution to pea seed rot problems because wrinkled-seeded peas generally yield more than smooth-seeded peas, and are much sweeter than smooth-seeded peas. Indeed, it is probably this very sought-after sweetness which is the cause of the greater spermosphere effect and hence the greater rot problem. Thus, growers with large acreages of peas have relied primarily on fungicide seed treatments for minimizing seed rot. However,

soaking peas prior to planting has been a practice of some home gardeners for many years, perhaps because soaked seeds rotted less frequently and plumules emerged more quickly than when seeds were untreated. Planting soaked seeds may also delay an increase in pathogen propagule inoculum in soils repeatedly cropped to peas. Mechanical devices are not currently available for commercially planting soaked and swollen seeds; however, planting soaked seeds would be feasible for those planting peas by hand, and might result in significantly reducing incidence of seed rot.

Recent research has revealed additional seed characteristics which affect incidence of seed and seedling decay. For example, the green color of pea and lima bean seeds occasionally fades as seeds mature, a process known as bleaching. When pea and lima bean seeds were planted in pathogen-infested soil, bleached seeds had a higher incidence of rot than non-bleached seeds. Bleached (yellow) peas exuded more pathogen-stimulating carbohydrate than non-bleached (green) peas; consequently, the spermosphere around bleached peas would likely be larger than around non-bleached peas. The mechanisms of bleaching in peas and lima beans have not been elucidated. Nevertheless, environmental factors rather than genetic factors are probably responsible for differences between bleached and non-bleached peas in susceptibility to seed-rotting organisms.