Etiology of Cephalosporium stripe in relation to the expression of resistance in cultivars of winter wheat (Triticum aestivum L.)
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A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Plant Pathology
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Abstract:
Systemic spread of the vascular pathogen, Cephalosporium gramineum was characterized relative to anatomical and developmental features of its winter wheat (Triticum aestivum L.) host. The pattern of foliar chlorotic striping and the distribution of fungal cells in vascular bundles within consecutive nodes were examined at various growth stages of winter wheat. Pathogen movement appeared to be restricted by xyIem maturation gradients between internodes, within nodes, and within leaves. Restriction of symptom development above that imposed by maturation gradients was exhibited by the winter wheat cultivar Crest LRC 40, This response was attributed to increased gelation and gummo-sis within the xyIem and/or to inhibition of fungal speculation. A descriptive model was developed that related pathogen invasion and colonization to symptom expression. All evidence suggested that this pathogen is incapable of actively penetrating living cells at any time during the disease cycle. The number and pattern of vascular bundles in different winter wheat cultivars and the temporal association between pathogen colonization and symptom induction formed the basis for a disease index rating system. Leaves were quantitatively rated on a scale of one to eleven, with one denoting one stripe per leaf and eleven denoting complete chlorosis. Both the movement and distribution of C. gramineum and the disease index rating system have practical implications in a germplasm development program. The first emphasizes the need to separate rate of disease development from rate of host development in evaluating winter wheat genotypes. The second provides a valuable tool for monitoring symptom expression within and among plants of different genotypes.

The pattern of stripe formation on Cephalosporium gramineum infected flag leaves of the susceptible winter wheat cultivar Marias was closely correlated with depression of relative water content, conductance, net photosynthesis, and chlorophyll content. All measurements were made in the field from paired healthy and infected plants. Regression analysis indicated that all four physiological parameters were interrelated, providing evidence that stripe formation coincides with localized restriction of lateral water movement, reduction in transpiration rate, suppressed photosynthetic activity, and loss of chlorophyll. Chlorosis around colonized vascular bundles is therefore attributed to effects of localized water deficits rather than a diffusible toxin. Highly significant correlations between all four parameters and the disease index suggested that visual scoring of infected leaves is an accurate indicator of physiological effects of symptom expression. The influence of pathogenesis on vegetative and reproductive growth patterns was followed throughout the ontogeny of three winter wheat cultivars, Marias, Crest LRC 40, and P.I. 278212. Internode elonaation was inhibited, but leaf exnansion remained unaffected by disease. Differential responses between stem and leaves was ascribed to the relationship between pathogen movement and host xyIem maturation gradients. Spikelet number was unaltered, seed number was reduced in Marias and P, I . 278212, and thousand kernel weight was sharply reduced in Marias and P. I . 278212 but only moderateIy reduced in Crest LRC 40. Thus, the effects of this disease are not pronounced until after anthesis during grain filling. Duration of photosynthesis, as measured by averaging COg exchange of ten flag leaves of each cuI -tivar over a 35 day period after anthesis, appeared to play a major role in seed weight reduction. Both Marias and P.I.

278212 are highly susceptible to Cephalosporium stripe based on reduction in seed weight, whereas Crest LRC 40 is more resistant.

Seven winter wheat cultivars representing established varieties, as well as selections from the USDA World Collection, were examined to identify possible phenotypes expressing resistance to Cephalosporium gramineum. Two types of resistance were observed: (l) exclusion of the pathogen such that successful colonization was prevented and (2) restriction of systemic spread of the pathogen following successful colonization of the host. The former was expressed as a reduction in the percentage of diseased plants. The latter was expressed as a reduction in the percentage of diseased tillers per infected plant and also as a reduction in the rate and severity of disease development. Both types of resistance were expressed independently. P.I. 278212 exhibited a low infection percentage, but was rapidly and completely invaded after successful ingress. Crest LRC 40, on the other hand, demonstrated a high percentage of diseased plants, but showed restricted infection between tillers and a moderate rate of systemic invasion. Each phenotype may be identified and evaluated separately if seeding rates are set to permit recognition of individual plants. Maximum resistance would be attained if both types of resistance were incorporated into a single agronomical Iy desirable genotype. Infection among plants occurred only in soils undergoing frost-heaving, suggesting that root breakage was essential for ingress of the fungus into the host. Differential responses between cultivars to pathogen exclusion could not be attributed to gross changes in either propagule levels in the soil or root mass. Cumulative effects of microhabitat interactions between the soil-root interface or differential responses to woundhealing are suggested as possible explanations for dissimilarities between cultivars.

ETIOLOGY OF CEPHALOSPORIUM STRIPE IN RELATION TO THE EXPRESSION OF RESISTANCE IN CULTIVARS OF WINTER WHEAT (*TRITICUM AESTIVUM* L.)

by

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Approved:

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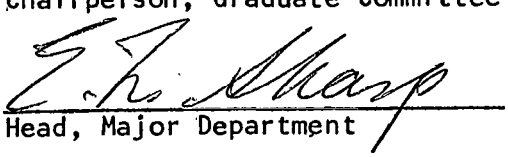

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ABSTRACT

Systemic spread of the vascular pathogen, Cephalosporium gramineum was characterized relative to anatomical and developmental features of its winter wheat (Triticum aestivum L.) host. The pattern of foliar chlorotic striping and the distribution of fungal cells in vascular bundles within consecutive nodes were examined at various growth stages of winter wheat. Pathogen movement appeared to be restricted by xylem maturation gradients between internodes, within nodes, and within leaves. Restriction of symptom development above that imposed by maturation gradients was exhibited by the winter wheat cultivar Crest LRC 40. This response was attributed to increased gelation and gummosis within the xylem and/or to inhibition of fungal sporulation. A descriptive model was developed that related pathogen invasion and colonization to symptom expression. All evidence suggested that this pathogen is incapable of actively penetrating living cells at any time during the disease cycle. The number and pattern of vascular bundles in different winter wheat cultivars and the temporal association between pathogen colonization and symptom induction formed the basis for a disease index rating system. Leaves were quantitatively rated on a scale of one to eleven, with one denoting one stripe per leaf and eleven denoting complete chlorosis. Both the movement and distribution of C. gramineum and the disease index rating system have practical implications in a germplasm development program. The first emphasizes the need to separate rate of disease development from rate of host development in evaluating winter wheat genotypes. The second provides a valuable tool for monitoring symptom expression within and among plants of different genotypes.

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INTRODUCTION

Cephalosporium gramineum, causal agent of Cephalosporium stripe, is the only fungal vascular pathogen of winter wheat. Consequently, study of the fungus, the winter wheat host, and most of all, the host-pathogen interaction is required to characterize etiological and epidemiological relationships of this disease.

The Pathogen. Most studies on the biology of *C. gramineum* have concerned (1) its morphological, cultural, and nutritional habit to disease incidence and development, and (2) its ability to colonize and inhabit plant residues in the soil. The first affects the pathogen's capacity to sporulate and invade the host's vascular network. The second influences the pathogen's overwintering ability, which determines inoculum potential in the soil from one growing season to the next.

C. gramineum is an imperfect fungus belonging to the order Moniliales and the family Moniliaceae. This classification is based on sporulating structures, in which conidiophores are separate with loose clusters of hyaline, one-celled conidia produced in a slime matrix at the tips. More recently, however, this fungus has been renamed *Hymenula cerealis* Ell. & Ev. of the family Tuberculariaceae because it produces conidiophores packed tightly together into a sporodochium under certain environmental conditions. This fruiting structure can develop in the laboratory as well as in the field.

C. gramineum grows slowly on a solid medium, with the optimum temperature for sporulation around 15°C. In a simple liquid medium

consisting of glucose, $NaNO_3$, and K_2PO_4, abundant conidial proliferation occurs by fission and budding.

In its saprophytic phase, C. gramineum is a poor competitor in the soil. Other fungi such as Trichoderma sp., Penicillium sp., and Fusarium culmorum are much more efficient colonizers of plant residues. Hence, survival of C. gramineum between seasons in the soil is contingent upon systemic invasion of the host during its parasitic phase. After ingress, conidia move passively with the transpirational stream. In this way, C. gramineum eventually colonizes all tissues of the winter wheat plant, from the culm and leaves to the glumes and awns of the head. Since Cephalosporium stripe is a simple interest disease in which infection of plants occurs only once during the growing season, it must rely upon the straw, stubble, and chaff remaining after harvest for inoculum carryover and dissemination.

The host range of C. gramineum is quite broad within the family Gramineae, when artificial inoculations with liquid conidial suspensions are employed. Under field conditions, however, winter wheat is the most common host. Barley, oats, and rye also have been reported to be naturally infected in some areas.

The Host. The conditions under which winter wheat is grown make it particularly susceptible to infection by C. gramineum. It is a fall-sown crop and establishes some tillers and adventitious root growth prior to onset of dormancy in the winter. Vernalization, which occurs

during the winter months, is a prerequisite for flowering. In the spring, frost-heaving conditions coincide with high inoculum levels in the soil. Thus, infectious propagules of C. gramineum are present around infection sites created by root breakage or wounding. Spring-sown crops do not encounter these conditions, and hence escape infection.

The Host-Pathogen Interaction. Most observations on host response to pathogen invasion have been made either late in the infection process or in non-vernalized winter wheat seedlings. In both instances, only events occurring in the infected leaves were examined extensively. The chlorotic and necrotic striping of leaves and leaf sheaths, severe stunting, and blighted heads are symptoms attributed to imposition of water deficits. Accumulations of fungal cells, gums, and gels were suggested as the cause of vascular dysfunction, which in turn prevented the vertical and lateral transport of water.

The study of host responses in relation to differences observed between winter wheat cultivars has been limited to white head counts in infected rows and yield data. While these parameters showed that differential responses between cultivars existed, they did not reveal the specific phenomena that caused these disparities.

The overall purpose of this thesis was to identify and evaluate phenotypic responses of selected winter wheat cultivars which responded differentially to infection by C. gramineum. In so doing, it was necessary to follow disease development throughout the ontogeny of the

host. This approach provided the opportunity to examine closely the dynamic nature of the host-pathogen interaction over time against the background of different host genotypes.

Chapter one relates microscopic movement and distribution of C. gramineum with macroscopic foliar symptom development throughout the ontogeny of two differentially responding winter wheat cultivars.

Chapter two relates foliar symptom development with physiological effects of pathogenesis on growth and yield of three differentially responding winter wheat cultivars.

Chapter three re-examines the role of root wounding on pathogen ingress, identifies three phenotypic responses attributed to two types of resistance, and evaluates deployment of this resistance in a germplasm development program.

CHAPTER I

RELATIONSHIP BETWEEN FOLIAR SYMPTOM DEVELOPMENT AND THE MOVEMENT AND DISTRIBUTION OF *CEPHALOSPORIUM GRAMINEUM* THROUGHOUT THE ONTOGENY OF ITS WINTER WHEAT (*TRITICUM AESTIVUM* L.) HOST

INTRODUCTION

Cephalosporium gramineum Nisikado & Ikata (= Hymenula cerealis Ell. & Ev.), a facultative soil-borne pathogen, is the causal agent of the only reported fungal vascular disease of winter wheat, Cephalosporium stripe. It has a wide host range within the Gramineae, but environmental conditions restrict infection to fall-sown crops or perennials (4,15). The pathogen enters the vascular system of the roots through wounds made by frost heaving of the soil or by wireworms (4,19). Subsequently, the fungus moves systemically through the vascular network and is confined there until the host is moribund (21,22). The rate and extent of disease development is dependent upon the movement and multiplication of conidia in the xylem (4,21).

Symptoms appear initially as discrete necrotic or chlorotic stripes on leaves and leaf sheaths. Ultimately, coalescence of these stripes results in chlorosis and necrosis of all foliar tissue followed by blighting of the heads (3,4,12). Nodal discoloration appears as foliar symptoms become more extensive (3).

Colonized vascular bundles are discolored and become occluded with conidia, mycelia, and gels (3,4,20,21). Interruption of the lateral flow of water has been suggested as the cause of stripe formation (16, 20,21). While the events leading to impairment of lateral water transport are unresolved, it is known that colonization by the pathogen precedes foliar symptom development (21). In addition to occlusion by the

fungus and by-products of the host-pathogen interaction, a polysaccharide produced by C. gramineum in culture has been hypothesized as a major contributor to vascular dysfunction (16,20).

To date, studies of the relationship between pathogen localization and foliar symptom expression have involved analysis of leaves from field-grown plants late in the season or from non-vernalized, wound-inoculated seedlings in the greenhouse (20,21). Even though infected leaves are obvious indications of the host-pathogen interaction, they still represent only a portion of the integrated vascular network within the entire plant body. Furthermore, a non-vernalized winter wheat plant is an atypical physical and chemical environment for C. gramineum, since infection normally occurs in the spring long after vernalization has occurred. In this study, therefore, we examined disease development in C. gramineum infected winter wheat under natural field conditions using controlled inoculum levels. The rate and distribution of the pathogen were followed in relation to the developmental growth stages of winter wheat. To aid in selecting resistant genotypes for an ongoing germplasm development program (12), a standardized disease index rating system based on anatomical features of a wheat leaf and on patterns of movement in relation to symptom expression was also developed.

MATERIALS AND METHODS

Histological processing. Standard paraffin methods were used in preparing excised leaf, node, and internode segments for light microscopic analyses (9). The segments were fixed in FAA (40% formaldehyde-glacial acetic acid-ethanol 5:5:90 v:v:v) for 48 hours, dehydrated in a graded series of tertiary-butyl alcohol solutions, embedded in paraffin (Paraplast, Scientific Products), softened in a glycerol-water-Tween 20 solution (30:69:1 v:v:v) for 5 days, and finally frozen in dry ice to prevent tearing of the paraffin away from the tissue segments during sectioning. Transverse sections 10-11 uM thick were cut with a Model 820 Microtome (American Optical) and placed on glass slides coated with Haupt's adhesive (2% gelatin). After dissolving the paraffin from the sections in xylene, they were stained with Safranin (1.0% aqueous) and Fast Green (0.5% ethanol).

Field planting and inoculation procedures. All cultivars in this study were planted in a randomized block design with four replications at the Montana Agricultural Experiment Station near Bozeman, Montana. The rows were 3.1 m long and spaced 30.5 cm apart. Each winter wheat line was planted in early September at a rate of 200 seeds per row. Early planting facilitated root growth which maximized infection. Twenty grams of oat kernels infested with C. gramineum were added as an inoculum source with the seed (11). Inoculation occurred naturally in the spring coincident with frost heaving.

Plant materials. The disease index rating system for measuring symptom severity was developed using six winter wheat cultivars which demonstrated differential yield responses when infected with C. graminum. Marias (C.I. 17595) and Lancer (C.I. 13547) were highly susceptible, Winalta (C.I. 13670) and Crest Line Row Component (LRC) 40 (MT 7579) were intermediate, and P.I. 094424 and P.I. 278212 were resistant (12). Marias and Crest LRC 40 were used to compare the movement and distribution of C. gramineum with symptom expression in infected tillers.

RESULTS

Disease index rating system for evaluating symptom severity. A system for rating symptom severity was developed for all winter wheat leaves regardless of their position on a plant or genotypic background. Ten leaves from each of six cultivars were selected randomly from different locations on primary tillers at heading. Each leaf exhibited a different striping pattern ranging from one discrete stripe to complete chlorosis. Leaves were photographed on a slide-viewing box, which provided the backlight illumination necessary to distinguish translucent appearing veins from the more opaque lamina. Hence, veins associated with chlorotic stripes could be identified readily (Figure I-1) A segment of each leaf was excised for histological processing and subsequently examined for (1) anatomical comparisons of vascular bundle distribution between cultivars, and (2) identification of vascular bundles colonized by C. gramineum and their distribution relative to the external striping pattern on intact leaves.

Patrick (13,14) classified wheat leaf vascular bundles into three categories. The order of earliest to latest maturing and of largest to smallest bundles were: the median bundle, the lateral bundles, and the intermediate bundles (Figure I-1). In this study, the bundle types in each leaf were identified and the number of each determined. Five lateral bundles were observed consistently on each side of the median bundle in all cultivars. The number of intermediates ranged from 33 to

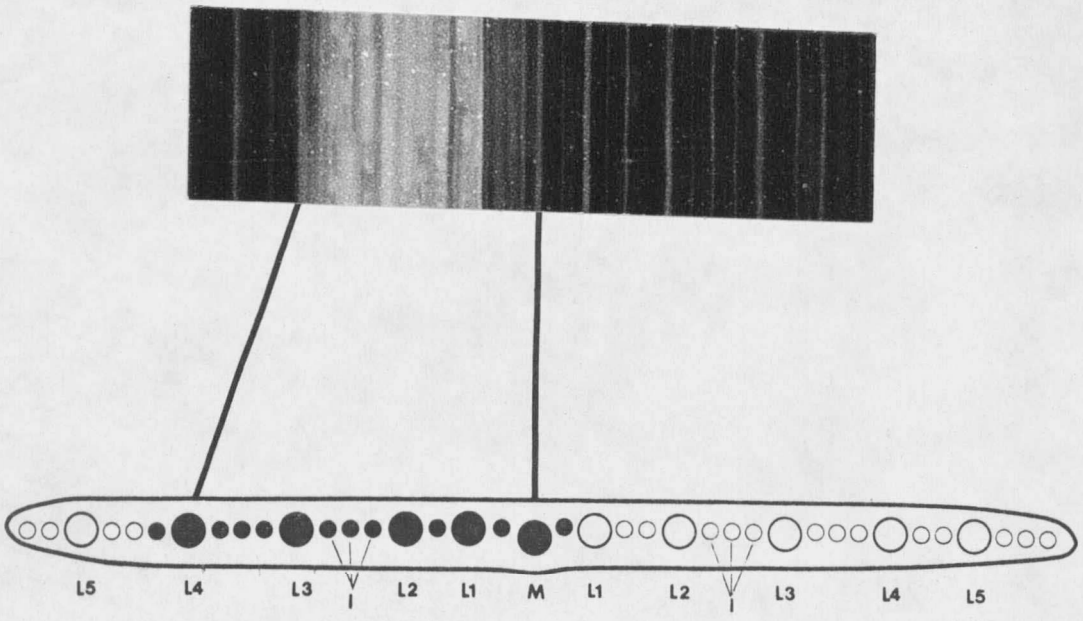


Figure I-1. Chlorotic striping pattern on a Cephalosporium gramineum infected winter wheat leaf and an illustration of a transverse section of that leaf showing the distribution of vascular bundles colonized. M = median vein, L = lateral vein, and I = intermediate vein. Ten lateral bundles were always observed in all wheat leaves examined.

43 per leaf between cultivars as well as between leaves within each cultivar.

The distribution of colonized vascular bundles was closely related to the pattern of external striping. Small quantities of the fungus in vascular bundles of both the stem and leaves produced no visible damage to surrounding tissues. With increased propagule levels, however, disruption of protoplasts in phloem and mesophyll cells bordering the vascular bundles was detected (Figure I-2A-C). Gels and gums appeared concomitant with increased colonization by the fungus and appearance of cellular degeneration in regions proximal to the infected vascular bundles. A marked decrease in chloroplast numbers within affected mesophyll cells was associated with external manifestations of chlorotic stripes. Zones of tissue disruption coalesced when adjacent vascular bundles were colonized.

Each stripe appeared to result from localized vascular dysfunction caused by fungal colonization. Hence, the striping pattern was used as an indication of the extent of systemic infection by C. gramineum throughout the leaf vascular system. The disease index quantified the spread of these chlorotic stripes on a leaf. A range of one to eleven was chosen, since all wheat leaves possessed a single median vein and ten lateral veins. The intermediate bundles, being numerous and variable between leaves, were not included. However, since the intermediates adjacent to a lateral bundle became infected soon after

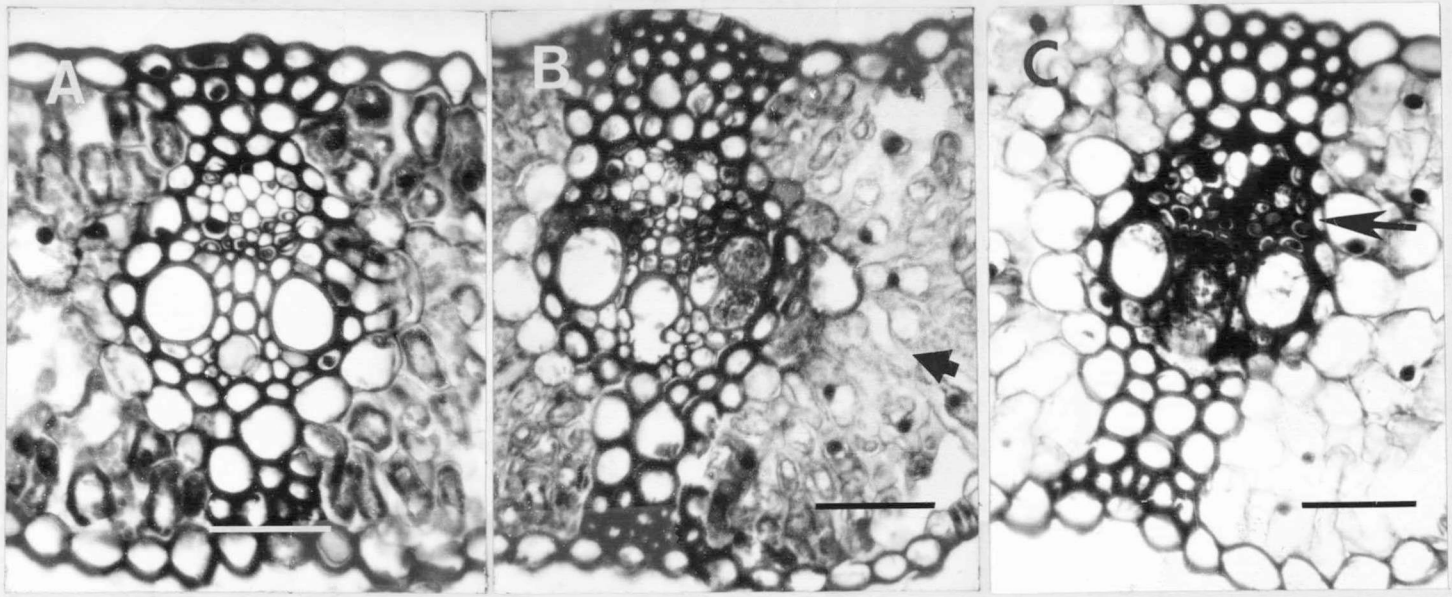


Figure I-2. The pathological effects of vascular bundle colonization by Cephalosporium gramineum in infected winter wheat leaves. A) An uninfected vascular bundle. B) An infected vascular bundle in the early stages of colonization. Some mesophyll deterioration is evident (arrow). C) An infected vascular bundle in late stages of colonization. Extensive deterioration of phloem (arrow) and surrounding mesophyll cells. Bars represent 50 microns.

colonization of the lateral, stripes induced by them were represented with the lateral bundle. A score of one denoted a single stripe on a leaf, which corresponded to invasion of a single lateral bundle and adjacent intermediates. A score of eleven indicated complete chlorosis, which corresponded to fungal establishment in most, if not all, vascular bundles of a leaf.

Systemic movement of C. gramineum between consecutive nodes. A node is the only region of the wheat stem where leaf traces interconnect to form a vascular continuum with the rest of the plant. As such, it is a critical region for successful systemic movement of the fungus acropetally in the plant. Each successive node represents a temporal gradient both with respect to the extent of infection and the extent of host development. Consequently, the focus of attention was on consecutive nodes of winter wheat culms at various developmental growth stages.

Fifty randomly selected diseased plants of the susceptible cultivar Marias were tagged in the spring when stem elongation of the main tillers began. Observations were restricted to the developmental stages between penultimate leaf emergence and anthesis (Feeke's scale 7.5 to 10.5) (10). At each of five collection dates corresponding to a change of 0.5 on the Feeke's scale, ten main tillers were harvested and the upper four leaves of each rated for symptom severity. Each leaf and its node of attachment were then excised and cultured on acidified cornmeal agar (HCMA) (Difco) to test for the presence of the fungus. Each nodal segment

extended from the apex of the internodal lacuna below the node to midway into the pulvinus above the node. This was termed the node-pulvinus segment to differentiate it from the pulvinus-internode segment, which consisted of the remainder of the pulvinus and 0.5 cm of the internode above. Both segments were excised from the upper two visible nodes at each growth stage and cultured on HCMA to determine the leading edge of fungal advancement.

Although the crowns of all tillers examined were invaded systemically by C. gramineum as determined by both visual blighting and fungal isolation from the lowermost leaves, the rate of movement throughout the entire tiller apparently was affected by the stage of host development (Table I-1). At growth stage 7.5, the fungus had advanced only as far as the fourth node (the node immediately above the crown). Only two nodes were visible at that time, the third being in a juvenile stage of development as judged by the diameter of stem and pulvinus. The fungus had progressed into the third node by growth stage 8, at which time the second node was visible, but still immature. This pattern of advancement continued through to the flag node (node 1 in Table I-1).

Resistance to fungal advancement was observed in immature node-pulvinus segments. For example, at growth stage 9.5, when the head was almost ready to emerge from the "boot", the fungus was isolated from only one edge of the flag node-pulvinus segment. It did not emerge from the pulvinus edge of the segment. In addition, the fungus was not

TABLE I-1. Relationship between foliar striping patterns and movement of Cephalosporium gramineum in consecutive leaves of infected Marias winter wheat plants at different developmental growth stages.

	Severity Score[a/]				
	Feekes scale[b/]				
Leaf[c/]	7.5	8.0	8.5	9.5	10.5
1	0	0	0	0	1.4*[d/]
2	0	0	0.1*	2.0*	4.4*
3	0	1.7*	4.1*	6.9*	10.1*
4	2.3*	9.4*	9.9*	10.8*	11.0*

[a/] Each value respresents the mean of ten tillers at each growth stage. Each leaf was assigned a severity score based on the number of stripes visible on a scale from one to eleven. 1 = one stripe/leaf, 11 = totally blighted leaf.

[b/] Each growth stage is represented by the Feekes scale, which partitions winter wheat development into a numerical scale from 1 to 11.

[c/] Leaf 1 represents the flag leaf, leaf 2 the penultimate leaf, leaf 3 the third leaf below the flag, and leaf 4 the fourth leaf below the flag.

[d/] Asterisk indicates successful isolation of the fungus from nodes cultured on acid cornmeal agar.

detected in the pulvinus-internode segment above the flag node.

To examine this phenomenon more closely, anatomical features of the node and pulvinus and the pattern of fungal movement within these regions were studied. Successive nodes and leaves of attachment were excised from six primary tillers of Marias at each growth stage and processed for histological examination. Serial sections were cut from the base of each node to midway through the pulvinus. Presence of the fungus in node-pulvinus regions and leaves of attachment was established by microscopic identification of conidia, mycelia, gel accumulations, or any combination of the three.

A node is composed of vascular bundles and interconnecting xylem strands from the leaf of attachment as well as from the two leaves immediately above. Patrick (13) classified vascular bundles in each node according to their leaf of origin. They can be identified by (1) morphological characteristics, (2) position relative to other bundles, and (3) pattern of branching through the node (Figures I-3, I-4). All of the vascular bundles originating in the leaf of attachment (designated L) are continuous and non-branching through the node, although "bridging strands" fuse with them. They are readily distinguished by their distinctive size and shape within the node. Lateral vascular bundles originating in the leaf above leaf L (designated L1) are continuous through the node and fuse with other xylem traces only near the base of the node. They are identified by their autonomy relative to

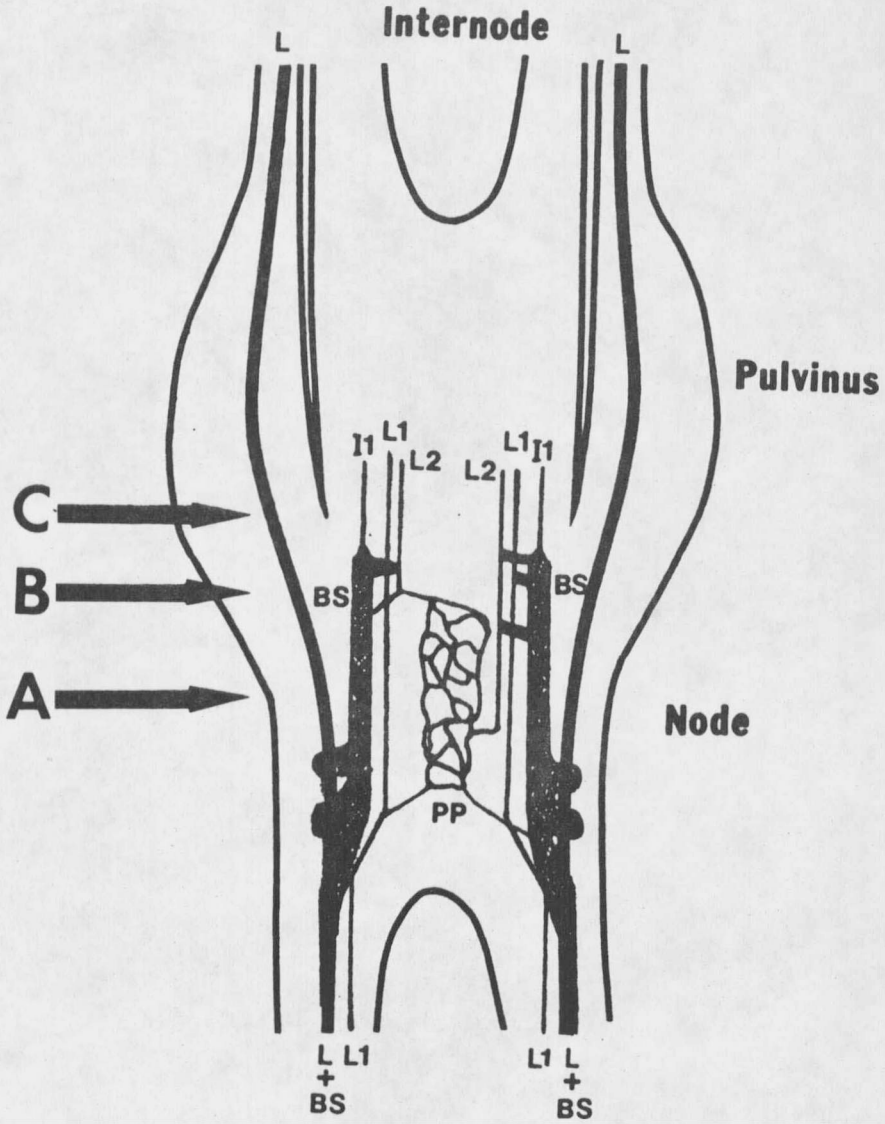


Figure I-3. Illustration of the vascular bundle types and their course through a winter wheat node. Redrawn from Patrick (13). L = lateral and intermediate bundles from leaf of attachment (LA), Ll = lateral bundles from leaf above LA, L2 = lateral bundles of second leaf above LA, BS = bridging strand network, PP = pith plexus. A-C refer to regions of the node shown in cross-section in Figure I-4.

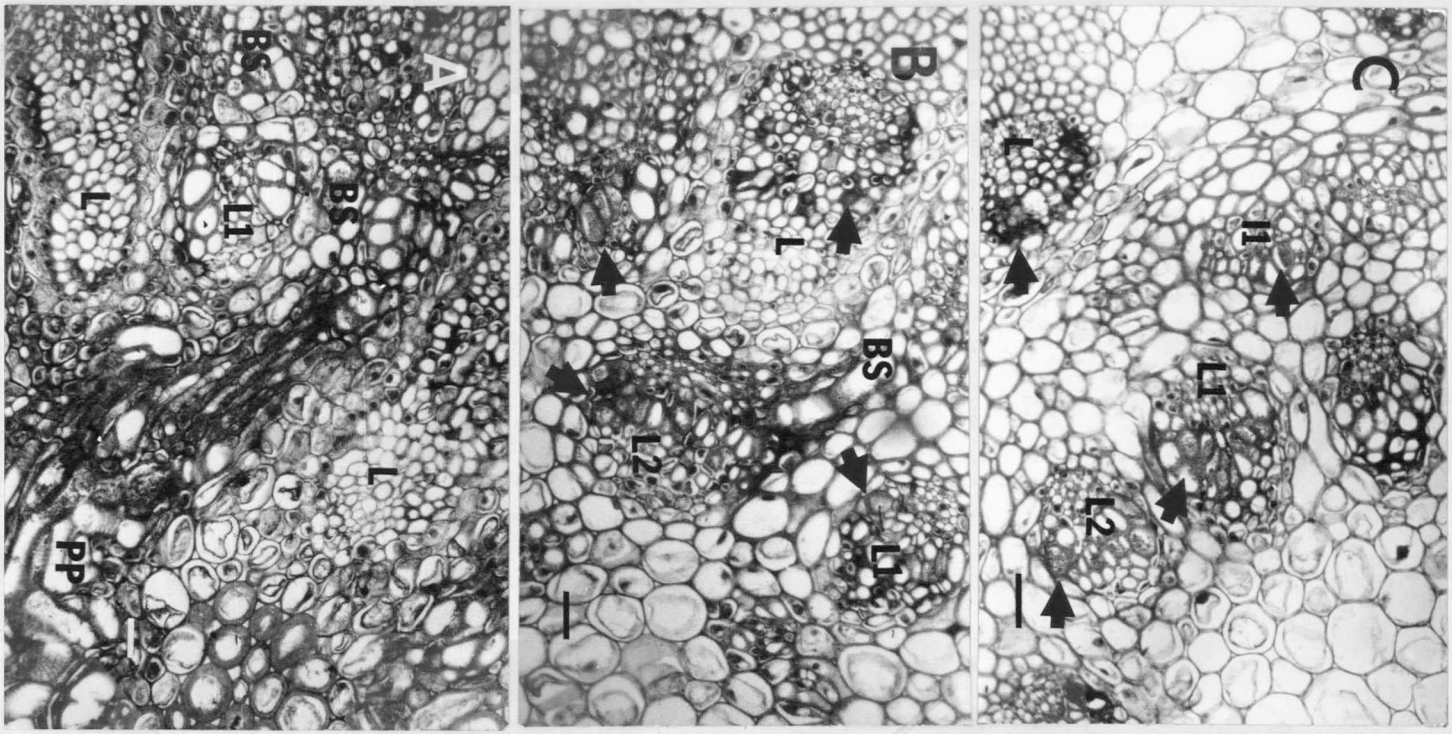


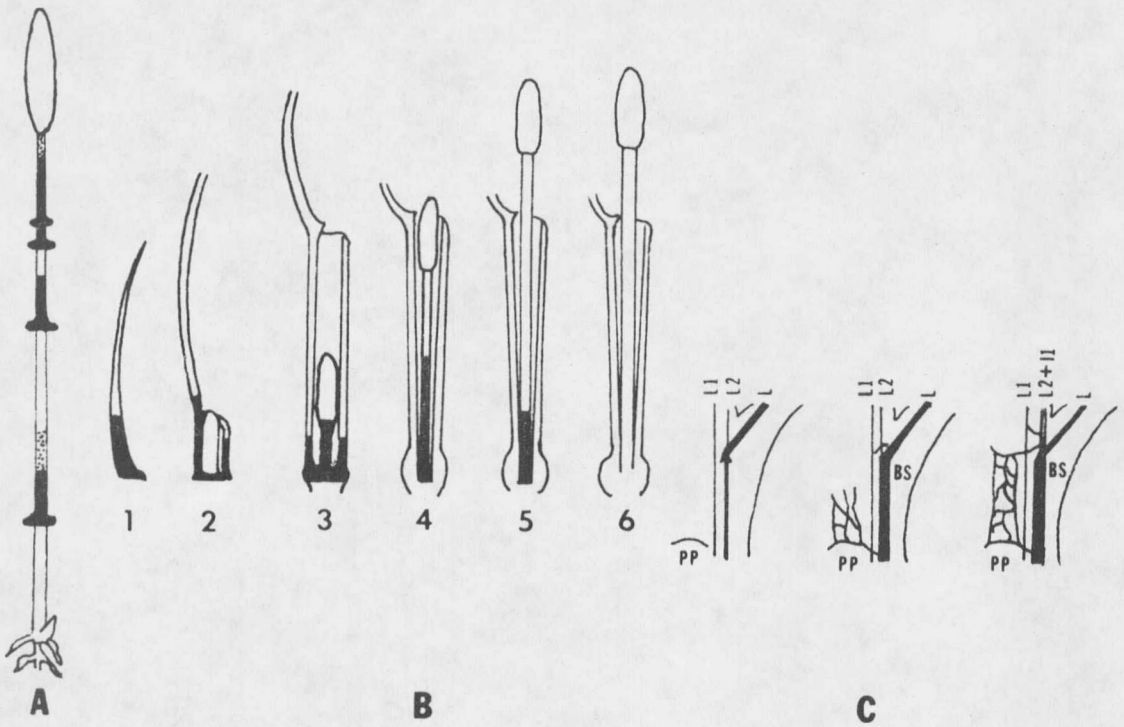
Figure I-4. Light micrographs of cross-sections through a winter wheat node infected with Cephalosporium gramineum, showing vascular bundle types and their distribution at levels A-C indicated by arrows in Figure I-3. L = lateral bundles from leaf of attachment, L1 = lateral bundles from leaf above leaf of attachment, L2 = lateral bundles from second leaf above leaf of attachment, I1 = intermediate bundles from leaf above leaf of attachment, BS = bridging strand network, PP = pith plexus. Arrows indicate colonization by Cephalosporium gramineum. Bars represent 50 microns.

other bundles and the "bridging strand network" that links with entering leaf traces to interconnect leaf and stem bundles. Lateral bundles of the leaf above L1 (designated L2) were difficult to distinguish readily, since they fuse with the "bridging strand network" and the pith plexus near the top of the node. The pith plexus connects with the bridging strands, making the xylem network continuous among all vascular bundles within the node (13).

When C. gramineum was identified in a specific bundle in one node, it often could be traced to the same bundle in the node below and the node above. For example, if the fungus was observed in a lateral L1 bundle in a node, it would be found also in an L2 or possibly a linking strand in the node below and in an L bundle in the node above. Thus, vertical movement of the fungus was followed from one node to the next at each growth stage. Its distribution within each node could then be related to distribution in other nodes.

Systemic advancement of C. gramineum at various growth stages was closely associated with xylem maturation gradients between nodes, xylem strand differentiation within nodes, and xylem strand differentiation within leaves. The grass stem matures vertically in a multinodal fashion, e.g. basipetally from the top of one node to the top of the node immediately below (Figure I-5A)(17). The leaf at each node matures basipetally from the tip to the base of the leaf sheath, with the median bundle developing first, followed by the lateral bundles, and

Figure I-5. Illustration of xylem maturation gradients in a winter wheat plant. A) Vertical maturation gradients in the internodal regions. B) Vertical maturation gradients within the flag node-leaf junction of the pulvinus. Six phases are represented, 1 = flag leaf emergence, 6 = full maturity of all tissues after heading. Both A and B were redrawn from Pratt (17). Filled areas indicate immature tissues. C) Redrawn from Patrick (14), this shows the sequence of vascular bundle differentiation within a node. L = lateral bundles of leaf of attachment, L1 = lateral bundles from leaf above leaf of attachment, I1 = intermediate bundles from leaf above leaf of attachment, L2 = lateral bundles from second leaf above leaf of attachment, BS = bridging strand network, PP = pith plexus.



finally by the smaller intermediate bundles (14). The last region to mature between nodes is the stem portion at the area of insertion into the pulvinus at the node-leaf junction. This maturation sequence is numerically partitioned into five phases (Figure I-5B)(17). Maturation of xylem strands within a node is acropetal. The differentiation sequence for different bundle types is the same through the node, but it is initiated first at the node base. The L, L1, and L2 bundles mature first but are not interconnected until differentiation of the bridging strand network. The pith plexus develops independently of the other xylem strands and is last to mature (Figure I-5C)(14).

C. gramineum consistently invaded nodes only after maturation of the xylem strands leading into them. Moreover, the fungus demonstrated selectivity for bundle types dependent upon their sequence of development within each node. The order of ingress followed closely the order of differentiation.

Fungal propagules were first detected in nodes at phase 5 (Figure I-5B) of the vertical maturation gradient. This stage of differentiation was characterized most often in node 3 at growth stage 8, node 2 at growth stage 8.5, and node 1 at growth stage 9.5. Histological examination of these node-pulvinus segments verified the maturation gradient among bundle types. The L, L1, and L2 bundles appeared to be fully mature through the node. Much of the bridging strand network and the pith plexus, however, were still juvenile. Although much of the

cytoplasm of these vessels had disappeared, the cell walls were not lignified. In the vascular bundles of the stem in the pulvinus, the metaxylem elements were still undifferentiated whereas the protoxylem elements had lost their protoplasmic contents and the cell walls were becoming lignified (Figure I-6).

Conidia and occasionally mycelia were detected only in the large L bundles of these nodes. Rarely were any signs of the fungus evident in L1 or L2 bundles. The fungus was never found in the bridging strand network, the pith plexus, or vascular bundles of the stem above the node. Interestingly, C. gramineum was never found in vascular bundles until all of the vessel elements within them had fully matured.

The pattern of pathogen distribution from fully mature nodes into developing nodes above at each growth stage of winter wheat development illustrates the restrictive influence imposed by both the vertical and the lateral maturation gradients (Table I-2). For example, node 3 at growth stage 10.5 is extensively colonized in all bundle types and linking strands. Once the fungus had penetrated into the pith plexus, it was able to invade all bundles in the node. After the fungus had successfully colonized all of the bundle types, it was afforded open channels into all of the bundles of node 2 directly above. If no restrictions were imposed on vertical or lateral movement, the fungus should have invaded most or all of the L, L1, L2 bundles, the bridging strand network, and the pith plexus of node 2 concurrently. Only some

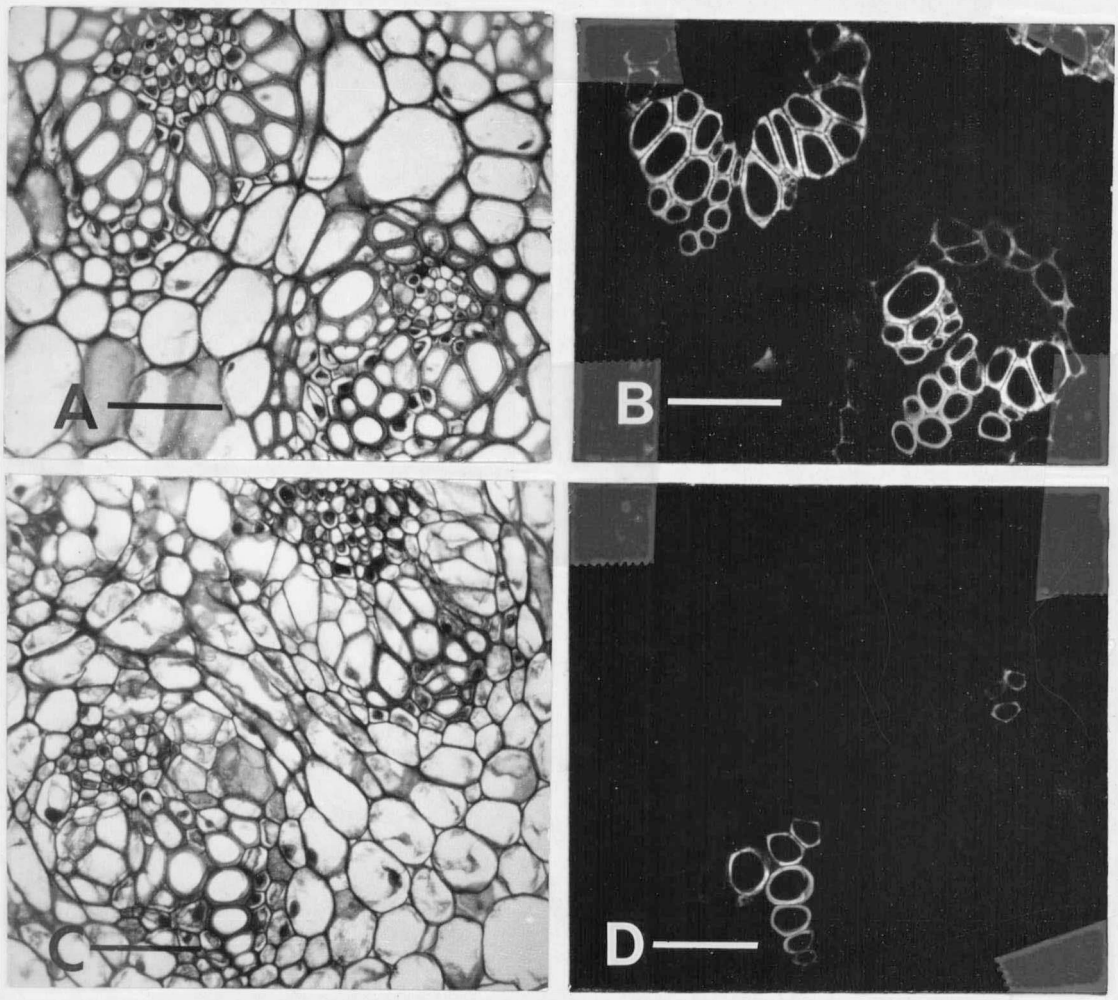


Figure I-6. Light micrographs showing xylem differentiation in response to a maturation gradient in the stem of a winter wheat plant. Bright-field optics of A) mature and C) immature vascular bundles in the stem of the pulvinus. Fluorescent optics of B) the same mature and D) the same immature vascular bundles showing the extent of lignification in vessel cell walls. Bars represent 50 microns.

TABLE 1-2. Distribution of *Cephalosporium gramineum* in each bundle type within consecutive nodes of two winter wheat cultivars at different growth stages.

	Proportion of Bundles Colonized[a]							
	MARIAS				CREST LRC 40			
Growth Stage (Feekes scale)	Bundle Type[b]				Bundle Type			
	L	L1	L2 & BS	PP	L	L1	L2 & BS	PP
7.5								
NODE 4[c]	1.7	0.8	0	0	1.2	0.2	0	0
NODE 3	0	0	0	0	0	0	0	0
NODE 2	0	0	0	0	0	0	0	0
NODE 1	0	0	0	0	0	0	0	0
8.5								
NODE 4	2.0	1.2	0.7	0	1.3	0.8	0.3	0
NODE 3	1.5	0.5	0	0	1.0	0.2	0	0
NODE 2	0	0	0	0	0	0	0	0
NODE 1	0	0	0	0	0	0	0	0
9.5								
NODE 4	3.2	2.5	2.1	1.2	2.1	1.5	0.5	0
NODE 3	2.3	2.1	1.5	0.5	1.0	0.5	0.3	0
NODE 2	1.2	0.8	0.2	0	0.3	0	0	0
NODE 1	0	0	0	0	0	0	0	0
10.5								
NODE 4	4.0	3.5	3.2	2.2	2.5	1.8	1.3	1.0
NODE 3	3.9	3.3	2.7	2.0	1.5	1.0	1.0	0.2
NODE 2	2.0	1.7	1.2	0.3	1.0	1.0	1.0	0
NODE 1	1.1	0.4	0	0	0.2	0.2	0	0

[a] Proportion of vascular bundles colonized within each bundle type. Each value represents the mean of 6 tillers. 1 = 0-25% bundles colonized, 2 = 25-50% bundles colonized, 3 = 50-75% bundles colonized, 4 = 75-100% bundles colonized.

[b] Bundles identified by leaf of origin in relation to each node. L = lateral bundles of leaf above leaf of insertion, L2 = lateral bundles of the second leaf above leaf of insertion, BS = bridging strand network, comprised of intermediate bundles from the leaf above leaf of insertion, PP = pith plexus, arising independently within the node.

[c] Node 4 is the lowermost node and node 1 is the flag node.

of the L, L1, and L2 bundles and the bridging strands linked to them were colonized, however, and the pith plexus was completely free of the pathogen. Additional constraint was in evidence for the bundles between node 2 and the flag node (node 1), for only several L bundles were sparsely colonized in the latter. Consequently, the fungus was observed moving up an infected stem in a sequential temporal pattern between nodes and between the bundle types in each node rather than in a rapid, unordered progression.

The pattern of pathogen distribution observed histologically between nodes was mirrored macroscopically in the pattern of stripe formation (Table I-1). At each consecutive growth stage after 7.5, the average number of stripes on leaves attached to recently matured nodes did not exceed two stripes, whereas the leaves immediately below averaged between four and ten stripes. Leaves further down the stem were usually completely blighted.

Thus, the movement and distribution of C. gramineum is closely associated with the xylem maturation gradients of developing winter wheat plants. Vertical movement in the stem is limited by the basipetal maturation gradient between nodes; movement between vascular bundle types is restricted by the acropetal and lateral differentiation of L, L1, L2, bridging strands, and pith plexus; and movement within the L bundles is regulated by the sequential differentiation of median, lateral, and intermediate bundles originating in the leaves.

Differential responses of two cultivars to spread of C. gramineum. Marias and Crest LRC 40 were compared with respect to the rate of symptom expression throughout their growth and development. Crest LRC 40 was chosen because it appeared to possess some mechanism by which complete blighting of all leaves occurred later than in Marias within the same time span (Chapter 3). Consequently, histological studies similar to those performed on Marias were conducted to determine any visual dissimilarities which might characterize this phenomenon. Identical sample sizes and harvesting dates were used, since both Marias and Crest LRC 40 have the same heading dates. The fungus invaded the different bundle types of Crest LRC 40's nodes in the same sequence observed in Marias, but its rate of invasion lagged considerably (Table 1-2).

To discern differences in host response to pathogen colonization, conidial, mycelial, and gel accumulations were quantified in colonized vascular bundles by rating each on a scale of one to three indicating trace (1-30% vessels/bundle filled), moderate (30-70% vessels/bundle filled), and abundant (70-100% vessels/bundle filled) quantities. The number of vessels in each vascular bundle within a node ranged from a minimum of 24 in L1 bundles upward to 139 in L bundles. No tyloses were ever observed in infected vessels, ruling them out as a significant host response. Only those nodes at various growth stages in which the fungus had progressed into the L1 and L2 bundles, but had not yet invaded the

pith plexus, were scored.

Substantial differences in conidia, mycelia, and gel accumulations appeared to exist between Marias and Crest LRC 40 (Table I-3). If Marias was assumed to lack any capacity for active restriction of pathogen movement, then Crest LRC 40 possessed the ability to elicit greater gel production, inhibit conidial proliferation, or a combination of both.

TABLE I-3. Reaction of two winter wheat cultivars to infection by Cephalosporium gramineum as related to accumulation of gels, conidia, and mycelia within the lumina of infected vascular bundles.

	Extent of Accumulation[a/]		
Cultivar	Gels	Conidia	Mycelia
Marias	1.0	2.4	1.1
Crest LRC 40	2.2	1.2	1.8

a/ Each value represents the mean of 42 nodes. Readings of all L and Ll bundles in each node were averaged. Only nodes in which C. gramineum had not yet progressed into the pith plexus were scored. 1 = trace amounts (1-30% vessels/bundle), 2 = moderate amounts (30-70% vessels/bundle), and 3 = abundant amounts (70-100% vessels/bundle).

DISCUSSION

All host-pathogen interactions may be characterized by examining components of the disease pyramid singly and together (2). This study placed equal emphasis on pathological effects of C. gramineum on the host and on physiological and morphological effects of the winter wheat host on pathogen movement. The temporal relationship between these two components was investigated by following disease development during the ontogeny of infected wheat plants. Both disease development and host development are initiated within a similar time frame, since field infection in Montana occurs at one time--in the spring when soil heaving causes root breakage (4,15). The exposed xylem elements provide sites for passive entry of conidia into the roots. Hence, disease onset is concomitant with induction of stem elongation in winter wheat.

Our study confirmed earlier reports that pathological effects in and around infected vascular bundles did not occur until after abundant colonization by C. gramineum. Consequently, a sequential relationship between invasion and colonization by the pathogen and foliar symptom expression was observed. The fungus was detected prior to external symptom development, but chlorotic striping was rarely visible in vascular regions devoid of the fungus. Impairment of lateral water transport resulting from vascular dysfunction has been postulated as the mechanism of stripe formation (16,20,21). Phloem and mesophyll deterioration within and around abundantly colonized vascular bundles could be a

manifestation of localized water stress caused by resistance to water movement to these cells. As adjacent vascular bundles became infected, the regions of tissue degeneration overlapped. Ultimately, when all bundles were colonized, the leaf became blighted.

This pattern of pathogen movement in relation to symptom expression formed the basis for a disease index rating system. Each stripe, ranging from one to eleven per leaf, indicated the extent of infection through a node and into its leaf of attachment. This disease index may be a useful tool for visually monitoring the rate and extent of pathogen spread upward through an infected winter wheat plant. Individual readings may then be extended to a population of plants within a single cultivar for evaluating differential responses between genotypes as well as selecting plants from a segregating population.

Many elements of the C. gramineum-winter wheat interaction are unique in comparison to other vascular pathogen-host interactions. With respect to the host, winter wheat is an annual, herbaceous monocot, and it differs significantly from woody monocots such as banana and dicots which are susceptible to other vascular pathogens. The vascular bundles of wheat lack a cambium for secondary growth and thus remain as discrete bundles of fixed sizes except in the nodal regions, where they form an interconnecting network. Traces from the leaf of attachment pass unbranched through the node and into the internode below. The vascular bundles in the leaf are of similar sizes to their counterparts in the stem. Thus,

movement of the fungus from several vascular bundles in the stem upward into the leaf results in localized stripes in the regions of colonization. No visible external evidence of physiological disorder caused by water stress was found in asymptomatic regions of the leaf.

In dicots, on the other hand, the petiole bundles branch from the interconnecting vascular network of the stem and are much smaller in size. Whereas localized occlusion in stem bundles does not alter water flow significantly, extensive dysfunction in the petiolar bundles results in almost a total cutoff of water flow (6,7). Consequently, vascular diseases associated with these plants often exhibit generalized wilting rather than discrete chlorotic patterns around infected bundles(7).

Winter wheat is also distinctive in its requirement for a vernalization period to initiate flowering. In comparing symptom development within wound-inoculated vernalized and non-vernalized winter wheat plants, Bruehl (4) observed that the latter were resistant to stripe formation. He suggested that "it is probable that the fungus is not particularly active until the host passes a certain stage of development". In our studies, we found that stripe formation in non-vernalized winter wheat was evident only in the mature outer leaves (Chapter 3). While Weise's (21) observations regarding events leading to symptom development within maturing leaves is similar to ours, his examination of only a specific organ, the leaf, did not reflect the impact of host ontogeny on disease development.

The unique nature of C. gramineum as a plant pathogen was discovered after studying the mutual interaction of host ontogeny and pathogen movement. The speed of fungal growth and sporulation, the structure and function of vessels in the plant, and the speed of host response all interacted to determine the rate and extent of systemic infection. In the highly susceptible cultivar Marias, systemic movement was restricted by the stage of vascular differentiation. Since the fungus could not be detected in vascular bundles in which any of the individual vessel elements retained protoplasmic components, it apparently lacked any capability to parasitize living cells. Vessels in their juvenile stage of differentiation are living cells since they contain intact, metabolically active protoplasts. In the mature state, however, they become devoid of the protoplasm and nucleus and thus may be considered as non-living cells. The pathogen was able to invade these non-living vessels readily.

Other aspects of disease etiology substantiate the inability of C. gramineum to penetrate living cells. These include the pathogen's requirement for root wounding for successful ingress and its restricted presence within the xylem until after all other plant tissues lose biological activity.

Bateman (1) recently partitioned plant pathogens into three categories: parasitic, perthophytic, and saprophytic. The first obtains nutrients from living cells directly, the second derives its food from

cells killed in advance of colonization, and the third seeks its food from non-living cells after colonization. The parasitic habit of C. gramineum does not fit into any of these convenient divisions. Rather, this pathogen exhibits characteristics common to both perthophytes and saprophytes, since it parastizes functionally non-living cells that are an integral part of a living system. Although other vascular pathogens, notably Fusarium and Verticillium, are also restricted to xylem throughout the lifetime of the host, they possess the ability to penetrate living cells of the root to initiate infection (8,18).

A descriptive model for C. gramineum movement and distribution through a node and above into the stem and leaf of attachment is presented here based upon the evidence for developmental sequence of bundle types within each node, the maturation gradient between nodes, and the sequence of invasion among bundle types after initial ingress into a node. As a node develops, the fungus gains ingress first into the large L bundles within which all of the vessel elements are fully mature. The fungus subsequently moves into the L1 bundles near the base of the node after maturation of the bridging strand network which interconnects them with the L bundles. With the acropetal maturation of additional bridging strands, the fungus gains access to the L2 bundles near the apex of the node. Since the pith plexus develops independently of all other bundle types, contact must be made with the bridging strands before the fungus can invade it. After successful colonization of the pith plexus,

the fungus has ready access to all other vascular bundles and linking strands thoughout the node which ultimately leads to complete systemic invasion. Macroscopically, this sequence appears as initial discrete stripes (L bundles) on leaves, followed by slowly widening stripes as the adjacent intermediate bundles become invaded. Stripes may coalesce depending upong the pattern of L bundle colonization in the node. Eventually, all lateral and intermediate bundles become colonized and blighting occurs. This sequence is repeated in each node and leaf of attachment as the fungus moves upward in the plant.

This model has important practical application to a germplasm development program. Because of the close temporal association between symptom expression and xylem maturation gradients, all parental and progeny lines require close monitoring of heading dates so that selection for late-maturing genotypes is not favored. Furthermore, selection for differential responses to symptom development is effective only after heading, since symptoms do not appear on the flag leaf until head emergence.

The rate of systemic spread of C. gramineum may be affected at two levels. The first, which is related to the capability for gaining successful ingress into new territory, is governed by the rate of growth and maturation of xylem tissue as discussed above for Marias. The second, which is related to the ability of the pathogen to overcome host defenses after successful ingress, is governed by genetic differences

between winter wheat cultivars. Such an interaction appeared to exist in Crest LRC 40, which delayed complete systemic infection by the fungus much longer than did Marias.

Two responses which could elicit this reaction are (1) the physical blockage of vertical and/or lateral movement of conidia by tyloses, gums, and gels, and (2) the synthesis of an inhibitory substance which slows sporulation. The first has been implicated as a resistance mechanism in several vascular diseases (7). However, unlike most of these host-vascular pathogen interactions, no tyloses or vessel collapse were observed in vessels infected with C. gramineum (5,7). Moreover, both gelation and gummosis were rarely detected in advance of conidial invasion, but occurred simultaneously with or subsequent to fungual proliferation in infected vessels.

Crest LRC 40 exhibited increased gelation and gummosis as compared to Marias. It is possible that these reactions precluded or retarded the lateral movement of conidia from colonized L and Ll bundles into the bridging strand network and pith plexus, which would limit systemic spread into all bundles of a node. Movement of C. gramineum into this interconnecting network of xylem strands lagged considerably behind the maturation gradient.

The apparent inhibition of conidial proliferation within colonized bundles of Crest LRC 40 suggested an interaction at the molecular level. Possibly, a toxic compound is synthesized by the cultivar which inhibits

sporulation sufficiently to retard systemic infection. Since either or both of these phenomena may be responsible for the retardation of systemic invasion by C. gramineum, each must be subjected to rigorous biochemical analyses to determine their relative roles in pathogenesis.

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CHAPTER II

PHYSIOLOGICAL EFFECTS OF CEPHALOSPORIUM STRIPE ON GROWTH AND YIELD OF WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS

INTRODUCTION

Alteration of water relations has been implicated in the interaction between the vascular pathogen, Cephalosporium gramineum, and its winter wheat host (5,6). All of the disease symptoms, which include blighted leaves and heads, stunted plants, and greatly reduced yields, are responses that can be attributed to disruption in water economy (14,21). Diseases of winter wheat caused by root and crown-infecting fungi have occasionally been confused with Cephalosporium stripe because of their resemblance to the white head stage of disease development (2,5,22). In such instances, symptoms were associated with water deficits imposed as a result of severe infection (22).

Most attempts at defining the disease physiology of the Cephalosporium stripe-winter wheat interaction have been indirect. Weise (31) histologically evaluated pathogen movement relative to foliar symptom development. He observed that invasion and colonization by the fungus preceded induction of foliar stripes. Moreover (Chapter 1), there seemed to be an association between the appearance of gums and gels, which accumulated as fungal colonization became more extensive, and the disruption of phloem within vascular bundles and mesophyll cells surrounding the vascular bundles. Stripes appeared in conjunction with cell collapse in these regions. Examination of eosin dye transport in excised infected leaves revealed that both vertical and lateral translocation of water were inhibited in symptomatic regions (27,31). Some

investigators have suggested that accumulations of a low molecular weight polysaccharide produced by C. gramineum in culture is responsible for stripe formation (24,27). Others have implicated a tetronic acid toxin, Graminin A, in vascular browning, although they do not postulate a mode of action (18).

The only attempt to directly determine the effects of pathogenesis on the water relations of winter wheat infected with C. gramineum was conducted by Spalding, et al (27). They measured the relative moisture content of all regions of winter wheat shoots after heading and found that desiccation was more pronounced in diseased than in healthy tissues. Their study, while substantiating that water deficits are induced during pathogenesis, did not evaluate the physiological effects of these water deficits on the host throughout disease development or determine whether the disease syndrome was due wholly or in part to water stress.

The purpose of this investigation was to obtain a more comprehensive analysis of disease physiology in relation to symptom expression and to determine its application to selection for disease resistance. The effects of pathogenesis on plant metabolic processes, on plant growth patterns, and ultimately on yield components from the onset of stem elongation through the grain filling period were examined in several differentially responding winter wheat cultivars.

MATERIALS AND METHODS

Three winter wheat cultivars, Marias (C.I. 17595), Crest Line Row Component (LRC) 40 (MT 7579), and P.I. 278212 were used in this study. They were planted in early September, 1978 at the Montana Agricultural Experiment Station near Bozeman, Montana in a randomized block design with four replications. Each cultivar was seeded in paired rows 3.1 m long and spaced 71 cm apart. To one row was added 20 grams of oat kernels infested with C. gramineum (20) simultaneously with the seed. A seeding rate of 200 seeds per row was used to maximize the number of infected seedlings. In the spring, diseased plants were identified in each row by striping on lower leaves and leaf sheaths and the main tillers were tagged. Each row was thinned manually until infected plants were spaced at least 15 cm apart. Seedlings in adjacent check rows were similarly thinned so that every infected plant was paired with a healthy plant.

Relative water content (RWC), conductance, net photosynthesis, and chlorophyll content were measured to evaluate the physiological relationships between water stress and symptom expression at various degrees of striping on flag leaves of diseased winter wheat plants. RWC and conductance were used as indicators of water stress; the former being a measure of total water potential in a leaf and the latter being a measure of transpirational behavior. Net photosynthesis and chlorophyll content were used as indicators of total photoassimilatory activity. For each

infected flag leaf, a healthy flag leaf was sampled concurrently. Thus, all four parameters could be expressed as percent of control to cancel out environmental factors common to both healthy and diseased plants in the field. Implicit in the results, therefore, was the assumption that observed differences are caused primarily by pathological responses. Symptom severity (number of stripes per leaf) was based on a disease index rating system previously described (Chapter I).

Leaves were sampled between 9 a.m. and noon on calm, clear days. On each sampling date, between ten and fifteen infected and healthy flag leaves were evaluated. The leaves ranged in symptom severity from one stripe to complete chlorosis. Ultimately, ten leaves of each severity rating were analyzed for all four physiological parameters. For one sample population, measurements were made in the following sequence: Net photosynthesis, conductance, and relative water content. Chlorophyll content was determined from a different, though equal sized, sample population, since RWC determinations were destructive.

Net photosynthesis. Carbon dioxide flux was measured on attached leaves in the field using a portable closed system adapted from a technique developed by Clegg, et al (9). The sample chamber was constructed of tubular plexiglass with a diameter of 2.6 cm and a length of 19 cm. The internal volume totalled 100 cm^3. The chamber consisted of two halves sealed by closed-cell insulation tape (Figure II-1). Thus, when both halves were clamped together, a tight seal was obtained. Glass

tubing 0.6 cm X 2.5 cm was attached to two portals positioned in the upper half of the tube and sealed off by rubber serum bottle caps. Gas samples were taken in plastic syringes (B·D multifit, 10 ml) inserted in the two glass tubes. The sample chamber was mounted at one end in a buret clamp holder attached to a metal ringstand. The angle of the sample chamber was continually adjusted so that the leaf blade was positioned perpendicular to the sun's incident rays. With both halves of the chamber clamped tightly at one end, there was a sufficient gap to insert a leaf from the opposite end. A 10 ml aliquot of gas was collected prior to sealing the leaf within the sample chamber to represent initial CO_2 levels around the leaf. The chamber was then clamped shut with a self-closing spring brass test tube wire clamp, and after two minutes, a second 10 ml aliquot of gas was taken from within the chamber. The halves of the chamber were left open a few minutes between leaf measurements to allow equilibration with ambient air. After gas sampling, the syringes were stored for 2-3 hours in a cool, shaded container until they could be transferred to the laboratory. The gas samples were injected into a Beckman IR 215 Infrared Gas Analyzer to determine their CO_2 concentrations. A carrier gas of known CO_2 concentration (320 ul/l) was passed through the system at a rate of 1l/min. A six inch drying column packed with drierite was inserted between the injection site and the IR analyzer. The IR analyzer was calibrated to read from 0 to 200 ul/l CO_2 using standard gases at 170 ul/l and 320 ul/l.

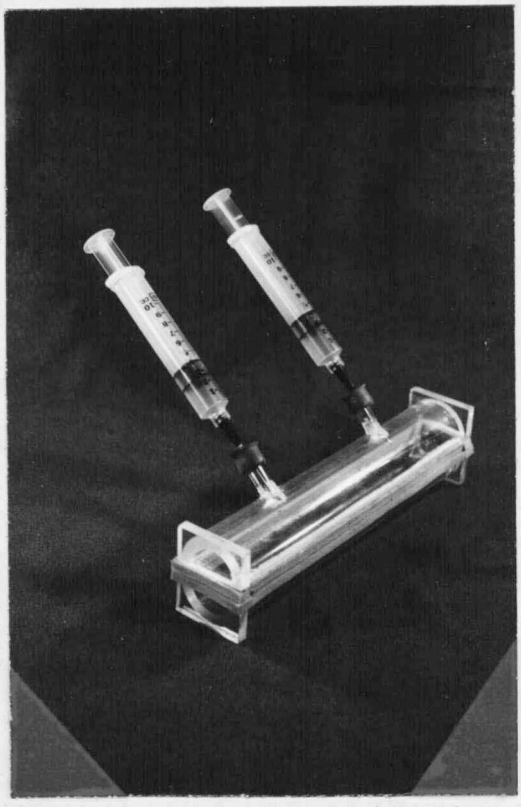


Figure II-1. Plexiglass tubular chamber for measuring carbon dioxide exchange in winter wheat leaves in the field. Two gas samples were collected in plastic 10 ml syringes at the beginning and end of a two minute interval and brought back to the laboratory for injection into an IR gas analyzer.

Differential CO_2 concentrations were recorded as peaks on a Beckman Model 93500 Recorder. Peak heights were measured to determine differential CO_2 assimilation rates between paired healthy and diseased leaves.

In converting ul/l CO_2 to $mgCO_2/dm^2/hr$, leaf area measurements were estimated using the formula, .905 L X W where L = leaf length and W = width of the leaf midway from the tip (16).

Conductance. Diffusive resistance to water vapor loss was measured with a diffusive porometer (Model LI 60, Lamda Instruments Co., Lincoln, Nebraska). The recommended precautions were observed to reduce sampling variation (17). The wide aperture vapor cup (1 X 2 cm) was used in these studies. Three readings were taken from the adaxial leaf surface, near the base, in the middle, and near the tip. The mean represented an estimation of overall diffusive resistance for each leaf. Readings were made after completion of photosynthetic measurements, which allowed at least 30 minutes for a leaf to return to a steady-state condition.

Leaf conductance, which was preferred to diffusive resistance because of its direct correlation with net photosynthesis, relative water content, and transpiration rate, was calculated from the formula, $C = 1/r_{ad}$ where r_{ad} is the adaxial leaf diffusive resistance.

Relative water content. After diffusive resistance had been measured, the leaves were severed below the ligule. A healthy leaf was paired with an infected leaf, and both excised leaves were immediately sealed together in a small plastic bag, which was placed on ice in a

chest cooler.

In the laboratory, a specific sequence for handling each leaf was employed to minimize sampling error. Only one leaf was removed from the bag at a time, the infected leaf first. Removal took place within an enclosed chamber lined with saturated paper towels to maintain high humidity. Four 1.5 cm segments were cut from each leaf using a pre-cut template. One segment was from the basal region, two from the middle region, and one from near the tip of each leaf.

Each leaf segment's fresh weight was determined immediately after excision and subsequently wedged between two strips of water-saturated open-celled polyurethane foam. This apparatus (Figure II-2) was modified from Catsky (8) to work with square wheat leaf segments instead of leaf discs. It consisted of a square plexiglass tray which enclosed a 2.5 cm layer of polyurethane foam. At 2 cm intervals, a 1.5 cm X 1 cm deep trough was cut for positioning the leaf segments. After insertion of all leaf segments, an opaque square piece of glass 0.5 cm thick was centered over the foam. It supplied enough downward pressure to ensure that all cut edges of the leaf segments were in direct contact with water. The entire apparatus was then placed in a clear plastic chamber containing 2.5 cm of water and sealed with a lid to maintain high relative humidity. The leaf segments were incubated at room temperature under illumination of 350 ergs/cm^2 for three hours.

Turgor weight was obtained by removal of each segment with flat

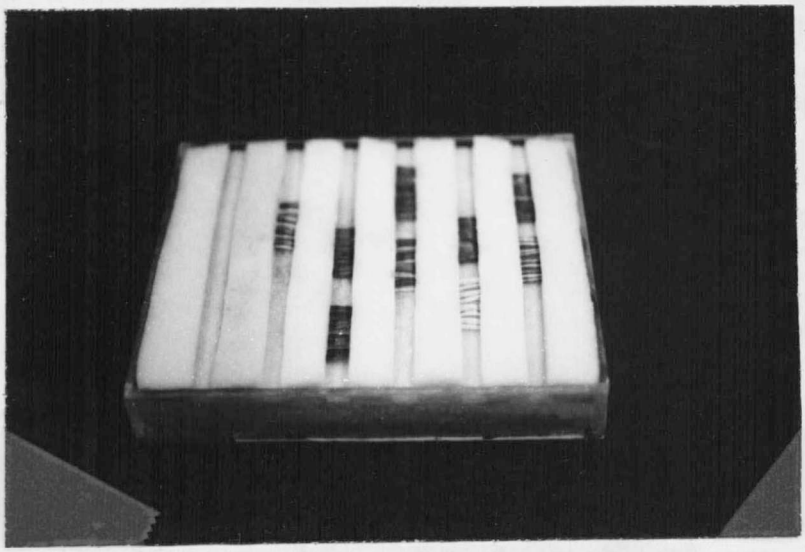


Figure II-2. Polyurethane foam device for saturating 1.5 cm X 1.5 cm segments cut from healthy and Cephalosporium gramineum infected winter wheat leaves.

tipped forceps, pressing it between six layers of Whatman No. 1 filter paper for 30 seconds, and weighing it. Leaf segments were incubated at 70°C in a mechanically convected drying oven for 24 hours and dry weights determined.

Relative water content was computed using the formula: (Fresh Weight - Dry Weight)/(Turgor Weight - Dry Weight).

Chlorophyll content. Chlorophyll was extracted from a separate population of paired healthy and diseased leaves using Arnon's procedure (1). Optical density readings of the chlorophyll-acetone supernatant were obtained from a Beckman Model 25 Scanning Spectrophotometer. Chlorophyll content was computed as milligrams of chlorophyll per unit leaf area. Leaf area was estimated as described above.

RESULTS

Cephalosporium gramineum infected flag leaves did not exhibit generalized wilting typical of other vascular diseases (11). Rather, reductions in water content, water vapor diffusion, CO_2 uptake, and chlorophyll content were linearly correlated with successive increases in the number of chlorotic stripes per infected leaf (Figure II-3). This and the highly significant correlations between all of the parameters with respect to symptom severity (Table II-1) suggested that the effects of vascular dysfunction led to localized water deficits only in the regions around heavily colonized vascular bundles. Non-invaded portions of the leaf continued to function in a normal manner.

No change in net photosynthesis was observed until after foliar symptoms appeared. The compensation point, where CO_2 uptake is balanced by CO_2 evolution, was attained when greater than 90% of the vascular bundles in a leaf were colonized. In completely blighted leaves, CO_2 exchange was expressed as a negative value. Thus, as symptom severity increased, respiratory activity rose in relation to photosynthesis until it predominated.

The consequences of water imbalance, along with the depression of photosynthesis, can significantly inhibit plant growth patterns and yield potential. Responses which measure these effects on vegetative growth include internode elongation and leaf expansion. Responses indicative of alterations in reproductive development include the number of

Figure II-3. The relationship between stripe formation in Cephalosporium gramineum infected flag leaves and net photosynthesis, relative water content, conductance, and chlorophyll content. Symptom severity was based on the number of stripes per leaf. 1 = one stripe per leaf, 11 = complete chlorosis. Each point is the mean of ten leaves.

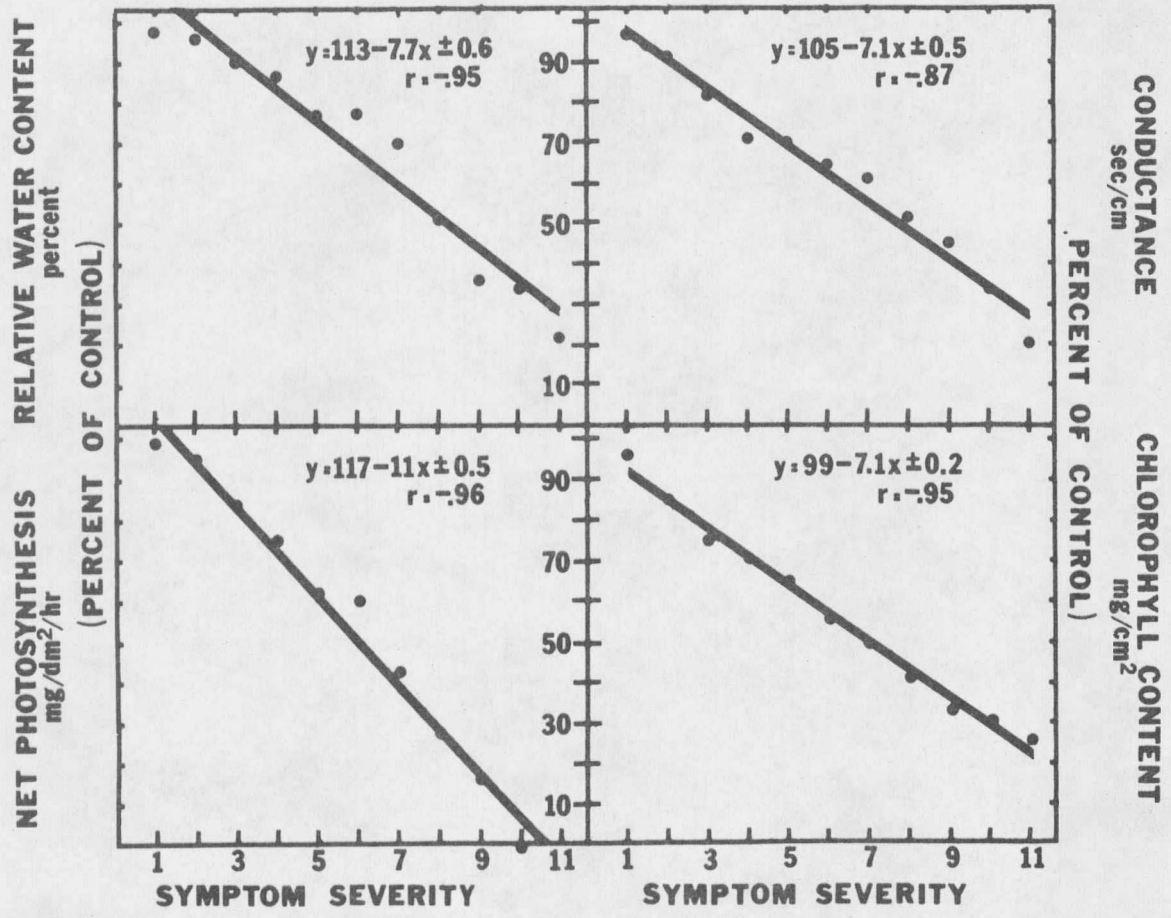


TABLE II-1. Correlations between net photosynthesis, relative water content, conductance, and chlorophyll content in Cephalosporium gramineum infected flag leaves of the susceptible winter wheat cultivar Marias.

	Correlation Coefficient[a]			
	A[b]	B	C	D
A[b]	---	.99	.98	.98
B	---	---	.96	.97
C	---	---	---	.96

[a] Calculated from the mean values represented in Figure II-2.

[b] A = net photosynthesis, B = relative water content, C = conductance, and D = chlorophyll content.

spikelets formed per head, the number of florets fertilized per head, and the extent of grain filling.

Internode elongation was severely restricted continuously throughout development of the two cultivars Marias and P.I. 278212 (Table II-2). Stunting was more pronounced during elongation of the internode between the penultimate and flag nodes and of the peduncle, suggesting that the cumulative effects of disease were most severe during head emergence. The response of Crest LRC 40 reflected a more moderate increase in rate of pathogen spread. Unlike stem elongation, there were no significant effects of pathogenesis on leaf areas in any of the winter wheat cultivars (Table II-3).

The heads of twenty primary tillers of each cultivar were examined to determine the effects of disease development on the yield components, spikelets/head, grains/head, and grain weight (thousand kernel weight)/head.

No change in spikelet number among the three cultivars indicated that the movement of C. gramineum had not progressed enough in the early stages of vegetative growth to evoke a stressed condition (Table II-4). By the time of flowering, however, disease severity had increased sufficiently to impose water stress on late maturing florets located near the apex and base of the heads. The most dramatic effect observed was a reduction in grain weight at plant maturity. Thus, the period of grain filling between flowering and senescence appeared to be most

TABLE II-2. Percent reduction with respect to healthy controls of consecutive internode lengths of winter wheats infected with Cephalosporium gramineum.

	Internode[a]					
Cultivar	I-4	I-3	I-2	I-1	Peduncle	Mean
Marias	35[b]	34	32	42	41	37
Crest LRC 40	4	6	11	17	16	11
P.I. 278212	28	28	27	47	40	35

[a] Internodes are numbered from below the flag node (I-1) downward.

[b] Mean percent reduction from healthy controls based on a sample size of 50 primary tillers of each cultivar.

TABLE II-3. Effect of *Cephalosporium gramineum* infection on leaf areas of three winter wheat cultivars.

	Leaf Area (cm^2)[a/]			
	Flag Leaf		Penultimate Leaf	
Cultivar	Infected	Healthy	Infected	Healthy
Marias	23.3	23.9	19.3	21.2
Crest LRC 40	17.1	18.0	20.5	20.8
P.I. 278212	33.5	34.1	30.8	31.6

[a/] The mean of ten leaves. Non-significant differences were observed at P = .05 using a paired T-test for all comparisons between healthy and infected leaves.

TABLE II-4. The effects of Cephalosporium stripe symptom development on winter wheat yield components and their relationship to the duration of photosynthesis of flag leaves following anthesis.

	Percent of Healthy Control[a]			
Cultivar	Spikelets/ Head	Seeds/ Head	Thousand Kernel Wt.	Duration of Photosynthesis[b]
Marias	100a[c]	96b	31a	35a
Crest LRC 40	100a	100a	65b	72b
P.I. 278212	100a	85c	33a	27a

[a] Mean of 20 primary tillers.

[b] Mean percent net photosynthesis over a 35 day period following anthesis from the data presented in Figure II-4.

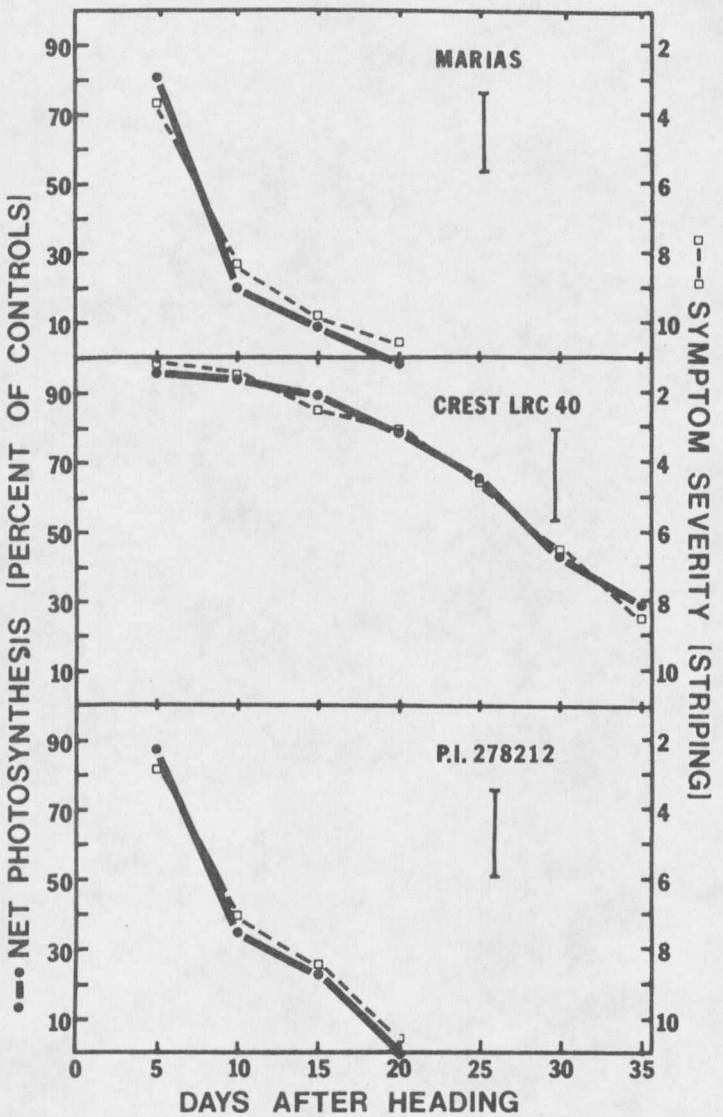
[c] For each column, values with the same letter are not significantly different at P = .05, according to Duncan's multiple range test.

seriously affected by pathogenesis. It is during this stage of plant development that symptom expression is manifested on the flag leaf, the peduncle, and the head (Chapter 3).

To monitor the rate of photosynthetic impairment in relation to increasing symptom severity, net photosynthesis was measured at five day intervals from the flag leaves of ten primary tillers of each winter wheat cultivar for 35 days following anthesis. Measurements were then terminated because of hail damage. Both Marias and P.I. 278212 exhibited a sharp decline in photosynthetic activity concomitant with the increase in chlorotic stripes (Figure II-4). By three weeks after heading, the flag leaves were completely blighted and photosynthesis was totally suppressed. The flag leaves of Crest LRC 40, however, continued to show photosynthetic activity, even 35 days after heading, although blighting eventually occurred. The close association between visual indexing of stripe development and net photosynthesis substantiates the close correlation between the two parameters illustrated in Figure II-3.

The duration of photosynthate production by the flag leaves of each cultivar was estimated by averaging the decline in net photosynthesis over the 35 day period (Figure II-4). CO_2 exchange was assumed to be zero after complete foliar blighting occurred, even though there were indications of some residual respiratory activity. Much of the lack in grain-filling in diseased plants as compared with healthy ones can be

Figure II-4. Relationship between stripe formation and net photosynthesis in Cephalosporium gramineum infected flag leaves of three winter wheat cultivars. Ten leaves of each cultivar were monitored for 35 days after heading. Symptom severity was scored by the number of stripes per leaf. 1 = one stripe per leaf, 11 = complete chlorosis. Vertical bars represent one standard deviation.



probably be attributed to the effects of disease development after flowering (Table II-4).

DISCUSSION

Disease-induced plant water deficits may be generated by alteration in the total plant water potential either directly, as in the case of increased resistance to water movement within the water conducting system, or indirectly as in the case of toxin-induced changes in membrane permeability, which alter solute potentials within affected cells (12). While vascular dysfunction in C. gramineum infected plants has been implicated in disease symptomatology (6,24,27,31), a toxin, Graminin A, also has been suggested as a cause of symptom development. Graminin A caused vascular discoloration and some chlorosis when it was administered to healthy plants (18).

The results of this study suggest that a toxin does not play a major role in disease development. The linear relationships between symptom expression and net photosynthesis, relative water content, conductance, and chlorophyll content, as well as the significant correlations between the four parameters throughout progressive stripe formation, indicate that only pronounced localized effects develop around extensively colonized vascular bundles.

The decline in RWC and conductance, which is controlled primarily by stomatal regulation (4,12,28), indicated localized internal water stress. Since both of these parameters were similarly affected by disease development, the main factor causing water deficits appeared to be reduced water supply. A diffusible toxin that altered membrane

permeability would have resulted in a poor correlation between RWC and conductance due to abnormal stomatal opening or closure (12,28). It remains unclear, however, whether the decrease in conductance immediately preceded or occurred simultaneously with the drop in RWC, since small temporal differences associated with the appearance of each new chlorotic stripe were difficult to detect. If diffusive resistance increased concurrently with the decline in RWC, toxin activity confined to the region around each colonized vascular bundle cannot be ruled out completely. It is doubtful, however, that the low molecular weight tetronic acid derivative isolated by Kobayashi and Ui (18) would be so spacially restricted. The temporal association between accumulation of fungal cells, gels, and gums with internal cell collapse and external chlorosis (Chapter 1, 31) suggests that the low molecular weight polysaccharide isolated from culture extracts of C. gramineum would be a more logical incitant of restricted lateral water movement (24,27).

Net photosynthesis can be suppressed by pathogen-induced water deficits in two ways. The first involves an increase in stomatal resistance, which restricts both the outward diffusion of water vapor and the uptake of carbon dioxide (4,28). The second involves localized disruption of chloroplasts, which would effectively alter the photochemical machinery of a leaf such that the Hill reaction, photophosphorylation, and the reductive pentose phosphate cycle are inhibited (4). Based on the highly significant correlations between net photosynthesis, RWC,

conductance, and chlorophyll content with respect to stripe formation, both responses are involved. Lawlor (19) determined that photosynthesis in wheat may be completely suppressed at a water potential of only -18 bars. Thus, localized water stress due to blockage of lateral water transport out of colonized vessels could be responsible for the drop in photosynthetic activity.

The linear relationships between symptom severity and the four physiological parameters provided indirect evidence that an interaction between successive leaves was not a significant host response. The penultimate and flag leaves of 15 primary tillers of the susceptible cultivar Marias were examined for decline in net photosynthesis and RWC in relation to symptom severity. Both leaves responded as predicted from the relationships shown in Figure II-3. Thus, each leaf responded independently of other leaves on a diseased tiller. Vertical movement of water in the culm was evidently not impaired, since wilting in leaves not yet invaded or in the early stages of infection was not observed. Such a response rules out high molecular weight substances as contributory toward water imbalance in this host-vascular pathogen interaction, since the extremely short vessel elements in the nodal regions (Morton, unpubl. observ.) would greatly facilitate a wilting response similar to that observed in elm trees treated with the high molecular weight toxin produced by Ceratocystis ulmi (30).

The collective disruptive effects of Cephalosporium stripe

development on the physiological processes of a wheat leaf were accurately reflected in the leaf's symptom severity score. This indicates that the disease index rating system is a valid indicator of visual disease severity. In comparing net photosynthesis with symptom development concurrently in three cultivars, the close relationship that existed between the physiological measurements and visual scoring of symptom expression makes either method suitable for delineating cultivar differences in disease severity. Evaluation of germplasm for resistance to Cephalosporium stripe, therefore, does not require the more complex and time-consuming procedures involved in measuring physiological responses. Rather, a direct visual scoring of symptom severity after heading suffices to accurately reflect the host's phenotypic response to infection.

Monitoring disease development at several sequential ontogenetic stages of host development was useful in partitioning the physiological effects of pathogenesis. Invasion by C. gramineum produced no visible effects on leaf expansion, yet caused a severe reduction in internode elongation. Both would have responded similarly if hormone imbalances (11) or generalized water stress (4,11) were involved. The differential responses elicited in the stem and leaves may be attributed to the temporal and spacial relationship between pathogen movement and host xylem maturation gradients (Chapter 1). By the time xylem in a leaf-node junction reaches maturity, the leaf has completely expanded. Thus,

the pathogen is effectively prevented from invading the leaf before it has attained maximum size. If the effects of pathogen colonization on host water balance result from localized lateral restriction of water movement, then there is no opportunity for water stress to be imposed by the pathogen in a developing leaf. Pathogen invasion of stem vascular bundles above a node, however, occurs during its period of elongation, so that reduced growth in that internode may be caused by cumulative water stress-induced localized effects around colonized vascular bundles. Undoubtedly, source-sink relationships involving the partitioning of photoassimilates between leaves and internodes during their respective periods of dominant growth also play a role in the differential response between plant organs (23). In fact, this competition for photosynthate may be more responsible for the severe reduction in elongation of the uppermost internode and peduncle than pathogen colonization of the stem. For the first 15 days after anthesis, the top internode competes for up to 50% of assimilates from the flag leaf (7). Foliar striping increases so rapidly in a susceptible cultivar that after 15 days, only 10% of the flag leaf is still actively photosynthesizing, thereby severely reducing the supply of photosynthate to both the top internode and to the peduncle. The moderate response by Crest LRC 40 may be due in part to colonization of fewer bundles in the internodal regions and also in part to more leaf area actively producing photosynthate during the first few weeks after heading.

The effect of Cephalosporium stripe infection on different stages of head development pinpointed the period in which pathogenesis most severely affected yield potential. Full expression of spikelet number per head is contingent upon the duration of photosynthetic area on lower leaves (10). The inability of disease to alter this yield component supported visual and histological evidence that pathogen movement and distribution was linked to host maturation gradients (Chapter 1). Source-sink relationships between consecutively expanding leaves and the differentiating head apparently kept apace with stripe formation. Complete genetic expression of grain number per head is dependent upon successful self-fertilization of each mature floret. Since floret development progresses from the middle of the head toward each end (3), the location of any abortive florets is an indicator of the stage during flowering at which detrimental effects of pathogenesis are felt the most. Reduction in seed number occurred only at both ends of the heads, indicating that water stress caused by disease was introduced only during late anthesis. Substantial reduction of carbohydrate storage in the grains of each diseased head suggested that the most severe effects of pathogenesis are expressed between flowering and senescence, when grain filling takes place. This verified earlier reports which concluded that yield reduction resulted from pathological responses late in host development (15,25). The flag leaf, peduncle, and head contribute up to 80% to carbohydrate production for grain filling (13,29), most of which

accumulates within the first four weeks after anthesis (26). It is not surprising, therefore, that the decrease in net photosynthesis concomitant with an increase in foliar striping of diseased flag leaves was largely responsible for the dramatic decline in thousand kernel weight of two susceptible cultivars. In addition, transport of assimilates to the head was undoubtedly affected along with inhibition of photosynthesis, both from the standpoint of reduced carbohydrate synthesis and also because of extensive phloem disruption in colonized vascular bundles (Chapter 1).

Seed weight reduction is visually manifested as reduced seed size. Consequently, seed size may be a useful selection tool, particularly when screening large bulk or recurrent selection populations. Selecting on the basis of seed size could be an effective, yet simple, means of identifying and evaluating resistance to Cephalsoporium stripe in winter wheat germplasm.

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CHAPTER III

IDENTIFICATION OF RESISTANCE TO CEPHALOSPORIUM STRIPE IN SELECTED WINTER WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS

INTRODUCTION

One of the most potentially damaging pathogens of winter wheat (Triticum aestivum L.), especially in regions practicing monoculture, is Cephalosporium gramineum, the causal agent of Cephalosporium stripe. Its potential for economic destruction is reflected in stunted plants, blighted leaves and heads, and reduced yields, which can reach 50% or greater (10,18). C. gramineum is a facultative soil-borne parasite, which overwinters as a saprophyte within infected plant residues carried over from the previous crop (3,4,22,23). Here it can remain viable in the soil for up to two years by producing a broad-spectrum antifungal antibiotic (4). Current control procedures have been oriented toward crop rotation, refuse destruction, and deep-plowing, all of which serve to reduce soil inoculum levels (3,11,17,24). Late planting, which minimizes root growth and hence infection sites available to the pathogen, also has been recommended (11,17). These cultural practices are not always dependable, however, since they rely upon favorable climatic conditions for successful implementation and are influenced by economic factors with respect to alternate crops. The most effective and desirable control method would be planting of resistant varieties.

Differential host susceptibility was first noted by workers in Japan (24). However, their observations were based on winter wheat materials planted in naturally infested fields where inoculum and virulence levels were unknown. In the United States, Bruehl (3) identified

four varieties as resistant to Cephalosporium stripe using hypodermic inoculations of a liquid conidial suspension into wheat culms above the crown, but they proved to be susceptible in the field under conditions of natural infection (19).

Until the development of oat kernel inoculum, genotypic differences, especially in large populations, were difficult to assess in the field. This technique called for addition of a defined quantity of oat kernels infested with C. gramineum isolates of known virulence with the seed at planting (12). Mathre and Johnston (14) used this inoculum to screen over 1000 hard red winter wheat cultivars from the major winter wheat growing areas of the world. Although most of these cultivars were of high or intermediate susceptibility, some promising sources of resistance were discovered. None of the cultivars tested, though, were immune to the disease.

The major criterion for evaluating susceptibility to Cephalosporium stripe has been reduction in yield potential (3,10,14,24). Other tests have included general visual disease readings (3,24), disease readings early in host development measuring the proportion of diseased tillers (12,14), and readings late in host development measuring the number of white heads (12,14). These parameters provided information necessary to differentiate cultivars across a graded series which extended from extreme susceptibility to high resistance (3,12,14,24). However, these criteria did not reveal the specific phenotypic response(s) expressed by

each genotype which, if recognized, might allow more discrete classification of cultivars into susceptible, intermediate, and resistant categories.

The purpose of this work was to identify the types and causative action of Cephalosporium stripe resistance elicited by selected winter wheat cultivars. Phenotypic expressions of resistance were evaluated in relation to important facets of the host-pathogen interaction and their value as selection tools in a germplasm development program.

MATERIALS AND METHODS

The seven hard red winter wheat cultivars chosen for this study varied in their susceptibility to Cephalosporium stripe and in their agronomic characteristics. Based on yield reductions, Marias (C.I. 27595) and Lancer (C.I. 23547) were rated as highly susceptible, Winalta (C.I. 13670) and C.I. 07638 were rated as intermediate, Crest Line Row Component (LRC) 40 (MT 7579) and P.I. 094424 were rated as moderately resistant, and P.I. 278212 was rated as highly resistant (14).

Unless otherwise specified, all tests were conducted at the Montana Agricultural Experiment Station near Bozeman, Montana. The cultivars were planted in early September of 1977 and 1978 at a seeding rate of 200 seeds per 3.1 m row. Each row was spaced 30.5 cm apart. A split plot experimental design with four replications was used, in which treatments comprised the main plots and the cultivars made up the sub-plots.

Inoculum consisted of either infested oat kernels or a liquid conidial suspension. The former was prepared by inoculating autoclaved oat kernels with a concentrated conidial suspension of C. gramineum, incubating them for 2-3 weeks, and then allowing them to air-dry (12). The oat kernels were added simultaneously with the seed at the time of planting. The liquid inoculum was prepared by growing the fungus in shake culture composed of modified Eckert's medium (20). One liter of 10^6 conidia/ml was added to each side of a 3.1 m row by directly pouring

the inoculum into a soil slice after cutting all of the roots with a sharp knife. Thus, two liters of inoculum were added to each row.

Populations of C. gramineum in field soil were quantified by dilution plating on selective green wheat agar (21). Soil samples were collected from the rhizosphere of Marias, Crest LRC 40, and P.I. 278212. Six replicates were obtained from six different rows planted in a randomized block design. Twenty gram subsamples were agitated in a Waring Blendor with 200 mls of distilled water for 20 seconds and subsequently diluted to 10^{-3} and 10^{-4}. Colony counts were read after 5 days incubation at 22°C.

Phenotypic expression of resistance to Cephalosporium stripe was scored according to (1) the number of tillers per row exhibiting disease symptoms 30 days after heading, (2) the number of tillers per plant exhibiting disease symptoms 30 days after heading, and (3) the rate and severity of disease symptoms at periodic intervals before and after heading. Symptom severity was rated on a scale of one to eleven, with one denoting a single stripe on a leaf and eleven indicating complete chlorosis (Chapter 1). To identify responses within individual plants, inoculated rows were thinned manually in the spring so that mitigating effects of different seeding rates were prevented.

Root growth of Marias, Crest LRC 40, and P.I. 278212 was measured by displacement in water and by dry weight. Single seeds of each cultivar were planted in a sandy-loam soil contained within Polyvinyl chloride

pipe sections 30.5 cm long and 3.8 cm in diameter. Four replications of five plants each were arranged in a randomized block design. The 60 pipe sections were enclosed in a rectangular-shaped enclosure, the floors and sides of which consisted of 2.5 cm wide styrafoam to remove temperature effects around the edges. The plants were grown for 60 days in an environmental growth chamber at 5/20°C (dark/light) with a 12 hour photoperiod (3.8×10^4 ergs/cm^2/sec combined incandescent and cool, white fluorescent light). After two months, the soil and roots were removed from the pipe sections by applying gentle pressure to one end. Much of the soil was first removed by careful washing with water, after which the roots were soaked in 0.05% sodium hexametaphosphate for five days. Calcium chloride was then added until the roots floated to the surface (H. Ferguson, personal comm.). The roots were collected, washed again by agitation in water, and then placed in a water-filled separatory funnel which was connected to a 10 ml pipette by rubber tubing. This allowed the determination of root displacement in water. Dry weights were obtained after incubating the roots in a drying oven at 70°C for two weeks.

RESULTS

Phenotypic expression of resistance. The manifestations of Cephalosporium stripe resistance were examined on a population basis (pathogen exclusion between plants) as well as on an individual plant basis (pathogen restriction within plants). The cultivars examined in this study demonstrated a differential response to the incidence of diseased tillers within inoculated rows (Table III-1). Although an almost two-fold difference in the number of infected tillers was observed for each cultivar between 1977 and 1978, the comparative differences between cultivars remained the same. Marias and Lancer were classified as susceptible. Crest LRC 40 and Winalta were intermediate, and C.I. 07638, P.I. 094424, and P.I. 278212 were resistant both years. Thus, environmental effects on infection between plants did not obscure inherent genetic differences between cultivars.

Five cultivars were employed to examine responses within plants to infection by C. gramineum. Thirty plants of each cultivar were rated for the incidence of infection among tillers of individual plants. Crest LRC 40 was the only cultivar which possessed the capability to prevent systemic invasion of all tillers within each plant (Table III-1). In all of the cultivars, late developing tillers occurred because of spacing effects. Some of these tillers did not express disease symptoms, which could account for much of the variation among cultivars. However, the significant reduction in disease incidence among tillers within

TABLE III-1. Differential responses of selected winter wheat cultivars to the incidence of infection by Cephalosporium gramineum.

	Pathogen Exclusion		Pathogen Restriction
	Diseased Tillers/Row[a/]		Diseased Tillers/Plant
Cultivar	1977	1978	1978
Marias	42a[b/]	77a	99a
Lancer	45a	--	--
Crest LRC 40	26b	53b	66b
Winalta	25b	49b	96a
C.I. 07638	15c	--	--
P.I. 094424	9c	27c	93a
P.I. 278212	5c	15c	90a

[a/] Values are represented as mean percentages of healthy controls across four replications.

[b/] For each column, values with the same letter are not significantly different at P = .05, according to Duncan's multiple range test.

plants of Crest LRC 40 cannot be attributed to this environmental effect, since many of the earlier maturing tillers were also disease-free.

Three representative cultivars were selected to examine responses within plants which could lead to a reduction in the rate and extent of symptom expression. Marias and P.I. 278212 were chosen because they represented the extremes of resistance to pathogen exclusion between plants. Crest LRC 40 was selected, not only because of its intermediate reaction to infection between plants, but also because of evidence that it restricts stripe formation on flag leaves during the first month after heading (Chapter 2).

Since the dynamics of this host response were more difficult to quantify accurately, varied approaches were taken to follow symptom development within infected plants. The rate of stripe formation was monitored by scoring the uppermost four leaves of primary tillers for symptom severity from three weeks prior to heading until two weeks after heading (Figure III-1). The extent of symptom development was determined by scoring thirty infected plants of each cultivar one month after heading. The severity readings for the uppermost four leaves of all tillers within each plant were averaged, so that a mean severity rating was obtained for each plant (Figure III-2). Measurement of height reduction and yield performance in relation to healthy controls provided indiations of differential responses to disease severity between cultivars (Table III-2).

Figure III-1. The rate of foliar stripe formation on the upper four leaves of primary tillers from three winter wheat cultivars infected with Cephalosporium gramineum. Symptoms were quantified using a severity index measuring the number of stripes per leaf. 1 = one stripe per leaf, 11 = complete chlorosis. Leaves numbered from flag leaf (L1) downward to fourth leaf (L4). A = Marias, B = Crest LRC 40, C = P.I. 278212.

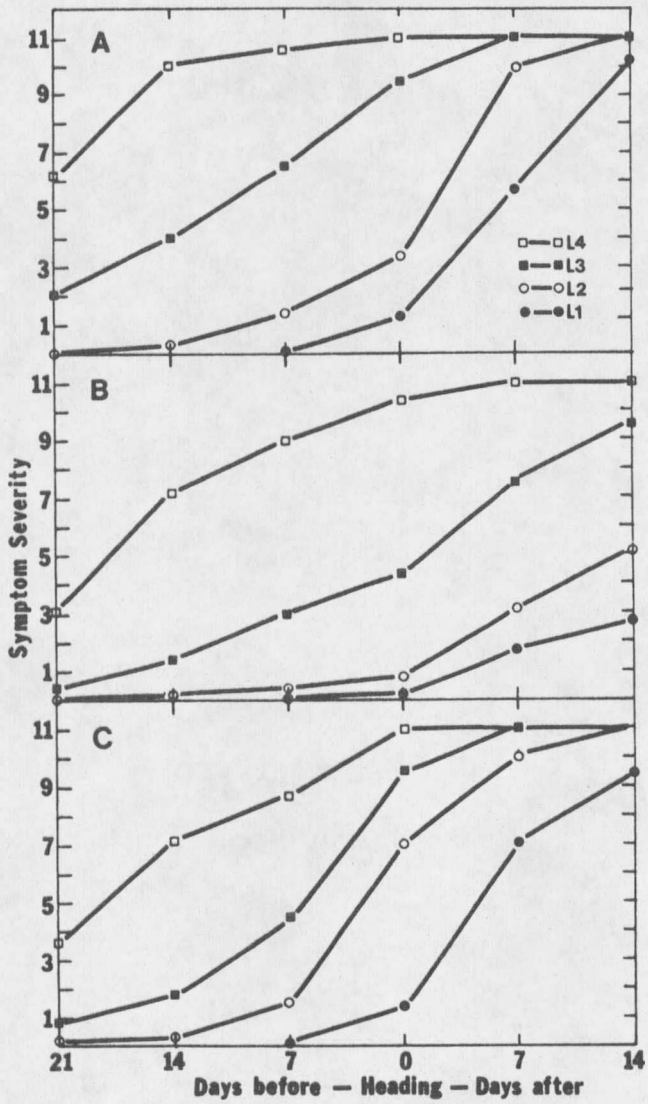

Figure III-2. The differential responses of three winter wheat cultivars to infection by Cephalosporium gramineum one month after heading. The uppermost four leaves of all tillers within each of 30 plants/cultivar were scored for symptom severity. These readings were averaged into a mean severity score for each plant. Severity was based on the number of stripes per leaf. 1 = one stripe per leaf, 11 = complete chlorosis. A = Marias, B = Crest LRC 40, C = P.I. 278212.

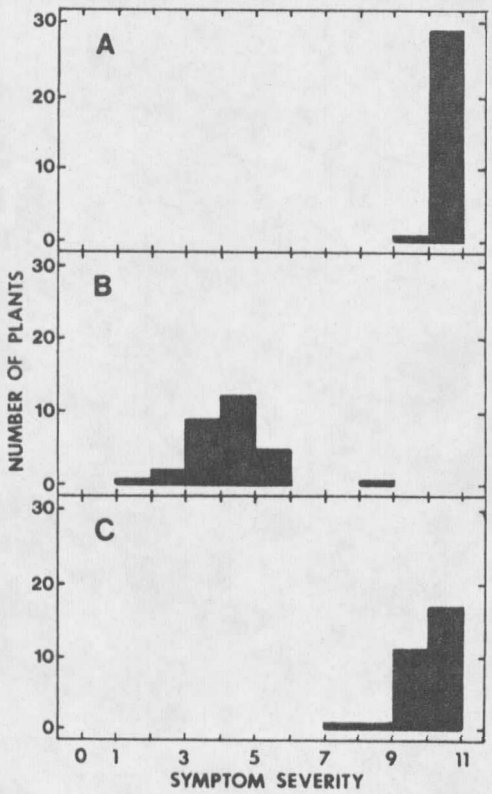

TABLE III-2. Effect of infection by *Cephalosporium gramineum* on height and yield of three winter wheat cultivars in 1978.

	Percent Reduction[a]	
Cultivar	Height	Yield
Marias	37a[b]	73a
Crest LRC 40	11b	34b
P.I. 278212	35a	79a

[a] Mean percentages with respect to healthy controls across four replications. Ten heads from infected main tillers were harvested within each replication.

[b] For each column, values with the same letter are not significantly different at P = .05, according to Duncan's multiple range test.

After successful invasion of the host occurred, both Marias and P.I. 278212 were equally susceptible to rapid systemic movement of C. gramineum throughout the vascular network. By fourteen days after heading, the flag leaves of the primary tillers of infected plants of both cultivars were nearly blighted. A similar temporal pattern occurred in all other tillers of these plants, as evidenced by the extent of symptom development a month after heading. In Marias, 29 out of the 30 plants exhibited total blighting. The remaining plant was almost as heavily affected, with a mean severity rating of 10. P.I. 278212 differed only slightly from Marias, as 93% of the infected plants had average severity ratings between 9 and 11. Both of these cultivars suffered substantial height and yield reductions.

Crest LRC 40, on the other hand, limited the rate of symptom expression to the extent that only the lowermost leaves of primary tillers were blighted 14 days after heading. Both the penultimate and flag leaves averaged severity scores of less than 5, indicating that less than half of each leaf expressed foliar chlorosis. Interestingly, the speed of stripe formation on the fourth leaf was similar to that observed on the fourth leaves of the two susceptible cultivars. Only as the pathogen moved up the plant did localizing responses become more effective. The extent of disease development in all tillers of Crest LRC 40 a month after heading was also greatly reduced as compared to Marias and P.I. 278212. Most of the plants displayed mean severity ratings between 3

and 6. In part, these lower scores were due to the significant number of disease-free tillers within each plant. The moderate response by Crest LRC 40 to height and yield reductions further substantiated this cultivar's tolerance to the physiological effects of pathogenesis.

Mechanisms of resistance. Two types of resistance appear to be involved in the expression of the three phenotypes identified in this study. The first, which is exemplified by P.I. 278212, prevents the successful invasion and colonization of the fungus in above-ground vascular tissues. It is termed pathogen exclusion. The second, which is exemplified by Crest LRC 40, confines the pathogen among tillers of individual plants and inhibits growth and sporulation within the vascular network after the fungus has initiated pathogenesis. It is termed pathogen restriction. Histological evidence that gelation, gummosis, or inhibition of sporulation activity curtail pathogen movement was presented in Chapter I.

Since C. gramineum is a soil-borne pathogen, exclusion of the fungus between plants may be due wholly or in part to soil-rhizosphere-pathogen interactions. Thus, the role of root wounding as a prerequisite for successful ingress of the fungus into the host was re-examined. Incidence of infection between plants of Marias, Crest LRC 40, and P.I. 278212 was evaluated in soils subjected to different environmental regimes both in the greenhouse and in the field.

The influence of soil microflora on infection in a controlled

environment in which mechanical root breakage did not occur was studied by transporting soil from the field into the greenhouse, where half of it was heat-sterilized. The three winter wheat cultivars were planted in a randomized design within each soil treatment block with four replications. To approximate seeding and inoculum rates in the field, 70 seeds and 7 grams of infested oat kernels were added together in each 1 meter row. In neither soil treatment was any substantial infection observed (Table III-3). Hence, the soils tested in this study did not appear to contain microorganisms capable of promoting infection by C. gramineum.

The possibility of root damage due to soil heaving in the field was eliminated by spring planting. Marias, Crest LRC 40, P.I. 278212, and a spring wheat, Lemhi, which is susceptible to Cephalosporium stripe when artificially inoculated, were planted with oat kernel inoculum in mid-April of 1977 at the Montana Agricultural Experiment Station near Bozeman. A randomized block design with four replications was used. Soil assays of rhizosphere propagule levels indicated that populations of up to 5×10^5 conidia per gram of soil were present during the first month after planting. Even with these high inoculum levels, less than 2% infection was evident. The winter wheat cultivars, being in a non-vernalized condition, expressed symptoms only on the outer, older leaves of the few plants that became diseased.

To substantiate the above findings under conditions where vernali-

colonization would occur, the three winter wheat cultivars were planted with oat kernel inoculum in early September of 1977 at Bozeman, Montana where severe winter conditions assured adequate root breakage in the spring from soil frost heaving; and at Davis, California in November of 1977, where a mild winter did not create the soil-freezing conditions required for soil heaving but did allow for plant vernalization. While extensive infection between plants was observed among cultivars at Bozeman, no infection was apparent at Davis (Table III-3). Assuming that soil frost heaving causes root breakage, these data provide evidence that root wounding is necessary for successful pathogenesis by C. gramineum.

This being the case, two mechanisms are envisioned which might affect the number of plants expressing disease symptoms: (1) physical and structural differences in root morphology or number, and (2) biochemical interactions between the host and fungal propagules in the rhizosphere. The first would affect the number of potential infection sites in the roots available to the pathogen, and the second would alter the inoculum potential in the soil around the roots.

Histological examination of root cross-sections using standard embedding, sectioning, and staining procedures (Chapter 1,9) and measurements of root mass by displacement in water and dry weight did not reveal any large differences in gross anatomy or in the extent of root growth between Marias, Crest LRC 40, and P.I. 278212. The number of

TABLE III-3. Effect of different soil environments relating to root injury or breakage on the percentage of plants infected with Cephalosporium gramineum.

Test	% Diseased Tillers/Row[a/]			
	Winter Wheat			Spring Wheat
	Marias	Crest LRC 40	P.I. 278212	Lemhi
	%	%	%	%
GREENHOUSE				
Sterile Soil	2	1	0	0
Non-sterile soil	1	0	1	1
FIELD				
Spring planted, Bozeman, Mont.	1	0	0	2
Fall planted, Bozeman, Mont.	77	53	15	--
Fall planted, Davis, Calif.	0	0	0	--

[a/] Values are represented as mean percentages of four replications.

roots produced by plants of each cultivar were not counted directly, but the lack of any dissimilarities in total root mass would suggest that physical differences in root growth patterns do not play a significant role in affecting infection percentages between plants.

To determine if these cultivars exhibited a differential response to mechanical root breakage, infection percentages were obtained from oat kernel inoculated rows exposed only to natural root wounding in the field and from oat kernel inoculated rows in which all of the roots were manually severed with a sharp knife in the spring after natural root wounding had occurred. Neither treatment differed substantially from the other (Table III-4), demonstrating that maximum root breakage probably occurred among all cultivars in the spring, regardless of physical factors such as root length or root tensile strength.

Inoculum levels in the rhizosphere of Marias, Crest LRC 40, and P.I. 278212 were monitored in the fall and spring, when propagule levels are high under natural field conditions (22). Wheat in each row was inoculated with 20 grams of infested oat kernels. Although propagule levels varied from 4×10^4 to 1×10^5 per gram of soil, no appreciable differences between cultivars were observed which could account for the disparities in pathogen exclusion between plants.

The effect of various inoculum levels on differential cultivar responses to infection between plants was examined by adding known quantities of infested oat kernels as an inoculum source to soil with

TABLE III-4. Effect of different inoculation procedures in the field on the percentage of tillers infected with Cephalosporium gramineum among three winter wheat cultivars in 1978.

Cultivar	Percent Infection[a] Natural wounding[b] & oat kernels	Root slice & oat kernels[c]	Root slice & liquid inoculum[d]
Marias	75	79	76
Crest LRC 40	48	52	77
P.I. 278212	21	17	76

[a] Mean percentages of tillers infected per row across four replications.

[b] Rows inoculated with 20 grams of infested oat kernels. Wounding due solely to soil frost heaving in the spring.

[c] Rows inoculated with 20 grams of infested oat kernels. In addition to root breakage from soil heaving, roots were severed on both sides of each row with a sharp knife in late March. No additional inoculum was added.

[d] Uninoculated rows sliced on both sides in late March with a sharp knife. Immediately after severing roots, two liters of 10^6 conidia/ml were poured into the soil slice.

no previous history of cropping to winter wheat. Under conditions of natural root wounding, a positive correlation existed between inoculum density and the percentage of tillers infected per row (Table III-5). The differential responses between cultivars, however, did not change. The same responses were noted when two isolates of the fungus were used, one of mild virulence (isolate #5) and one of high virulence (isolate #17). Thus, an inoculum density of 20 grams of oat kernels per 3.1 m row, which produced up to 1 X 10^5 propagules per gram of soil in the spring, cannot override the resistance mechanism affecting pathogen exclusion. Only when two liters of liquid inoculum concentrated to 10^6 conidia/ml were added immediately after manually severing the roots in the spring was the differential response between cultivars obliterated (Table III-4).

TABLE III-5. Effect of *Cephalosporium gramineum* inoculum density on infection of three winter wheat cultivars of differing susceptibility.

	% Diseased Tillers/Row[a]		
	Inoculum Density[b]		
Cultivar	5 gms	10 gms	20 gms
Marias	39	57	73
Crest LRC 40	30	38	50
P.I. 278212	14	18	24

[a] Mean percentages of four replications.

[b] The quantity of oat kernels infested with *C. gramineum* applied with the seed to a 3.1 m row.

DISCUSSION

Three phenotypic responses to Cephalosporium stripe by selected winter wheat cultivars were identified in this study. The first is expressed as a reduction in the number of diseased plants in a population. Soil conditions, the root system of the host, and infectious propagules of the fungus interact in a manner which excludes the pathogen from ingress into the host. The second causes a reduction in the number of diseased tillers within plants and the third reduces the rate and severity of disease development. Both of these latter responses involve host-fungus interactions after successful ingress such that the pathogen is restricted within the host. Based on the differential responses among cultivars, the exclusion and restriction types of resistance are independent of each other. P.I. 278212 is highly resistant in its ability to exclude the pathogen, e.g. a low percentage of plants become infected, yet it is highly susceptible to systemic movement of the pathogen, e.g. all tillers/plant become diseased and infected plants are severely blighted. Crest LRC 40, however, is susceptible to pathogen entry, e.g. most of the plants become infected, but it is moderately resistant to systemic movement of the pathogen, e.g. low percentage of tillers/plant become infected and the leaves and heads of diseased plants are only moderately blighted.

In heavily seeded rows, the percentage of tillers infected in a row reflected the percentage of plants infected/row for those cultivars in

which most or all of the tillers in each plant were diseased. In the case of Crest LRC 40, though, the percentage of tillers infected/row reflected differences to infection not only between plants, but between tillers of each plant as well. Therefore, the intermediate between plant reaction to pathogen infection by Crest LRC 40 may actually be a susceptible response which was biased toward a more resistant phenotype because of errors in distinguishing reductions in diseased tillers per plant. In order to effectively differentiate all three phenotypes in cultivars such as Crest LRC 40, lower seeding rates are required so that individual plants could be discriminated. This would partition pathogen exclusion responses from pathogen restriction responses.

Resistance, as manifested by low infection percentages between plants, was first attributed to root and/or crown regions of the winter wheat plant (13). Several cultivars, which were resistant when artificially inoculated through the roots, expressed susceptibility after stem inoculations. Reductions in infection percentages, concomitant with reduced inoculum densities, further indicated that the factor(s) governing resistance was inherent to the roots or crown. Resistance to root injury and/or reductions in root mass do not appear to be major factors influencing the number of potential infection sites available to the fungus. Mathre and Johnston (13) determined that the optimum period following root injury in which conidia can successfully gain ingress into the roots is one day. Possibly, cultivars may respond

to the rate of wound-healing, thus affecting the incidence of root infection by closing off infection sites. Otieno (15) observed high levels of conidia proximal to roots and root hairs prior to infection of seedlings germinated on petri plate cultures of C. gramineum. Similar localized pockets of conidia were observed next to roots collected in the field early in the spring (Morton, unpublished observ.). This distribution of infectious propagules could be altered significantly if cultivars differed in their capacity to synthesize the mucilaginous coating around the roots, which might serve to concentrate propagules around potential infection sites. The ability of liquid conidial suspensions to override the resistance response to pathogen exclusion could be attributed to high concentrations of propagules in the immediate vicinity of roots at the time of wounding. While this study failed to delineate major changes in the soil-root-pathogen interaction, the results do not rule out smaller, more significant localized effects within microhabitats in and around the soil-root interface.

Reduction in the percentage of tillers infected within plants was observed only in Crest LRC 40, which was also unique in its ability to restrict vertical and lateral movement of C. gramineum throughout its vascular network (Chapter 1). If the close association between phenotypes is more the circumstantial, then the same responses which slow down movement of the fungus in the culms and leaves of infected tillers might also be responsible for retarding systemic invasion of the fungus

between tillers. Limiting invasion between tillers of a plant may be loosely analagous to the dwarf bunt (Tilletia controversa)-winter wheat interaction, in which resistance is expressed by inability of the fungus to successfully colonize the growing point before the onset of stem elongation (6). In the case of the Cephalosporium stripe-winter wheat interaction, the fungus may need to enter the vascular system of each tiller prior to a restrictive host response. The crown region of a winter wheat plant is a complex aggregation of compressed nodes (16), which the fungus must traverse in order to invade developing tillers. The complex linkages and short vessel elements would influence pathogen movement and distribution in themselves. An active host response would impose additional barriers to the pathogen.

Differential responses between cultivars to percentage of diseased plants and to localization of pathogen movement have been identified in other vascular wilt diseases (1,8). It is doubtful, however, that the mechanisms regulating phenotypic responses are the same. Whereas other vascular pathogens of herbaceous annuals, such as Fusarium oxysporum and Verticillium dahliae, are capable of actively penetrating the root system of their respective hosts at different ontogenetic stages of development (7), C. gramineum passively enters the winter wheat root system only after it has been injured by soil heaving conditions in the spring. None of the responses related to hyperauxiny such as tyloses formation, vessel collapse, or cell proliferation (2,5) have been

observed in the vascular network of winter wheat plants infected with C. gramineum. Such significant differences in various facets of disease etiology suggest that Cephalosporium stripe cannot be compared readily with other vascular diseases. It follows, therefore, that the mechanisms and/or the inheritance of resistance may also not be comparable.

Many aspects of Cephalosporium stripe etiology have relevance to germplasm evaluation, especially in the context of selection procedures. Yield reductions have often been measured by harvesting all plants within individual rows (10,12,14). This procedure obscures phenotypic differences expressed as percentage of plants infected, percentage of tillers infected within plants, and alterations in the rate and severity of symptom development. All of these phenotypes are lumped together into a single measurement. Early visual disease readings have been recorded at a given time in the field without regard to differential heading dates between cultivars (12,14). This scoring procedure failed to account for these differences in maturation rates, which are closely associated with symptom development, regardless of susceptibility to disease (Chapter 1). For example, Marias and P.I. 278212 are equally susceptible to systemic invasion by C. gramineum after successful infection has occurred. When symptom severity is evaluated without knowledge of heading dates, the later maturing P.I. 278212 would appear to be more resistant than the earlier maturing Marias. In a germplasm development program, this would have two major drawbacks. First, it would obscure

genotypic differences ascribed solely to an active host response similar to that exhibited by Crest LRC 40. Secondly, it would favor the selection of late maturing cultivars at the expense of the more agronomically preferable early maturing cultivars. The white head readings made several weeks after heading (12,14) more accurately reflect genotypic differences in host response to disease incidence between plants.

The identification of distinct phenotypic responses to infection by C. gramineum establishes the groundwork with which parental materials and subsequent segregating populations may be more carefully screened for different manifestations of resistance. Both pathogen exclusion and pathogen restriction, being independently expressed, might be effectively combined to produce a superior genotype with the maximum potential to prevent, as well as check, infection by C. gramineum.

If this goal is to be attained, each phenotype must be recognized separately. To examine disease incidence between plants, white head counts or tallys of the number of diseased plants per row must be made. Disease incidence between tillers within infected plants, however, must be enumerated on space-planted materials. Both of these phenotypes should be appraised at least one month after heading, when disease symptoms are fully expressed. Yield performance may be used to measure cultivar responses to symptom severity, but only if infected plants within a row are examined. More definitive gauging of this phenotype would include evaluation of seed size, which reflects the extent of

grain-filling after anthesis, height reduction, or the rate of symptom development on flag leaves using a disease index rating system.

Mathre (unpub. results) observed up to 90% infection in inoculated rows planted to a susceptible cultivar at a seeding rate of approximately 50 seeds per 3.1 m row, suggesting that reduced seeding rates are not mitigating with respect to infection between plants. Therefore, by space-planting, identification of each phenotype and evaluation of cultivar responses are facilitated. Screening for parental materials requires space-planting, so that phenotypes are well defined prior to crossing. In addition, pedigree analysis and early generation testing currently employed at Montana State University (12,14) necessitates space-planted progeny rows. There is some question whether such an approach is the most effective method for increasing resistance, however, since the genetics of Cephalosporium stripe inheritance are unknown. If resistance is quantitatively expressed, then later generation testing of progeny populations may be more reliable. In such a program, some selection pressure may be applied on earlier bulk progeny populations by screening for seed size, which selects for disease-free plants as well as plants more tolerant to disease development. This approach would keep populations manageable over a number of selfing generations. Screening for seed size may also be effective in a recurrent selection program, should stable, genetic male-sterile sources in winter wheat be developed which could facilitate out-crossing.

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SUMMARY AND CONCLUSIONS

The etiology of Cephalosporium stripe was examined throughout the developmental growth stages of seven winter wheat cultivars, which were differentiated on the basis of the influence each had on various stages of the disease cycle. The maximum expression of disease symptoms is governed by optimal conditons for two biotic components, the host and the pathogen, and for two abiotic components, the soil and the atmosphere. Thus, the disease cycle may be partitioned temporally and spacially into two sets of interactions. The first takes place in early spring within the rhizosphere and is influenced by events surrounding the host-pathogen-soil system. The second takes place throughout the remainder of the growing season within the plant and is influenced by the host-pathogen interaction.

Exclusion of the pathogen, such that the percentage of diseased plants per row is reduced, is a type of resistance which may be intricately associated with events in the host-pathogen-soil system. Root mass, which would affect the number of infection sites available to the pathogen, and propagule levels in the rhizosphere, which would affect inoculum potential, were not significantly different between cultivars. These results, however, do not rule out the possibility of less discernible differential responses such as the rate of wound-healing in roots or localized propagule levels in the vicinity of the root-soil interface. Any influence on events associated with root breakage, which is

essential for ingress of the pathogen into the root system, would also affect this type of resistance. Of the cultivars examined, P.I. 278212, P.I. 094424, and C.I. 07638 were resistant to disease by excluding the pathogen while Marias, Lancer, Crest LRC 40, and Winalta were susceptible.

Restriction of the pathogen, such that either disease incidence among tillers of infected plants is reduced or the rate and severity of symptom expression is curtailed, represents a type of resistance associated with events in the host-pathogen system. In all cultivars, the systemic movement of C. gramineum was limited by maturation of the xylem network. At no time was the fungus observed penetrating immature, living vessel elements. In the case of Crest LRC 40, however, additional curtailment of the pathogen was evident between tillers, vertically up the culm, and laterally in the leaves. This active host response was thought to result either from localization of fungal cells by gelation or gummosis, from inhibition of conidial proliferation, or a combination of these two phenomena.

C. gramineum invades the various vascular bundle types of each node and its leaf of attachment in the same sequence. This repetitive pattern of colonization is visually apparent in foliar stripe formation. The pathogen was rarely observed in vascular bundles exhibiting no symptoms. Hence, chlorotic stripes were closely associated with proliferation of the fungus within colonized vascular bundles. Reduction in

relative water content, conductance, net photosynthesis, and chlorophyll content were directly correlated with stripe formation. Taken together, this data suggests that fungal colonization and proliferation interacts with the host to cause localized vascular dysfunction around and within infected bundles, which in turn inhibits the physiological activity of adjacent phloem and mesophyll cells.

The development of a disease index rating system, which quantified the number of chlorotic stripes on a leaf at a given time, allowed experimental relationships between symptom development and physiological activity of infected leaves to be monitored throughout the ontogeny of the host plants.

Identification of the two types of resistance to Cephalosporium stripe and their subsequent effects on the host-pathogen interaction have important applications in a germplasm development program. Exclusion of the pathogen and restriction of the pathogen are independently expressed among cultivars. This is not surprising if each exerts its influence at different times during the disease cycle. Each phenotype should be recognized and evaluated separately when screening for parental materials, but selected together in segregating populations so that both types of resistance are combined together into a single genotype.

Foliar symptom expression provides a powerful selection tool for evaluating all phenotypic responses, since it accurately reflects the physiological state of the leaf. Thus, more complicated and time

consuming measurements of physiological activity such as photosynthesis and water deficits are unnecessary.

Selection procedures must be used which recognize that the rate of symptom development is affected, not only by active resistance to the pathogen movement, but by the host's maturation gradients as well. Recording heading dates and making selections at a standardized interval after heading would ensure that the mitigating influences of host development are removed from consideration.