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.

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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.