Factors Affecting Inoculum Development and Seed Transmission of Helminthosporium gramineum

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ABSTRACT

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Seed transmission of *Helminthosporium gramineum* was increased by soil temperatures below 12 C, and reduced or prevented above 15 C, in field plantings of naturally infected seed. The critical stage for infection of the germinating embryo began when the coleoptile reached the apex of the seed and continued until the seedling emerged from the soil. Inoculum located in the pericarp and seedcoat over the embryo was most effective in producing seed-transmitted stripe. A laboratory assay for presence of the fungus in barley seed demonstrated that the percentage of seed transmission was always less than the percentage of infected seed. Developing barley seed was susceptible to infection during any stage of development from before head emergence through the soft dough stage. Infection of developing seed occurred over a temperature range of 10 to 33 C, and free moisture was not required.

Additional key words: floral infection, soil temperature, *Hordeum vulgare,* epidemiology, cereal diseases.

Barley stripe disease, caused by *Helminthosporium gramineum* Rab., is a seed-borne single cycle disease that may occur wherever barley is grown. The incidence of stripe is influenced by soil temperature during germination and early growth (4, 5, 6, 7, 8, 9, 11), but optimum temperatures for disease development have not been clearly defined. Early studies on effects of soil temperature on stripe incidence were inconclusive because low and variable percentages of diseased plants were obtained from naturally infected seed. Leukel et al. (7) reported that disease incidence increased when naturally infected barley seed were germinated in soil below 15 C, and reduced or eliminated above 15 C.

The nature of seed transmission is not completely understood. Barley seed becomes infected during development in the spike, but reports conflict regarding the stages of kernel development that are susceptible (2, 14, 15) and the importance of moisture effects on the infection process (4, 12, 14). Embryos of mature seed are not infected, but the fungus is present in the hull, pericarp, and seedcoat (8, 11, 13, 15). Surface contamination is considered a relatively unimportant source of inoculum (11, 15). Histological studies by Skoropad and Arny (10) reported that the fungus entered the embryo during germination by direct penetration of the coleorhiza. Because the dormant mycelium is located in nonliving tissues, the mature seed could be considered infested rather than infected. In this paper, barley seed harboring the stripe fungus as a consequence of infection during development will be referred to as naturally infected seed, and the subsequent infection of embryos during germination that results in systemic stripe infection will be referred to as seed transmission.

Artificial inoculation methods generally involve the placement of healthy seed in contact with actively growing mycelium (1, 4, 5, 9). These methods nullify the effect of reduced temperature on embryo infection during germination because invasion of the embryo often occurs during inoculation and prior to planting in cool soil. Nevertheless, the effect of soil temperature on incidence of disease can be demonstrated using artifically inoculated seed because the fungus may have topically infected the embryo, but may not have established systemic infection of the seedling at the time of planting. However, low disease incidence, usually associated with warm temperatures, is often obscured. For instance, intact barley seed germinated at 6 to 30 C on agar cultures of *H. gramineum* produced similarly high percentages of diseased plants regardless of soil temperatures or planting date (3). Fifty to 90% infected plants may result from artificially inoculated seed (3, 9), whereas rarely more than 20% stripe occurs in California barley fields planted with naturally infected seed (12).

The understanding of the epidemiology of stripe disease would be improved by more information on the effects of temperature on seed transmission in plants from naturally infected seed. This report is concerned with factors required for infection of developing seed and the effect of temperature on seed transmission in plants grown from naturally infected seed.

METHODS AND RESULTS.—*Detection of Helminthosporium gramineum in seed lots.—Helminthosporium gramineum* was detected in seed lots by a method that induced sporulation of the fungus on barley leaf piece agar (BLPA). To prepare BLPA, fresh barley leaves were pressed and dried, cut into 3- to 4-cm pieces, autoclaved dry for 30 minutes, and placed on solidified 1.5% water agar in Pyrex petri dishes. One seed was placed on each leaf piece and any fungi associated with the seed allowed to grow over the leaf pieces for 4 days at room temperature. The cultures then were exposed to cool-white fluorescent light at an

intensity of .0338 cal cm^{-1} sec^{-2} for 24 hours followed by 24 hours at 15 C in the dark. A sample of 100 seed was tested, and each experimental seed lot was indexed at least twice.

The laboratory assay consistently detected *H. gramineum* in naturally infected seed lots, but the percentage of infected seed indicated by the assay was always greater than the percentage of seed-transmitted stripe that developed in field plantings under conditions favorable for disease development.

Effect of soil temperature on seed transmission.—Infected barley seed was planted at three locations on several different planting dates to determine the effect of soil temperature on seed transmission. Each trial consisted of four to six replications in a randomized complete block design. Each plot was a single 2.4-meter row with 50 to 60 plants. The incidence of stripe was determined at the rosette stage, and again shortly after the heads emerged.

Soil temperatures were recorded continuously at a depth of 4 cm at Davis and Kings County, and at 10 cm at Tulelake, California. Average maximum and minimum soil temperatures were calculated for the first 28 days after planting.

Naturally infected seed lots were developed by inoculating barley cultivars Briggs (C. I. 13682), Grande (C. I. 1758), and Numar (C. I. 13683) grown in the field at Davis. Inoculum was prepared from leaves on which the fungus was sporulating. The leaves were agitated vigorously in water and the spore suspension was strained through two layers of cheesecloth. Soon after heading, cultivar Numar was inoculated once (Numar 1X) and cultivar Briggs on each of 2 days (Briggs 2X) by spraying with the spore suspension. Inoculation was followed by 20 minutes overhead irrigation in late afternoon and evening for 2 days in succession. Cultivar Grande was inoculated by injection of the spore suspension with a hypodermic syringe onto the heads while still in the boot and the heads were covered with glassine bags until harvest. The seed lot designated Grande-2 was grown at Davis, and seed lot Grande-5 was grown at the University of California Field Station at Tulelake. Mature grain of each seed lot was hand-harvested, threshed, and thoroughly mixed.

The percentage of seed-transmitted stripe varied in plantings of the same seed lot, but seed planted in cool soil produced more stripe-infected plants than seed planted in warm soil. Selected data from trials at three locations of seed lot Briggs 2X are presented in Table 1. Seed grown in soil below 12 C produced 3.3 to 40.9% stripe-infected plants, but little or no stripe developed when soil temperatures exceeded 15 C.

Samples from naturally infected seed lots were planted in December, February, March, and May of 1972-73 and October, December, January, February, and March of 1973-74. Incidence of disease decreased as soil temperature increased in 1972-73 plantings (Fig. 1), and similar results were obtained from 1973-74 trials. Only small amounts of stripe developed in plantings of any seed lot when either maximum or minimum soil temperatures were above 15 C, and disease did not occur in plantings sown in May 1973 when average maximum and minimum soil temperatures were 32.4 and 24.2 C, respectively.

Effect of temperature and time of exposure on disease incidence.—The time at low temperature during germination which resulted in high disease incidence was determined by planting Briggs 2X seed in moist soil in pots that were maintained at 6 C in a refrigerated dark chamber. Ten pots with 12 to 14 plants each were moved to a greenhouse bench where temperatures ranged from 19 to 24 C after 4, 7, 14, 21, and 28 days at 6 C. Seedlings kept at 6 C for 21 or 28 days were just emerging from the soil when transferred to the greenhouse, whereas those moved to the greenhouse prior to 21 days emerged from the soil within 2 or 3 days after transfer.

The groups of plants germinated at 6 C for 4 to 7 days produced 4.1 and 4.6% seed transmission (Fig. 2). Each additional 7-day exposure to 6 C resulted in increased percentages of infected plants, with a maximum of 47.3% stripe in the group held at 6 C for 28 days. Laboratory assay of Briggs 2X seed revealed that about one-half of the seed were infected; thus, a stripe incidence of 47.3% was near the expected maximum. The control treatment that received no exposure to 6 C, but was planted and

TABLE 1. Variation in incidence of barley stripe disease in different plantings of one naturally infected seed lot of cultivar Briggs

Location planted in California	Average soil temperature[a] (C)		Plants with stripe (%)
	Maximum	Minimum	
Kings County	11.2	4.5	40.9
Davis	11.3	6.9	20.4
	9.2	5.4	15.4
	9.0	6.4	3.3
	16.5	12.1	1.9
	18.1	12.5	0.8
	32.4	24.2	0
Tulelake	5.2	4.7	11.7

[a]Measured for the first 28 days after planting.

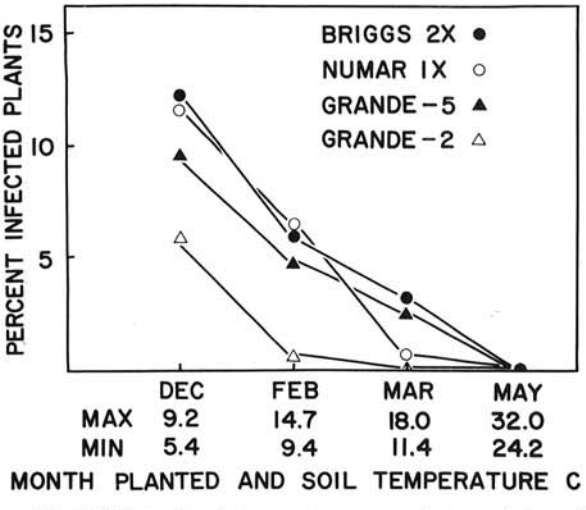

Fig. 1. Effect of soil temperature on seed transmission of barley stripe in the field from four naturally infected seed lots planted at Davis, California, in 1972-73. Average maximum and minimum soil temperature calculated from measurements during the first 28 days after planting.

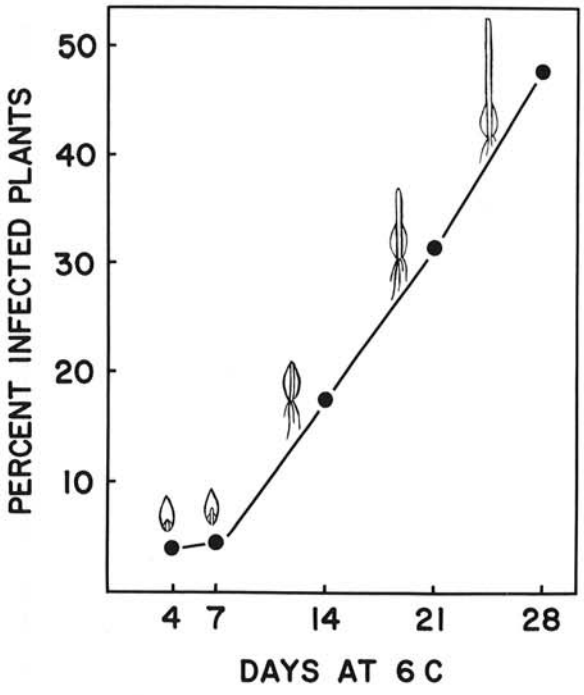

Fig. 2. Incidence of stripe disease in greenhouse plantings from naturally infected barley cultivar Briggs seed germinated at 6 C for 4 to 28 days prior to transplanting to soil in greenhouse at temperatures of 19 to 24 C. Approximately 125 plants per treatment. Diagrams represent stages of seedling growth when removed from 6 C. No significant difference at $P = 0.05$ between 4 and 7 days; significant difference at $P = 0.05$ between 7, 14, 21, and 28 days.

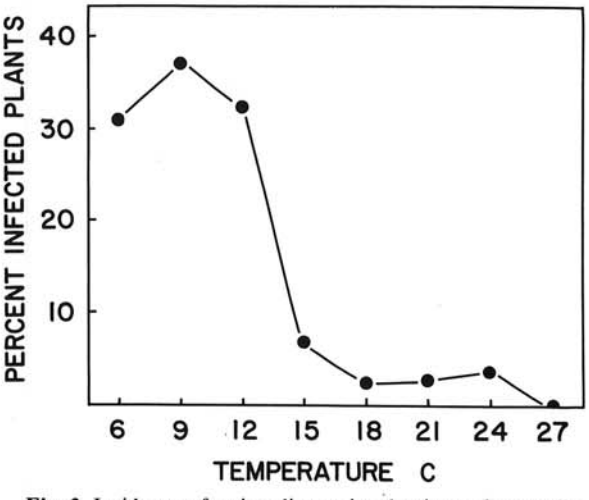

Fig. 3. Incidence of stripe disease in plantings of naturally infected barley cultivar Briggs seed germinated at different temperatures to same stage of growth then grown in the greenhouse at 19 to 24 C. Approximately 125 plants per treatment. Seedlings required 4 to 25 days to reach the same stage of development when germinated at 27 or 6 C, respectively. Combined data for the 6 to 12 C treatments were significantly different at $P = 0.01$ from the combined data of the 15 to 27 C treatments.

grown in the greenhouse for the duration of the experiment, had 1.2% diseased plants.

A high incidence of stripe resulted from slowly germinated seedlings, but not from those which grew rapidly, suggesting that a slow growth rate resulting from low temperatures during early stages of development increased the amount of seed transmission. To determine the critical stage of seedling development during which slow growth and cool temperatures affect seed transmission, the following experiment was conducted.

Briggs 2X seed were germinated in the dark on moist filter paper in petri dishes for 4, 8, 12, 16, 24, 36, 48, 72, and 96 hours at 21 C, a temperature that promoted rapid germination. At the end of each time period, 130 seedlings were transplanted to soil in pots and grown at 6 C for 4 weeks, then moved to the greenhouse. To avoid etiolation, seedlings transplanted after 48, 72, and 96 hours at 21 C were transferred to the greenhouse after 3 weeks at 6 C.

Stripe infection ranged from 27.1 to 42.8% in groups germinated rapidly for 4 to 48 hours at 21 C before transfer to 6 C. Rapid germination at 21 C for longer than 48 hours resulted in a significant decrease in the incidence of stripe with 17.9 and 7.0% stripe in the 72- and 96-hour treatments. The check population planted and grown in the greenhouse for the entire time developed 4.9% stripe in contrast to 31.2% in a check series held at 6 C for 28 days prior to planting in the greenhouse. In the previous experiment, a slow growth rate from planting until the coleoptile was the same length as the seed (14 days at 6 C) followed by an increased growth rate at warm temperatures, resulted in a relatively low stripe incidence of 17.2%. However, this experiment showed that rapid germination at 21 C through the same stage (48 hours at 21 C) did not prevent infection when low temperature prevailed following the first 48 hours of rapid growth. Thus, it appears that the critical stage of seedling development for establishment of disease was from about the time the coleoptile reached the apex of the seed until it emerged from the soil.

The temperature range favoring disease establishment in naturally infected seed was determined by germinating Briggs 2X seed in petri dishes at 3-degree increments from 6 to 27 C. About 130 seedlings from each temperature treatment were transplanted to soil in pots in the greenhouse when the first leaf had just emerged from the coleoptile. Four to 25 days, depending upon the temperature, were required to reach this stage of development. The highest percentages of stripe, 30.8 to 37.5, occurred in groups germinated at 6, 9, and 12 C, whereas only 7.5 to 0% infection developed in groups germinated at 15 through 27 C (Fig. 3). The experiment was repeated with similar results.

Seedling invasion by Helminthosporium gramineum in naturally infected barley seed.—Helminthosporium gramineum is not present in the embryos of naturally infected seed. Therefore, the fungus must grow from other seed tissues into the developing seedling to produce systemic infection. Because the percentage of plants with systemic stripe infection increased with time of exposure to low temperature during germination and rapidly germinated seedlings escaped infection, it was postulated that the pathogen should be detectable in greater numbers of embryos with increased germination time at 6 C, but

TABLE 2. Influence of temperature on invasion by *Helminthosporium gramineum* of barley seedlings from naturally infected seed of cultivar Briggs

Germination time and temperature	Number of seedling parts infected with *H. gramineum*[a]							
	coleorhiza		apex[b]		stem		scutellum	
	exp 1	exp 2	exp 1	exp 2	exp 1	exp 2	exp 1	exp 2
Days at 6 C:								
7	15	6	0	0	0	0	11	0
14	10	10	0	1	1	3	8	5
21	18	11	5	1	13	2	13	1
28	3	19	17	9	10	10	23	18
Hours at 24 C:								
18	1		0		1		3	
48	9		0		0		0	
96	0		0		0		1	

[a]Sixty seed per treatment.
[b]Apex includes apical meristem, embryonic leaves, and small portion of stem tissue.

not in those that germinated rapidly at higher temperatures. To test this hypothesis, the following experiment was conducted.

Briggs 2X seed were germinated at 6 C in petri dishes for 7, 14, 21, and 28 days. Another series was germinated at 24 C for 18, 48, and 96 hours producing seedling growth stages approximating those at 7, 14, and 28 days at 6 C. Invasion of barley seedlings by *H. gramineum* was determined by excision of seedlings into four parts which were cultured on BLPA and incubated to induce sporulation as described for the laboratory assay. The four seedling parts cultured from each seedling were the coleorhiza, stem section (first and second internodes), scutellum, and apex. The apex here refers to the apical meristem and embryonic leaves with some upper stem tissue. Sixty seedlings were excised and tested for each temperature treatment. The sample size was considered reliable because plants produced from 60 Briggs 2X seed (germinated in petri dishes for 28 days at 6 C prior to transplanting to the greenhouse) showed 36.6% seed-transmitted stripe.

Helminthosporium gramineum was present in the coleorhiza and scutellum after 7 days at 6 C, but not in the stem or apex (Table 2). The total number of seedling pieces invaded after 28 days of germination at 6 C was distinctly greater than the number invaded after 7 days. The greatest increase in the number of infected apex and stem sections occurred between 21 and 28 days of germination at 6 C. The pathogen colonized low numbers of coleorhizae and scutella of seedlings germinated at 24 C, and was present in the stem of only one seedling and in none of the apices.

Inoculation of healthy barley seed at different stages of seedling development during germination.—Slow growth of naturally infected barley seedlings at low temperature perhaps favors seed transmission because time is required for the seed-borne fungus to resume growth and reach the infection court. If so, rapidly growing seedlings may become infected when seed are inoculated with mycelium at the optimum location for infection of the embryo. To test this possibility, a small portion of the lemma over the embryos of dry healthy barley seed (cultivar CM67) was cut away and the seed germinated in petri dishes at 24 C for 4, 18, and 48 hours. The embryo of each seed was exposed by removing the pericarp-seedcoat layer over the embryo. Small bits of agar from the margins of 4- to 7-day-old cultures of *H. gramineum* were placed on the coleorhizae of germinating embryos in one series, and on the scutella in another. For each germination time period, 125 seed inoculated on the coleorhiza were placed at 24 C for 3 days, and an additional 125 inoculated seed were grown at 6 C for 21 days prior to being transplanted to soil in the greenhouse. Seed inoculated on the scutellum received the same treatment.

TABLE 3. Effect of removal of lemma or lemma, pericarp, and seedcoat on incidence of stripe in plantings of naturally infected and artificially inoculated healthy barley seed

Treatment[a]	Plants with stripe (%)	
	Inoculated CM67	Naturally infected Briggs
Intact seed	8.6 s[b]	18.4 y
Lemma removed	13.5 s	9.3 z
Lemma, pericarp and seedcoat removed	41.5 t	1.9 z
LSD ($P = 0.05$)	13.93	7.8

[a]All treatments germinated at 6 C for 28 days then transplanted in greenhouse.
[b]Data sharing common letters are not statistically different, $P = 0.05$.

Inoculation of the coleorhiza and scutellum produced 9.0 and 2.5% diseased plants in the 4 and 18 hours at 24 C germination treatments, and no stripe-infected plants developed in the groups germinated for 2 days at 24 C when seedlings were grown at 6 C for 21 days after inoculation. Stripe infection did not occur in any treatment returned to 24 C instead of 6 C before planting in the greenhouse. Thus, the requirement for cool temperatures and slow growth for infection was not overcome by inoculation of exposed embryos on either the coleorhiza or scutellum.

Inoculation of intact barley seed.—A high incidence of stripe was consistently produced when seed were inoculated by artificial methods that placed germinating seed in contact with high inoculum levels. The susceptibility of intact healthy barley seed to a small amount of artificially introduced inoculum was tested by

TABLE 4. Effect of hull removal, surface contamination and surface sterilization on seed transmission of *Helminthosporium gramineum* in several barley cultivars grown in the field

Treatment	Plants with stripe (%)		
	Location		
	Riverside	Davis	Kings County
CM67, intact	0	0	0
CM67, intact, contaminated[a]	0	0.7	3.3
CM67, hulled, contaminated[a]	6.1	15.5	45.8
Briggs 2X[b], intact	15.0	20.4	40.9
Briggs 2X[b], hulled	0.6	14.7	4.3
Grande-5[c], intact	6.7	10.0	17.9
Grande-5[c], hulled	0	1.6	1.7
Surface sterilization			
Briggs 2X[b], untreated	15.0	20.4	40.9
NaOCl soak 5 minutes	5.3	15.5	23.1
4 hours	0	1.0	2.4
10 hours	0	0.9	1.9
Water soak 10 hours	12.4	8.2	27.2
Grande-2[c], untreated	2.5	6.6	2.7
NaOCl soak 5 minutes	4.2	3.9	1.6
4 hours	0	0	0
10 hours	0	0	0
Water soak 10 hours	3.3	2.2	9.7

[a]Seed dusted with infected leaf debris and spores of *Helminthosporium gramineum*.
[b]Naturally infected cultivar Briggs.
[c]Naturally infected cultivar Grande.

placing pieces of agar containing mycelium of the fungus on the lemma directly over the embryos. The inoculated seed were germinated in petri dishes for 1, 2, 3, and 4 weeks at 6 C before being transplanted to soil in the greenhouse. The percentages of plants infected with stripe were 0, 6.6, 25.4, and 16.9%, respectively. Unlike other artificial inoculation methods using high inoculum levels that produce unnaturally high percentages of infected plants, the results of this experiment were similar to those in field plantings of naturally infected seed.

Since artificial inoculation of the exposed coleorhizae or scutella of germinating barley seed failed to produce many striped plants, but similar inoculation of the intact seed did, the influence of the lemma and pericarp-seedcoat layer on infection was tested in the following manner.

Three groups of 125 seed each of Briggs 2X and healthy cultivar CM67 were allowed to imbibe water at 6 C for 4 hours to cause swelling of the embryo. For each cultivar, one group of seed was left intact, the lemma over the embryos was removed from the second, and the lemma and pericarp-seedcoat layer were removed from the embryos of the third group. Cultivar CM67 was inoculated by placing small pieces of agar with mycelium over the embryos in all three groups. The naturally infected Briggs 2X seed were not inoculated. All six groups were incubated in petri dishes at 6 C for 28 days, and then transplanted to soil in the greenhouse.

The highest percentage of stripe (41.5%) that developed in CM67 inoculated directly on the exposed embryo was significantly greater ($P = 0.05$) than that which developed in the other two inoculation groups (Table 3). The unsuccessful coleorhiza inoculations previously described were made at later stages of germination than the inoculations in this experiment. Seed transmission in naturally infected Briggs 2X was significantly less ($P = 0.05$) in groups grown from seed which had the lemma or the lemma and pericarp-seedcoat layer removed from the embryo than in the group grown from intact seed. The group which had the lemma and pericarp-seedcoat layer removed had significantly less stripe ($P = 0.10$) than the group from which only the lemma was removed.

Effect of hull removal and surface treatment of seed on disease incidence.—Numerous experiments were conducted in the field to determine the relative amounts of seed transmission following surface contamination, hull removal, or surface treatment of seed. Inoculum used for surface contamination was prepared by inducing *H. gramineum* to sporulate on BLPA. The leaf pieces with spores were stripped from the agar, air-dried, and ground in a Wylie mill (dry inoculum). Seed of naturally infected Briggs 2X and Grande-5 and healthy cultivar CM67 were hand-hulled, and intact and hulled CM67 seed were rolled in dry inoculum. Naturally infected Briggs 2X and Grande-5 seed were surface sterilized in 0.5% NaOCl for 5 minutes, 4, or 10 hours; rinsed three times in distilled water; and dried before planting. Naturally infected seed also were soaked in distilled water for 10 hours. The effect of exogenous nutrients on seed transmission was tested by soaking Briggs 2X and Grande-5 seed in solutions of amino acids, or in extracts from barley seedlings or leaves. All treatments were planted at Riverside, Kings County, and Davis.

Less seed transmission occurred in plantings of hulled, naturally infected Briggs 2X and Grande-5 seed than in plantings of intact seed (Table 4). Surface contamination of intact healthy seed with dry inoculum resulted in few or no infected plants, but relatively high percentages of

TABLE 5. Incidence of seed transmission in field plantings of cultivar CM67 seed from spikes inoculated in the field at different stages of flower and kernel development

Stage of seed development when inoculated	Plants with seed-transmitted stripe (%)[a]			
	Davis seed source		Tulelake seed source	
	Wet[b]	Dry[c]	Wet	Dry
In boot	3.8	51.8	2.9	13.9
Emerged, not pollinated	8.5	33.3	2.2	18.5
Pollinated	3.8	38.4	2.5	11.1
Milk	2.3	6.6	...	...
Soft dough	4.2	15.5	3.3	2.7
Hard dough	0	3.0	...	...
Average air temperature (C)[d] Max	32.6		28.7	
Min	13.8		6.8	

[a]Planted at Davis, California, January, 1974.
[b]Heads sprayed with a mist of water following inoculation.
[c]Heads not sprayed with water after inoculation.
[d]Measured for the first 10 days after inoculation.

infection occurred in plantings of surface contaminated hulled healthy seed. Surface sterilization of naturally infected seed for 4 and 10 hours in 0.5% NaOCl greatly reduced or eliminated seed transmission. Addition of surface nutrients such as amino acids, glucose, or barley leaf and seedling extracts to naturally infected seed had no effect on seed transmission.

Inoculation at different stages of floral and seed development.—Barley seed becomes infected with *H. gramineum* during its development in the spike. The effects of moisture and temperature on infection at different stages of seed development were studied in the field and laboratory.

Inoculations of cultivar CM67 spikes in the field were conducted at Davis and Tulelake. The stage of flower or seed development to be inoculated was determined for each spike by inspection of one floret from near the center of each head and the heads were tagged accordingly. Flowers at three stages of development and developing seed at three stages were selected for inoculation. The floral stages were: (i) spike still in boot, (ii) spike emerged prior to pollination, and (iii) embryo just fertilized; and the stages of seed growth were (iv) milk, (v) soft dough, and (vi) hard dough. Dry inoculum prepared as described, was dusted by means of a deVilbis insufflator onto the spikes, and in the case of spikes at stage i, the leaf sheath was rolled back to facilitate inoculation. The spikes were covered with glassine bags either immediately (dry inoculation) or after being sprayed once with water (wet inoculation) and remained covered until harvest. Similar experiments were performed in the laboratory. Container-grown plants of cultivar CM67 grown in the greenhouse until headed were transferred to controlled environment cabinets at 10, 15, 21, 27, and 33 C 24 hours prior to inoculation. The determination of stages i through vi of floral and seed development and an additional stage, mature, and the method of dry inoculation were performed as previously described. Wet inoculation was accomplished by misting the spikes with water for a 24-hour period following inoculation and prior to enclosure in glassine bags. Plants were returned to the temperature chambers immediately after inoculation, and kept there for 48 hours before being transferred to the greenhouse where they were maintained until maturity. The glassine bags were removed about 2 weeks after the plants were transferred to the greenhouse. Mature seed from each treatment was hand-harvested separately, threshed, and each was thoroughly mixed. To test for seed transmission, samples of each treatment were planted in the field at Davis in December and January 1973-74.

Seed transmission resulted from seed inoculated at all stages of development from before head emergence through soft dough and in both wet and dry inoculations made in the field (Table 5). Dry inoculation was superior to wet inoculation for producing infected seed under field conditions at both Tulelake and Davis. Seed transmission from the Davis dry-inoculated seed source indicated that the earlier stages of seed maturation were more susceptible to infection than the later stages. Laboratory inoculations produced considerable variation in the amount of seed-transmitted stripe in seed inoculated at stages i through v. There was no apparent difference associated with stage of seed development when inoculated, whether wet- or dry-inoculated, or with different incubation temperatures.

DISCUSSION.—These studies corroborate reports that low soil temperature during germination and early growth of seedlings from naturally infected barley seed increases seed transmission of stripe. Because the critical stage of seedling growth for infection of embryos begins when the coleoptile is about the same length as the seed and ends near time of seedling emergence, soil temperature during the later stages of germination has the greatest effect on seed transmission. Seed transmission is reduced or eliminated when naturally infected seed are sown in warm soil, but relatively high percentages of infection result when artificially inoculated seed is similarly planted. Thus, it seems probable that reduction in seed transmission in warm soil is due to disease escape during germination rather than resistance.

Low soil temperature is apparently not the sole factor affecting disease incidence. Naturally infected seed germinated at 6 C for 28 days, and grown during late spring or summer in the greenhouse, produced much less seed-transmitted stripe than comparable plantings in late

fall or winter. The somewhat warmer greenhouse temperatures, and long days in summer, adversely affected barley growth indicating that environmental conditions other than soil temperature may affect development of systemic stripe after seedling emergence.

The location of the inoculum in infected seed also may affect seed transmission. Placement of fungus inoculum over exposed embryos of healthy seed which had just begun to imbibe water resulted in high percentages of diseased plants, and many infected seedlings were stunted or killed. When the pericarp-seedcoat layer was removed from over the embryos of naturally infected seed, seed transmission was significantly reduced. It was observed that the pericarp-seedcoat layer split away from most expanding embryos of rapidly germinating seedlings soon after germination began but remained appressed to the shoot-root juncture of slowly germinating seedlings even after 28 days at 6 C. These observations suggest that inoculum in the pericarp-seedcoat layer in the vicinity of the embryo may be in a near-optimum position for effecting seed transmission.

Inoculation of developing barley seed demonstrated that infection can occur at any stage of development from before head emergence through the soft dough stage, and over a wide range of temperature and moisture conditions. The earliest stage at which infection can occur was not established because inoculum introduced early in spike development may not have caused infection until some time later. There is no reason to suppose that all stripe-infected seed have uniform amounts of inoculum, or that the inoculum is always located in the same place on the seed. However, infection during early stages of flower or kernel development would probably result in extensive colonization of the growing seedcoat, pericarp, and hull tissues. Thus, the stage of seed development at the time of infection, and the extent to which the tissues are invaded, also may affect the amount of seed transmission.

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