Physiologic Leaf Spot of Winter Wheat

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ABSTRACT

Smiley, R. W., Gillespie-Sasse, L.-M., Uddin, W., Collins, H. P., and Stoltz, M. A. 1993. Physiologic leaf spot of winter wheat. Plant Dis. 77:521-527.

A leaf spot of unknown etiology damages winter wheat in the northwestern United States. The symptoms are similar to Septoria leaf blotch and tan spot, but the causal agents of these diseases are not present. Leaf spot symptoms are described. Dominant microbial colonists of the spots were *Cladosporium herbarum, C. macrocarpum, C. cladosporioides,* and *Alternaria* species. Leaf spot symptoms could not be induced on fresh leaf tissues with any of the inoculation or incubation procedures examined. Fungicides toxic in vitro to the dominant fungal colonists failed to suppress disease or increase grain yields in field experiments. Regression analyses of leaf spot severity and yield indicated that leaf spot reduced yield potential by 10% during each of 2 yr. No evidence is presented to indicate that this leaf spot is of microbial origin. Retention of the name physiologic leaf spot is suggested.

A leaf spot of unknown etiology has been present in the semiarid inland Pacific Northwest for 30 or more years. The disease is called physiologic leaf spot or "no-name" disease, and it recently became more pronounced than usual on winter and spring wheat (*Triticum aestivum* L. and *T. durum* Desf.) cultivars. Disease incidence and severity differ among cultivars, crop management practices, and seasons (27). Physiologic leaf spot was particularly severe in numerous fields during 1990, when as much as 60% of the flag leaf area became necrotic soon after flag leaves became fully extended. Lower leaves often became totally necrotic and senesced early. Leaf spots of similar description (33) on wheat include Alternaria leaf blight (*Alternaria triticina* Prasad & Prabhu), Septoria leaf blotch (*Septoria tritici* Roberge in Desmaz.), tan spot (*Pyrenophora tritici-repentis* (Died.) Drechs.), and bacterial leaf blight (*Pseudomonas syringae* pv. *syringae* van Hall).

Physiologic leaf spot is often presumptively identified as Septoria leaf blotch even though pycnidia are not present and cannot be induced by clinical methods. Although some cultivars of wheat become infected by *S. tritici* and exhibit typical symptoms without supporting development of pycnidia (33), the mycelium of the pathogen is associated with early development of tissue necrosis. There is no histological evidence to suggest an association of mycelia with the necrotic spots for the leaf spot in question. However, Septoria leaf blotch, identified by production of pycnidia with characteristic pycnidiospores, is occasionally present at nondamaging levels on the lower leaves of physiologic leaf spot-affected wheat cultivars in the inland Pacific Northwest, and infrequently causes economic damage in irrigated fields of the same cultivars. Moreover, the dominant wheat cultivars produced in the inland Pacific Northwest are highly susceptible to Septoria leaf blotch in subhumid western Oregon, and they support extensive development of pycnidia in that environment.

Physiologic leaf spot is also presumptively diagnosed as tan spot, even though tan spot has apparently never been confirmed in the inland Pacific Northwest. Conidiophores and conidia of *Pyrenophora tritici-repentis* are not present or inducible on necrotic or chlorotic tissues diagnosed as physiologic leaf spot, and the pseudothecia of this pathogen have not been observed on wheat straw in this region.

Bacterial exudates are also absent and cannot be induced on the leaf spots in question. The leaf spot that occurs in the inland Pacific Northwest does not appear to be Septoria leaf blotch, tan spot, or bacterial leaf blight. Nevertheless, presumptive field identifications of physiologic leaf spot as Septoria leaf blotch or tan spot have led to applications of fungicides to approximately 5,000 ha annually in the Pacific Northwest between 1989 and 1992. Benomyl and propiconazole were applied most frequently, generally once during the spring (May) before flag leaf emergence. It was not known if the fungicides reduced damage to wheat leaves or improved grain yield, or if the leaf necrosis from this leaf spot suppressed grain yields.

"Physiologic" leaf spots (5,9,21,24,28) with variable symptoms and local names have been recognized on wheat for at least 50 yr. The name is probably a generic term referring to leaf spots for which the etiology is not yet understood. It also likely designates different diseases in different regions. Chester (5) used the name to describe a disease apparently caused by the drying of water-soaked tissues after particularly sensitive genotypes were subjected to high humidity for a long time. Sallans and Tinline (24) used similar descriptive terminology to define a "splotch" associated with nitrogen deficiency. Vanterpool (32) described a similar nonparasitic condition of wheat in the greenhouse as "dry leaf blotch." Another disease of similar appearance and apparently of nonparasitic origin is called gray speck of wheat and dry leaf spot of oats (9). Physiologic leaf spot in the semiarid Pacific Northwest has not been described. A description of symptoms and additional etiologic inquiries were considered important in view of the disease severity during recent years.

The objectives of this investigation were to characterize the symptoms of physiologic leaf spot of winter wheat, to identify the microflora associated with tissue necrosis, to attempt to demonstrate pathogenicity by one or more microbial colonists, to evaluate the effects of fungicides on fungal colonists and of fungicides and a bactericide on grain yields, and to estimate yield damage caused by this disease.

MATERIALS AND METHODS

The experiments and observations were performed in north central and northeastern Oregon and south central Washington from 1989 to 1992. The warm-temperate climate of these regions has warm-to-hot summers and cool-to-cold winters, with most precipitation (230–700 mm) occurring during winter and spring. Soils at the experimental sites were silt loams of the Walla Walla, Ritzville, and Palouse series (coarse-silty mesic Typic Haploxerolls) with about 1–2% organic matter, a surface horizon pH (in 0.01 M $CaCl_2$) of 5.0–6.5, and deep, well-drained profiles. Winter wheat was rotated with summer fallow, and tillage involved either a moldboard plow with subsequent management of low-

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Oregon Agricultural Experiment Station Technical Paper 9997.

Accepted for publication 14 December 1992.

residue fallow or a chisel plow with subsequent management of high-residue fallow.

Characterization of symptoms. Leaf spot symptoms on winter wheat were characterized during 1990–91. Leaves were examined closely over time for size, shape, pigmentation, partitioning of chlorotic and necrotic zones, and signs of pathogenic agents. Microscopy was utilized when appropriate.

Isolation of fungi. Samples were collected from several hundred experimental plots and commercial fields in Oregon and Washington. Collections were made throughout the spring and summer of both 1990 and 1991, when plants were in the late tillering to head emergence stages. Isolations of fungi were attempted from nontreated, washed, and surface-disinfested (1.25% sodium hypochlorite for 30–60 sec) leaf segments removed from the margins of spots. Isolations were from leaf spots differing widely in size and appearance, and were taken from different cultivars, leaf positions, and times of the growing season.

Fungi were isolated by placing leaf segments on 2% water agar with or without 50 μg/ml of rifampicin, or on half-strength potato-dextrose agar medium (PDA). Emerging isolates were transferred onto half-strength PDA. Identifications were based on morphology and the development of anamorphs. Fungi infrequently sporulated on leaf tissue collected from the field. These fungi were identified directly or were transferred onto plates for further diagnostic procedures. Affected leaves were also incubated in a moist chamber for up to 15 days at 22 C to encourage sporulation for direct identification.

Fungi were isolated in 1990 from 11 entries in a winter wheat yield testing nursery near Helix, Oregon. Entries included the cultivars Dusty, Hill 81, Hyak, Madsen, Malcolm, Moro, Oveson, Stephens, Tres, and selections OR855 and 87-684. From 40 to 44 leaf segments of each entry (460 leaf segments) were tested. Small necrotic lesions or margins and central regions of larger lesions were placed on water agar, half-strength PDA with or without rifampicin, or half-strength tryptic soy agar (TSA). Emerging fungi and/or bacteria were transferred to individual plates of PDA (for fungi) or TSA (for bacteria), which were incubated at 24 C on an open laboratory bench. Isolated fungi and bacteria were identified to genus and, in some instances, to species.

Isolations in 1991 were performed on samples collected over a broad geographic region in eastern Oregon and Washington. The samples represented a wide range of commercial and experimental variables, including tillage, precipitation zone, crop history, and fertility. Fungi were identified, but proportions of genera and/or species were not quantified.

Isolation of bacteria. Flag leaves from 10 leaf spot-affected plants (cultivar Stephens) were cut into 1-mm segments. Five nonsterile leaf segments were placed on duplicate plates of 0.1% TSA and King's medium B (KB). The remaining leaf segments (1 g) were shaken for 30 min in 0.85% saline. The saline was then diluted serially and spread onto plates of sucrose casein hydrosylate, sucrose peptone, TSA, and KB. Bacterial colonies were selected from each plate and grouped by morphological characteristics (i.e., color, size, shape, margins, etc.); of these, 46 representative isolates were selected for further characterization. Bacterial isolates were assumed pure after subculturing three times on TSA.

Representative isolates were tested for aerobic utilization of 95 different substrates with a commercially available microtiter plate test system. The test system was used as recommended by the manufacturer, except that the inoculum was grown in nutrient broth yeast extract (NBY) on a rotary shaker at 25 C rather than on solid TSA medium. After 24 hr of growth, the cultures were centrifuged and washed three times in 0.85% sterile saline before inoculation of the microtiter plate. Inoculated plates were incubated in the dark at 25 C instead of at the 33–35 C recommended.

Other etiologic studies. Three fresh tissue samples of plants affected by physiologic leaf spot were sent to the Plant Disease Diagnostic Laboratory at Oregon State University. Thin sections across necrotic lesions were evaluated by electron microscopy to determine whether mycoplasmalike bodies or viruses were present. Expressed liquids were also evaluated for evidence of a virus.

Nine air-dry samples of physiologic leaf spot and two samples of apparently healthy winter wheat were collected in Oregon and Washington and sent to Microbial ID, Inc. (MIDI), Newark, Delaware. Affected tissues were subjected to gas-liquid chromatography for identification of fatty acid methyl esters (7,25). Fatty acid profiles were examined by principal-component analysis and pattern-recognition software to determine whether bacteria or fungi were or had been present in necrotic tissue, even though they could not be cultured by our isolation procedures.

Inoculation experiments. Cultures of fungi and bacteria were inoculated into or onto highly susceptible cultivars of wheat produced and maintained in controlled-environment chambers, a greenhouse, and in the field. Plants were subjected to various stress or nonstress conditions before or after inoculations. Examples included drought stress or ample water for plants at ambient temperatures (3–15 C or 8–20 C), with or without simulated frost damage (0 to −1 C for 10 min daily). Photoperiods were generally 12-hr light and dark cycles, and relative humidities varied from ambient to 100%.

Inoculation procedures included spraying, injecting, or rubbing spore and mycelial suspensions (approximately 10^5 spores per milliliter) onto asymptomatic wheat leaves previously incubated under high (>95%) or low (30%) humidity, high (20–30 C), low (3–15 C), or diurnal (3–15 C or 8–20 C) temperatures, low or high (100–800 μmol/m²/sec) light intensity, and low or high (−10 to −500 J/kg) matric potential. After inoculation, half of the seedlings in one diurnal temperature experiment were incubated for 20 min at −2 C on two successive days before initiating the cold cycle. Wheat leaf surfaces were damaged by abrasion with Carborundum or were undamaged. Incubations were performed under various conditions described above, either by leaving inoculated plants under one incubation condition or by moving them from one to another, as often as daily. Leaf spots from fresh leaf tissue were also attached directly to asymptomatic wheat leaves and incubated under various controlled conditions to encourage the transfer of an agent, if one was present. Inoculations were also made in the field on plants already moderately affected by leaf spot. Plants in the field were inoculated under such conditions as drought stress or nonstress; periods of cool, wet weather; or dry, warm-to-hot periods with prolonged leaf wetness from dew and guttation.

Fungicides. *Sensitivity of fungi in vitro.* A laboratory study was performed to examine the sensitivity of the dominant fungi isolated from symptomatic leaves to nine fungicides: benomyl, chlorothalonil, fenarimol, flutolanil, mancozeb, prochloraz, propiconazole, tolclofos-methyl, and triadimefon. Isolates of each fungus were grown on half-strength PDA for 7 days. Agar plugs (3 mm in diameter) were removed and placed on PDA amended with fungicides at rates of 0, 1, and 10 μg a.i./ml. Plates were incubated at 24 C on a laboratory bench. Measurements of radial growth rates were made daily. Isolates were characterized by fungicide concentrations that completely inhibited growth (ED_{100}) or reduced it by half (ED_{50}).

Single foliar application. Fungicide applications timed to protect the flag leaf were conducted on commercial fields near Pendleton and Helix, Oregon, during 1990, and at Helix again during 1991. Fungicides applied to Stephens winter wheat during the spring of 1990 included chlorothalonil, mancozeb, and propiconazole. Additional treatments during 1991 included benomyl and a broad-spectrum fungicide/bactericide containing elemental sulfur + tribasic copper sulfate (50% S, 4.4% Cu; "TopCop" manufactured by Stoller, Inc.). All treatments (rates are

provided in Table 1) were applied in 100 liters of water per hectare when the flag leaf was emerging (Haun growth stage 6.7) or was fully extended. At the time of application, leaf spot was not present on the flag leaf (F) or the next two lower leaves (F-1 and F-2), but was increasing rapidly on the third (F-3), fourth (F-4), and fifth (F-5) leaves below the flag. Plots were 1.5 × 10 m, replicated three to five times, and arranged in randomized complete block designs. Leaf spot ratings (percent necrotic area on each leaf of 100 main stems per plot) were made once after head emergence. Grain yields were measured with a plot combine during August.

Multiple foliar applications. Field studies were established on Stephens wheat at the Helix site during 1991 and 1992 to determine whether preventative applications of fungicides could provide protection not achieved with a single application to the flag leaf. All treatments were as described for the single-application study in 1991, except for multiple applications of most treatments from December 1990 to May 1991 and from December 1991 to April 1992. Leaf spot ratings on main stems and the two oldest tillers were made twice during 1991, once after head emergence and again during the soft dough stage. Only the latter timing was used for leaf spot ratings during 1992. Grain yields were measured with a plot combine during August 1991 and July 1992.

Yield damage assessment. The relationship between leaf spot severity (individual main stem leaves) and grain yield (whole plots) was evaluated (30) on Stephens winter wheat in the multiple-application fungicide trials during 1990–91 and 1991–92. Regression analyses were performed separately for each leaf (F, F-1, F-2, and F-3), based on ratings made when the flag leaf was fully emerged in May (Feekes scale 10).

RESULTS

Characterization of symptoms and signs. When leaf spot occurs on leaves in the upper plant canopy, the field appears drought stressed (Fig. 1A). Such regions of the field become dull and have a brownish tint compared to nearby less affected stands.

Leaf spot typically becomes evident on the oldest (lowest) leaves of susceptible cultivars during midspring (March, Feekes scale 3–5) and progresses to successively younger leaves after they have fully emerged. Affected leaves typically become chlorotic (Fig. 1B) and die prematurely. For some cultivars and fields, and during some growing seasons, leaf spot does not advance from older to younger leaves (F, F-1, or F-2).

This leaf spot is characterized by several lesion types with variations in color, size, and shape. Initial symptoms are small (1–3 mm in diameter), oval, dark brown spots (Fig. 1D) or small necrotic lesions (Fig. 1E and 1F). These spots or lesions may or may not expand into spots with necrotic, light tan centers and/or narrow (<2 mm wide) chlorotic halos (Fig. 1F). As leaf spots enlarge to 5 × 10 mm, they remain oval or become irregularly shaped, with a light or dark brown necrotic center and with or without a narrow (<2 mm wide) chlorotic halo (Fig. 1G). The appearance of each lesion apparently depends on undefined interacting factors such as lesion age, wheat cultivar, weather, and perhaps others. When leaf spot is present on flag or older leaves, the flag leaf is also frequently affected by tiny chlorotic spots (<1 mm in diameter), with or without brown centers (Fig. 1C), that are presumed by the authors to be hypersensitive reactions to unsuccessful infections by rust fungi (9,33).

Attempts to observe and/or induce the development of pycnidia, perithecia, or bacterial exudates were unsuccessful on leaf segments previously diagnosed with physiologic leaf spot. Plants provisionally diagnosed with bacterial leaf blight (based on leaf necrosis, water soaking, and the presence of a surface "varnish") readily produced exudates from leaf spots when tissue sections were incubated in a humid chamber.

Fungal colonists. The identity and proportion of isolates from each of the 11 wheat cultivars in the yield trial near Helix in 1990 were nearly uniform. Fungi were isolated from 89% of the surface-disinfested segments, and bacteria from 5%. The dominant fungi (58% of the lesions, range 35–86%) were *Cladosporium* species, consisting of a 5:4:1 proportion of *C. herbarum* (Pers.:Fr.) Link, *C. cladosporioides* (Fresen.) G.A. De Vries, and *C. macrocarpum* G. Preuss. Other fungi included species of *Alternaria* (13%, range 5–30%), *Stemphylium* (5%, range 0–15%), *Penicillium* (5%, range 0–14%), *Fusarium* (3%, range 0–8%), *Drechslera* (trace, range 0–3%), *Mucor* (trace, range 0–2%), and an unidentified white nonsporing fungus (7%, range 0–14%). *Cladosporium* species occasionally (<5% of lesions) grew as visible colonies from necrotic tissue on leaves in the field and in high-humidity cultures. *Septoria, Pyrenophora,* and other notable pathogens of wheat foliage were not detected.

During 1991, fungi were isolated from leaf spots over a broad region encompassing eastern Oregon and Washington. Although proportions of fungi isolated were not quantified, they were similar to those detected at Helix during 1990. Species of *Fusarium* (especially *F. graminearum* Schwabe) were present on nondisinfested leaf tissues but were not isolated from surface-disinfested leaf segments. Isolates of 159 fungi were collected from disinfested tissues. The isolates were identified as *C. herbarum* (42%), *C. cladosporioides* (29%), and *C.*

Table 1. Incidence and severity of physiologic leaf spot on main stem leaves and grain yield of winter wheat plants treated one or more times with foliar-applied fungicides

Treatment	Rate (kg/ha)	Application Dates: 1991–92						Necrotic area[a] (%)				Yield (kg/ha)
		Dec.	Jan.	Feb.	Mar.	Apr.	May	F	F-1	F-2	F-3	
Control	...							0	2	12	26	4,769
Benomyl	1.1	X						0	1	5	20	5,779
Benomyl	1.1		X					0	1	5	20	4,901
Benomyl	1.1	X	X				X	0	1	7	23	5,541
Chlorothalonil	1.2	X	X				X	1	2	10	35	5,217
Chlorothalonil	1.2			X		X		0	1	7	22	5,088
Mancozeb	1.8	X		X			X	0	1	7	16	4,883
Mancozeb	1.8			X		X		0	1	6	22	5,191
Propiconazole	0.1	X						0	1	8	26	4,729
Propiconazole	0.1			X				0	1	7	23	5,350
Propiconazole	0.1	X		X			X	1	1	6	25	5,345
S + $Cu_3(SO_4)_2$	3.5S + 0.3Cu	X	X				X	0	1	10	23	4,968
S + $Cu_3(SO_4)_2$	3.5S + 0.3Cu			X	X	X		0	1	5	16	5,243
S + $Cu_3(SO_4)_2$	3.5S + 0.3Cu	X	X	X	X	X		0	1	5	15	4,955
LSD ($P = 0.05$)								NS	NS	5	NS	659

[a] Flag leaf (F) and first (F-1), second (F-2), and third (F-3) leaves below the flag leaf.

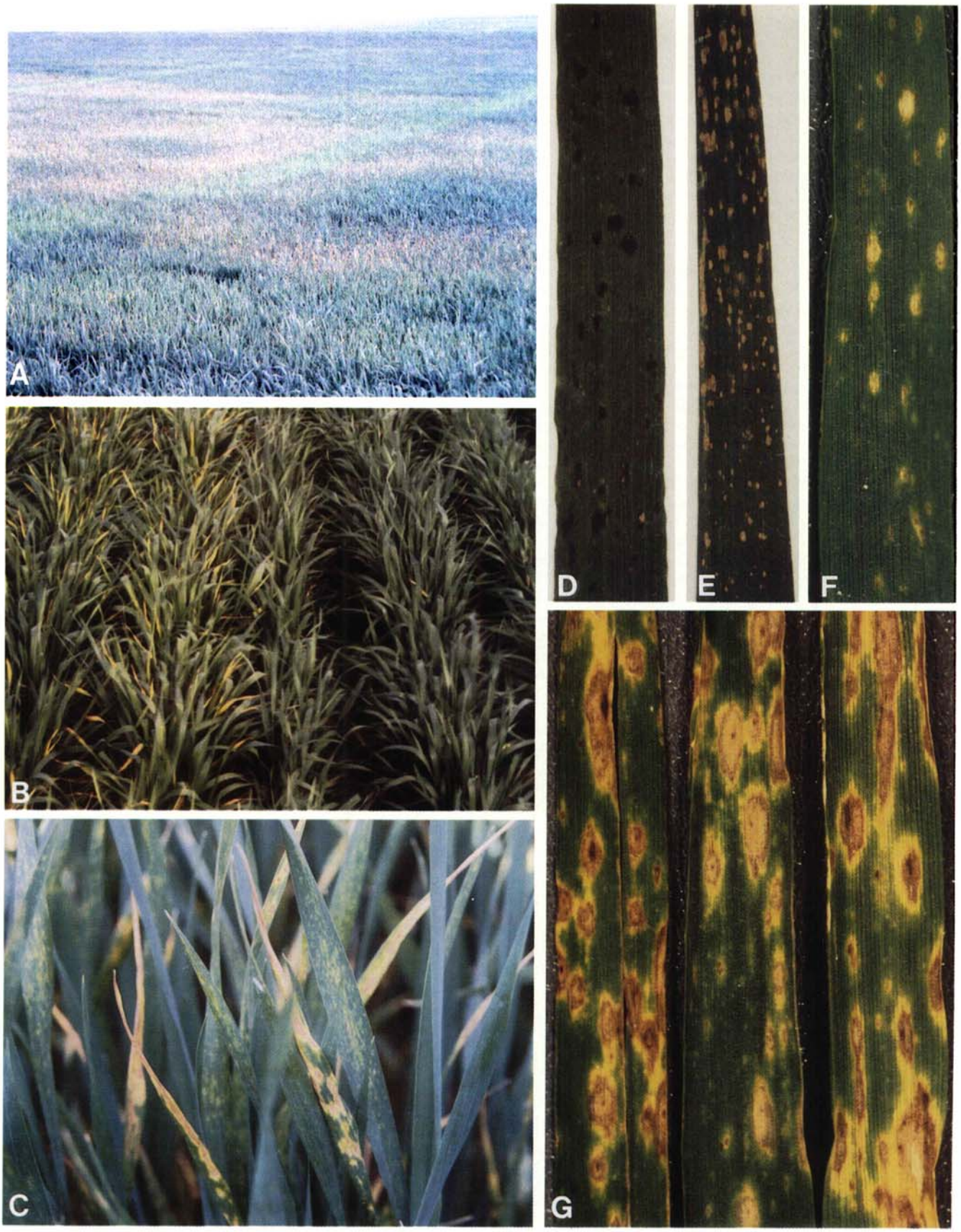

Fig. 1. Physiologic leaf spot of winter wheat: **(A)** drought-like appearance of severely affected field (greenish bands correspond to overlaps of nitrogen fertilizer applicator), **(B)** chlorosis and necrosis of oldest leaves (left, susceptible cultivar; right, resistant cultivar), **(C)** older leaves with necrotic lesions and younger leaves with tiny chlorotic spots presumed to be hypersensitive reactions to unsuccessful infections by rust fungi, **(D)** initial brown spots that appear on some cultivars, **(E,F)** initial necrotic lesions that appear on some cultivars, and **(G)** enlarged necrotic lesions with chlorotic halos.

macrocarpum (9%), and species of *Alternaria* (11%), *Ulocladium* (5%), and other dematiaceous fungi (4% *Stemphylium, Drechslera,* and perhaps others).

Bacterial colonists. A total of 46 bacterial colonies representing 11 morphological groups were isolated from culture plates at 10^{-2} dilution. No colonies on any medium were present below 10^{-2} dilution, and growth was never detected on KB, suggesting that *Pseudomonas* populations were not present or were below the level of detectability for the leaf segments evaluated. Individual isolates of each group were characterized as short gram-negative rods, except for isolate H-1, which was gram-variable. Table 2 provides colony characteristics for the 11 morphological groups identified.

The BIOLOG GN plate consists of simple sugars, amino acids, organic acids, and complex polymers and polysaccharides. The percentage of substrates used by test isolates ranged from 0 to 70%. Three distinct nutritional groups of aerobic heterotrophic bacteria were identified on the phylloplane of winter wheat. Nutritional group I, characterized by isolate H-4, gave negative results to all test substrates, suggesting a requirement for unknown growth factors. Therefore, identification of group I isolates by the BIOLOG test system was not possible. Group II consisted of species (e.g., isolates H-1, NSP1-4, and H-2) that used only simple sugars (fructose, galactose, glucose, maltose, mannose, and sucrose), amino acids (proline), and organic acids (succinate and hydroxybutyrate). Group II isolates were determined to be species of *Pseudomonas* and *Xanthomonas*. In addition to the substrates used by group II isolates, members of group III used complex polymers (e.g., dextrin and glycogen), polysaccharides (e.g., cellobiose and trehalose), and a myriad of amino acids and organic acids. Group III bacteria were identified as species of *Pseudomonas*. None of the group II and III species (e.g., *P. cepacia* (Burkholder) Palleroni & Holmes, *P. glathei* Zolg & Ottow, *X. campestris* pv. *oryzae* (Ishiyama) Dye, and others) were recognized as pathogens of wheat.

Other etiologic studies. Neither mycoplasmas nor viruses were detected in expressed sap or cross sections of leaves evaluated by electron microscopy. Likewise, evaluations of fatty acid compositions of affected tissue and subsequent comparisons with principal-component analysis and pattern-recognition software suggested an absence of bacteria or fungi in tissues affected by physiologic leaf spot.

Inoculation experiments. Transferability of this disease was not demonstrated by these experiments (*data not shown*). No inoculation or incubation procedure led to the development of leaf spots similar to those observed under natural conditions in the field. Minor leaf spots resulted from inoculations with certain isolates of *C. herbarum, C. macrocarpum, Alternaria* species, and *Pseudomonas*. However, the lesions were small, nonspreading, and without the characteristic dark brown pigmentation either inside or at the margins. As such, the induced leaf spots were not typical of those observed in the field.

Fungicides. *Sensitivity of fungi in vitro.* Radial growth of *C. herbarum* and *C. macrocarpum* were completely inhibited (highly sensitive, $ED_{100} < 1$ μg/ml) by the presence of prochloraz or propiconazole in the growth medium. The isolates were moderately sensitive ($ED_{50} < 1$ μg/ml, $ED_{100} < 10$ μg/ml) to chlorothalonil, fenarimol, and triadimefon; sensitive ($ED_{50} > 1$ μg/ml, $ED_{100} < 10$ μg/ml) to mancozeb; moderately tolerant ($ED_{50} < 1$ μg/ml, $ED_{100} > 10$ μg/ml) of benomyl; and tolerant ($ED_{50} > 1$ μg/ml, $ED_{100} > 10$ μg/ml) of flutolanil and tolclofos-methyl. In contrast, *C. cladosporioides* was not highly sensitive to any fungicide; was moderately sensitive to fenarimol, prochloraz, and propiconazole; moderately tolerant of tolclofos-methyl; and tolerant of benomyl, chlorothalonil, flutolanil, mancozeb, and triadimefon. The *Alternaria* species was moderately tolerant of fenarimol, prochloraz, propiconazole, and tolclofos-methyl; and tolerant of benomyl, chlorothalonil, flutolanil, mancozeb, and triadimefon. Overall, these fungi were most sensitive to fenarimol, prochloraz, and propiconazole.

Single treatments. Leaf spot on flag leaves during 1990 ranged from 10 to 60% necrosis (mean of 30%) at Pendleton, and from 0 to 40% necrosis (mean of 15%) at Helix. During 1991 at Helix, the range of damage was 0–25% necrosis (mean of 7%). There were no differences between test plots, and the surrounding commercial crop and leaf spot symptoms were uniform among treatments.

A single application of fungicide in three field tests failed to suppress leaf spot or increase grain yields ($P > 0.10$). Grain yields at Pendleton during 1990 ranged from 3,807 to 5,323 kg/ha, with the highest yield occurring in the control. Yields at Helix ranged from 4,575 to 4,804 kg/ha in 1990, and from 4,125 to 4,597 kg/ha in 1991. The autumn and winter were abnormally dry both years, and late-season (April to June) rainfall was well below average during 1990, but above average during 1991.

Multiple treatments. In 1991, leaf spot was first observed on plants on 29 March (Feekes scale 2). On 15 May (Feekes scale 10), when plants were collected for evaluation, all were of uniform size and appearance, and 50–80% of the leaves exhibited symptoms of leaf spot. At that time the disease was moderately severe in the lower plant canopy, with leaf necrosis mostly limited to the F-2 and F-3 leaves (Table 1). Leaf spot incidence on the F-2 was reduced ($P < 0.05$) by benomyl, mancozeb, propiconazole, and sulfur + copper sulfate. No pesticide reduced disease incidence on the F-3, and there was no significant relationship between disease incidence and severity on any of the leaves. Severity of leaf spot for a specific leaf on the main stem did not differ ($P > 0.05$) from the severity on the same leaf type for the two oldest tillers (*data not shown*). Likewise, the severity of disease on each leaf type was always correlated ($P < 0.01$) with the severity on each of the other leaves (*data not shown*). Benomyl was the only fungicide that improved yield (Table 1), but this was unrelated to disease suppression, as indicated by the failure of other fungicides to increase yield at equivalent levels of disease suppression. No fungicide altered test weight ($P = 0.59$, *data not shown*) or protein content ($P = 0.17$, *data not shown*) of grain.

Fungicides did not reduce leaf spot severity or increase grain yield during 1991–92 (*data not shown*). On 2 June 1992, the necrotic area ranged from 7 to 10% on the flag leaf and from 14 to 20% on the F-1. Many (4–25%) of the F-1 leaves were dead at the time of evaluation on 2 June, when heads were emerging from the "boot" (Feekes scale 10.3). Grain yields ranged from 5,336 to 5,542 kg/ha, with no significant differences ($P = 0.97$) among treatments.

Table 2. Morphological characteristics of dominant bacterial colonies isolated from the phylloplane of winter wheat displaying symptoms of physiologic leaf spot

Isolate	Color	Form	Elevation	Margin	Size (mm)[a]
H-1	White	Circular	Curved	Entire	0.8
NSP1-3	White	Circular	Convex	Undulate	1.0
NSP1-5	White	Circular	Raised	Undulate	2.2
NSP1-19	White	Irregular	Flat	Lobate	8.0
AR-1, AR-2[b]	White	Circular	Convex	Undulate	1.6 (mucoid)
NSP1-1	Gray/white	Circular	Umbonate	Entire	1.4
NSP1-4	Gray	Irregular	Raised	Undulate	2.9
H-4	Translucent/white	Circular	Convex	Entire	0.5
H-5a	Translucent/white	Circular	Raised	Entire	0.3
H-2, H-3, H-5[b]	Gold/yellow	Circular	Convex	Entire	0.5
NSP1-26	Yellow	Circular	Raised	Entire	1.0

[a] Determined after 3 days growth on tryptic soy agar medium.
[b] Isolates had similar morphological but different physiological characteristics.

Crop loss assessment. Although fungicide treatment differences were not detected in field experiments, variability occurred for leaf spot and grain yield in individual plots. Regression analyses for data collected during 1991 revealed a significant correlation between grain yield and leaf spot severity on the F-2 leaf ($y = 5{,}419$ kg/ha—41[percent necrotic leaf area]; $P = 0.02$, $r^2 = 0.29$). This regression predicted a 41-kg/ha reduction in yield for each percent necrosis on the F-2 when sampling occurred on 15 May (Feekes scale 10). This corresponds to a 10.3% reduction in yield at this experimental site during 1991; the control treatment had 12% necrotic leaf area and a yield of 4,769 kg/ha. Yield-loss predictions were also important but less precise when based on damage to the F-1 (189 kg/ha/percent necrosis, $P = 0.06$) and F-3 (10 kg/ha/percent necrosis, $P = 0.09$) leaves. At the time of evaluation on 15 May, leaf spot was mostly absent on the F, less than 2% on the F-1, and intermediate (5–12%) on the F-2. The F-3 was severely affected (15–35% necrosis) and becoming chlorotic, and the F-4 was fully chlorotic and either fully or mostly necrotic.

The disease was more severe in 1992 than in 1991, and the most reliable data again came from leaves exhibiting intermediate levels of damage. The regressions predicted yield losses of 53 and 31 kg/ha/percent necrotic area on the F ($r^2 = 0.31$) and the F-1 ($r^2 = 0.20$), respectively. Each relationship was significant at $P < 0.002$. On 2 June, necrotic leaf area in the control treatment was 10% for the F and 19% for the F-1, and the grain yield was 5,336 kg/ha. These relationships predict yield losses of 9.9 and 11.0%, which correspond well with losses predicted during 1991.

DISCUSSION

The evidence presented supports the retention of the name physiologic leaf spot for a disease which causes a spot-type leaf necrosis on winter wheat in semiarid regions of the Pacific Northwest. Either the microbial colonists isolated from symptomatic tissue and inoculated onto apparently healthy plants were not the principal incitants of this leaf spot, or the environments used were not favorable for expression of pathogenicity. No isolate was considered atypical of the normal phyllosphere microflora (8,12,13). Therefore, we present no evidence that a microbial agent incites this disease. Although numerous interactions were examined in our experiments, the possibilities were not exhausted, and we remain unconvinced that a genetic dysfunction can be responsible for the pattern of leaf necrosis exhibited by this disease.

Cladosporium species were isolated with high frequency in our experiments. It is well known that *Cladosporium* species are common epiphytes of wheat leaves (19,21), are colonists of cereal residues remaining after harvest (15), are disseminated readily through the air (3,10,11), and are capable of accelerating the rate of wheat-leaf senescence (14,31). Some members of this genus are also pathogenic to onions (16), timothy (29), wheat (2), and other crops. When *C. macrocarpum* invades the stomata of barley leaves, an induced resistance reaction similar to that for pathogenic fungi occurs (6). Special attention was therefore given to the association of these fungi with physiologic leaf spot. Many of our inoculation procedures followed those used to demonstrate the pathogenicity of *Cladosporium* species to onions, timothy, and wheat (3,16,29). In our experiments, these techniques failed to demonstrate the pathogenicity of *Cladosporium* species to winter wheat. We were, however, successful in inducing small, pale chlorotic spots on wheat leaves by inoculating the leaves with spores of *C. herbarum* and *C. macrocarpum*. This response appears identical to the hypersensitive response described for barley (6) and also to the well-known response of resistant wheat genotypes to incipient infections by rust fungi (33). It is possible, therefore, that the small chlorotic specks often found on flag leaves of winter wheat in eastern Oregon may be caused by other fungi in addition to rust fungi. A similar role for *Cladosporium, Alternaria,* and *Ascochyta* species was suspected in Germany (23). It is also possible that plant stresses that reduce plant resistance may enable *Cladosporium* species to cause a higher level of damage than occurs in healthy plants (1,2,14). However, because *Cladosporium* species were particularly sensitive to propiconazole, its failure to reduce the severity of leaf spot in the field provides circumstantial evidence that *Cladosporium* species are not primary incitants of this disease. Similar reasoning suggests that the disease is not Septoria leaf blotch or tan spot (22,33).

Wheat diseases of biotic origin (33) that most closely resemble the symptoms reported for physiologic leaf spot in the semiarid Pacific Northwest include tan spot (17,18) and Alternaria leaf blight (26). Although the species of *Alternaria* isolated in our study was mostly intolerant of fungicides, it was isolated at low frequency and did not induce leaf spot in inoculation experiments. Furthermore, sporulation of *Alternaria* species was not observed on fresh tissues collected from the field. Triticale is affected by Alternaria leaf blight (4), but triticale entries in winter wheat yield nurseries in Oregon are highly resistant or immune to the leaf spot (27). Collectively, these observations suggest that this leaf spot is not caused by *Alternaria.*

Physiologic leaf spot appears to be a generic term for leaf spots for which the etiology is not yet understood. The term "splotch," as used by Sallans and Tinline (24) to describe symptoms associated with nitrogen deficiency, does not fit the description of physiologic leaf spot in the inland Pacific Northwest. Splotch becomes more severe in the upper than in the lower canopy, and the reverse occurs with the physiologic leaf spot addressed in this study. During our study, we also noted that plants grown to maturity in small pots (10–15 cm in diameter) in the greenhouse developed symptoms similar to those observed in the field, but usually of lesser severity. Vanterpool (32) described a similar nonparasitic condition of wheat in the greenhouse as "dry leaf blotch." In view of the name recognition established in the Pacific Northwest, we propose to retain the name physiologic leaf spot.

The reason for an increase in damaging occurrences of physiologic leaf spot during recent years in the inland Pacific Northwest remains unknown. There have been no significant changes in wheat cultivars during the past 15 years. Stephens wheat was introduced in 1977 and rapidly gained dominance in the region. As much as 90% of the winter wheat acreage has been planted with Stephens during the past decade, and although declining in importance, this cultivar is still planted on 70% of the acreage. Typically, spring wheats are not grown consecutively in the same fields or portions of fields; yet they too have sustained high disease severity in recent years. Tillage procedures and crop rotations have not evolved significantly during the last decade. Nitrogen management levels appear to have increased, but application procedures and timing have remained static. The region is experiencing a sustained period of below-average precipitation, and was in the third year of continuing drought when leaf spot became highly damaging in 1990. The drought continued during the course of our study. It is unlikely, however, that drought is directly responsible for the increase in leaf spot, because this disease also occurs on nearby irrigated fields in an arid region that receives 200–230 mm of annual precipitation.

The economic importance of physiologic leaf spot and the strong effects on symptom expression of genotypes, cultural managements, and growing seasons (27) indicate the need for further studies to determine whether this disease is caused by a specific physiologic dysfunction or a biotic agent. If the cause is genetic, there must be a strong environmental effect on symptom expression; because susceptible cultivars exhibit great variability in symptoms from season to season, field to field, and locale to locale within the Pacific Northwest. Likewise, if imbalances in mineral nutrition are responsible, it should be possible to utilize the symptom expression in

highly susceptible cultivars as presumptive evidence that foliar analyses, soil tests, and/or corrective actions are needed. For example, the occurrence of a leaf spot similar to tan spot, but not responsive to tan spot management procedures, led to the discovery that zinc deficiency caused necrotic lesions on wheat in the Darling Downs wheat region of Australia (20). If a biotic agent is responsible for physiologic leaf spot, it must attain a large enough population to invade foliage on a massive scale without developing colonies or growths visible by routine microscopy. Further, it must maintain this high population density long enough to invade successive leaves after they become fully extended.

ACKNOWLEDGMENTS

We thank Lori Carris (Washington State University, Pullman), Paul Koepsell (Oregon State University, Corvallis), and Myron Sasser (Microbial ID, Inc. "MIDI", Newark, Delaware) for contributing taxonomic and diagnostic services; Robert Correa, Sandra Ott, Melanie Houser, Karl Rhinhart, and Elizabeth Smiley for technical assistance; Gloria Eidam and Judy Elliott for clerical assistance; and Clinton Reeder, Calvin Spratling, and Stanley Timmermann for donations of land for these experiments. We also appreciate special assistance provided by Pendleton Grain Growers, Walla Walla Grain Growers, McGregor Chemical Co., Hill Brothers Chemical Co., Brogoitti Elevator, Wilbur-Ellis, Pure-Gro, Ciba-Geigy, Farm Chemical Service, Stoller, and others. This study was a component of Oregon Agricultural Experiment Station Project 268, and was funded by the Oregon Wheat Commission and the USDA-CSRS-Pacific Northwest STEEP Competitive Grants Program.

LITERATURE CITED

1. Ajayi, O., and Dewar, A. M. 1983. The effects of barley yellow dwarf virus, aphids and honeydew on *Cladosporium* infection of winter wheat and barley. Ann. Appl. Biol. 10:57-65.
2. Arya, H. C., and Panwar, K. S. 1955. Some studies on a virulent strain of *Cladosporium herbarum* on wheat. Indian Phytopathol. 8:176-183.
3. Caretta, G., del Frate, G., della Franca, P., Guglielminetti, M., Mangiarotti, A. M., and Savino, E. 1986. Studies on the occurrence of fungi in a wheat field. II. Fungi on the phylloplane and fungal air spora. Bol. Micol. 3:71-79.
4. Chaudhuri, S., Maiti, S. S., and Saha, P. 1976. Leaf blight of triticale caused by *Alternaria triticina.* Plant Dis. Rep. 60:133-134.
5. Chester, K. S. 1944. A cause of "physiological leaf spot" of cereals. Plant Dis. Rep. 28:497-499.
6. Christiansen, S. K., and Smedegaard, V. 1990. Microscopic studies of the interaction between barley and the saprophytic fungus, *Cladosporium macrocarpum.* J. Phytopathol. 128:209-219.
7. DeBoer, S. H., and Sasser, M. 1986. Differentiation of *Erwinia carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atroseptica* on the basis of cellular fatty acid composition. Can. J. Microbiol. 32:796-800.
8. Dickinson, C. H. 1976. Fungi on the aerial surfaces of higher plants. Pages 293-324 in: Microbiology of Aerial Plant Surfaces. C. H. Dickinson and T. F. Preece, eds. Academic Press, London.
9. Dickson, J. G., 1956. Diseases of Field Crops. 2nd ed. McGraw-Hill, New York.
10. Eversmeyer, M. G., and Kramer, C. L. 1975. Air-spora above a Kansas wheat field. Phytopathology 65:490-492.
11. Eversmeyer, M. G., and Kramer, C. L. 1987. Vertical concentration of fungal spores above wheat fields. Grana 26:97-102.
12. Fokkema, N. J. 1976. Antagonism between fungal saprophytes and pathogens on aerial plant surfaces. Pages 487-506 in: Microbiology of Aerial Plant Surfaces. C. H. Dickinson and T. F. Preece, eds. Academic Press, London.
13. Hislop, E. C. 1976. Some effects of fungicides and other agrochemicals on the microbiology of the aerial surfaces of plants. Pages 41-74 in: Microbiology of Aerial Plant Surfaces. C. H. Dickinson and T. F. Preece, eds. Academic Press, London.
14. Jachmann, H.-Th., and Fehrmann, H. 1989. Effects of phyllosphere microorganisms on the senescence of wheat leaves. Z. Pflanzenkrankh. Pflanzenschutz 96:124-133.
15. Jooste, W. J. 1978. The mycoflora of wheat field debris. III. [*Ulocladium botrytis, Drechslera australiensis, Cladosporium macrocarpum,* Fungi]. Bothalia 12:437-440.
16. Jordan, M. M., Burchill, R. T., and Maude, R. B. 1990. Epidemiology of *Cladosporium allii* and *Cladosporium allii-cepae,* leaf blotch pathogens of leek and onion. II. Infection of host plants. Ann. Appl. Biol. 117:327-336.
17. Lamari, L., and Bernier, C. C. 1989. Evaluation of wheat for reaction to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. Can. J. Plant Pathol. 11:49-56.
18. Lamari, L., Bernier, C. C., and Smith, R. B. 1991. Wheat genotypes that develop both tan necrosis and extensive chlorosis in response to isolates of *Pyrenophora tritici-repentis.* Plant Dis. 75:121-122.
19. Magan, N., and Lacey, J. 1986. The phylloplane microflora of ripening wheat and effect of late fungicide applications. Ann. Appl. Biol. 109:117-128.
20. Manning, G. K. 1989. Distinguishing zinc deficiency from yellow spot in wheat by foliar symptoms. Queensl. Agric. J. (Mar-Apr):106-107.
21. Meunier, S., and Meyer, J. A. 1985. Modification de la flore épiphytique de feuilles de froment d'hiver suite à des traitements fongicides. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 50:1039-1043.
22. Olvang, H. 1988. Sensitivity of *Drechslera teres* and *Septoria nodorum* to sterol-biosynthesis inhibitors. Neth. J. Plant Pathol. 94:57-68.
23. Pfister, J. A. 1989. Incidence and control of fungal diseases and aphids on durum wheat. Gesunde-Pflanzenschutz 41:49-51.
24. Sallans, B. J., and Tinline, R. D. 1962. Splotch, a physiological leaf disease of wheat. I. Nutritional studies. Can. J. Plant Sci. 42:403-414.
25. Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. Technical note 101. Microbial ID, Inc., Newark, DE.
26. Shukla, R. 1987. Effect of atmospheric temperature, relative humidity and age of host plant on the development of Alternaria leaf blight of triticale. Indian J. Mycol. Plant Pathol. 17:335-336.
27. Smiley, R. W., Uddin, W., Zwer, P. K., Wysocki, D. J., Ball, D. A., Chastain, T. G., and Rasmussen, P. E. Influence of crop management practices on physiologic leaf spot of winter wheat. Plant Dis. In press.
28. Straib, W. 1935. Untersuchungen über erbliche Blattnekrosen des Weizens. Phytopathol. Z. 8:541-587.
29. Sundheim, L., and Aarvold, O. 1969. Inoculation experiments with the timothy eyespot fungus *Cladosporium phlei.* Meld. Nor. Landbrukshoegsk. 48(26):1-10.
30. Teng, P. S., and Shane, W. W. 1984. Crop losses due to plant pathogens. CRC Rev. Plant Sci. 2:21-47.
31. Tolstrup, K., and Smedegaard-Petersen, V. 1984. Saprophytic leaf fungi on barley and their effects on leaf senescence and grain yield. Växtskyddsnotiser 48(3-4):66-75.
32. Vanterpool, T. C. 1943. Head discoloration (non-parasitic). Page 7 in: Can. Dep. Agric., Can. Plant Dis. Surv., 1942.
33. Wiese, M. V. 1987. Compendium of Wheat Diseases. 2nd ed. American Phytopathological Society, St. Paul, MN.