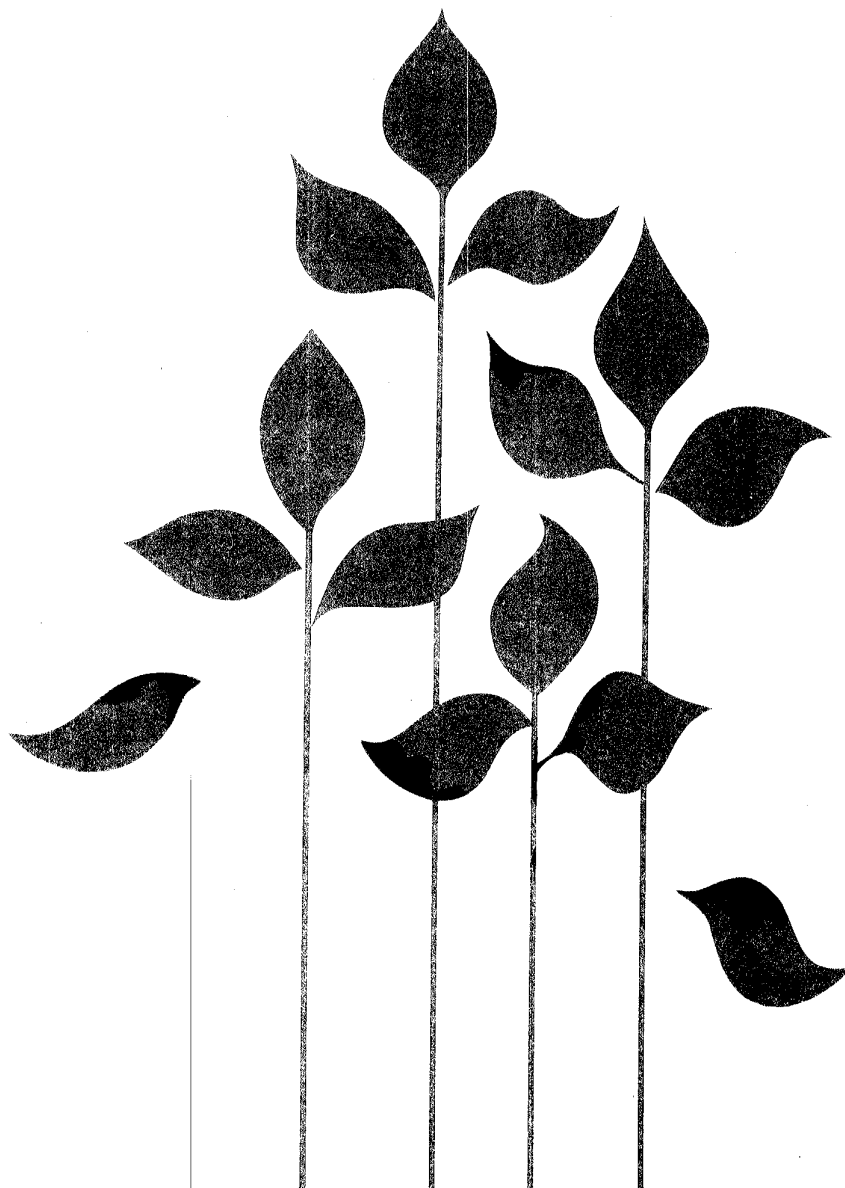


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The *Canadian Plant Disease Survey* is a periodical of information and record on the occurrence and severity of plant diseases in Canada and on the assessment of losses from disease. Other original information such as the development of methods of investigation will also be accepted. Review papers and compilations of practical value to plant pathologists will be included from time to time.

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L'Inventaire des maladies des plantes au Canada est un périodique d'information sur la fréquence des maladies des plantes au Canada, leur gravité, et les pertes qu'elles occasionnent. La rédaction accepte d'autres communications originales, notamment sur la mise au point de nouvelles méthodes d'enquête. De temps à autre, l'inventaire inclut des revues et des synthèses de rapports d'intérêt immédiat pour les phytopathologistes.

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ANNOUNCEMENT

Due to a recent policy change the Canadian Plant Disease Survey will no longer accept articles relating to the evaluation of new materials for disease control. Papers of this nature should be submitted either to the Pesticide Research Report, or if the research content is deemed sufficient, to a refereed journal.

THE EDITOR

Role of toxins in etiology of spot blotch disease of barley¹

Ross B. Pringle²

Filtrates of 46 different isolates of *Bipolaris sorokiniana* (*Helminthosporium sativum*), grown on liquid synthetic media, produced necrosis consistently on 27 different lines of barley but only on parts of detached leaves that had suffered previous mechanical damage; an effect that could be reproduced by solutions of organic acids. Chlorosis extending beyond the necrotic area was produced by some culture filtrates and occasionally by conidial inoculation; an effect that could be reproduced by methionine sulfoximine but not by organic acids. These separate and distinct effects suggest that two types of toxins may be involved in the etiology of spot blotch of barley.

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Les filtrats de 46 isolats différents de *Bipolaris sorokiniana* (*Helminthosporium sativum*) mis en culture dans des milieux synthétiques liquides ont provoqué de la nécrose sur 27 lignées d'orge, encore que seulement sur les parties de feuilles excisées qui avaient subi des dommages mécaniques (effet qui peut être produit artificiellement par des solutions d'acide organique). Des symptômes de chlorose s'étendant au-delà des plages nécrosées ont été obtenus par quelques filtrats et, à l'occasion, par inoculation au moyen de conidies (effet reproductible par la sulfoximine de méthionine mais pas par les acides organiques). Ces observations laissent soupçonner l'intervention distincte de deux types de toxines dans l'étiologie de la bipolariose de l'orge.

Introduction

Spot blotch of barley has been a chronic problem since the beginning of modern agriculture. In 1909, Pammel published the first description of a disease of barley characterized by irregular brownish lesions on the leaves and in 1910, with King and Bakke, described *Helminthosporium sativum* n. sp. as the inciting agent (11,12). Some 10 years later, Stakman established, by artificial inoculations, that *H. sativum* was the inciting agent, not only for spot blotch of barley, but also root rot and seedling blight of wheat and rye (18). As the area of cereal cultivation expanded, these diseases became increasingly troublesome and are now responsible for major losses.

Variation in this fungus has been studied for many years (21). In 1922, Christensen reported that a laboratory and greenhouse examination of plants infected by *H. sativum* did not yield consistent or reproducible effects (3). He found that leaf spot occurred usually as numerous dark-brown, oval or irregular blotches but that the appearance of the lesions was highly variable. Frequently, two or even more types and sizes of lesions appeared on the same leaf. He also stated that no general description of the morphological or cultural

characteristics of the fungus could be given because transfers from the same culture produced different types of growth and a wide variation in size of spores on different media and under different environmental conditions. Cook and Timian claimed that reproducible lesions appearing as small necrotic spots with chlorotic halos resulted when susceptible barley plants were incubated at 20°C with limited moisture, using inoculum prepared at one time, from only two isolates, and stored dry on talc at 4°C (4). Recognizing that reactions of whole barley plants fluctuated markedly to this fungus, Morton developed a procedure for infecting detached leaves in test tubes and found at 25°C or higher, under constant illumination, specimens became chlorotic and senescent much more rapidly than at lower temperatures (9). Mumford also inoculated detached leaves of barley in test tubes but at daily cycles of 20°C in the light and 16°C in the dark and did not report finding chlorosis although he noted dark pigment around the margin of lesions on resistant lines (10). Hrushovetz examined monoconidial and hyphal tip isolates and still found numerous physiological and morphological variants and found spores contained as many as 30 nuclei in a single cell, claiming that heterokaryosis with subsequent nuclear dissociation probably accounted for the abnormally high variability (8). In 1949, Tinline, Sallans and Simmonds reported the production of perithecia in paired monoconidial cultures of *H. sativum* (22) and the fungus was referred to as *Cochliobolus sativus* (Ito & Kurib) Drechsler ex Dastur (20). Hosford, Solangi and Kiesling examined 200 randomly isolated ascospores from a single cross of *C. sativus* and reported three to four genes were associated with virulence to barley (7). They found the most virulent progeny of this cross

¹ Contribution No. 1006 Chemistry and Biology Research Institute, Agriculture Canada, Ottawa, K1A 0C6. This is paper No. XVIII of a series on the phytopathogenic fungus, *Cochliobolus* (*Helminthosporium*, *Bipolaris*). For paper XVII see *Can. J. Bot.* 55, 1801-1806 (1977).

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produced large necrotic spots with chlorotic halos on barley and wheat but found fertility in mating varied among isolates and giving references where other workers reported low fertility, suggested that factors in addition to mating type limited sexual reproduction in this fungus (7).

Over the course of the last 20 years, it has become increasingly apparent that toxins play an important role in the etiology of plant diseases. The most striking examples involve cereals and the genus *Cochliobolus* [for historical references see part XVII of this series (14)]. In the case of the host-specific toxin produced by *H. victoriae*, the fungus which incites Victoria blight of oats, the toxin will produce all the symptoms characteristic of the disease; sensitivity to the toxin is correlated with susceptibility to the pathogen and toxin production by the pathogen is directly related to its ability to cause disease (16). The evidence that toxins are important pathogen-produced disease determinants in the root rot phase of *Bipolaris sorokiniana* (*H. sativum*) incited diseases has been summarized in a recent publication (14). Although no host-specific toxin, nor any great quantity of any toxic substance, was found in culture filtrates of 26 different isolates, the amount of the toxic metabolite, victoxinine, produced by these strains, may be correlated generally to their aggressiveness towards wheat (14).

Although the zone of chlorosis extending beyond the infection court of the fungus seen in spot blotch of barley strongly suggests that toxin also plays an important role in the etiology of this disease, among the numerous workers who have looked for toxins produced by *B. sorokiniana* only Gayed reported the effect of culture filtrates on aerial parts of barley (5). He found brownish necrotic spots and wilting where cut shoots were placed in undiluted sterile culture filtrates for 48 hr but did not report chlorosis; instead the shoots became dark green in color. All varieties tested, both susceptible and resistant to the disease, developed necrotic spots in 1957-58 but when other varieties were tested the following year, using filtrate from the same strain of the fungus, no necrosis was produced (5). In another test of filtrates involving 6 different isolates of the fungus Gayed found varying amounts of chlorosis in 3 of 6 tested as well as necrosis and wilting and claimed two different toxins were involved, one responsible for necrosis and the other for wilting (6). He suggested that chlorosis may be due either to a third toxin or to the interaction between the other two toxins or with the metabolites of the host tissues (6).

These observations and the appearance of the disease in the field (often a zone of sterile chlorosis extends beyond the infection court of the fungus) suggest strongly that a toxin plays an important role in the etiology of this disease. Also, in a general way, this pattern of leaf necrosis surrounded by sterile chlorosis bears some resemblance to the wildfire disease where a toxin has

been isolated (23) and characterized (19). However, to date, such a spot blotch toxin has not been isolated. To search systematically for this hypothetical toxin, it is necessary first to identify lines of barley, isolates of the fungus and conditions where chlorosis can be demonstrated consistently. Unfortunately, it has not been possible to satisfy these needs under greenhouse or growth room conditions, in Canada. It was hoped that a screening of detached leaves of representative lines of barley, tested with a variety of fungal isolates might indicate a combination where the effect could be reproduced by culture filtrates. In addition, although a number of toxic metabolites have been isolated from culture filtrates of *B. sorokiniana* (14), the effect of these substances on host leaves has not been studied systematically. To be meaningful, the production of these toxic materials must be related to disease susceptibility of the host and virulence of the pathogen producing them. The work reported here was undertaken in an attempt to develop a quick, simple test for susceptibility of barley lines to spot blotch. This would be very useful as an aid to breeding for resistance to this disease.

Materials and methods

The following lines of barley (*Hordeum vulgare*) were used in this study:

2-rowed

1. Centennial
2. Hector
3. Herta
4. Volla

6-rowed

5. OAC 21 C.I. 1470
6. Bonanza
7. Conquest
8. Gateway
9. Jubilee
10. Keystone Can 292
11. Larker
12. Manker M 16
13. Nordic
14. Olli
15. Paragon
16. Parkland
17. Swan Can 270
18. Galt
19. Beacon
20. Dickson

Although no clear cut susceptibility or resistance to spot blotch has been demonstrated, the following lines are generally considered to be quite susceptible in the field; Centennial, Conquest, Larker, Paragon, Galt. In addition to these commercial lines of barley the following lines, in process of being evaluated, were tested:

O.B. 123-34, O.B. 150-3, O.B. 160-3, O.B. 174-20,

O.B. 187-6, Q.B. 79-6, Q.B. 136-39.

These lines, became severely infected following inocula-

tion in growth chambers, although their reactions varied from test to test¹.

Seed of all plant cultivars was sown in 8'' plastic pots in soil-vermiculite mixture and grown in a growth chamber under 16 hr illumination per day. The plants were watered daily with Hoagland's solution. Wheat (*Triticum aestivum* cv. Manitou, Kharkov or Capelle) and oats (*Avena sativa* cv. Park, Rodney, or Garry) were included with the various barley cultivars. For testing, the youngest full leaf was cut with scissors from plants 2 to 3 weeks old, then recut near the basal end, under water, with a sharp scalpel.

Isolates of *Cochliobolus sativus* (Ito & Kurib) Drechsler ex Dastur identified as *Helminthosporium sativum* Pamm., King, and Bakke or as *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. used in this study included:

(a) Twenty different isolates from diseased leaves of barley exhibiting typical spot blotch lesions, collected from different fields in Eastern Canada.

(b) The 26 monoconidial or monoascosporic isolates from wheat and hosts other than barley, described in previous work (14).

These various fungal isolates were maintained on sterile barley straw on Fries' No. 3 basal agar without sucrose in Petri plates (15).

A number of different media were tried in an effort to demonstrate toxin production in culture. These included potato dextrose agar, Sach's agar, Tinline's minimal medium with various amino acid supplements (21), Fries' modified No. 3 basal medium (15), Fries' modified basal medium No. 3 without sucrose, using sterilized barley straw as a carbon source and fresh barley leaves autoclaved in Fries' No. 3 basal medium without sucrose.

The reaction of detached cereal leaves to spores or culture filtrates from the various fungal isolates was tested in 18 x 150 mm test tubes in high humidity growth chambers at 25°C under continuous illumination. Two ml of water or dilutions of the solution to be tested was placed in each tube. In certain tubes benzimidazole (50 µg/ml) was added for its cytokinin-like effect in prolonging the retention of chlorophyll by the leaves. Two leaves were placed in each tube of water or solution and were inoculated by touching a sterile needle, or sterilized cotton swab, dipped in a suspension of conidia (ca 10⁶/ml), prepared from slants that had grown at 25°C, 3 days in the dark followed by 2 days under fluorescent light, against the leaf about 1 cm above the surface of the water. This very drastic method of inoculation was used to bring out any differences in susceptibility or virulence, nevertheless care was taken to use the smallest amount of inoculum possible as sloughed off spores would drop into the solution and infect the leaves below the water line. Leaves of the

same cultivar, pricked with a sterile needle, were placed in water, as checks. The test leaves were examined at hourly intervals. Culture filtrates were tested by smearing a loopful of the dilution to be tested onto areas of 12 cm long barley leaves that had been scraped slightly to remove wax and to allow the liquid to adhere. Two treated leaves were placed in a tube with the scraped area 2 cm above the surface of water or benzimidazole solution. Leaves of the same cultivar, treated mechanically in a similar way with distilled water, were used as checks and all were examined at 24 and 48 hours. Another method of testing culture filtrates and solutions was to use barley culms, excised and freshly recut at the base under water. The excised culm was pricked with a sterile needle about 15 mm from the basal end and immersed into the solution to be tested with the needle prick about 10 mm below the surface of the liquid. The assays were read after 24 and 48 hours under continuous illumination and 100% humidity and were scored on a zero to four plus scale as follows:

- 0 = no lesion
- + = small lesion <1 cm with no chlorosis
- + + = large lesion >1 cm with some chlorosis
- + + + = chlorotic spot or halo
- + + + + = systemic chlorosis

For all these tests, 4 replicates were made per cultivar per treatment, repeated at least 3 times and in cases where chlorosis appeared, up to 10 times.

The crude culture filtrates of *H. sativum* isolates were subjected to the following series of tests in order to obtain information on the chemical and physical nature of the toxic factor(s): To measure the heat stability, the filtrates were boiled for 5 minutes and assayed in comparison with an unboiled sample of the same culture filtrate. To determine the volatility of the factor, 500 ml of culture filtrate was concentrated one tenth in a rotating vacuum evaporator and the residue and condensate were compared with an untreated sample of the same culture filtrate. To determine the molecular size of the toxic factor(s), 100 ml of the crude culture filtrate, in a 20 mm diameter dialysis sack, was dialyzed against running cold tap water and the nondialyzable portion was compared, after 15 minute intervals, with the original culture filtrate, taking into account the dilution produced by dialysis.

Culture filtrates were concentrated and fractionated by procedures similar to those already published (14,15,-16). The various fungal isolates were grown at 25 ± 1°C, in the dark, in 1000 ml lots of the different media (without agar) described above, in still culture in 5-1 Blake-type diptheria-toxin bottles, prepared, inoculated and handled as described previously (13,16). After incubation for 28 days, the culture solutions were harvested by filtration, first through several layers of cheesecloth and then through filter paper (Whatman No. 541). This filtrate was concentrated by evaporation under reduced pressure below 40°C in a rotating evaporator to 100 ml (1/10 original volume). This

¹ Dr. R.V. Clark, Ottawa Research Station, Agriculture Canada, personal communication.

concentrate was cooled in an ice bath and an equal volume of chilled methanol was added to precipitate protein and other high molecular weight constituents. The mixture was allowed to stand overnight at 5° C and the precipitated material was removed by filtration, washed with cold 50% methanol and resuspended in a small volume of water for assay. The filtrate from the methanol precipitation was further concentrated to 80 ml under reduced pressure to remove methanol. The solution was clarified by filtration through filter paper (Whatman No. 541) and extracted in a separatory funnel with low-boiling petrol ether (3 x 80 ml), ethyl acetate (3 x 80 ml), diethyl ether (3 x 80 ml) and n-butanol (3 x 80 ml). The solvents were removed by evaporation under reduced pressure and residues resuspended in 20 ml of water for bioassay and further processing.

The effect of known toxins was examined using the bioassays described above. Victoxinine prepared according to the procedures already published (13) was tested as the hydrochloride salt. Prehelminthosporol and 9-hydroxyprehelminthosporol, prepared according to the procedure of Aldridge and Turner (1) and L-methionine-DL-sulphoximine (Calbiochem.) were tested in aqueous solutions.

Results

After leaf inoculation, spores were visible at the inoculation site under a hand lens (14 power). Visible growth was seen after 24 hours as a brown patch of mycelium. After the brown mycelial colony had grown 2 or 3 mm across (these patches of growth were irregular in shape) occasionally a chlorotic halo appeared around the infection court which might extend 10 or 15 mm in diameter, and when it did so, usually followed the transpiration stream to form a chlorotic streak distal to the lesion, occasionally extending to the tip of the leaf. The production of chlorosis was an erratic event and was not particularly consistent or repeatable.

Results were more consistent when culture filtrates were used but it was very rare that a +++ or ++++ reaction occurred. When culms, cut under water, were placed in serial dilutions of culture filtrate, pronounced necrotic flecks appeared under the surface of the solutions surrounding the needle puncture. This was seen at dilutions of 1:2, 1:4 and 1:8 but not at 1:16 or higher dilutions. In these experiments there was no sign of chlorosis or evidence of toxin being taken into the transpiration stream. The necrotic area was generally 2 or 3 mm in diameter after 24 hours, spreading from the needle prick. Necrosis also spread from the cut base of the culm. Under low power magnification (14X) the necrotic spot was seen to consist of collapsed and empty cells. The border of the necrotic spot was sharp and well defined, sometimes being surrounded by a fringe of dark green. When the bioassays were carried out in the dark, the culms became somewhat etiolated so the fringe of dark green surrounding the necrotic fleck became more noticeable. Controls did not show such necrosis.

The growth of *B. sorokiniana* in culture was extremely variable as noted by previous investigators (3,12,18,-21). In addition to changing color and colonial morphology, sectoring frequently and growing at variable rates, some strains grew preferentially along the edges of the agar, following the interface between agar and glass. However, this adherence to glass like so many other characteristics of the fungus was variable and did not occur consistently.

No consistently different responses could be detected when the different cereal lines and cultivars were inoculated with spore suspensions. In some cases fungal growth was confined and dense with minimal damage to the leaf as a whole. In other cases fungal growth spread thinly over the leaf surface and necrosis with chlorosis was wide spread. However, the results were not consistently reproducible. Detached leaves of wheat and oats responded in a manner similar to barley, although perhaps there was a tendency for fungal growth to be more confined and dense. However, some of the barley cultivars were as little affected in some trials. Particular attention was paid to the cultivar Centennial, which is considered susceptible to spot blotch. Although the fungus spread readily over Centennial leaves, in most tests no quantitative differences could be detected when compared to Beacon or other cereals in paired tests. After growing on autoclaved barley straw suspended in Fries's medium without sucrose, each of the 46 isolates of *H. sativum* tested was capable of invading and damaging the leaves of all the barley, wheat and oat cultivars tested. No consistent differences in virulence could be detected. Although growth of strains freshly isolated from naturally diseased barley was more vigorous in culture with more abundant sporulation, if care was taken to use an equivalent number of spores in the inoculation, no differences in infectivity could be detected between freshly isolated strains and those that had been maintained in culture for 30 years.

The effect of culture filtrates from each of the isolates grown on the various liquid media described showed no noticeable differences. After the fungus had made appreciable growth in the medium, the necrosis and occasional chlorosis described appeared, at dilutions of the culture filtrate up to 1:8. Growth varied according to the history of the isolate. Fungi, freshly isolated from naturally diseased plants, grew more rapidly in liquid culture than old specimens that had been passed through culture for many years. The crude culture filtrates of *H. sativum* appeared to contain at least two kinds of toxins that damaged barley leaves. Necrosis was produced by all isolates under all conditions; chlorotic was not produced consistently by any isolate under any of the conditions tested.

Using the bioassay of necrosis described above, making readings at 24 and 48 hours under constant illumination, the nature of the agent responsible was studied. It was found to be heat stable because boiled and fresh filtrates evoked similar responses up to 1:8 dilution but not at 1:16 dilution. The toxicity of a crude culture

filtrate was not diminished by concentration to 1/10 its original volume. The original culture filtrate was active at a dilution of 1:8 and after concentrating to 1/10 volume, was equally active at a dilution of 1:80 when tested at the same time under similar conditions. The condensate from the evaporation showed no toxicity when tested full strength. The toxicity of culture filtrates was quickly lost on dialysis. After one hour of dialysis, no toxicity was found at full strength in a sample of the solution taken from inside the dialysis sack.

When 1000 ml lots of the different culture filtrates were processed as described, a complete retention of activity was found after concentration, methanol precipitation and reconstitution. No toxicity was found associated with the high molecular weight fraction. Leaves of barley tested in a concentrated suspension of this material showed no signs of toxicity. No activity was extracted from the concentrated culture filtrate by petrol-ether and the very small amount of oily residue remaining after evaporation of the petrol-ether was not toxic. Less than 25% of the activity of the concentrate of culture filtrates was extracted by ethyl acetate and no further loss of activity occurred on subsequent extraction with diethyl-ether. The remainder of the activity was extracted by n-butanol. When the extracted aqueous remainder was concentrated under reduced pressure to remove butanol and bioassayed full strength, no toxicity was found. When the residue remaining after evaporation of the butanol extract was resuspended in a volume of water equal to the original concentrate (80 ml) and compared to an aliquot taken from the original concentrate before extraction, it was found that about 1/2 the original toxicity was recovered from the butanol extract.

Known toxic metabolites from *B. sorokiniana* did not account for either the chlorotic or necrotic effects shown by culture filtrates. Victoxinine-HC1 tested at 2×10^{-3} M (10 times the concentration required to produce 50% inhibition of the rate of growth of barley roots) did not produce any visible changes in barley, wheat or oat leaves. A crude mixture of prehelminthosporol and 9-hydroxyprehelminthosporol (10 mg/ml) was likewise without effect. Large chlorotic halos were produced by an aqueous solution of L-methionine-DL-sulphoximine. A very dilute solution (1×10^{-6} M) produced chlorosis which extended to 50% of the leaf area by 96 hrs.

Material obtained from the residue after evaporating the butanol extract was active down to a concentration of 2.8 mg/ml. When the solution was adjusted to a pH of 8.4 with NaHCO_3 , the material was no longer toxic but toxicity returned when the pH was lowered to 3.5 with 0.1 N HC1. The material passed through a dialysis membrane and was stable to autoclaving for 30 minutes at 15 lb/sq in. Toxic activity was retained for 6 months at least when the material was stored in aqueous solution at 4°C.

The effect of common organic acids was examined along with the toxic substance obtained from the butanol extract. Acetic acid was active down to a concentration

of 4×10^{-2} M (3.1 mg/ml). Tartaric acid was more active; 1.7×10^{-3} M (1.25 mg/ml); as was citric acid, 4×10^{-3} M (0.78 mg/ml).

Discussion

A microorganism is capable of inciting infectious disease if it produces substances which have been defined as disease determinants (17). The most easily recognized of these disease determinants are host-specific toxins which have been found associated with strains of pathogens causing severe diseases of specific host genotypes. These toxin-producing strains have caused dramatic epiphytotics, usually involving newly introduced cultivars, quite distinct from the chronic fungal diseases that plague agricultural crops (16). The etiology of these chronic infections, such as root rot and spot blotch, is more difficult to unravel than the sudden, acute episodes.

Circumstantial evidence had indicated that toxins were important in the spot blotch phase of *H. sativum* infection of barley. The present study with that of Gayed (5,6) indicates that at least two toxins may be involved. Because necrosis was produced consistently by all isolates of the fungus and by all culture filtrates; an effect that could be reproduced by organic acids, the involvement of an acidic necrosis-producing toxin may be presumed. Because chlorosis was produced occasionally by filtrates both in this and work previous and because the extent of chlorosis was not proportional to the extent of necrosis (large chlorotic areas were observed around relatively small necrotic areas) the presence of a second chlorosis toxin may be presumed. Because no concentrated necrosis-producing fraction or any organic acid tested produced chlorosis, the two effects may be presumed to be due to two separate toxins. It was also noted that methionine sulfoximine produced massive chlorosis with very little necrosis. Contrary to the findings of Gayed (5,6) no wilting was noted in these experiments. This is due probably to the high humidity and senescence-inhibiting action of benzimidazole used. Because wilting could be the result of damage caused by either or both of the toxins described, it does not seem necessary, on the basis of present knowledge, to postulate a third, wilting toxin.

In any case, in this disease, it appears that these postulated toxins would be the sort that are generally regarded as secondary determinants which increase the severity of a disease but are not absolutely necessary for its inception (17). Toxins which are acidic are fairly common (2). The best known are fusaric acid produced by *Fusarium oxysporum* and alternaric acid produced by *Alternaria solani*. Acids released from the vacuoles of plant cells disrupted by the action of other toxins may contribute to further plant cell damage. The growing fungus may select preferentially the basic nitrogenous bases from electrolytes released from damaged cells to satisfy the nitrogen requirements of the growing mycelium. This would also contribute to a lowering of the pH in the environment where this takes place. Neither can a

chlorosis-producing toxin be regarded as a primary determinant (17) because lesions and disease are common in the field without it and it has not been possible to produce consistent chlorosis experimentally. In fact, only very rarely has chlorosis been produced by culture filtrates. The reason for this is not apparent although observations suggest wild type fungi, freshly isolated from diseased plants in the field, are more apt to produce chlorosis than cultures that have been grown for any length of time on artificial media. Presumably, the ability to produce toxin is very quickly lost in culture, possibly because of the heterokaryotic nature of the fungus. Because toxin production is an energy-requiring process that is not needed in culture, other variants with lesser energy requirements might quickly outgrow the toxin producers and become dominant.

Chlorosis-producing toxins are commonly produced by phytopathogenic bacteria. These are usually non-specific in nature. Although the bacterium *Pseudomonas tabaci* will only attack tobacco, the isolated toxin, tabtoxin, will produce chlorosis when tested on the leaves of any plant. Also the synthetic compound, methionine sulfoximine will produce chlorosis on many different leaves, including barley. There is, as yet, no evidence that *H. sativum* produces similar compounds and other nitrogenous bases produced by *H. sativum* do not cause chlorosis on barley leaves. Another disease determinant that may be of importance in the etiology of spot blotch of barley, the sporeling adhesion factor, will be the subject of a future communication.

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Cleistothecia of *Sphaerotheca macularis* on strawberry leaves in Nova Scotia

C. O. Gourley

Cleistothecia of the powdery mildew fungus, *Sphaerotheca macularis*, were found on leaves of the strawberry cultivar Micmac in September 1978. This is a new record for Nova Scotia and the only previous report of cleistothecia on this host in Canada was from British Columbia.

Can. Plant Dis. Surv. 59:4, 80, 1979.

Des cleistothèces de *Sphaerotheca macularis*, champignon de l'oidium (blanc) du fraisier, ont été observées en septembre 1978 sur les feuilles du cultivar Micmac. C'est la première fois qu'on constate cette forme en Nouvelle-Ecosse; auparavant la seule autre province au Canada où l'on avait signalé la présence de cleistothèces était la Colombie-Britannique.

In Nova Scotia the *Acrosporium* (= *Oidium*) conidial state of the powdery mildew fungus, *Sphaerotheca macularis* (Wallr. ex Fr.) P. Magn., occurs annually on many of the strawberry cultivars used for commercial production. Mildew symptoms are most noticeable on the foliage and infections often become severe during August and September. Cleistothecia, the ascocarps of *S. macularis*, have been reported on strawberry leaves in Canada only from British Columbia, DAOM 118253, (2).

Strawberry cultivars vary in their susceptibility to powdery mildew and in the expression of symptoms on leaves. On some cultivars the first evidence of mildew is a reddish or necrotic, generally circular area on the surface of the leaf resulting from aggregated subsurface infections. On other cultivars the only evidence of infection is fungal mycelium ramifying over the undersurface of the leaf. The mycelium may aggregate on the undersurface and produce conidia in the vee areas formed by the junction of leaf ribs. On the most susceptible cultivars, conidia may form anywhere on the undersurface of the leaves.

In September, 1978, throughout the province, cleistothecia of *S. macularis* occurred in abundance on the undersurface of powdery mildewed leaves of Micmac, a recently introduced cultivar from the Kentville breeding program (1). Micmac foliage provided an ideal substrate for the formation and maturation of the cleistothecia of this obligate parasite, a phenomenon not observed on other cultivars in this province. Although the conidial state of *S. macularis* occurs wherever strawberries are grown, this is the first report of the formation of cleistothecia on strawberry leaves in Nova Scotia.

Specimens were deposited in the mycological herbarium at the Biosystematics Research Institute, Ottawa, as DAOM 170962 and 170963.

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Leaf blotch on Laurier barley

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An unusual leaf blotch has been observed on Laurier barley at several locations in eastern Canada. It was prevalent in late June and early July 1976 when high daytime temperatures and, in Ontario, high relative humidity levels were experienced. Other cultivars were free from the leaf blotch symptoms. The characteristic blotches appeared as irregular clusters of small necrotic spots surrounding a central brown spot, sometimes giving the appearance of an eyespot lesion, with varying amounts of chlorotic or light brown tissue. Attempts to identify a causal agent and to reproduce the symptoms by inoculation with affected leaf tissue were unsuccessful. Somewhat similar symptoms have been noted in certain barley cultivars affected by various stresses under conditions of high humidity. Examples include Laurier affected by powdery mildew, Perth affected by physiological brown spot, and Vanier treated with maneb or pirimicarb.

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Des symptômes exceptionnels de taches foliaires ont été observés sur le cultivar d'orge Laurier à plusieurs endroits de l'est du Canada. Ils étaient très répandus en fin de juin et au début de juillet 1976, période caractérisée par des températures diurnes chaudes et, en Ontario, par des taux d'humidité relative élevés. Les autres cultivars ne portaient pas ces symptômes. Les taches se présentaient en grappes irrégulières de petits points nécrotiques entourant une plage centrale brune, et lui conférant parfois un aspect de lésion ocellée, le tout accompagné de plus ou moins de tissus chlorosés ou brun pâle. Les essais d'identification de l'organisme causal ou de reproduction des symptômes par inoculation au moyen de tissu foliaire infecté se sont révélés vains. On a constaté des symptômes approchants chez certains cultivars d'orge exposés à divers stress en milieu de végétation très humide. Il y a, notamment, les cas de Laurier à la suite d'infection d'oïdium (blanc), de Perth atteint de la tache brune d'origine physiologique et de Vanier sous l'effet de traitement au manèbe ou au pirimicarbe.

In 1976 an unusual leaf blotch was observed on the recently released 6-rowed barley (*Hordeum vulgare* L.) cultivar Laurier (4) at several locations in eastern Canada. The leaf spot most commonly found on barley in the field in eastern Canada is spot blotch caused by the fungus *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur, stat. imperf. *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. Symptoms of spot blotch on leaves include dark brown, elongate spots with definite margins, the spots often coalescing to form irregular stripes (Fig. 1A). The blotches occurring on Laurier appeared first as clusters of small necrotic flecks or spots, usually surrounding a central dark brown spot (Figs. 1B, 1C). As the blotches enlarged the necrotic spots often formed a more definite ring and the surrounding tissue became chlorotic (Fig. 1E) or light brown (Fig. 1C). The blotches occurred on leaves of all ages but were more prominent on upper leaves.

In Ontario the condition was seen first at Ottawa in mid June in test plots of Laurier barley when plants were at the heading stage. At Brantford, the condition was prominent during the first week of July at the milky ripe stage of growth in a 0.5 ha planting of Laurier located within a large field of Herta barley; similar plantings of

Conquest and Vanier barley in adjacent blocks and of Herta in the large field were free from the symptoms. The unusual symptoms were also observed in test plots of Laurier at Kippen, Elora, and Arkell but were not seen at Douglas, where only typical spot blotch symptoms were observed (Fig. 1A). Late in the growing season spot blotch and barley yellow dwarf became severe on all cultivars, and it was much more difficult to distinguish symptoms at that time. Consequently the effect of the blotch on yield could not be assessed. The unusual symptoms were not seen on Laurier in Ontario in 1977 but were evident in trace amounts in 1978.

In late June and early July 1976 a similar condition was evident in plot and field plantings of Laurier barley in Prince Edward Island and Nova Scotia (Figs. 1D, 1E). As in Ontario, the blotch symptom (referred to locally as "splat") was common on and unique to Laurier. Other leaf spots were scarce in the Maritimes in 1976 so that this symptom was particularly striking. In 1977 the symptom was very rare on Laurier in P.E.I., but it was present in a plot of the cultivar Zumpakei (C.I. 692). Christensen (1) in 1933 also described symptoms on Zumpakei that were similar to those found on Laurier in Ontario and P.E.I. in 1976. In Quebec similar symptoms, described as large concentric lesions, were observed in Laurier in 1976 and 1977 (G.J. Pelletier, personal communication).

Isolation and inoculation tests

Attempts to isolate pathogenic fungi and bacteria from surface sterilized Laurier leaves showing blotches were

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unsuccessful. Under moist conditions *B. sorokiniana* sporulated profusely on the surface of many of the older senescent leaves from Ontario plants but did not develop on green leaves showing the unusual blotch symptoms. Samples of the leaf tissues and of sap expressed from green leaves with blotches were examined with the transmission electron microscope but no evidence of causal agents was detected.

In a series of tests conducted in growth cabinets (light period 16 h, 32,000 lux, 22°C; dark period 8 h, 16°C) affected leaves of various ages from Ontario fields were placed on the surface of a soil potting mixture in pots of Laurier and Vanier barley plants at the 4- to 5- leaf stage. Some of the test plants were enclosed in polyethylene bags for 4-6 days to provide conditions of high relative humidity and elevated temperatures. No symptoms developed in test plants growing in proximity to detached green leaves showing only the unusual blotch symptom, and there was no indication of *B. sorokiniana* development on the detached leaves. However in enclosed pots containing detached senescing leaves, symptoms of spot blotch developed on the test plants and *B. sorokiniana* sporulated profusely on the detached leaves. In some of the enclosed pots containing older necrotic leaves, powdery mildew (*Erysiphe graminis* DC. ex Mèrat) developed on susceptible test plants; in a few leaves of Laurier the mildew-affected areas expanded to form light brown blotches surrounding a central dark brown spot (Fig. 1F). Plants not enclosed in polyethylene bags were free from symptoms except for the development on a few plants of symptoms similar to physiological brown spot described by Dickson (2).

In inoculation tests at Charlottetown, blotched leaf tissues from field-grown Laurier barley were ground up with distilled water and the suspension was sprayed on plants of Laurier barley in the greenhouse. Similar preparations from leaves showing no symptoms were used as checks. The plants were kept at 23°C and 60-75% relative humidity (RH) during the day and at 15°C and 98% RH during the night. No symptoms developed on any of the test plants.

Other blotch symptoms

Other unusual leaf blotches have been observed recently at Ottawa in barley cultivars in the field, greenhouse, and growth room following application of pesticides and/or exposure to periods of high humidity. In field plots of Vanier barley, blotch symptoms (Fig. 2A) were observed in late June 1977, a few days after the plants had been sprayed with maneb 80% WP, 7.3 kg active ingredient (a.i.) per hectare, at the boot stage. The fungicide application was preceded and followed by several periods of wet, humid weather accompanied by high daytime temperatures; blotch symptoms developed only on the lower leaves and none developed on unsprayed plants or later when maneb was applied under less humid conditions.

In a greenhouse test, similar symptoms developed at heading time on plants of Vanier barley that had been

sprayed a few days earlier with the insecticide pirimicarb 50% WP, 0.25 g a.i. per litre, for aphid control. Following treatment, one group of plants was kept on a greenhouse bench, another was exposed to one 36 h period of 100% relative humidity at the late tillering stage, and a third group was exposed to two periods of high humidity, at the late tillering stage and 10 days later at the early flowering stage. Greenhouse temperature ranged from 15.6°C to 24°C but occasionally reached 38°C for short periods. Blotch-like symptoms developed on Vanier plants in the three groups but were more pronounced on plants exposed to the two periods of high humidity (Fig. 2B). Plants of three other cultivars in the test, Beacon, Bonanza, and O.B. 123-34, did not develop symptoms.

Recently in growth room plantings of the barley cultivars Massey and Perth, all plants developed nonparasitic brown spot symptoms (Figs. 2C,2D) at heading time under standard growing conditions. Plants of Perth that were placed in polyethylene bags for 4 days prior to heading to provide high humidity developed unusual leaf blotch symptoms (Figs. 2E,2F), but plants of Massey did not.

Discussion

The purpose of this report is to illustrate the unusual symptoms observed recently in Laurier and other barley cultivars growing under a variety of conditions. While at present no positive explanation of the factors responsible for the leaf blotches is evident, our observations suggest that the unusual symptoms are expressed by certain cultivars under conditions of variable temperature and high humidity. Factors triggering development of these blotches apparently may be parasitic or nonparasitic.

Physiological disease problems are often associated with unusual weather patterns. In Ontario records for the Douglas, Ottawa, Brantford, and Elora areas indicate that the weather in early June 1976 was very dry and hot; however, from mid June to mid July rainfall, humidity, and daytime temperatures were unusually high and night-time temperatures were low, especially at Brantford during the 4th week of June. In Prince Edward Island and Nova Scotia, the June 1976 weather was also hot and dry while July continued hot but with considerable rain; in 1977 all locations were generally cooler and wet, especially toward the end of the growing season.

Extensive studies by Christensen (1) indicated that nonparasitic leaf spots in barley could be cultivar specific, as in the case of the cultivar Zumpakei, but that they could also be related to nutrition and soil toxicity. None of the cultivars or lines grown with Laurier in the field in 1976 developed similar blotch symptoms, indicating that the reaction of Laurier to an unidentified stress, possibly mildew, is specific to that cultivar. However, the differential reactions observed with other cultivars under conditions of high relative humidity suggest that similar symptoms may be induced in cultivars such as Vanier and Perth.

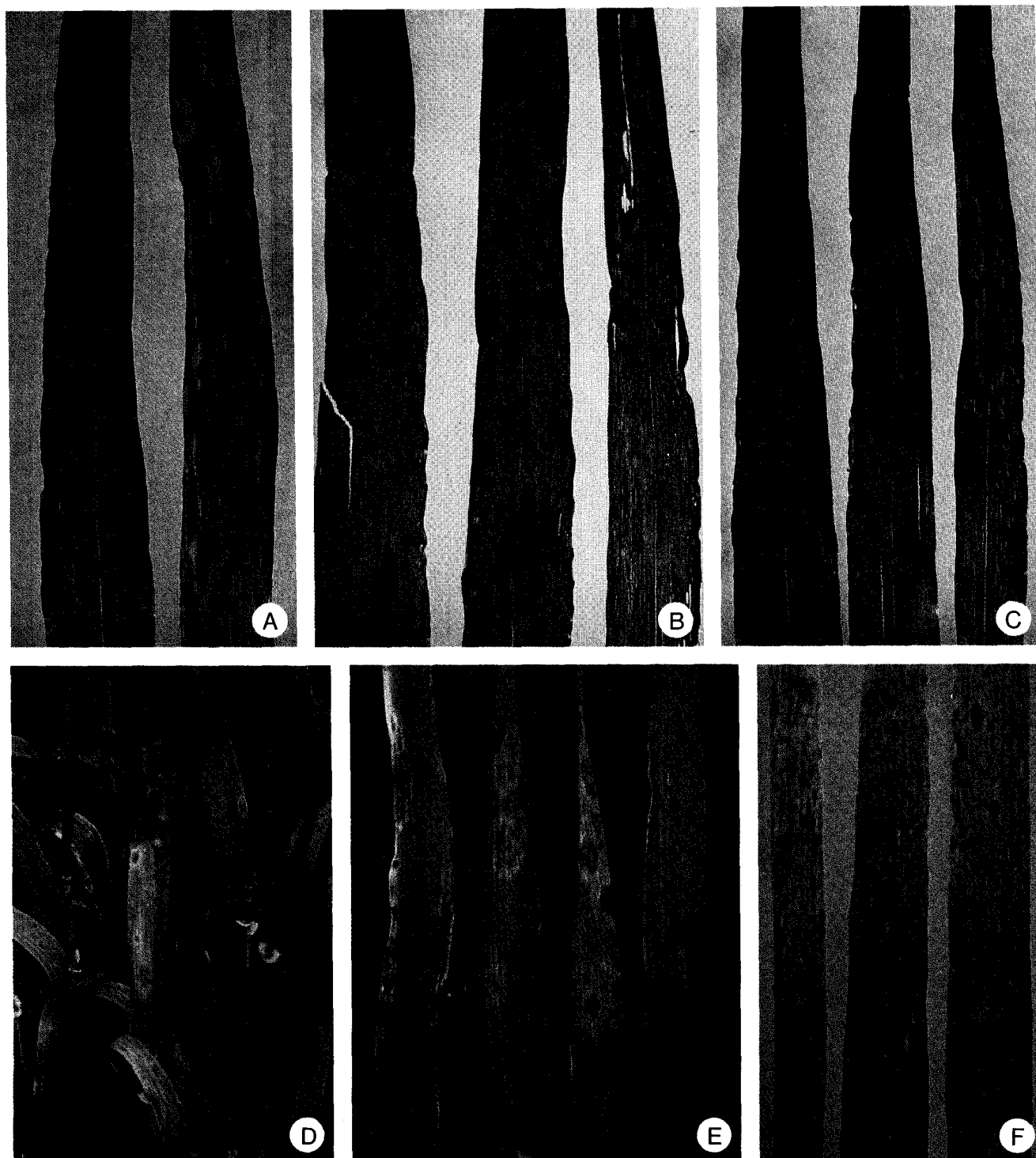


Figure 1 (A to F). (A) Spot blotch (*Cochliobolus sativus*) on Laurier barley, Douglas, Ontario, July 1976. (B and C) Leaf blotch on Laurier barley, Brantford, Ontario, July 1976. (D and E) Leaf blotch on Laurier barley, Charlottetown, P.E.I., July 1976. (F) Laurier barley affected by powdery mildew in a growth cabinet. Under conditions of high humidity many lesions included an area of chlorotic or light brown necrotic tissue not usually associated with the disease, and some of the lesions enlarged to form light brown blotches around atypical dark necrotic spots.



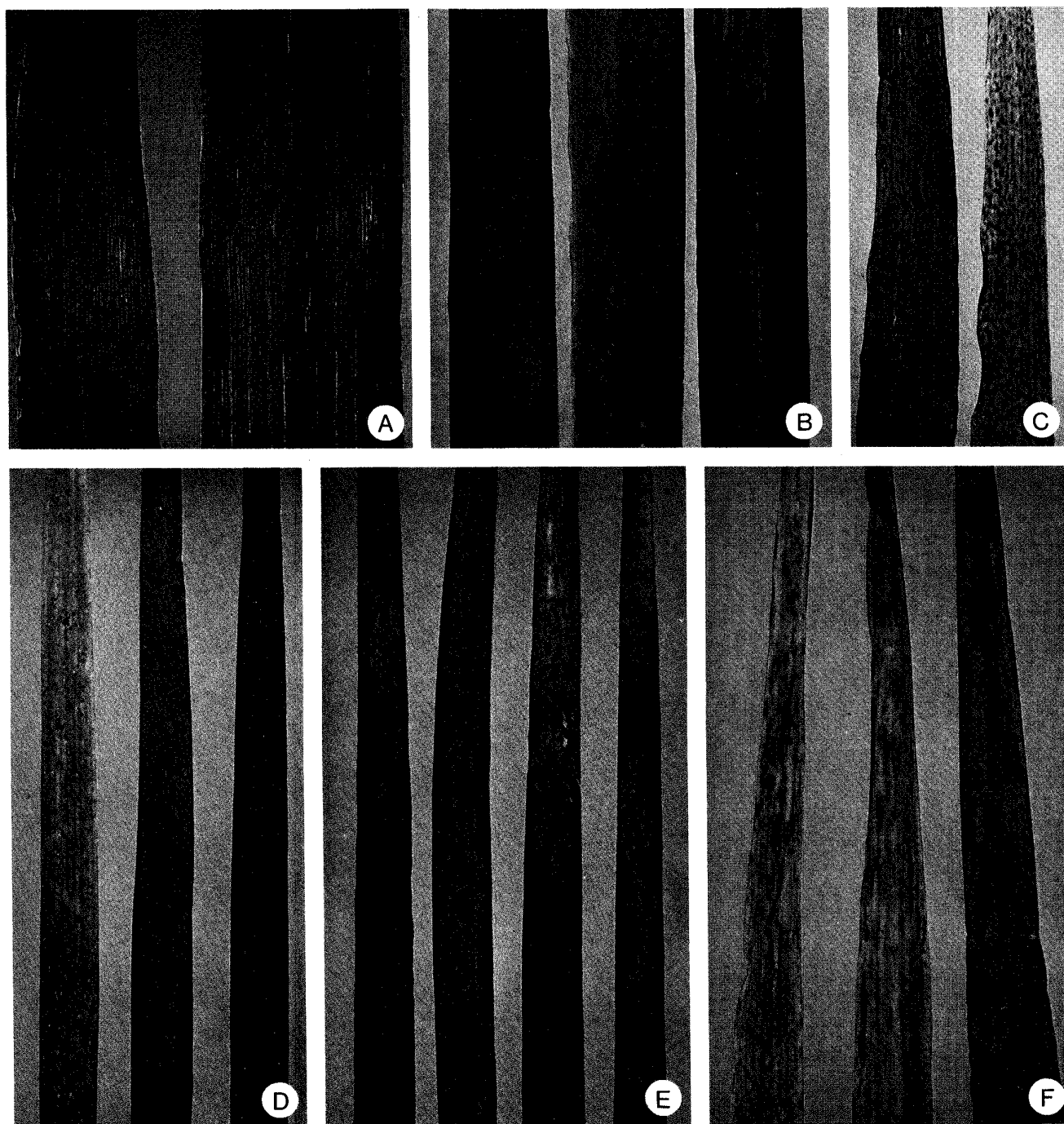
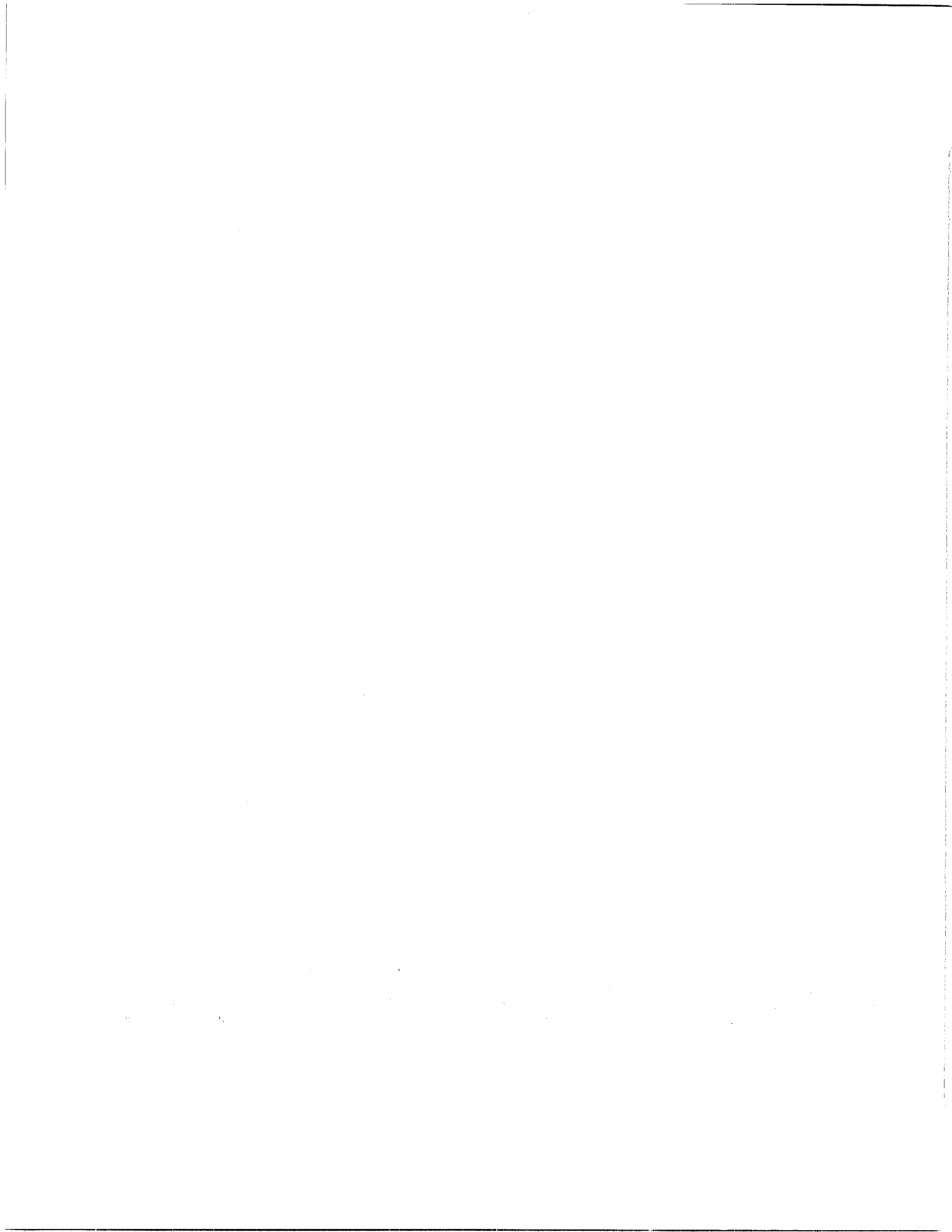


Figure 2 (A to F). (A) Leaf blotch on Vanier barley sprayed with maneb fungicide in field plots. (B) Leaf blotch on Vanier barley sprayed with pirimicarb insecticide and subsequently exposed to two periods of high relative humidity in the greenhouse. (C and D) Physiological brown spot on (C) Massey barley and (D) Perth barley at time of heading under standard growth cabinet conditions. (E and F) Leaf blotch on Perth barley grown under the same conditions as those in (D) except that plants were exposed to a 4-day period of high humidity just prior to heading. Similar blotches did not develop on Massey.



Recently Faris (3) reported that nonparasitic spotting of barley leaves before and after heading has considerable effect on seed yield, especially if the spotting is severe following heading. Thus the extensive blotching of upper leaves observed on Laurier in eastern Canada in 1976 may have had considerable effect on plant development and subsequent seed yield.

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