Interactions of Puccinia striiformis and Mycosphaerella graminicola on wheat
by Ricardo Burrows Madariaga

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology
Montana State University

Abstract:
Puccinia striiformis and Mycosphaerella graminicola are frequently found attacking the same wheat leaf. The effect of one pathogen upon the other, and the effects of the interactions between pathogens upon the host-pathogen interactions are the subjects of these studies.

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Wheat leaves infected by P. striiformis remained green longer and were heavier than leaves infected by both pathogens. This may have been due to the sequestering effect known to be characteristic of rusts. It is possible than M. graminicola interferred with the redirection of translocation of assimilates that is a usual effect of rust.

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APPROVAL

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Ricardo Patricio Madariaga Burrows

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Puccinia striiformis and *Mycosphaerella graminicola* are frequently found attacking the same wheat leaf. The effect of one pathogen upon the other, and the effects of the interactions between pathogens upon the host-pathogen interactions are the subjects of these studies.

Seedlings of four spring wheat cultivars were inoculated at different time combinations of *P. striiformis* and *M. graminicola*. In susceptible cultivars, inoculations with both pathogens resulted in similar host tissue damage to the damage obtained by inoculation with each organism separately. The hypersensitive cultivar Anza responded in the same way to inoculation with *M. graminicola* regardeless or whether or not *P. striiformis* was present. Data from field plots inoculated with *M. graminicola* and naturally infected with *P. striiformis* confirmed data obtained in the glasshouse and growth chamber. A smaller amount of leaf area was damaged by *P. striiformis* when both pathogens were present than when this pathogen was present alone. Caution is needed in reading plants for stripe rust if septoria tritici blotch is present.

Wheat leaves infected by *P. striiformis* remained green longer and were heavier than leaves infected by both pathogens. This may have been due to the sequestering effect known to be characteristic of rusts. It is possible than *M. graminicola* interferred with the redirection of translocation of assimilates that is a usual effect of rust.

INTRODUCTION

"For good reason, research pathologists tend to study the effect of one disease at a time. In the real world, the occurrence of one disease at a time is the exception rather than the rule." (Zadoks and Schein, 1979).

Several disease interaction systems have been noted in wheat, such as root rot disease and speckled leaf blotch by Bensaude (1926), and Sprague (1950); stripe rust and glume blotch by Hyde (1978) and Van der Wal (1970); and septoria tritici blotch and powdery mildew by Brokenshire (1974). The presence of more than one pathogen at the same time on the same leaf can be seen as having additive or synergistic effects which result in less, the same, or more damage to the host, as compared to the effect of each pathogen acting separately. This effect can be measured as yield loss or in the epidemiological behavior of each pathogen.

The leaf tissue colonized by one pathogen is modified. Consequently, the substrate for a secondary infection is different from the original tissue. Hyde (1981), discussing the interaction between Septoria nodorum and Puccinia striiformis, reported that areas

first attacked by S. nodorum are probably no longer suitable for infection by the obligate parasite, P. striiformis. Furthermore, areas attacked by P. striiformis may possibly be infected by S. nodorum but such double infection of the same tissue has not been recorded.

Yarwood (1959), stated that plant pathogens are like other factors such as chemicals or mechanical damage which can predispose plants to infection by secondary unrelated plant pathogens.

Rust is one of the diseases of wheat which has received much attention in the literature. This is explained by its economic importance throughout the world. Hendrix (1967) reported P. striiformis in epiphytotic proportions in 1960 and 1961 in the Pacific Northwest Region of the USA. In commercial fields, losses were estimated to range from 20 to 75 percent. He reported losses in California, Montana, Utah and Wyoming of up to 40%. According to Doodson et al. (1964), the stripe rust epidemic which occurred in Holland on Heines VII in 1955 resulted in an estimated yield loss of 15 to 20 percent, in addition to the loss of quality.

Referring to the economic importance of Septoria diseases, Shipton et al. (1971), noted that few critical

studies have been conducted to establish the losses attributable to them. The same author estimated that S. nodorum together with S. tritici were responsible for up to a ten percent yield loss in fifty percent of the wheat belt of Western Australia in 1966. In autumn sown crops in the Canterbury province of New Zealand, reduction in yield of up to 40 percent has been attributed to S. tritici by Sanderson (1978).

Several countries reported heavy losses caused by S. tritici. Bahat et al. (1980), reported it as "a major wheat disease in many parts of the world, particularly on the Mediterranean sea coast, in South America, in the highlands of East Africa, and in Australia".

Volin (1971), noted that P. striiformis, in comparison with other rusts that attack cereals, is adapted to lower temperatures. The cool environmental characteristics of coastal and intermountain regions are favorable for disease development. The description of favorable conditions for stripe rust have similarities to those S. tritici requires and this coincidence explains the occurrence of these two diseases together in the field as reported by Mork (1982), in Oregon.

Most of the genetic improvement of cultivars is directed toward introduction of resistance to one

pathogen at a time. However, in field conditions cultivars must show simultaneous resistances to many pathogens in order to maintain high yield potentials.

The aim of this research was to study the behavior of Mycosphaerella graminicola with and without the presence of Puccinia striiformis in its common host, wheat.

REVIEW OF LITERATURE

Septoria tritici blotch

Taxonomy

Speckled leaf blotch, leaf spot or nebular spot are common names which have been used to describe the disease caused by *Septoria tritici* Rob. ex. Desm. The first name was coined by Weber (1922). He referred to the "dark colored and prominent" pycnidia which produced a speckled appearance. There was an agreement at the Septoria diseases workshop in Bozeman, Montana (August 2-4, 1983) to refer to the common name of the disease as septoria tritici blotch (Anonymous 1983). Correct citation of the deuteromycetes-anamorph state of the pathogen is *Septoria tritici* Rob. ex Desm.

The telomorph was not described until 1972 when Sanderson isolated it from New Zealand wheat stubble and was able to reproduce symptoms of this disease starting with ascospores born in pseudothecia. The two celled ascospores used measured 10 to 15 x 2.5 to 3 millimicrons and were referable to the genus Mycosphaerella (Sanderson, 1972). Correct citation of this Ascomycetes-telomorph stage is *Mycosphaerella graminicola* (Fuckel) Schroeter.

Environment and infection process

Overseasoning

After crop harvest *S. tritici* inoculum will remain in the stubble for a time, depending on the moisture conditions which, in a wet environment, will cause total discharge of pycnidiospores. The spores, in the absence of volunteer wheat or other susceptible species, will die.

The hypothesis of Septoria attacking other plant species has been studied by several authors. However, most of the testing of *S. tritici* on other plant species has been done by artificial inoculation without further studies of the epidemiological implications of a broadened host range (Table 1).

According to Sanderson and Hampton (1978), the ascospores of *M. graminicola* in New Zealand are first released six weeks after harvest. Therefore pseudothecia are the main structures that allow the fungus to survive the last part of the summer and to produce the primary inoculum for the next crop.

Table 1. Plant species reported as susceptible to *Septoria tritici* Rob. ex. Desm.

Plant species	Author
Agropyron repens	Teterevnikova and Bokhyan (1970).[2/]
Agrostis tenuis	Williams and Jones (1973).
Alopecurus pratensis	Derevyankin (1969).[2/]
Arrenatherum elatior	Brokenshire (1975).
Bromus mollis	Williams and Jones (1973)-b.
Bromus sterilis	Williams and Jones (1973)-b.
Dactylis glomerata	Zaprometoff (1926).[2/]
Festuca arundinaceae	Brokenshire (1975).
Holcus lanatus	Williams and Jones (1973).
Hordeum murinum	Brokenshire (1975).
Hordeum vulgaris	Brokenshire (1975).
Poa annua	Brokenshire (1975). Williams and Jones (1973)-b.
Poa pratensis	Brokenshire (1975), Weber (1922), Williams and Jones (1973).
Poa secunda	Sprague (1944).[2/]
Poa trivialis	Williams and Jones (1973).
Secale cereale	Derevyankin (1969).[2/] Sprague and Fischer (1952)[2/], Weber (1922).
Stellaria media [1/]	Prestes (1976).
Vulpia bromoides	Brokenshire (1975).

[1/] Only non Gramineae (Caryophyllaceae).
[2/] As cited by Prestes (1976).

Dissemination

Natural dissemination of the anamorphstate

Bahat et al. (1980), noted that all stages of the infection cycle, from pycnidiospore liberation, dispersal, penetration, and lesion development through pycnidial formation are dependent on moisture in the form of rainfall and dew.

Shearer and Smith (1978), using multiple regression, found that variation in rainfall was responsible for most (75%) of the variation in sporulation of S. tritici. Removal of rainfall from the model reduced the coefficient of determination to 13 percent. Murray and Martin (1978), found that the amount of rain in September and October at the Australian Agricultural Research Station in Temora gave the highest correlation with severity of S. tritici.

Scharen (1966) reported that spore exudation of S. nodorum commenced soon after wetting of leaves or straw and continued for three hours, then steadily diminished until cessation after six to seven hours. A similar situation was observed with S. tritici where after pycnidia formation, moisture induced pycnidiospore extrusion until the pycnidia were depleted. (Eyal, 1971).

According to Shipton et al. (1971), S. nodorum is disseminated by seed-borne pycnidia and pycnidiospores while seed-borne inoculum has not been demonstrated in S. tritici.

Artificial dissemination of the anamorph state

It is possible to induce an artificial epiphytotic of S. tritici by spreading wheat straw infested with pycnidia containing viable pycnidiospores, just after seedling emergence. (Bahat et al. 1980). Another method used to spread inoculum artificially is to use inoculum prepared in synthetic media as was reported by Shaner and Finney (1982). Conidia were collected from petri dishes of potato dextrose agar. The spore suspension was strained through two layers of cheesecloth and diluted to 10^6 spores per milliliter. Jenkins and Jones (1981), also used 10^6 and 5×10^6 spores per milliliter. Gough and Merkle (1977), used 6×10^6 spores per milliliter.

Natural dissemination of the telomorph state

One of the most important differences between pycnidiospore and ascospore dissemination is that the latter can be moved by air currents. Even in the absence of rain, Sanderson and Hampton (1978) found that free water during night time was sufficient to induce spore

release. The same authors noted that ascospores can serve as efficient inoculum of septoria tritici blotch. Eight months after harvest, pseudothecia were still capable of releasing spores. Sanderson and Hampton (1978) concluded that wind-borne ascospores initiated infections on young crops growing at a distance of several kilometers from wheat stubble.

Germination and latent period

Pycnidiospores and ascospores of M. graminicola germinate well in water. However, there is no reference in the literature which correlates the variables humidity, temperature and light to spore germination or to the latent period of this pathogen. Shaner (1976) noted that laboratory data were insufficient to support a model that would relate weather to disease progress in the field for septoria tritici blotch.

Weber (1922), reported the shortest latent period to be 11 to 15 days in May and June. Fellows (1962) noted that at 22 C, the lesions and first fruiting bodies appeared after 21 to 30 days. Holmes and Colhoun (1975), suggested that the latent period was as long as 60 days under winter conditions.

Shearer and Zadoks (1972), studying the latent period in S. nodorum, found that, apart from the variance

between the first appearances of sporulating pycnidia, there was also a variance within leaves with respect to appearance of successive sporulating pycnidia. In their work they noted, "These variances have been conveniently neglected..."; however, this point demonstrated the complications encountered in the study of latent periods.

Germination, penetration and latent period are determined by the interaction of humidity, temperature and other factors which are not well understood but which can modify the expression of these phenomena. Aust and Hau (1981), found that 45 percent of the variability in the latent period of S. nodorum could be explained by temperature, 12 percent by inoculum density, and only 3 percent by wetting periods. The remaining 40 percent could not be accounted for.

Environment

Light

Benedict (1971) studying the effect of light intensity on M. graminicola infection in wheat, found that between 500 and 4000 lux gave conditions favorable for pycnidia formation than other magnitudes of illumination. Intensities between 3,000 and 8,000 lux were optimal for early growth of hyphae in the substomatal chamber. Eight thousand to 15,000 lux induced

faster conidial germination and penetration as compared to a lower range of 500 to 4,000 lux and a higher range of 20,000 to 24,000 lux. According to Shaner (1976), 24,000 lux is 20 percent of the maximum illumination on a bright day at sea level, meaning that the fungus has a very low, but definite requirement for light intensity.

Humidity

Many authors agree that high moisture seems to be the most important environmental factor governing development of S. tritici. Free water is necessary for spore germination and for the spread of this pathogen to other leaf tissues. Soaking of the leaf also seems to be important to mycelial spread within the tissue (Eyal, personal communication). Hilu (1956), used a three to four day postinoculation dew period to obtain S. tritici symptoms. Hess and Shaner (1983) reported increases in severity of disease after increases in the postinoculation moisture period until up to 72 hrs, after which there was no effect.

Temperature

According to Hilu (1956), the incubation period of S. tritici is temperature dependent and can have a range of seven to 16 days, being longer at lower temperatures.

In summary, the information relating to M. graminicola and temperature present in the literature is shown in Table 2. Bahat et al. (1980) noted that certain phases in pathogen development of S. tritici are probably temperature dependent. However, specific information on the relationship between disease development and temperature during the wheat growing season is lacking.

Table 2. Effect of temperature on Mycosphaerella graminicola development.

Temperature C	Occurrence of feature
3	Minimum temperature for conidia germination Weber (1922) and Georghies (1974).
7	Two consecutive days at this temperature inhibited infection Renfro and Young (1956).
16-21	Infection favored Morales (1957). as cited by Shipton et al., 1971).
16-27	Disease development proceeded equally well Morales (1957) as cited by Shipton et al., 1971).
21	Optimum temperature for infection Fellows (1962) and Renfro and Young (1956).
22-24	Favored conidia germination (Weber, 1922).
27	Infection greatly reduced. (Narvaez, 1957) as cited by Shaner, 1976).
33-37	Maximum temperature for conidia germination (Weber, 1922) and (Georghies, 1974).

Penetration

Weber (1922) found that germ tubes penetrated the cuticle at a point directly above adjacent walls of the epidermal cells, suggesting that there was some evidence

of stomatal infection; however, such instances were quite rare and mycelium entering the stomata were not found to be directly connected to pycnidiospores of septoria. However, Hilu (1956) reported that the fungus usually penetrated either the opened or closed stomata and that direct penetration was observed only occasionally. Straley (1979) found that S. nodorum could penetrate closed or open stomata with or without the formation of appressoria. Direct penetration was also observed. Harrower (1978) noted that subsequent to spore germination, penetration by both septorioid fungi followed appressorium production above the junction of adjacent epidermal walls, and also reported penetration through either open or closed stomata.

Assessing of symptoms and signs

According to Khan (1978), three commonly used techniques were noted in the literature for assessment of S. tritici: 1) The foliar disease scoring scale of the CIMMYT Information Bulletin Number 38; 2) Rosielle's scale; and 3) a scale of percentage leaf area affected. A diagram of this percentage scale was made by K.M. Harrower. Eyal et al. (1983) compiled a diagramatic percentage scale to assess head and leaf coverage, a Septoria progression coefficient. This scale rates plant

and disease height and is composed of the Eyal-Brown scale to estimate pycnidial densities and the Clive James scale, which also related a percentage scale to leaf area covered by septoria tritici blotch.

Percent necrotic leaf area and pycnidial density per square millimeter were visually estimated 21 days after inoculation by Shaner and Finney (1982). Bahat et al. (1980), assessed S. tritici by estimation of disease severity as the percentage of green area of each leaf covered by S. tritici pycnidia and the maximum height (cm) at which pycnidia of S. tritici could be found on green plant tissue. Rufti et al. (1980), comparing symptoms on seedlings grown in a growth chamber to symptoms of mature plants infected with S. nodorum grown in the field, found that seedling and adult plant susceptibility were highly correlated (r^2=0.64 P< 0.01).

Physiology of the diseased plant

According to Harrower (1978), after S. tritici penetrates plant tissue, hyphal growth is intercellular and tissue response is characterized by chlorosis and subsequent necrosis of colonized tissue as well as an advance of the zone of colonization. He also noted that, prior to pycnidial production, hyphal aggregation occurs in the substomatal cavities of necrotic tissues.

Malcolm (1979), reported that the S. tritici infection process interferes with both translocation and photosynthesis, and concluded that the disruption of either in the plant must affect yield. To support this contention, he isolated a toxin(s) from a broth culture which caused symptoms characteristic of septoria disease in wheat. The toxin(s), because it was rich in serine, threonine, glucose, galactose and a methyl pentose and because it failed to pass through either a Diaflo ultrafilter or a standard dialysis membrane, was identified as a glycopeptide.

According to Malcolm (1978), this toxin acts directly upon the plant cell protoplasts, while causing little damage to cell walls. The death of the cells may be explained if the toxin is involved in the necrotic tissue caused by S. tritici. Fungal hyphal growth in the intercellular spaces can occur without cell penetration.

Bird and Ride (1981) reported that lignification probably reduced fungal growth of S. nodorum by reduction in penetration and in the rate of fungal colonization. In more resistant cultivars lignification may be even more effective. They noted that the administration of cyclohexamide, a translation inhibitor, or the application of ultraviolet light to unwounded leaves

prior to inoculation, reduced varietal differences making all varieties more susceptible. This suggests that the defense mechanisms may be induced and or may be dependent on protein synthesis.

Stripe rust

Taxonomy

Puccinia striiformis West. is classified in the class Basidiomycetes, the club fungi, order Uredinales. The name *Puccinia glumarum* (Schm.) Erikss. and Henn. was often used in early literature and is still frequently used in Europe. No alternate host is known.

Environment and infection process

Oversummering and overwintering

Sharp and Hehn (1963) found no evidence of stripe rust overwintering in native grasses adjacent to infected wheat fields in the Flathead Lake area of Western Montana. They suggested that these grasses were of minor importance to the epidemiology of stripe rust on wheat in this area. Dormant mycelia, which can survive on fall-infected leaves, were important only if these leaves survived.

According to Tollenaar and Houston (1967), stripe rust of wheat was found to oversummer in the Sierra

Nevada area of California, at altitudes of 1800 meters or more in wild grasses belonging to Elymus spp., Hordeum spp., and Sitanion spp. (Table 3).

Since P. striiformis is not known to have a sexual stage or an alternate host, the survival and production of the primary inoculum for the next season must be from uredomycelia on volunteer wheat or possibly by cross infection from susceptible grasses.

Dissemination

Natural dissemination occurs by air-borne uredospores originating from uredomycelia. Different methods have been suggested to induce artificial infection. Cartwright (1981) applied a fine covering of a mixture of P. striiformis spores and talc (1:5) with a paint brush to the adaxial surface of leaves of both seedling and adult plants. Mares and Cousen (1977) used the same mixture but sprayed the plant first with water and then dusted on the inoculum. Tollenaar and Houston (1967), used a nonquantitative method of rubbing uredospores on the newly unfolded primary leaves

Table 3. Hosts attacked by *Puccinia striiformis* West. in California. [1/].

Agropyron cristatum	*Hordeum leporinum*
Bromus carinatus	*Hordeum vulgare*
Bromus marginatus	*Phalaris minor*
Bromus unioloides = *Bromus catharticum*	*Poa pratensis*
Elymus condensatus	*Sitanion hanseni*
Elymus glaucus	*Sitanion hystrix*
Hordeum brachyantherum	*Sitanion jubatum*
Hordeum depressum	*Triticum aestivum*
Hordeum jubatum	

[1/] Compiled by Tollenaar and Houston (1967).

Uredospore germination

According to Sharp (1967), uredospores of *P. striiformis* are extremely sensitive to environmental fluctuations. The atmospheric ion concentration can cause lower uredospore germination during periods of moderate air pollution. During these periods, ions of larger size (mobility 0.0064 to 0.03 cm^2 $volt^{-1}$ sec^{-1}) were highest in concentration.

Macko et al. (1977) noted that the variation several authors observed in the germination characteristics of stripe rust uredospores could be related to the age and maturity of the spores, environmental and host conditions during spore formation, concentration and type of air ions and the ratio and levels of endogenous stimulators and inhibitors of germination. The self-inhibitor methyl

cis-3,4-dimethoxycinnamate, was isolated by Macko et al. (1977).

Environment

Light

Mares and Cousen (1977), reported that growth of rust colonies was favored by low light intensities, and stated that "weather conditions were unfavorable for rust development with clear, sunny days." Stubbs (1967) found that light intensity influenced the response of some wheat varieties to *P. striiformis*. More susceptible infection types were a result of a reduction in light intensities.

Humidity

Sharp (1965) noted that differences in saturation deficits (relative humidities) for different temperature conditions did not appreciably affect the infection process. Previous hydration of uredospores was an important factor to successful germination. Germination was highest when the spores were hydrated under saturated conditions for 24 hrs prior to inoculation. In this case, spore weight was approximately doubled due to water uptake. Hydration was equally effective when done in the dark or the light.

Temperature

Temperature seems to be the most determinant environmental factor in the P. striiformis infection process as indicated in Table 4.

Table 4. Effect of temperature on Puccinia striiformis West. as reported in the literature.

Temperature C	Effect
Subzero	Uredial state survived inleaves. (Eriksson and E. Henning, 1896) as cited by Tollenaar and Houston (1967).
Above zero	Minimum temperature for germination (Manners, 1950) and Newton and Johnson, 1936). 1/
2-5	Highest germination in dark(McCracken and Burleigh, 1962). 1/
10-13	Optimum temperature for in vitro germination (Manners, 1950); (Newton and Johnson, 1936), (Straib, 1940). 1/
12-14	Optimum temperature for yellow rust epidemics (Zadoks, 1965).
18-19	Growth of rust colonies are favored (Mares and Cousen, 1977).
18-22	Maximum temperature for germination depending upon the physiologic race (Straib, 1940). 1/
22.3	Ten day period at this mean temperature is lethal to P. striiformis (Tollenaar and Houston, 1967).
22-35	Unfavorable for P. striiformis (Mares and Cousen, 1977).
32.4	Ten day period at this mean maximum temperature is lethal to P. striiformis (Tollenaar and Houston, 1967).
34-42	P.striiformis was killed after 14 days (Kupriyanova and Zhokhova, 1981).

1/ As cited by Sharp (1965).

Penetration and fungal growth.

According to Hendrix (1967), the processes of germination and penetration take place over a six to 14 hour period. Penetration occurs via stomata. Direct penetration does not seem to occur. There is no formation of a distinctive appressorium (Sharp, personal communication). Mares (1979) suggested that the haustorium of the yellow rust fungus, like the haustorium of wheat stem rust, flax rust and several other rusts which have been studied, penetrated the host cell wall and caused invagination, but not penetration, of the host plasmamembrane. Cartwright (1981), noted that haustoria were produced more frequently in seedlings than in adult plants.

The direction of germ tube growth was reported by Cartwright (1981) to be predominantly across the axis of the leaf. Similar results were reported by Mares and Cousen (1977), who found that rust uredospores on the adaxial surfaces of the leaves, germinated by sending out a narrow germ tube which grew along the leaf surface at right angles to the leaf veins.

Cartwright (1981) analyzed fungal growth in different stages of the host. From the fifth leaf of unvernalized plants onwards, the size of the vascular

bundles appeared to discourage the growth of transverse infection hyphae. This restricted the fungus to the tissue between bundles, so that the characteristic longitudinal "striped" infection patterns developed between veins. In the second and third leaves of the seedlings, the direction of hyphal development was essentially random and non polar. However, on the fourth and subsequent leaves there was an increasing tendency for hyphae to grow toward the base of the leaf. Cartwright concluded that in the adult plant hyphal growth of any sort towards the tip of the leaf was rare and, if it did occur, it stopped after a very short distance.

Mares and Cousen (1977) noted a critical dependence of infection on leaf age at the time of inoculation. Under field conditions, the rate of disease spread was severely reduced on older leaves.

After penetration, according to Cartwright (1981), secondary, transverse runner hyphae crossed vascular bundles and started new infections in previously unpenetrated leaf areas. In susceptible host plants, allowing optimal development, germ tube growth as fast as 75 micrometers per hour and intercellular hyphae growth of five to ten micrometers per hour were observed

(Mengen, 1981). In some areas of the mesophyll, the growth of infection hyphae became particularly dense (Cartwright, 1981). Numerous haustoria were produced and a "bed" of uredia was formed. The formation of a pustule starts with the septation of a mother cell. The origin of uredospores seemed to be directly related to, and a consequence of, this septation (Cartwright, 1981).

Growth of P. striiformis mostly occurred in the mesophyll beneath the adaxial epidermis and pustules were found, therefore, mainly on the adaxial surface of the leaf. Sporulation on the abaxial surface occurred only in very advanced stages of infection. Even then the density of pustules was, on average, 60 percent lower than on the adaxial leaf surface. Spore production continued until such areas of the leaf were completely withered and dry (Cartwright, 1981).

Since P. striiformis infection is closely dependent on the presence of suitable stomata for penetration, the the number of stomata present on the adaxial surface will greatly affect the number of pustules that develop on that surface. Hayward (1948) as cited by Briggle (1967), reported that stomata occur in a ratio of about ten to seven on the adaxial and abaxial surface respectively. However, Salisbury and Ross (1978) suggested that

"...grasses usually have about equal numbers of stomata on both sides" of the leaves.

Physiology of the diseased plant

Mares (1979) summarized the host-pathogen relationship for stripe rust as follows, "...fungal components and infected host cells in a particular section of the leaf showed a series of morphological changes which correlated with a phase of active fungal growth, a phase of accumulation and storage of fungal and host reserve material, and a period of export of nutrient to the developing reproductive structures of the fungus." This haustorial activity may be modified by the gradual senescence of host leaf cells, by the export of metabolites from the leaf by a change in plant hormone levels, temperature and light intensities, and metabolites diffusing from host cells penetrated in an early stage of infection.

It has been shown that rust infection accelerates host tissue senescence (Mares, 1979). However, apart from the presence of a collar around the neck of some haustoria, there was little morphological evidence of host cell injury. The same author, in combination with Cousen (1977), reported host cell collapse to be confined to the mesophyll and usually to one or a small number of

isolated cells in the colony. Deposition of a collar of material at the point where the fungus enters the host cells is another common response of the host to the presence of haustoria. Little is known of the composition of the collar and its relationship to the host cell walls (Chong and Harder, 1982).

The rust pustule acts as a focus for the accumulation of many metabolites (Medgen, 1981). The fungus derives nutrients by alteration of the direction of normal phloem transport. It is also reported by MacDonald and Strobel (1970), that the starch content of P. striiformis infected wheat leaves increased in nine to twelve days, to twice that of healthy leaves. Micrographs of cross sections of these infected leaves indicated that starch accumulated in the chloroplasts of host cells adjacent to fungal hyphae.

To study the nutrient uptake of the rust fungi, the experimental approach must consider that the fungal mycelium grows only in restricted areas within the leaf (Medgen, 1981). The nutritional requirements of a rust fungi can be studied starting with the rust mycelium in axenic cultures. This method has defined the basic needs of a rust fungus (e.g., a balanced mixture of amino acids, a sulfur amino acid, different sugars, inorganic

nutrients and some minor nutrients such as steroids). Burrell and Lewis (1977), as cited by Medgen (1981), considered serine and alanine to be important in the successful establishment and maintenance of the rust fungus in the host.

It was reported by Strobel and Sharp (1965), that a difference existed in protein content of susceptible and resistant leaves of the wheat variety, Rego. This reaction was induced by incubation of inoculated plants at different temperature profiles.

Mares and Cousen (1977), suggested that host cell damage in incompatible interactions may be caused by a toxic product which diffuses from the site of the host cell-fungus interaction into adjacent host tissue. This idea has not been proven.

III. Interaction of microorganisms in wheat

Yarwood (1959) reported that plants attacked by one pathogen may be predisposed to attack by a second, unrelated microorganism. There are several reports in the literature regarding pathogen interactions in T. aestivum (Table 5).

Table 5. Microorganism interactions occurring in Triticum aestivum reported in the literature.

Presence of microorganism		Affected the incidence of	Author
1/	2/		
Gg	>	Mg	Bensaude (1926). Sprague (1950).
Rs + Gg	>	Mg	Sprague (1950).
Mg	<	Pr	Chester (1944)
Eg	>	Mg	Brokenshire (1974).
BYDV	>	Mg	Sanderson (1964). (Cited by Brokenshire 1974.)
Ln	-	Mg	Jenkins and Jones (1980).
Ph	>	Ln	Jones and Jenkins (1978).
Pr	>	Ln	Van Der Wal (1974).
Pr	-	Ln	Hyde (1978).
Ps	-	Ln	Hyde (1978).
Xt	-	Ln	Jones and Roane (1979).
Pc	<	Ln	Jones and Roane (1979).
WSMV	>	Hs	Adlakha and Raychaudhuri (1975).
F.spp	<	Hs	Tinline (1967).

1/ Gg=Gaeumannomyces graminis var. tritici
Mg= Mycosphaerella graminicola
Rs= Rhizoctonia solani
Es=Erysiphe graminis
Ln= Leptosphaeria nodorum
Ph= Pseudocercosporella herpotrichoides
Ps= Puccinia striiformis
Xt= Xanthomonas translucens
Pc= Pseudomonas cepacia
WSMV= Wheat Streak Mosaic Virus
F.spp= Fusarium species.

2/ >, <, -, indicates that presence of the first pathogen increased, decreased or did not affect the incidence of a second pathogen, respectively.

Plants infected by Gaeumannomyces graminis or G. graminis and Rhizoctonia solani were reported to be more susceptible to S. tritici infection by Bensaude (1929) and Sprague (1950) respectively (cited by Shipton et al. 1971). However, these field observations have not been confirmed in the laboratory.

Hyde (1981), in concluding his studies on the interaction between Leptosphaeria nodorum and Puccinia striiformis, noted that "...it becomes more evident that the possibility of interactions occurring between the effects of different pathogens attacking one host is a very complex phenomenon. The sites and timing of inoculations, level of infection, host age and many other host and pathogen characters probably affect the outcome, and methods of experimentation and assessment must also be important factors in interpreting the results." As a summary of several experiments, he noted that in leaves infected with both pathogens the proportion of leaf infected or killed was less than was predicted by adding the effects of the two pathogens occurring separately.

The only interaction between M. graminicola and a Puccinia spp. reported is by Chester (1944) in Oklahoma on the cultivar Cheyenne, which is susceptible to leaf rust (caused by P. recondita). The low incidence of leaf

rust that year was attributed to an early attack of S. tritici which destroyed leaf tissues first. However, the benefit of Septoria in diminishing leaf rust was greatly outweighed by its destruction of wheat leaf tissues.

The interaction between Septoria species (M. graminicola and L. nodorum) was studied by Jenkins and Jones (1980). They found no significant interaction between pathogens as determined by disease scores or by grain yield.

Sanderson and Hampton (1978), reported that the two pseudothecial states, Mycosphaerella and Leptosphaeria, commonly occurred on the same leaf. However, Jenkins and Jones (1980) noted that while speckled leaf blotch occurred typically at the earlier growth stages of the crop, glume blotch infected the flag leaf and ear. Results of work by Sanderson and Hampton (1978), showed that pseudothecia of Leptosphaeria nodorum matured earlier than those of Mycosphaerella graminicola.

A triple interaction was reported in oats by Pelletier et al. (1974), where the pathogens studied were Barley Yellow Dwarf Virus (BYDV), Puccinia coronata and Septoria avenae f. sp. avenae. Results indicated that plants inoculated with BYDV were infected at higher severity levels by S. avenae f. sp. avenae but P.

coronata was diminished. They explained that effect as due to the fact that *P. coronata* and BYDV are obligate parasites, while *S. avenae* is a facultative parasite and can use nutrients from senesced leaves.

When considering the simultaneous action of two or more plant pathogens such as *M. graminicola* and *P. striiformis*, one must evaluate not only the individual effects of the separate organisms, but also the effect of each on the action of the other.

MATERIALS AND METHODS

General procedures

Wheat cultivars having a suitable range of resistance and susceptibility to both *P. striiformis* and *M. graminicola* were selected for these interaction studies. Cultivars 'Lemhi', CI 11415; 'Baart', CI 1697; 'Anza', CI 15284; 'Wampum', CI 17691; and 'Lakhish' (Israeli), were used.

Plants were grown in a glasshouse maintained at 17± 2 C in a one to one mixture of sand and sterile clay loam soil in aluminum pans 20 x 20 x 5 cm. Supplemental light was provided by 400 watt metal halide lamps to assure a 12 hr photoperiod. Each pan was planted with 15 seeds of each cultivar along the border of the pan using an equal area for each cultivar. Plants were grown for 6-12 days and inoculated when they reached growth stage 12 (Tottman,1979). After disease symptoms appeared, nine plants per cultivar per pan were evaluated by assessing total non green area present on the oldest leaf. The presence of pycnidia also was recorded. Control plants which were not inoculated were maintained in all experiments.

A culture of *M. graminicola* (ORG-82076-1), obtained

from Hyslop Farm, Oregon Agricultural Experiment Station, was used exclusively. The Bozeman isolate of *P. striiformis* used in all experiments was collected in 1979 from a field of the wheat cultivar, Itana. Spores were maintained at 5 C in vacuum sealed tubes at the Montana Agricultural Experiment Station.

M. graminicola was increased in liquid media and adjusted to a concentration of 10^7 spores per ml of suspension. The liquid medium was prepared using 9g yeast extract + 9g sucrose and 900 ml distilled water. After five days on a shaker (Burrel Wrist-Action Shaker) at ambient laboratory temperature, abundant sporulation of the fungus was obtained. Plants were inoculated using a diaphragm pump connected to a DeVilbiss atomizer according to the method developed by Eyal et al. (1983).

P. striiformis tubes were opened two hours before inoculation and the spores were placed on a slide in a humid chamber at room temperature to allow hydration. Inoculation was performed by discharging a CO_2 gun loaded with 40 mg of hydrated uredospores twice in a settling tower to induce a slow and uniform precipitation of the spores on the plants located on the mobile surface below. After the first shot, this mobile surface was moved 180 degrees from its original position to ensure a more

uniform dissemination of the uredospores. Disease incidence was assessed by visual estimation of symptoms and signs of the disease using the modified Cobb scale. Reactions were coded as resistant (R) in the absence of pustules; moderately resistant (MR) when small pustules were surrounded by necrotic tissue; moderately susceptible (MS) when pustules were surrounded by chlorotic tissue; and susceptible (S) when no defense reaction in the plant was detected and when pustules developed freely.

A coefficient of infection was calculated using the infection types as multipliers S=1, MS=0.8, MR=0.4 and R=0.2. The number assigned to the infection type was multiplied by the percentage of leaf covered as estimated using the Cobb scale to obtain the coefficient.

Specific Procedures

Interaction experiment A

This experiment was designed to study disease progression in five spring wheat cultivars having different levels of susceptibility to the pathogens *P. striiformis* and *M. graminicola*. The wheat plants were inoculated at the same time with measured amounts of inoculum of each pathogen.

A suspension of budding conidia of *M. graminicola*

was adjusted to a concentration of 10^7 spores per ml of inoculum. Ten ml of inoculum was sprayed on each experimental unit containing five wheat cultivars. Plants were inoculated at growth stage 12 with both pathogens at the same time. M. graminicola was applied first; then the plants were placed in the settling tower for the P. striiformis inoculation.

The experimental design was a completely randomized experiment with four disease treatments, five cultivars and three replications. Disease treatments were: a) P. striiformis alone, b) both pathogens together, c) M. graminicola alone and d) control.

Three days after inoculation, plants were moved from the 10 C dew chamber to a growth chamber set at 4.5 C (dark) and 10 C (light). Twenty days after inoculation, plants were moved again to a growth chamber set at 16 C (dark) and 21 C (light).

At the time of rust inoculation, polyethylene squares 3 x 3 cm were placed at the same level as the plants at the base of the settling tower to determine the number of spores disseminated per unit area and spore germination percentages. The polyethylene squares remained in the dew chamber for 24 hours. They were removed and cut into five strips for microscopic

examination. Twenty one microscopic fields were counted on each strip. The number of germinated spores was related to the area of the microscopic field and the number of viable spores per area of leaf was determined.

Interaction experiment B

This experiment was designed to study the colonization patterns of a second pathogen when it was inoculated on tissues already infected by a primary pathogen. Seven treatment combinations and four cultivars (Lakhish, Anza, Lemhi, and Baart) were organized in a split-plot design with three replicates as follows: 1) no inoculation, coded 00 00; 2) M. graminicola inoculated at time 1, coded S0 00; 3) P. striiformis inoculated at time 1, coded 00 R0; 4) M. graminicola inoculated at time 1 and P. striiformis inoculated at time 2, coded S0 0R; 5) M. graminicola inoculated at time 2 and P. striiformis inoculated at time 1, coded 0S R0; 6) both pathogens inoculated at time 1, coded S0 R0; 7) both pathogens inoculated at time 2, coded 0S 0R. The first inoculation was made as seedlings reached growth stage 12, and the second was 17 days later. Incubation was in a growth chamber set at 18.5 C (light) and 2 C (dark) with a 12 hr photoperiod. Assessments were made 21, 26 and 31 days after the

initial inoculation.

After the last assessment, the same nine leaves which had been used to assess the symptoms were removed by clipping all of them equally at the auricle. They were then placed in an oven at 50 C for 72 hr and then weighed to determine dry matter.

Interaction at spore germination level

Germination of spores in the inoculum of both pathogens was observed on the polyethylene sheets as previously described, with treatment as follows: 1) uredospores of P. striiformis alone; 2) uredospores of P. striiformis in the presence of M. graminicola conidia; 3) uredospores of P. striiformis in the presence of sterile media in which the M. graminicola was grown. This experiment was performed using a completely randomized design with three treatments and three replications.

Field observations

Using natural infection of P. striiformis, which occurred in Bozeman during 1983, it was possible to collect information in two groups of spring and winter wheats inoculated with M. graminicola. Rows three meters long were planted on September 20, 1982 (winter wheats) and on April 25, 1983 (spring wheats). Each row, planted one per genotype, was divided in the middle; half for M.

graminicola inoculation and half kept as a control. To inoculate, a back-pack sprayer was used containing a concentration of 10^7 spores per ml of suspension. Five inoculations were made on the winter genotypes and four on the spring genotypes on a weekly basis. Inoculations were performed in the evening. To increase the dew on the leaf surface, the inoculated rows were covered for eight to ten hours on three consecutive nights after each inoculation using a plastic tarpaulin. Winter genotypes were evaluated on July 27, 1983 and spring genotypes on August 10, 1983. Most of the plants were between growth stages 65 (mid-anthesis) and 75 (medium milk development). *P. striiformis* was assessed using the modified Cobb scale and *M. graminicola* by recording a visual estimation of the necrotic leaf area on the flag leaves of each genotype. Results obtained were analyzed using regression analysis.

RESULTS

Interaction Experiment A

Results of previous studies (Madariaga, unpublished) indicate the importance of warm temperatures on shortening the latent period of *M. graminicola*. In this experiment a latent period of 23 days for *M. graminicola* was obtained by moving the plants from a cold regime of 4.5 to 10 C to a warm regime of 14 to 21 C.

Successful inoculation was obtained with both pathogens. Necrosis with the presence of pycnidia caused by *M. graminicola* was distinguishable from uredospore pustules of *P. striiformis* colonizing the same leaf. Stripe rust pustules close to necrotic areas of septoria tritici blotch had a tendency to change from the normal yellow-orange to a black-brownish color.

P. striiformis uredospore germination was variable. In this experiment the germination was 37.3 % with approximately 7.3 viable uredospores per mm^2 of leaf. Since the amount of leaf area affected by *P. striiformis* had a significant disease x cultivar interaction it was necessary to analyze each cultivar separately. In Table 6 results are presented of leaf area affected by the rust when the different cultivars were inoculated only with

Table 6. Percentage leaf area affected and coefficients of infection of five wheat cultivars infected by *Puccinia striiformis* in the presence or absence of *Mycosphaerella graminicola*.

	Days after inoculation					
	23		25		32	
Cultivar	Percent leaf area	Coeff. inf.[1/]	Percent leaf area	Coeff. inf.	Percent leaf area	Coeff. inf.
Lakhish	11 A[3/]	9 A	6 A	5 A	29 A	23 A
Lak. Mg. 2/	4 A	3 A	5 A	4 A	26 A	21 A
Anza	15 A	4 A	12 A	2 A	32 A	6 A
Anz. Mg.	9 A	2 A	10 A	3 A	1 B	0 A
Lemhi	40 A	40 A	54 A	54 A	71 A	71 A
Lem. Mg.	18 B	18 B	33 B	33 B	68 A	68 A
Baart	27 A	27 A	52 A	52 A	81 A	81 A
Baa. Mg.	13 A	13 B	19 B	19 B	56 A	56 B
Wampum	16 A	7 A	31 A	13 A	57 A	23 A
Wam. Mg.	3 A	1 A	3 B	1 B	19 B	8 A
C.V %	62	62	37	33	36	28
Comparison using orthogonal contrast:						
All cultivars	22 A	17 A	31 A	25 A	54 A	40 A
All cul. Mg.	9 B	7 B	14 B	12 B	34 B	31 B

1/ Indicates adjusted values using the coefficient of infection. Lakhish MS= 0.8. Anza R= 0.2, Lemhi and Baart S= 1.0 and Wampum MR= 0.4.

2/ Mg= indicates *P. striiformis* infection in presence of *M. graminicola*

3/ Pairs of values vertically followed by the same letter are not significantly different as determined by LSD($P< 0.05$) or T-test ($P< 0.05$) in case of orthogonal comparison.

P. striiformis (treatment a) and with *P. striiformis* + *M. graminicola* (treatment b). Also included are the adjusted values using the infection type observed in each cultivar.

There was a progressive increase of leaf area affected by rust after inoculation on the five cultivars studied. Thirty-two days after inoculation, susceptible cultivars Lemhi and Baart showed higher coefficients of infection (71 and 81 respectively) than the moderately susceptible Lakhish and Wampum (23) and resistant Anza (6) as determined by the Duncan's New Multiple Range Test ($P < 0.05$).

The significant interaction between *P. striiformis* and *M. graminicola* was evident in the lowest amount of leaf area affected by *P. striiformis* in some of the cultivars when *M. graminicola* was also present. With the cultivar Lakhish, it was not possible to detect an interaction difference when it was infected by *P. striiformis* alone or in the presence of *M. graminicola*. The cultivar Anza, 32 days after inoculation, showed differences in percent leaf area affected. However, this cultivar produced a resistant reaction to the Bozeman isolate of *P. striiformis*. It is possible that when leaves colonized by both pathogens were assessed,

necrotic tissue caused by *P. striiformis* was counted as being caused by *M. graminicola*. In the absence of pustules and pycnidia it was not possible to conclude whether the necrotic and chlorotic symptoms were due to *P. striiformis* or *M. graminicola*. The cultivar Lemhi showed a decrease in leaf area affected by rust in the presence of *M. graminicola* in the first two disease assessment dates, but differences disappeared by the last evaluation. With the cultivar Baart, there was a significant difference on all assessment dates in coefficients of infection. The level of *P. striiformis* on the cultivar Wampum tended to show a similar pattern to the other cultivars with a lower amount of rust when *M. graminicola* was also present. When all the cultivars were analyzed together, the presence of *M. graminicola* caused lower amounts of leaf area to be affected by *P. striiformis* and lower coefficients of infection by *P. striiformis* than when these cultivars were infected by *P. striiformis* alone.

In Table 7, the percentages of plants for each cultivar for each *P. striiformis* infection type are given. The presence of plants in category 0, the absence of disease, was possibly due to the method of uredospore inoculation. In some cases a few plants in the pans

Table 7. Percentage of plants in each *Puccinia striiformis* infection type inoculated alone or in combination with *Mycosphaerella graminicola*.

Cultivar	Infection Type 0 1/	R	MR	MS + S
Lakhish	33	0	41	26
Lak. Mg. 2/	44	0	52	4
Anza	22	78	0	0
Anz. Mg.	26	74	0	0
Lemhi	7	0	0	93
Lem. Mg.	19	0	0	81
Baart	0	0	4	96
Baa. Mg.	11	0	56	33
Wampum	7	0	82	11
Wam. Mg.	74	7	19	0

1/ *P. striiformis* infection type categories. 0= absence of disease; R= resistant; MR= moderate resistant; MS= moderate susceptible and S= susceptible.
2/ Response to *P. striiformis* in the presence of *M. graminicola*.

apparently did not receive inoculum. However, the presence of M. graminicola caused increases in the percentages of plants in category 0 in all cultivars studied. This may be the result of inhibition of uredospore germination caused by conidia of M. graminicola or other unknown reasons. In general, changes occurred in the percentages of plants in each P. striiformis infection type when M. graminicola was present. This tendency was more apparent in the first rust assessment 23 days after inoculation before the pathogens caused abundant necrosis. Except in the case of Anza, a resistant cultivar to the Bozeman isolate of P. striiformis , percentages of plants either susceptible or moderately susceptible to P. striiformis were reduced by the presence of M. graminicola.

In Table 8, the percentages of leaf area affected by M. graminicola are given. These percentages tended to increase, with time, in all the cases. Thirty-two days after inoculation Baart and Wampun showed significantly greater amounts of leaf area affected by M. graminicola than did Lakhish and Anza as determined by Duncan's New Multiple Range Test ($P < 0.05$). Leaf area affected in Lemhi, was not significantly different from the other cultivars.

Table 8. Percentage leaf area affected by *Mycosphaerella graminicola* in the presence or absence of *Puccinia striiformis*.

Cultivar	Days after inoculation 23	25	32
	2/		
Lak. Ps. 1/	0 A	2 A	19 A
Lakhish	0 A	1 A	12 A
Anz. Ps.	0 A	4 A	27 A
Anza	1 A	4 A	24 A
Lem. Ps.	4 A	11 A	25 A
Lemhi	2 A	5 A	46 A
Baa. Ps.	4 A	18 A	39 B
Baart	0 B	7 B	67 A
Wam. Ps.	3 A	15 A	34 B
Wampum	3 A	12 A	71 A
C.V. %	129	52	37
Comparison using orthogonal contrast:			
All cul. Ps.	2 A	10 A	29 B
All cultivars	1 A	6 B	44 A

1/ Indicates presence of *M. graminicola*.
2/ Pairs of values vertically followed by the same letter are not significantly different as determined by LSD ($P < 0.05$) or T-test, ($P < 0.05$) in case of orthogonal comparison.

Significant interactions between *M. graminicola* and *P. striiformis* were observed for Baart, and in the last disease assessment, for Wampum. At the first two disease assessments of Baart this interaction was positive with an increase in the amount of *M. graminicola* in the presence of *P. striiformis*. At the last disease

assessment the interaction was negative. It is possible that the first cold regime of incubation (4.5 to 10 C) favored *P. striiformis* and the shifting to the warm profile (16 to 21 C) induced greater activity in the necrotrophic *M. graminicola*.

Cultivar variability was observed in the ability to produce pycnidia of *M. graminicola* (Table 9), in the presence or absence of stripe rust. Wampum and Lakhish showed a tendency to have lower numbers of plants with pycnidia in comparison to the susceptible cultivars Lemhi and Baart as determined by Duncan's New Multiple Range Test ($P < 0.05$). The cultivar Anza showed a consistent absence of plants with pycnidia in this experiment. The presence of *P. striiformis* seemed to decrease pycnidial production significantly only in Lemhi 32 days after inoculation. At that time, fewer plants inoculated with both pathogens showed pycnidia (0.5) than plants inoculated with *M. graminicola* only. In the analysis of all the cultivars together there were fewer plants with pycnidia when plants where colonized with both pathogens (0.2 and 0.3), though differences were not significant at the 0.05 level.

Table 9. Ratio of plants bearing pycnidia of Mycosphaerella graminicola to total plants in the presence and absence of Puccinia striiformis.

Cultivar	Days after inoculation 23	25	32
	2/ 3/		
Lak. Ps.	0.0 A	0.0 A	0.1 A
Lakhish	0.0 A	0.0 A	0.2 A
Anz. Ps.	0.0 A	0.0 A	0.0 A
Anza	0.0 A	0.0 A	0.0 A
Lem. Ps.	0.1 A	0.2 A	0.5 B
Lemhi	0.2 A	0.3 A	0.9 A
Baa. Ps.	0.2 A	0.5 A	0.7 A
Baart	0.0 A	0.4 A	0.9 A
Wam. Ps.	0.0 A	0.1 A	0.2 A
Wampum	0.0 A	0.4 A	0.2 A
LSD 5%	0.2	0.3	0.3
C.V.	24	43	59
Comparison using orthogonal contrast:			
All cul. Ps.	0.6 A	0.2 A	0.3 A
All cultivars	0.6 A	0.2 A	0.4 A

1/ Ratio was calculated by dividing the number of plants with obvious pycnidia by the total number of plants in the treatment.
2/ Indicate presence of P. striiformis.
3/ Pair of values vertically followed by the same letter are Not significantly different as determined by LSD ($P < 0.05$) or T-test ($P < 0.05$) in case of orthogonal comparisons.

There was a relationship between the leaf area affected by M. graminicola and the number of plants with

pycnidia when analyzing all results. This relationship was positive and significant 23 and 32 days after inoculation (r^2= 0.6 and 0.5, respectively, $P < 0.05$) and indicates that leaf area affected by M. graminicola is associated with higher production of pycnidia.

Interaction experiment B

The aim of this experiment was to study the behavior of P. striiformis and M. graminicola when they were inoculated onto tissues which had been colonized first by the other pathogen. Different treatment combinations of the pathogens were inoculated at time one or time two. The results indicate that the cultivars used have different responses to these pathogens. The information collected therefore was analyzed separately for each of the four cultivars.

Cultivar Lakhish

Necrosis and chlorosis of leaf tissue eventually occured in plants without inoculation (Table 10). Compared to the control significant differences appeared in the first two disease assessments of treatment 00 R0 and the first disease assessment of S0 R0, both of which include P. striiformis inoculation at time 1. M. graminicola (S0 00) did not cause significantly greater nongreen area of Lakhish as compared with the control,

which confirmed a previous report (Eyal, personal communication) that this cultivar was resistant to this particular isolate of *M. graminicola*.

Table 10. Effect of different inoculum combinations of *Mycosphaerella graminicola* and *Puccinia striiformis* on nongreen area, rust severity and leaf dry weight of cultivar Lakhish.

Treatment Code	Nongreen area % Days after inocu. 21	26	31	*Puccinia striiformis* severity % Days after inocu. 21	26	31	Leaf dry weight mg.
00 00 [1]	15	30	65	-	-	-	43
S0 00	29	31	60	-	-	-	46
00 R0	38 * [2]	49 *	64	38	49	64	71 *
S0 0R	32	36	55	-	-	-	49
0S R0	14	37	47	14	37	47	52
S0 R0	37 *	39	61	19	14	20	54
0S 0R	9	37	62	-	-	-	44
LSD	20	19	17	31	32	22	21
C.V.	61	27	20	69	38	40	10

1/ First two characters indicate presence (S) or absence (0) of *M. graminicola* inoculated in time 1 or time 2. Second two characters indicate presence or absence of *P. striiformis* inoculated in time one or time two.
2/ Significantly different from 00 00 (control) as determined by LSD ($P < 0.05$).

As in interaction experiment A, the amount of leaf area affected by *P. striiformis* (S0 R0) in the cultivar Lakhish tended to be less when both pathogens were

inoculated together at time one compared with *P. striiformis* alone, (Table 10). The inoculation of *M. graminicola* at time two (treatment OS RO) did not significantly modify the leaf area affected by rust.

There was a change in the leaf dry weight of stripe rust infected and sporulating Lakhish leaves. This may have been due to a sequestering effect induced by *P. striiformis*. The leaf dry weights ranged between 43 and 71 mg, with the lowest figures being the control and the *M. graminicola* treatments and the highest figures being from inoculation with *P. striiformis* only (OO RO). It was noted that the moderate susceptibility to *P. striiformis* of Lakhish (OO RO) was expressed as a leaf weight increase of 68 % over the control. However the increase of the leaf weight of treatment SO RO was only 27 % indicating that there was an interaction between *M. graminicola* and *P. striiformis*. In the absence of *M. graminicola*, *P. striiformis* caused a greater increase in leaf dry weight. *M. graminicola* alone did not cause significant changes in the dry weight of leaves. The percentage calculations are shown in Appendix Table 20.

In Table 11 the leaf areas affected by *M. graminicola* are presented. Though no significant differences were found, the treatment of both *M.*

Table 11. Effect of different inoculum combinations of Mycosphaerella graminicola and Puccinia striiformis on leaf area affected and pycnidia produced by M. graminicola on Lakhish.

Treatment Code	% leaf area affected by M. graminicola			Pycnidia production Ratio 1/		
	Days after inoculation.			Days after inoculation.		
	21	26	31	21	26	31
S0 00 2/	29	31	60	0.9	0.7	0.6
S0 0R	32	36	55	0.7	0.2	0.6
S0 0R	18	25	41	0.7	0.5	0.3
LSD	21	17	25	0.3	0.4	0.3
C.V.	38	18	17	35	24	30

1/ Ratio= Number of plants with pycnidia / totalnumber of plants in the experiment.

2/ First two characters indicate presence (S) or abscence (0) of M. graminicola inoculated in time one or time two. Second two characters indicate presence or absence of P. striiformis inoculated in time one or time two.

M. graminicola and P. striiformis inoculated together at time one (S0 R0) tended to cause decreased amounts of leaf area with symptoms attributed to M. graminicola. The maximum number of plants with pycnidia was observed 21 days after inoculation. This was possibly the result of the longer dew period required for two inoculations. It has been observed that when the leaves were soaked with water, the amount of tissue affected by M. graminicola

and the number of plants with pycnidia both increased (Eyal personal comunication). In the later disease assessments the larger amounts of necrosis made pycnidia counts more difficult. This could explain the tendency to find fewer plants with pycnidia.

Cultivar Anza (Table 12).

Significant differences, compared to the control, were found for nongreen leaf area in most of the disease treatments. However, the presence of both pathogens inoculated at the same time (SO RO and OS OR) did not cause a significantly greater nongreen leaf area, with the exception of SO RO, 21 days after inoculation.

The amount of leaf area affected by P. striiformis was difficult to assess on cultivar Anza. When the pathogens were inoculated separately (SOOO and OORO), symptoms which appeared were presumed to be caused by the organism inoculated. However, with both pathogens together, one could not discriminate how much or what type of symptoms belonged to which pathogen since similar necrosis appears to have been caused by both fungi. Furthermore, there was no change in the behavior of the P. striiformis (pustule production) and / or M. graminicola (pycnidial production) when the pathogens were together in the same leaf.

Table 12. Effect of different inoculum combinations of *Mycosphaerella graminicola* and *Puccinia striiformis* on nongreen area, rust severity and leaf dry weight of cultivar Anza.

Treatment No.	Code	Nongreen area % Days after inoculation. 21	26	31	*Puccinia striiformis* severity % Days after inoculation. 21	26	31	Leaf dry weight mg
	1/							
00	00	12	24	70	-	-	-	43
		2/						
S0	00	44 *	63 *	87	-	-	-	45
00	R0	53 *	59 *	89	53	59	89	44
S0	0R	34 *	62 *	88 *	-	-	-	47
0S	R0	33 *	49 *	85 *	-	-	-	43
S0	R0	44 *	39	82	-	-	-	44
0S	0R	12	48	86	-	-	-	46
LSD		20	19	17				21
C.V.		61	27	20				10

1/ First two characters indicate presence (S) or abscence (0) of *M. graminicola* inoculated in time one or time two. Second two characters indicate presence or absence of *P. striiformis* inoculated in time one or time two.

2/ Significantly different to 00 00 as determined by LSD, $P < 0.05$.

Anza was resistant to the Bozeman isolate of *P. striiformis* (Table 12). There were no significant differences in the leaf dry weights. The absence of sporulation of *P. striiformis* in this cultivar could explain why no increase in the leaf dry weight occurred. Also there were no changes in the leaf weights of plants inoculated with *M. graminicola*.

Results obtained for the cultivar Anza inoculated with *M. graminicola* at time one are presented in Table 13. Significantly lower amounts of leaf area were affected by *M. graminicola* in combination with *P. striiformis* inoculated at time one (SO RO) than *M. graminicola* alone (SO OO) in the first two disease assessment dates. However, since the method of assessment used did not allow discrimination between the symptoms caused by the different pathogens, the analysis af the interaction was limited to the total nongreen leaf area and the leaf dry weight determinations.

The number of plants with pycnidia increased in the cold temperature regime of 2/18.5 C on Anza to a maximum of 20 percent observed 21 days after inoculation in treatment SO OR. Cold temperatures and a dew period of six days may have caused the unexpected pycnidial formation in this cultivar. In the treatment SO OR, once

again a decrease in the number of plants with pycnidia was observed. There were very few pycnidia per leaf produced in Anza.

Table 13. Effect of different inoculum combinations of *Mycosphaerella graminicola* and *Puccinia striiformis* on leaf area affected and pycnidia produced by *M. graminicola* on cultiver Anza.

Treatment Code	% leaf area affected by *M. graminicola*			Pycnidia production Ratio 1/		
	Days after inoculation			Days after inoculation		
	21	26	31	21	26	31
S0 00 2/	45 * 3/	63 *	87	0.0	0.0	0.0
S0 0R	34	62 *	88	0.2	0.1	0.0
S0 0R	14	39	82	0.0	0.1	0.0
LSD 5 %	21	17	25	0.3	0.4	0.3
C.V.	38	18	17	35	24	30

1/ Ratio= Number of plants with pycnidia/ total number of plants.
2/ First two characters indicate presence (S) or abscence (0) of *M. graminicola* inoculated in time one or timetwo. Second two characters indicate presence or absence of *P. striiformis* inoculated in time one or time two.
3/ significantly different to S0 R0 as determined by LSD, $P < 0.05$.

Cultivar Lemhi (Table 14).

Disease treatments generally caused significantly greater amounts of nongreen leaf area than were present on the control (00 00). Thirty-one days after inoculation the control showed a lower amount of nongreen leaf area (25%) than Lakhish and Anza (65% and 70% respectively). The presence of an interaction between pathogens on Lemhi was demonstrated in the significantly lower amount of P. striiformis when both pathogens were together (S0 R0) compared with P. striiformis alone (00 R0) (34% and 64% respectively). There was a significantly lower amount of leaf area affected by P. striiformis when this pathogen was inoculated at time two as compared with time one. This was possibly because older tissue was less susceptible to infection by P. striiformis.

Lemhi was the only cultivar in this study that showed a nonsignificant decrease (4 %) in leaf weight when M. graminicola (S0 00) was inoculated. In most disease inoculations there were significant increases in leaf weight of from 22% (S0 0R) to 84% (0S R0) (Appendix Table20). The interaction between pathogens was exemplified by a greater increase in leaf weight when plants were inoculated only with rust (00 R0) than when both pathogens were inoculated together (S0 R0).

Table 14. Effect of different inoculum combinations of *Mycosphaerella graminicola* and *Puccinia striiformis* on nongreen area, rust severity and leaf dry weight of cultivar Lemhi.

Treatment Code [1/]	Nongreen area % Days after inocu.			*Puccinia striiformis* severity % Days after inocu.			Dry leaf weigth mg
	21	26	31	21	26	31	
00 00	1	1	25	-	-	-	65
S0 00	16	51 *	79 *	-	-	-	62
00 R0	30 * [2/]	50 *	64 *	30	50	64	106 *
S0 0R	17	40 *	66 *	-	-	2	79
0S R0	25 *	49 *	74 *	25	49	74	119
S0 R0	26 *	53 *	75 *	13	25	34	90 *
0S 0R	0	1	54 *	-	-	19	88 *
LSD 5 %	20	19	17	31	32	22	21
CV.	61	27	20	69	38	40	10

1/ First two characters indicate presence (S) or abscence (0) of *M. graminicola* inoculated in time 1 or time 2. Second two characters indicate presence or absence of *P. striiformis* inoculated in time 1 or time 2.
2/ Significantly different to 00 00 as determined by LSD ($P < 0.05$).

Inoculation with *P. striiformis* always caused significant increases in leaf dry weight with the exception of treatment S0 0R, in which *M. graminicola* was inoculated at time one. Necrosis caused by *M. graminicola* could have prevented abundant sporulation and sequestering of photosynthates by *P. striiformis*.

Table 15. Effect of different inoculum combinations of *Mycosphaerella graminicola* and *Puccinia striiformis* on leaf area affected and pycnidia produced by *M. graminicola* on cultivar Lemhi.

Treatment Code	% leaf area affected by *M. graminicola*			Pycnidia production Ratio 1/		
	Days after inoculation			Days after inoculation		
	21	26	31	21	26	31
SO OO 2/	16	51 * 3/	79 *	0.9	1.0	1.0
SO OR	17	40	65	0.9	1.0	1.0
SO RO	13	29	41	0.7	1.0	1.0
LSD	21	17	25	0.3	0.4	0.3
CV.	38	18	17	35	24	30

1/ Number of plants with pycnidia/ total number of plants in the experiment.
2/ First two characters indicate presence (S) or abscence (O) of *M. graminicola* inoculated in time one or timetwo Second two characters indicate presence or absence of *P. striiformis* inoculated in time one or time two.
3/ Indicate means are significantly different to SO RO as determined by LSD, $P < 0.05$.

M.graminicola alone (SO OO) caused more leaf area to be affected, than when this pathogen was in combination with P. striiformis (SO RO) (Table 15.). Twenty-one days after inoculation, most of the Lemhi plants had abundant pycnidia. There were no significant differences in the number of plants with pycnidia after tissues were colonized by P. striiformis. Lemhi showed the greatest number of plants with pycnidia at the first disease assessment. There was no decrease in the number of plants with pycnidia as was shown in interaction experiment A in tissues colonized by both pathogens. The longer dew period utilized in this experiment could have accounted for this result.

Cultivar Baart (Table 16).

Baart showed similar behavior to Lemhi, with significantly greater nongreen leaf area in most of the disease inoculations than that exhibited by the control. The amount of leaf area affected by P. striiformis inoculated at time one (OO RO, OS RO and SO RO) was greater than when inoculated at time two (SO OR and OS OR). As in interaction experiment A, there were fewer P. striiformis pustules on plants inoculated with both pathogens together. Also, leaf weights of treatments which included P. striiformis inoculated at time one

(00 RO, OS RO and OS OR) tended to be higher than other treatments. P. striiformis inoculated alone (00 RO) caused an increase in leaf weight of 86% compared with 34% when these pathogens were inoculated together (SO RO) (Appendix Table 20).

Table 16. Effect of different inoculum combinations of Mycosphaerella graminicola and Puccinia striiformis on nongreen area, rust severity and leaf dry weight of cultivar Baart.

Treatment Code	Area nongreen % Days after inoculation			Puccinia striiformis % Days after inoculation			dry leaf weight mg
	21	26	31	21	26	31	
00 00 [1/]	0	2	31	-	-	-	80
SO 00	25 * [2/]	52 *	77 *	-	-	-	81
00 RO	36 *	60 *	77 *	36	60	77	148 *
SO OR	20	43 *	65 *	0	0	3	88
OS RO	40 *	72 *	81 *	40	72	81	169 *
SO RO	23 *	43 *	68 *	7	23	36	107 *
OS OR	1	1	58 *	0	0	13	105 *
LSD	20	19	17	31	32	22	21
C.V.	61	27	20	69	38	40	10

1/ First two characters indicate presence (S) or abscence (0) of M. graminicola inoculated in time one or timetwo. Second two characters indicate presence or absence of P. striiformis inoculated in time one or time two.
2/ Significantly different from 00 00 as determined by LSD ($P < 0.05$).

Leaf areas affected by M. graminicola are given in Table 17. The two pathogens together at time one (S0 R0) produced a significantly lower amount of M. graminicola for the last two disease assessments than for M. graminicola alone. In addition, fewer plants with pycnidia in treatment (S0 R0) were observed (21 days after inoculation) as compared with M. graminicola alone (S0 00).

Table 17. Effect of different inoculum combinations of Mycosphaerella graminicola and Puccinia striiformis on leaf area affected and pycnidia produced by M. graminicola on cultivar Baart.

Treatment Code	% leaf area affected by M. graminicola Days after inoculation			Pycnidia production Ratio 1/ Days after inoculation		
	21	26	31	21	26	31
S0 00 1/	25	52 * 2/	77 *	1.0 *	0.9	1.0
S0 0R	20	43	61	1.0	1.0	1.0
S0 R0	18	20	32	0.6	0.8	0.9
LSD	21	17	25	0.3	0.4	0.3
C.V.	38	18	17	35	24	30

1/ First two characters indicate presence (S) or abscence (0) of M.graminicolainoculatedintime one or timetwo. Second two characters indicate presence or absence of P. striiformis inoculated in time one or time two.
2/ significantly different to S0 R0 as determined by LSD ($P < 0.05$).

Interaction during spore germination

This assay was conducted to test the hypothesis that the presence of bud spores of M. graminicola would cause inhibition of P. striiformis uredospore germination. Uredospores of P. striiformis had 18 % germination when inoculated alone, 16 % germination in the presence of the M. graminicola medium and only 5 % in the presence of bud-spores of M. graminicola. Using an orthogonal comparison, the germination of the uredospores inoculated alone was significantly higher than when bud-spores were also present on the membrane. This indicated that the interaction between P. striiformis and M. graminicola possibly starts at the time of germination. (Appendix Table 21).

Field observations

The natural infection of P. striiformis in Bozeman, Montana in 1983 was very uniform and severe. Information was recorded pertinent to the interaction between P. striiformis and M. graminicola. The latter pathogen was inoculated and was restricted to the rows on which it was inoculated. Low relative humidity and cold temperatures present in this area were not favorable to the natural spread of M. graminicola.

In general, on winter wheats, the leaf area affected by P. striiformis decreased when M. graminicola was also present in the tissue (Table 18). The average infection level of M. graminicola was greater than that measured for P. striiformis. A result concordant with growth chamber studies was observed when rust infection types were separated into different categories. There was a decrease in the number of plants of the susceptible infection type (17 to 7 %) and an increase in the number of plants with no disease recorded (62 to 75 %) when readings were taken on plants infected only with rust compared to those when rust developed in the presence of M. graminicola.

On spring wheat, the relationship between P. striiformis and M. graminicola was negative and significant (Table 19). The levels of both rust and M. graminicola were slightly higher compared to the winter genotypes. The general trend, however, was a decrease in the amount of natural P. striiformis infection in the presence of M. graminicola, which was similar to results obtained with the winter wheat.

Table 18. Mean and range of percentage of leaf area affected by *Puccinia striiformis* and *Mycosphaerella graminicola* on 84 winter wheat cultivars. Bozeman 1983.

Part 1.

	P. striiformis Mg. present. 1/ A	*P. striiformis* Alone B	*M. graminicola* Ps. present. 1/ C
Mean	5	13	37
Maximum	40	80	80
Minimum	0	0	5

Correlations A vs. C = -0.12 (Non-significant)
A vs. B = 0.52 (significant at P< 0.05)

Part 2.

Treatment	Percentage of the total number of plants in each rust infection type. 2/ 0	R	MR	MS	S
Ps. and Mg.	75	0	7	11	7
Ps. only	62	1	1	19	17

1/ Ps., Mg. *Puccinia striiformis* and *Mycosphaerella graminicola* respectively.
2/ 0= absence of disease, R= resistant reaction, MR= moderate resistant reaction, MS= moderate susceptible reaction and S= susceptible reaction.

Table 19. Mean and range of percentage of leaf area affected by Puccinia striiformis and Mycosphaerella graminicola on 83 spring wheat cultivars. Bozeman 1983.

Part 1.

	P. striiformis Mg. present 1/ A	P. striiformis alone B	M. graminicola Ps. present 1/ C
Mean	13	27	39
Maximum	80	99	90
Minimum	0	0	0

Correlations A vs. C = -0.43 (Significant P< 0.05).
A vs. B = 0.70 (Significant P< 0.05).

Part 2.

Treatment	Percentage of the total No. of plants in each rust infection type. 2/ 0	R	MR	MS	S
Ps. and Mg. 1/	64	1	14	18	2
Ps only	36	2	12	30	19

1/ Ps. and Mg. Puccinia striiformis and Mycosphaerella graminicola respectively

2/ 0= absence of disease, R= resistant reaction, MR= moderate resistant reaction, MS= moderate susceptible reaction and S= susceptible reaction.

There was a decrease in the number of plants which showed infection type S (19 to 2 %) and an increase in the plants with infection type O (36 to 64 %).

The plastic tarpaulin which was used to cover the plots (inoculated with M. graminicola) to induce dew on the leaves sometimes caused unfavorably high temperatures. To measure this factor, a thermograph recorder was placed in the middle of the row in each treatment, covered and uncovered. The temperature measurement was made on July 22, a day without rain or wind and one in which the soil had been irrigated a day before. A maximum temperature of 17 C, which was observed in the covered treatment, was not unfavorable to rust since in susceptible cultivars large amounts of leaf area were infected. The minimum temperature recorded in the uncovered treatment (7 C) could explain the limited amount of natural spread of M. graminicola.

DISCUSSION

Wheat cultivars in the field will always be confronted with many constraints usually including several pathogenic microorganisms. Some pathogens have similar environmental requirements, and are, therefore, often found together. When two or more pathogens and diseases converge on a single wheat plant, they may interact to produce an outcome different from that which would occur if each were present alone. That is the case with *M. graminicola* and *P. striiformis*, the subjects of these studies.

Septoria tritici blotch, the disease caused by *M. graminicola*, is an important wheat disease in those parts of the world where a cool, wet environment exists during the vegetative portion of plant development. Stripe rust of wheat, caused by *P. striiformis*, occurs and causes damage in some of the same areas of the world where septoria tritici blotch is a problem. Sometimes the two diseases occur together on the same plants (Mork, 1982; Dubin, 1983) confounding research and causing losses in farmers' crops.

The restrictive cool environment (12 to 14 C) that favors epidemics of stripe rust (Zadoks, 1979) and the

ability of *P. striiformis* to survive at less than 0 C usually reduces the concurrent development of other economically important wheat leaf pathogens. Intercellular mycelia rapidly colonize leaf tissue, ramifying throughout the leaf, but favoring the newly developed tissue near the axil of leaf and sheath.

M. graminicola can infect tissues and grow in the exact same environment as *P. striiformis*, but does not move as rapidly through the tissue, often remaining confined to the tissues first penetrated when free moisture is limited. When moisture is adequate, however, *M. graminicola* can cause lesions that expand rapidly and occupy large areas of the wheat leaf (Chester, 1944).

Reactions of wheat cultivars used in these studies were varied when they were inoculated with *M. graminicola*. Pycnidia were formed in susceptible cultivars, but appeared in a "green island" on Baart, a phenomenon also observed by others (Beach, 1919). Inoculation of Anza resulted in a hypersensitive reaction exemplified by chlorosis and necrosis, but not pycnidia except after 30 days at temperatures of 4 to 10 C, leading one to suspect a toxic reaction.

Some isolates of *M. graminicola* are known to produce phytotoxins (Malcolm, 1978). The often observed early

onset of senescence may be a toxigenic reaction. Hypersensitive resistance, as in Anza, may be susceptibility to a toxin, while more compatible host-pathogen interactions may reflect a lesser response to a toxin, as was observed with infection of Baart.

When P. striiformis and M. graminicola were inoculated together, similar area was affected by the resultant disease to that obtained when either pathogen was inoculated alone. If the wheat was resistant or hypersensitive to either or both pathogens as in the case of Anza the results were not consistent. The interaction of the two pathogens was best demostrated on the susceptible cultivars Baart and Lemhi.

In Baart, most of the plants (93 %) showed a moderately susceptible infection type in the absence of M. graminicola. When this pathogen was also present in the tissue the percentage of plants with moderately susceptible reactions was only 33 %. Therefore with M. graminicola present, plants subsequently inoculated with P. striiformis exhibited a trend away from moderately susceptible toward moderately resistant or even further to no disease symptoms. The reduced germination of P. striiformis uredospores in the presence of M. graminicola conidia could, in part, account for this result.

However, cross protection by induction of general resistance mechanisms or nutrient depletion or imbalance are other possibilities. The interaction between *P. striiformis* and *M. graminicola* could have been an increase in resistance to *P. striiformis* due to some unknown mechanism triggered by the presence of *M. graminicola*. The result observed was simply premature destruction of the substrate by the necrotrophic *M. graminicola*.

In the resistant cultivar Anza, without *M. graminicola* inoculation, most of the plants (78 %) showed a resistant reaction (R) to *P. striiformis*. The presence of *M. graminicola* decreased this percentage slightly (74 %), but *P. striiformis* pustule production was not observed in either case. There was no modification in the ability to produce pycnidia in the resistant cultivar Anza due to the presence of *P. striiformis*. Brokenshire (1974) reported that the presence of *Erysiphe graminis* could modify the incidence of *M. graminicola* as a secondary pathogen. In this case, *M. graminicola* sporulated freely on a resistant wheat cultivar (Elite Lepeuple) when the powdery mildew fungus was present.

With a post-inoculation dew period of three days, 85 % of Lemhi plants inoculated with *M. graminicola* alone

produced pycnidia, while 45 % of plants produced pycnidia after inoculation with M. graminicola and P. striiformis. When the same cultivar was simultaneously inoculated with both pathogens, followed by a six day dew period, the difference in number of plants with pycnidia was lessened. This suggested that pycnidial production was associated with leaf wetting. In the longer wet treatment, 93 % and 70 % of the plants produced pycnidia 21 days after inoculation, when M. graminicola was inoculated alone and in combination with the stripe rust organism, respectively.

Pycnidial production for M. graminicola and uredospore and uredomycelia production for P. striiformis are some of the mechanisms which perpetuate the pathogen from one life cycle to another. Production of uredospores is related to higher demands of energy which result in a rise in respiration, a drop in C_6/C_1 ratio and abolition of the Pasteur effect (Daly, 1976). There is evidence that plants colonized by rust alter phloem transport with materials moving to more toward infected areas (Medgen, 1981; Mares 1979 and MacDonald and Strobel, 1970). Results of this work indicate that, for P. striiformis, the susceptible wheat cultivars Baart and Lemhi and for moderately susceptible Lakhish, the presence of stripe

rust increased leaf dry weight by 86 %, 64 % and 68 %, respectively . The presence of septoria tritici blotch and stripe rust caused an increase in dry leaf weight of only 34 %, 40 % and 27 %, respectively, when both pathogens were inoculated at the same time. The leaf dry weight of the resistant cultivar Anza was not significantly modified by infection with either pathogen alone or with both at the same time.

Hendrix et al. (1965) reported that stripe rust was more harmful than mechanical defoliation in spring wheats. This result is consistent with the sequestering effect which deprives nutrients from other plant parts by moving them to sporulating tissues.

In this work leaf area affected by P. striiformis was correlated with increased dry weight. Furthermore, it is possible that since M. graminicola interrupted the sequestering effect caused by the rust, the total effect of the pathogens in the host could be less damage than the effect caused by stripe rust alone.

This information suggests a danger in using multiple infection methods as proposed by Kilpatrick (1981) and Tomerlin et al. (1984) without further research on the physiology of the interactions. If the sequestering effect occurs by deprivation of nutrients from other

plant tissues, these plant parts will probably show a different behavior toward pathogens as compared to healthy tissues.

The relationship between leaf area affected by P. striiformis and dry leaf weight was different for each cultivar studied. The dry leaf weight of the resistant cultivar Anza was not changed according to the amount of leaf area colonized by either fungus . Leaf weight was significantly altered in the case of cultivars Lakhish, Lemhi and Baart. When stripe rust was the sole disease, or the first occurring, leaves were heavier than non-diseased controls. When septoria tritici blotch was first established in the leaves before P. striiformis was inoculated, leaves were not heavier than those from non-inoculated control plants.

The great variation that authors (Macko, 1977 and Sharp, 1972) have noted in the germination of stripe rust uredospores was also observed in this study. The presence of M. graminicola bud-spores caused a decrease in uredospore germination which could explain, in part, the reduced stripe rust observed in tissues colonized by both pathogens. An alternate explanation might be that since both organisms are stomatal penetrators, competition for stomatal openings may occur. The poor germination of P.

striiformis in the presence of M. graminicola might be a survival mechanism for the uredospores which do not germinate until suitable conditions are present. Alternatively, the uredospores could have been inhibited directly by a product of the metabolism of M. graminicola (toxin) or by other compound(s) produced in the host-pathogen interface.

With field observations it was possible to corroborate some of the results obtained from laboratory studies. In 1983, the Bozeman Montana fields were naturally infected with P. striiformis. Chester (1944) found that the presence of septoria tritici blotch caused a decrease in the leaf area affected by leaf rust. These results were similar to those found in Bozeman in 1983. Measuring flag leaf areas affected by P. striiformis showed that a decrease occurred in the area affected by P. striiformis, when the tissues were also infected with M. graminicola.

In the analysis of the plants as a population, without separating genotypes, a reduced number of plants of susceptible infection type was observed when these plants had been inoculated with both pathogens. It is possible that the presence of M. graminicola in the tissue of some genotypes does induce a "pseudoresistance"

reaction to *P. striiformis*.

Concluding remarks

By using seedlings of cultivars Lakhish, Anza, Lemhi and Baart the interaction between *P. striiformis* and *M. graminicola* was observed and studied. Temperature profiles favorable to stripe rust development also favor the development of septoria tritici blotch. The interaction of the two pathogens exists since they compete for the same plant tissue. When each pathogen is measured separately each produces more symptoms of disease than when the two pathogens occur together.

The peculiar pathogenic system of the biotrophic *P. striiformis* causes an increase in the dry leaf weight of infected seedlings that have sporulating pustules on their leaves, and ables them to sustain life and normal shape even longer than equivalent plants in control treatments with no inoculation. Necrotrophic *M. graminicola* causes a decrease in the leaf area affected by stripe rust, diminishing also its effect on leaf weight.

CONCLUSIONS

1. *P. striiformis* and *M. graminicola* are pathogens of wheat that can colonize the same tissue simultaneously.
2. The presence of *M. graminicola* caused a decrease in the leaf area affected by *P. striiformis*.
3. The pustules of *P. striiformis* changed color from yellow-orange to black-brownish when they were close to lesions of *M. graminicola*.
4. Presence of *M. graminicola* possibly caused a decrease in infection of *P. striiformis*. There were more plants that apparently escaped infection by *P. striiformis* when both pathogens were inoculated together.
5. Conidia of *M. graminicola* caused inhibition of germination of *P. striiformis* uredospores.
6. Anza showed few pycnidia in response to the Oregon isolate of *M. graminicola* and only when exposed to low temperature and a long period of time. The presence of *P. striiformis* did not change this result.

7. With one dew period of 72 hours at 10 C, Lemhi infected with M. graminicola alone tended to exhibit a greater number of plants with pycnidia than when P. striiformis was also present in the tissue.
8. If the tissues were colonized first by necrotrophic M. graminicola, susceptible cultivars Baart and Lemhi showed a reduced colonization by P. striiformis. This only occurred in new tissues toward the base of the leaf.
9. P. striiformis caused increases in dry weight of seedling leaves. M. graminicola did not. When P. striiformis and M. graminicola were together at the same time the increase was smaller than that caused by P. striiformis separately.
10. P. striiformis infection caused greater increases in dry leaf weights of the susceptible cultivars Lemhi and Baart than in the moderately susceptible Lakhish and resistant Anza.

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APPENDIX

Table 20. Increase in leaf weight expressed as percentage of the control (00 00) induced by the pathogens Mycosphaerella graminicola (S) and Puccinia striiformis (R) in four spring wheatcultivars.

Treatment Code 1/	Cultivar Lakhish	Anza	Lemhi	Baart
00 00	0	0	0	0
S0 00	8	6	-4	2
00 R0	68 *	3	64 *	86 *
S0 0R	14	4	22	11
0S R0	22	1	84 *	112 *
S0 R0	27	10	40 *	34 *
0S 0R	3	8	37 *	32 *
Mean	20	5	35	39

1/ The first two digits of the code indicate Mycosphaerella graminicola inoculated on time one or time two (S present, 0 absent). The third and fourth indicate Puccinia striiformis inoculated on time one and time two (R present, 0 absent).

* Significantly different from the control as determined by LSD ($P < 0.05$).

Table 21. Analysis of variance and orthogonal comparisons of percent germination of *Puccinia striiformis* uredospores tested on polyethylene membranes.

Number	Treatment	Mean % of germination n=9
1	Uredospores alone. (R)	18.4
2	Uredospores in presence of bud-spores of *Mycosphaerella graminicola.* (RS)	5.0
3	Uredospores in presence of liquid media.(Yeast extract+ sucrose) (RM)	15.8

Analysis of variance

Source of var.	d.f.	M.S.	F	P
Treatment	2	456.0	2.77	0.08
Residual	24	164.5		

	Orthogonal contrast			
	R vs RS		R vs RM	
Contrast	-13.44		2.68	
SE. contrast	6.05		6.04	
Sum Squares	813.40		32.00	
T (24)	-2.22	*	0.44	
P- Value	0.04		0.66	

* Significant at the 0.05 level of probability.