Etiology of Cephalosporium stripe in relation to the expression of resistance in cultivars of winter wheat (Triticum aestivum L.)
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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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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.

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 elongation was inhibited, but leaf expansion remained unaffected by disease. Differential responses between stem and leaves was ascribed to the relationship between pathogen movement and host xylem 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 moderately 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 CO_2 exchange of ten flag leaves of each cultivar 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.

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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. gramineum. 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

<u>Disease index rating system for evaluating symptom severity</u>. 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 <u>C. gramineum</u> 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

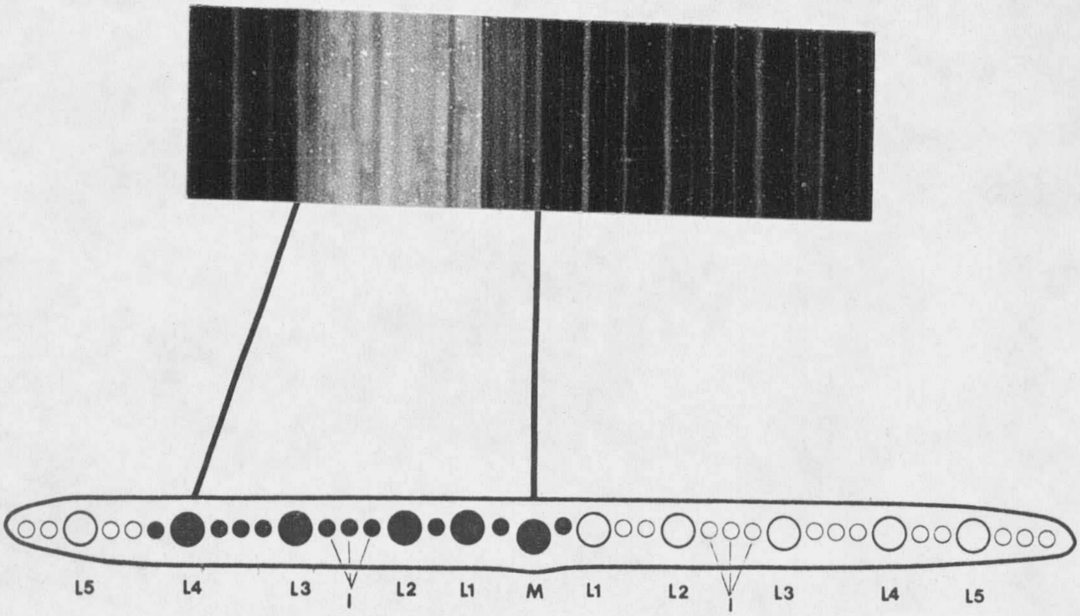

Figure I-1. Chlorotic striping pattern on a Cephalosporium gramineum infected winter wheat leaf and an illustration of a transverse section of that leaf showing the distribution of vascular bundles colonized. M = median vein, L = lateral vein, and I = intermediate vein. Ten lateral bundles were always 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 1-2A-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